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O-10-48-2

Phenotypic effects of transformed osmotin gene on the resistance of salt stress in Nicotiana tabaccum

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Osmotin has been classified as a member of the PR-5 type proteins of tobacco. Several environmental and hormonal signals are known to control the expression of plant genes. The osmotic gene, which was originally identified as salt (NaCl) induced, and later recognized as an osmotically induced. Patogenise-realted genes are regulated by a multitude of environmental and hormonal signals. These signals include adaptation to salt (NaCl), salt shock, ABA, ethylene, wounding, desiccation, UV light, cold, TMV and Fungi. Only few of signals such as adaptation to osmotic stress, treatment with ethylene and the fungal infection result in protein accumulation. We obtained osmotin gene from Kailash Banzal in biotechnology center-IARI, New Delhi which is supported with 35S promoter. This construct was transferred to Agrobacterium Tumefaciens and introduced to Nicotiana tabaccum by A. Tubefasens-mediated tobacco leaf disk transformation. Plants were regenerated directly from the leaf disks. Several independent primary transformant were obtained and examined by PCR. Transgenic plant was reproduced and then it was exposed to MS with 150,250,320 and 370 mM NaCl. We studied effects of salting on germination, rhizogenesis and callus formation. We put leaf discs in potent tissues of reproduction of rhizogenesis (0.1mg/l BAP, 1mg/l NAA), shoots (1mg/mlBAP.0.1mg/mlNAA) and callus formation (0.2mg/mlBAP, 2mg/mlNAA) with nontransgenic plants. We displayed that transgenic callus and root can growth in up to 150mMNaCl but with 320 and 370mMNaCl only transgenic shoots can growth. For examining we put transgenic roots, callus and shoots into the MS with 370mM NaCl. After two weeks, callus and roots were destroyed, only shoots survived. Results display that defiance and regeneration of shoot is more than callus and root.

Keywords: osmotin gene, salt stress, Nicotiana tabaccum, NAA, BAP

O-10-91-1

An "anti-cancer" herpes virus: Using one human enemy against the other!

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The Ras signal transduction pathway is a central hub for a variety of pro-oncogenic events with a fundamental role in normal and neoplastic physiology. In this work we were interested in linking Ras activation to HSV-1 replication in a direct manner in order to generate a novel oncolytic herpes virus which can target cancer cells. To establish such link, we developed a mutant HSV-1 in which the expression of ICP4 (infected cell protein-4, a viral protein necessary for replication) is controlled by activation of ELK, a transcription factor down-stream of the Ras pathway and mainly activated by ERK (extracellular signal-regulated kinase, an important Ras effector pathway). This mutant HSV-1 was named as Signal-Smart 1 (SS1). A series of prostate cells were infected with the SS1 virus. Cells with elevated levels of ELK activation were preferentially infected by the SS1 virus, as demonstrated by increased levels of viral progeny, herpetic glycoprotein C and overall SS1 viral protein production. Upon exposure to SS1, the proliferation, invasiveness and colony formation capabilities of prostate cancer cells with increased ELK activation were significantly decreased ($p<0.05$), while the rate of apoptosis/necrosis in these cells was increased. Additionally, high Ras signaling cells infected with SS1 showed a prominent arrest in the G1 phase of the cell cycle as compared to cells exposed to parental HSV-1. The results of this study reveal the potential for re-modeling the host-herpes interaction to specifically interfere with the life of cancer cells with increased Ras signaling.

Keywords: Ras, oncogenes, HSV, oncolytic herpes, prostate cancer

P-10-91-2

Silencing epidermal growth factor receptor variant-III (EGFRvIII) as a therapeutic strategy against cancer

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Epidermal growth factor receptor variant-III (EGFRvIII) is a constitutively active mutant form of EGFR that is expressed in 40-50% of gliomas and several other malignancies. Here, we describe the therapeutic effects of silencing EGFRvIII on glioma cell lines in- vitro and in-vivo. A SiRNA molecule against EGFRvIII was introduced into

EGFRvIII-expressing glioma cells (U87D) by electroporation resulting in complete inhibition of expression of EGFRvIII as early as 48 hours post-treatment. During EGFRvIII silencing a decrease in the proliferation and invasiveness of U87D cells was accompanied by an increase in apoptosis ($p<0.05$). Notably, EGFRvIII silencing inhibited the signal transduction machinery downstream of EGFRvIII, as evidenced by decreases in the activated levels of Ras and extracellular-signal regulated kinase (ERK). A lentivirus capable of expressing anti-EGFRvIII short RNA was also able to achieve progressive silencing of EGFRvIII in U87D cells in addition to inhibiting cell proliferation, invasiveness and colony formation in a significant manner ($p<0.05$). Silencing EGFRvIII in U87D cultures with this virus reduced the expression of factors involved in epithelial-mesenchymal transition (EMT) including N-Cadherin, B-Catenin, Snail, Slug and Paxillin, but not E-Cadherin. The anti-EGFRvIII lentivirus also affected the cell cycle progression of U87D cells with a decrease in G1 and increase in S and G2 fractions. In an in-vivo model, tumor growth was completely inhibited in SCID mice ($n=10$) injected subcutaneously with U87D cells treated with the anti-EGFRvIII lentivirus ($p=0.005$). We conclude that gene specific silencing of EGFRvIII is a promising strategy for treating cancers which contain this mutated receptor.

Keywords: EGFRvIII, SiRNA, silencing, glioma, therapy,

P-10-91-3

Ral overactivation in malignant peripheral nerve sheath tumors

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Overactivation of the Ras signal transduction pathway plays an important role in the development of malignancies. Increased Ras activity is thought to be involved in the pathology of neurofibromatosis 1 (NF1) and its lethal complication, malignant peripheral nerve sheath tumor (MPNST), caused by lack of neurofibromin (a Ras-GTPase activating protein). In this study, we show that overactivation of Ras signaling and many of its down-stream effectors occurs only in a fraction of MPNST cell lines. An exception to this variability is the active form of Ral, which was increased in all of the studied MPNST cells and tumor samples as compared with non-transformed human Schwann cells. Importantly, silencing of Ral expression with short-interfering RNA (siRNA) caused a significant ($p<0.05$) reduction in the proliferation rate and invasiveness of MPNST cells, which was reversible upon cessation of Ral silencing. Ral silencing also resulted in loss of invasiveness associated with down-regulation of epithelial-mesenchymal transition (EMT) markers such as B-Catenin, Snail and E-Cadherin, whereas the levels of N-Cadherin decreased. Inhibiting the activation of Ral by expression of a dominant negative version of Ral (Ral S28N) also resulted in a significant decrease in the proliferation rate and invasiveness of MPNST cells. In the next step, we attempted to elucidate the mechanism of Ral overactivation. We hypothesized that lack of neurofibromin may result in activation of Ral given the well-known GTPase function of neurofibromin for Ras and the high degree of relatedness between the Ras proteins and Ral. To study this, we exposed human MPNST cells to a retrovirus expressing NF1-GTPase related domain (NF1-GRD). Such treatment caused a significant decrease in the level of Ral-GTP and was accompanied by reduction in the proliferation rate and invasiveness of MPNST cells. Also, the fraction of MPNST cells in the S phase of the cell cycle was decreased, but the rate of cell death in these cells increased. Further evidence for involvement of Ral in the biology of MPNST cells was provided by our results showing that gene specific silencing of Ral also results in significant reduction in tumor size in a subcutaneous SCID mouse model of MPNST ($p<0.01$, $n=10$). We consider these results as the first reported for overactivation of Ral in MPNST, its involvement in EMT and the role of neurofibromin in decreasing Ral activation. Therefore, this study introduces Ral activation as a novel mechanism involved in the biology of NF1-driven malignancies and introduces the possibility of targeting this signaling pathway for treatment of this malignancy via different approaches such as gene therapy and small molecule inhibitors.

Keywords: overactivation, Ras signal transduction, malignancy

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O-10-102-1

Human feeder support prolonged undifferentiated growth of embryonic stem cell

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Mouse embryonic fibroblast (MEFS) has been used to support the growth of mouse embryonic stem cells (mESc) and human embryonic stem cells (hESCs). Prolonged propagation of mESCs is currently achieved by co culture with MEFs serving as feeder cells. The presence of uncharacterized rodent cells or crude extracts imposes a risk to the clinical applications of hESCs or mESCs. Embryonic stem cell were expanded using human USSC, and then expression of CD146, CD29, CD49, VEGFR2, FLK1 were evaluated by flow cytometry and expression of Stat3, BMP4, REX1, Oct4, SOX2, Nanong, Brachyury, Tert, LIF, LIFR Fgf4, were evaluated by RT-PCR and protein expression of Oct4 were evaluated by immunohistochemestry. Mouse Es cell colonies cultured on inactive hUSSCs were ampilfied >600 fold during 80-day continuous culture (in 30 passage). The expanded mES cells displayed the unique morphology and molecular markers characteristic of undifferentiated mEs cell as observed when they were cultured with MEFs. They expressed oct-4, BMP4, REX1, Nanong, Brachyury, Tert, LIF, LIFR, and but not SOX2, Stat3, Fgf4. Expanded mES cells on hUSSCs retained unique differentiation potential in culture and a normal diploid karyotype. Our results indicated that co-culture of ESC on cord blood stem cell (USSC) significantly maintained ESCs in the undifferention state. Well–studied hUSSCs may provide a clinically and ethically feasible method to expand hEs cells for novel cell therapies.

Keywords: stem cell, embryonic stem cell, cord blood, flow cytometry

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O-10-104-1

Antiproliferative agents against anaplastic thyroid carcinoma cells

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Anaplastic thyroid carcinoma (ATC) is a highly aggressive, fatal disease for which there are no effective therapies currently and patients die within six months of initial diagnosis. Among the new approaches in the treatment strategies of ATC is the use of substances that can alter the proliferation of anaplastic thyroid cancer cells. This work describes the application of different antiproliferative agents and elucidates their effect on anaplastic thyroid cancer cells. Sphingolipids, such as the metabolites of sphingomyelin modulate several cellular processes e.g.

survival, migration and differentiation in almost all cells, and are in addition implicated in some pathological processes such as cancer. Sphingosylphosphorylcholine (SPC) is an active sphingolipid derivative, which is a naturally occurring lipid in serum, and is produced as a result of pathological disturbances in lipid metabolism. The G protein-coupled receptors 4 (GPR4) and OGR1, putative SPC-specific receptors, are shown in this study to be expressed in human anaplastic thyroid cancer cells (FRO cells). In these cells SPC evokes a concentration-dependent increase in intracellular free calcium concentration ([Ca2+]i) in a calcium-containing, but not in a calcium-free buffer. The entry of calcium is shown to be mediated by the phospholipase C (PLC)/ protein kinase C (PKC) pathway. Furthermore, SPC attenuates the proliferation of FRO cells in a concentration-dependent manner and is shown to be an effective suppressor of thyroid cancer cell migration. This effect is, in part, mediated by inhibition of both the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB), or Akt, and the mitogen-activated protein kinase (MAPK) signaling pathways. The phorbol ester, phorbol 12-myristate 13-acetate (PMA) is another antiproliferative agent used in this study. In various cell models, PMA is known to induce its effect by activating different PKC isoforms (i.e. α, βI, βII, γ, δ, ε, η, θ). Our findings suggest that incubating FRO cells with PMA induced a strong antiproliferative effect in a concentration-dependent manner. The results indicate that PMA is an effective inhibitor of thyroid cancer cell proliferation and migration by a mechanism involving PKCδ-MAPK/ Akt and forkhead box O (FOXO) transcription factor signaling. The role of human ether á-go-go related gene (HERG) potassium channels in thyroid cancer cell proliferation and migration is also described in this work. Our results indicate that HERG protein is expressed in different thyroid cancer cells and that blockade of HERG protein inhibits thyroid cancer cell proliferation and migration. Overall, in this study, three different approaches are introduced, which seem to be effective in regulating anaplastic thyroid cancer cell proliferation and migration and can be considered in the treatment strategies of this type of aggressive cancer.

Keywords: thyroid carcinoma, sphingolipids, PKC, HERG

P-10-140-1

Malathion induces oxidative damage in brain mitochondria

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Malathion [S-(1,2-dicarbethoxy)ethyl O,O-dimethylphosphorodithioate] is one of the most widely used organophosphate pesticides for agriculture and public health programs. The aim in this study was to investigate the effects of malathion on oxidative damage in rat brain mitochondria. Rats received moderate toxic dose of malathion (200 mg/kg/day) for one week. At the end of the experiment, the brain was removed and mitochondrial of brains isolated. The activity of enzymatic scavengers, catalase (CAT), superoxide dismutase (SOD) and TBARS as indicator of lipid peroxidation in brain tissue and brain mitochondria and biomarkers of oxidative stress were analyzed. Malathion significantly increased CAT activity and TBARS level compared to control group in brain and brain mitochondria. Thus subacute malathion exposure has the potential to disrupt antioxidant defense system and mitochondrial function.

Keywords: malathion, mitochondria, oxidative stress, lipid peroxidation

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P-10-136-1

Cloning & characterization of asparaginase from Klebsiella pneumoniae ATCC10031

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L-asparaginase (L-asparagine amidohydrolase, E.C.3.5.1.1) has been shown to be an effective antilymphoma agent in human. Both the substrate and the product of this enzymatic reaction play important roles in a number of metabolic processes in all organisms, from bacteria to mammals. The anti-tumor activity of this enzyme is based on the dependence of certain tumor cells on an extracellular supply of L-Asn. Unlike normal cells, some malignant cells could synthesize L-Asn slowly, due to their deficiency in L-asparagine synthetase. In this research Klebsiella pneumoniae ATCC10031 was chosen to survey the existence of L-asparaginase type II similar gene. The genomic DNA of this bacterium has been extracted with High Pure PCR Template Preparation Kit (Roche). Forward & reverse primers were designed for this locus according to Genbank sequence. Then this locus was amplified by PCR reaction. A locus about 1000bp was observed on gel electrophoresis. Then this gene was cloned in pTZ57R/T vector by use of T/A cloning kit (Fermentase). The cloned plasmid was isolated and then sequenced by MWG Company. This sequence was compared with the Genbank homolog gene sequence. There was high similarity between them but in some nucleotides were different. The sequence of asparaginase in E.coli which be used in treatment of ALL has about 40 nucleotide more than the same locus in klebsiella. Then the sequence of this gene was submitted in genbank which can be used for more researches

Keywords: L-asparaginase, Klebsiella pneumoniae, pTZ57R/T vector

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P-10-119-1

Cloning of mouse PPARγ1cDNA into pEGFP-C1

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Peroxisome proliferator-activated receptors are a group of nuclear hormonic receptors. There are three different isotype of PPAR (alpha, beta and gamma), that function at the transcription level. PPARγ is a member of these receptors and plays a role on cell metabolism and differentiation. It is found as 2 isoforms: PPAR (γ1, γ2). In this study cloning of PPARγ1cDNA was done for further purposes. The amplification of PPARγ1cDNA was done by RT-PCR procedure using total cDNA which were obtained from a mouse fat tissue. Amplified PPARγ1cDNA was placed in pEGFP-C1 plasmid at SacI – KpnI restriction sites. Recombinant plasmid was obtained after bacterial cell

transformation and sequence check. Here we describe the cloning stages of PPARγ1cDNA.

Keywords: PPAR, receptors, cDNA, cloning, plasmid

P-10-93-1

Genetic relationship and the possibility of inter species breeding between domestic sheep (Ovis Aries) and wild sheep (Ovis Orientalis Isphahanica)

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Cloning an individual by transferring somatic nuclei into enucleated recipient oocytes has already been well established. This technology also offers new opportunities to restore threatened species with interspecies somatic cell nuclear transfer (SCNT). However, few mammalian species have been studied for their reproductive biology whereas huge differences have been observed between these species. This study evaluated the similarities and genetic relationship of germ lines and the reproductive biology between domestic (Ovis aries) and threatened wild sheep (Ovis orientalis isphahanica). Six populations of wild and domestic sheep were sampled and analyzed for chromosome number, interbreeding capability and fecundity. Resulted hybrids (male or female) were investigated for survival, karyotyping and fertility. Both the domestic and wild sheep uniformly exhibited a 2n of 54 and were able to crossbreed and induce sustainable pregnancy into the counterpart species. The resultant hybrids (male or female) which were produced by either wild ram × domestic ewe or domestic ram × wild ewe had identical chromosome number (2n=54). Normal genital apparatuses and fertile conditions were observed in both types of adult hybrids. The results indicated a very close generational relationship between the wild and domestic sheep species and also the possibility of domestic species to be used as an abundant genetic background for saving endangered wild sheep via SCNT.

Keywords: nuclear transfer, interspecies cloning, hybrid, wild sheep, karyotype

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P-10-125-1

An in vitro investigation of weak electric field on the growth and maturation of mouse oocytes

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Progress in the treatment of human and animal infertility is directed towards the development of effective culture conditions for in vitro maturation of oocytes. Mouse is the only species in which whole process of oocyte maturation was successfully performed. The efficacious effects of electric field under the certain field parameters like intensity have been reported for various cells, tissues and molecules. For recent analysis, intact preantral follicles were isolated from ovaries of 6 weeks-old female Syrian mice using 25-gauge needles and cultured in TCM-199 medium supplemented with sodium pyruvate (2 mM), glutamine (2 mM), penicillin G (75 µg/ml) and streptomycin (50 µg/ml). Following analyses were done: I) 1, 3 and 6 mV electric field was applied to 30 follicles in the presence and absence of 100mIU/ml FSH. Electric field of 6 mV intensity showed 56% follicle survival and 48% oocyte maturation. While, the same field showed 88% follicle survival and 75% oocyte maturation in the presence of 100 mIU/ml FSH ($p<0.05$). Electric fields of 1 and 3 mV did not show significant results in the presence or absence of FSH. ($p\leq0.05$). So, it is concluded that weak electric field induces in vitro maturation of mouse oocytes.

Keywords: electric field, in vitro growth, oocyte maturation

O-10-207-1

Cloning and expression of tetanus toxin fragment C

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Fragment C is C-terminal domain of tetanus toxin heavy chain that can promote immune response against lethal dose of tetanus toxin, thus it can be considered as a vaccine candidate against tetanus infection, occurs by Clostridium tetani. In this study DNA of tetc was prepared, cloned into pTZ57R, subcloned into pET expression vector and the recombinant protein (fragment C) was over expressed in E. coli. Bacteria were grown in Modified Mueller's Media, the total DNA (cells and plasmids) was prepared for PCR and by using specific primers, tetc DNA was amplified and ligated into pTZ57R, subcloned into pET vectors (22b and 28 a). The recombinant vectors were transferred into competent E.coli strain BL21 (DE3) pLysS and after selection of proper colony, which carried the target DNA within the vector; cells were cultured and induced with IPTG, in order to express protein (TetC). The cultures were tested for presence of TetC by SDS-PAGE. Molecular techniques such as PCR and sequencing which showed exact defined size of the tetc (1356 bp) and SDS-PAGE of over expressed protein, confirmed the correct cloning and expression of TetC. In this research, full length of tetc was obtained from Harvard CN49205 strain of C.tetani, vaccinal strain of Razi institute. Sequencing this fragment showed 100% identity with Harvard and Massachusetts strains in Genbank and 99% identity with other sequences. Over expression in E. coli which, can be used for further studies, including purification and neutralizing antibody production against TetC.

Keywords: fragment C, Clostridium tetani, pTZ57R, pET vectors, IPTG, SDS-PAGE

P-10-204-1

Possible role of alpha5 beta1 integrin-fibronectin interaction in Large Cell Carcinoma with neuroendocrine differentiation metastasis

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Large cell carcinoma with neuroendocrine differentiation (LCCND) lacks neuroendocrine morphology but has neuroendocrine markers both by electron microscopy and immunohistochemistry. Cellular interaction with extracellular matrix proteins (ECM) provides a possible mechanism

by which cancer cells adhere, invade and metastasize. The integrins are a major family of adhesion molecules that bind to ECM components that contain the RGD (Arg-Gly-Asp) sequence, namely fibronectin (FN) and vitronectin. In this study we investigated the expression of some integrin subunits such as alpha4, alpha5 and beta1 which are generally considered closely associated to tumour metastasis in some cancerous cell lines. Mehr-80 as human large cell lung cancer showed no expression of integrin alpha4 by RT-PCR and flow cytometry to compare with the other cell lines used in this study for instance HMA375 (highly metastatic human melanoma cell line), HFLF (human fetal lung fibroblasts) and MRC-5 (human lung fibroblasts). High expression of integrin alpha5 beta1 in the Mehr-80 cell line may be crucially involved in cell adhesion and metastasis mediated by FN and 50K fragment of FN. Inhibition of cellular adhesion to the matrix reduced the invasive potential of cancerous cells. Anti-integrin beta1 and alpha5 antibody were significantly capable of inhibiting Mehr-80 cell adhesion or spreading on different fragments of FN. As integrin antibodies inhibit cell invasion in vitro, the integrins may be of potential value as antitumour therapeutic agents. This result also provide clear evidence that integrin alpha5 beta1 is positively correlated with Mehr-80 metastasis and might be an anti-tumour target.

Keywords: integrins, fibronectin, LCCND, cell adhesion

P-10-187-1

Study of expression of pdx-1 in mouse embryo

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Several transcription factors are known as critical regulators of pancreatic development in mouse. Expression patterns of transcription factors limit the boundaries of developing pancreas and determine the differentiation programs of individual cell lineages. Pdx-1 gene is an important regulator in pancreas development. The importance of pdx-1 gene is its role in early development of pancreas and differentiation of pancreas endocrine cells, especially β cells. In this research morphology of the developing pancreas and expression of pdx-1 were studied. Serial section of formalin fixed, paraffin embedded mouse embryos was prepared. Total RNA of developing pancreas was extracted. RT-PCR with specific primer of pdx-1 was done and expression of pdx-1 was studied. There was pdx-1 excretion in developing pancreas.

Keywords: transcription factor, development, pancreas, pdx-1 gene, β cells

O-10-233-1

Producing GST-p53 recombinant protein and polyclonal antibody against it

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P53 is a key tumor suppressor gene that is targeted for inactivation during human tumorigenesis. Point mutations or deletions in the p53 gene are found in approximately 50% of all cancers. In normal cells p53 plays a crucial role in the negative regulation of cell growth in response to DNA damage and other stress-activated signaling pathways. In this study, GST-p53 recombinant protein and polyclonal antibody against it were generated for further experiments. The pCDNA3 plasmid containing p53 gene was digested with EcoRI and XhoI enzymes and sub-cloned into the corresponding sites of a pGEX-4T1 vector to generate p53 - glutathione S- transferase (GST) fusion protein. Protein expression-plasmid was transferred into BL21 competent cells for the expression of GST fusion protein. The GST- p53 protein was purified by affinity chromatography using Glutathione-agarose. Subsequently, polyclonal anti-p53 was produced by immunizing rabbits with GST-p53 fusion protein. The antibody was purified and then it was tested by sandwich enzyme-linked immunosorbent assay (ELISA) and Immunobloting. The results showed polyclonal anti-p53 with 1:2000 dilutions can recognize p53 protein in ELISA and immunoblot analysis. To our knowledge, this is the first report on the production of polyclonal antibody from GST-p53 fusion protein. We will evaluate this antibody in other experiments such as chromatin immunoprecipitation assay. Also we can use GST-p53 recombinant protein and its antibody in protein- protein interactions studies in many cancer cell lysates.

Keywords: GST-p53, recombinant protein, cloning, polyclonal antibody, ELISA

O-10-231-1

Cloning and expression of second and third extracellular domain of VEGFR-2 in E.coli

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Vascular endothelial growth factor receptor-2 (VEGFR-2) functions as the major signal transducer of angiogenesis, mediating VEGF induction of angiogenesis. The therapeutic inhibition of VEGFR-2 action is now having an impact in the clinic for the treatment of a number of diseases. We have cloned and analyzed expression of the second and third extracellular domains of VEGFR-2 in E.coli TG1. The cDNA of the second and third extracellular domains of VEGFR-2 was obtained from the total RNA extracted from human umbilical cells by using RT-PCR, and was cloned to vector Pet22b(+). The recombinant expression plasmid Pet22b(+)/VEGFR-2(2,3) was constructed and transformed to E.coli TG1. The positive colonies were screened by colony PCR and their expressions were induced by IPTG. With the aids of SDS-PAGE and Western blot, a specific band being same to the reports near 20 kD was found. The second and third extracellular domains of VEGFR-2 were successfully expressed. The study lays a foundation for further application of the expressed product in the treatment of vasoformation related diseases, such as tumor and diabetic retinopathy.

Keywords: VEGFR-2, complementary DNA, polymerase chain reaction, cloning, expression, immunoblotting

O-10-204-2

Effects of chemokines, CXCL12 and CXCL8 on expression of fibronectin receptors in prostate cancer cell lines

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Metastasis of prostate cancer requires invasion through the basement membrane that surrounds the epithelial cells, which must be breached by tumor cells invading into surrounding tissues. The integrins are a major family of cell adhesion molecules that bind to extracellular matrix protein and have major role in invasion and metastasis. The CXC-chemokines, which have been shown to promote the migration of neutrophils and carcinoma cells, are candidates to influence prostate carcinoma cell invasion by altering expression or activity of integrins. In this study we investigated the effect of chemokines, CXCL12 and CXCL8 on expression of some integrin subunits such as alpha4, alpha5 and beta1 which are generally considered closely associated to tumor metastasis in some cancerous cell lines by Flow cytometry analysis and semi-quantitative RT-PCR assay. The surface expression and mRNA level of integrin subunits beta1, alpha4 and alpha5 in PC3 and DU145, prostate cancer cell lines, were demonstrated. In contrary to PC3 cells, there is no expression of integrin alpha4 subunit in DU145 cells. Among the chemokines, CXCL12 has an effect on PC3 cells by suppressing expression of alpha4 integrin. Also this chemokine reduced Du145 cell line's beta1 and alpha5 subunits in the both level of mRNA signal and surface expression. We found that CXCL8 could increase surface expression of beta1 subunit in DU145 cells. DU145 and PC3 cell lines metastasize to brain and bone respectively. Therefore the different effects of the chemokines on expression of fibronectin receptors can help to understand how these cells have different targets.

Keywords: integrins, fibronectin, CXCL12, CXCL8, prostate cancer

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P-10-215-1

RA-induced PeP elevation during P19 cell neural differentiation

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A, large number of factors are sequentially expressed during neurogenesis, suggesting the existence of a complex interacting set of events that control the developmental fate of differentiated cells. One of the proteins that is imported to the peroxisomal matrix, named Peroxisomal Protein (PeP), whereas in adult mice, strong expression is also found in the brain. To study the function of PeP during neurogenesis, we here employed P19 cells as an in vitro model of neural differentiation. Expression pattern of PeP was investigated under distinct steps of differentiation by RT-PCR. We found an elevated expression of PEP gene was markedly increased after the RA-induced stages of neural differentiation of P19 cells, However differentiation of P19 cells without RA treatment did not increase expression of PEP gene.

Keywords: neural differentiation, peroxisomal protein, P19 cells

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P-10-318-1

Synthesis of a water soluble Schiff base, N, N'-bis {5-[(triphenylphosphonium)-methyl] salicylidine}-o-phenylenediamine, and DNA binding studies

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The water soluble Schiff base, SF, (SF=N,N'-bis{5-[(triphenylphosphonium)-methyl]salicylidine}-o-phenylenediamine), has been synthesized and characterized by spectroscopic (1H NMR, Uv-Vis and IR) and elemental analysis techniques. Binding interaction of this Schiff base with calf thymus DNA (CT-DNA) has been investigated by emission, absorption, circular dichroism and viscosity studies. In fluorimetric studies, the dynamic enhancement constants (KD) and bimolecular enhancement constant (KB) were calculated at different temperatures and showed that the fluorescence enhancement is not initiated by a dynamic process and static process involves complex-DNA formation in the ground state. Furthermore, the enthalpy and entropy of the reaction between SF and CT-DNA showed that the reaction is exothermic and enthalpy favored (ΔH=-153.51 kJ/mol; ΔS=-427.67 J/mol K). The experiments proved that the interaction mode between SF and CT-DNA was intercalation.

Keywords: DNA, intercalation, Schiff base

P-10-333-1

Effects of different doses of LIF on IVM rate and cumulus expansion in mouse oocytes

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Leukemia inhibitory factor (LIF) is a pleiotropic cytokine of interleukin-6 family with a remarkable range of biological actions such as proliferative effects on the granulosa and theca cells. But its role in oocyte maturation is unknown yet. The aim of this study was to evaluate the effects of different doses of LIF on GVBD and MII development rate and cumulus expansion. Immature mice superovulated with HMG and GV oocytes obtained from ovary 48 hours after. The GV oocytes were cultured in TCM199 with 100, 500 and 1000 µg /ml LIF. Cumulus expansion was analyzed with two examiners and after that number of MII oocytes were recorded. For denuding the oocytes hyaloronidase was used. Our results showed that rate of GVBD and MII development increased in groups with LIF compared to control group. Rate of MII development with 1000 µg/ml LIF was significantly higher than control group ($P<0.05$). Cumulus expansion in group with 1000 µg/ml LIF improved significantly compared to control group ($p<0.05$). Our results showed that LIF could improve IVM rate in a dose dependant manner. Also cumulus expansion improved in group with LIF and increased oocyte quality.

Keywords: LIF, IVM, Cumulus expansion, mouse

P-10-333-2
gp130 gene expression during in vitro maturation of mouse oocyte

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LIF is a 45-56 kDa glycoprotein that has important role in proliferation and embryo implantation. LIF exerts its function via its receptors, such as gp130. Effects of LIF in oocyte maturation and expression of its receptor in oocytes is unknown. Immature mice superovulated with HMG and GV oocytes obtained from ovary 48 hours after. The GV oocytes were cultured in TCM199 with 5% Co2 for obtaining MII oocyte. For Real-time RT-PCR, Total RNA from GV and MII oocytes were extracted by Trizol reagent the quantity and quality of RNA were determined by spectrophotometry and electrophoresis, respectively. Reverse transcription was performed by SuperScript III reverse transcriptase with 1 µg of total RNA followed by DNaseI treatment and heat inactivation. Expression of gp130 was determined by Real time RT-PCR. Our results showed that gp130 is expressed neither in GV nor in MII oocytes during in vitro maturation of mouse oocytes. It is proven that gp130 is expressed in human oocyte but is not expressed in mouse oocytes. It is suggested that in mouse, LIF could affects the oocyte via another receptor. However its details must be elucidated.

Keywords: IVM, LIF, gp 130, oocyte, mouse

P-10-297-3
Study of the effects of sulfur mustard on rat liver tissue active and inactive chromatin

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Sulfur mustard is a blistering agent with electrophilic property, which reacts with such cellular macromolecules as DNA, RNA, and protein. It affects nuclear function such as DNA–histones interaction significantly. In this study, after preparing the nuclei, rat liver tissue chromatin digested with micrococcal nuclease. Then fractions obtained from enzymatic digestion, S2 (active chromatin) and P2 (inactive chromatin), were treated with various concentrations of SM (25-1000 µM) and the samples were investigated based on using UV/VIS spectroscopy, DNA and SDS gel electrophoresis. Results obtained from U.V spectroscopy studies show that the absorbance were increased at concentrations lower than 500 µM for S2 chromatin and 750 µM for P2 chromatin at 260 and 230 nm and at higher concentrations were decreased. S2 showed a higher degree of absorbance reduction. These results were confirmed by gel electrophoresis. The data suggest that the effects of sulfur mustard on active chromatin are more than inactive chromatin and indicate two kinds of different mechanisms. At concentrations lower than 500 µM, it unfolds DNA and releases histone proteins, while at concentrations higher than 500 µM, probably induces compaction and precipitation of chromatin by producing cross-links between DNA - protein or protein - protein and subsequently inhibits DNA synthesis.

Keywords: sulfur mustard, active and inactive chromatin, micrococcal nuclease, rat liver tissue

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P-10-297-4
Study of the effects of sulfur mustard on DNA and chromatin of rat liver cells

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Sulfur mustard (SM) is a chemical warfare vesicant that rapidly penetrates the skin due to its hydrophobicity. It reacts with membranes, RNA and proteins. DNA is one of the main target molecules for SM. SM alkylates DNA, leading to DNA strand breaks and cell death in a variety of cell types and tissues. In the present study, after purification of DNA and chromatin from rat liver cells, the effect of various concentration of SM (25-1000 µM) was investigated using UV/VIS spectroscopy, DNA and SDS gel electrophoresis. Results obtained from U.V spectroscopy studies show that the absorbance was increased at concentrations higher than 50 µM for DNA at 260 nm and at concentrations higher than 100 µM for chromatin at 230 and 260 nm. Also, at higher concentrations of SM (>250 µM), DNA fragmentation represented a smear pattern on agarose gel electrophoresis. The results suggest that the effect of SM is dose dependent. At higher SM concentrations, alkylation of DNA leads to DNA strand beaks and the nature of the DNA fragments produced suggested necrotic form of cell death.

Keywords: sulfur mustard, DNA, chromatin, rat liver tissue

P-10-325-1
Evaluation of gene-finding programs on mouse genomic DNA

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Six gene-finding programs i.e. Genscan, GeneMark hmm., HMMgene, GenView 2, FGENESH and FGENESH+ were evaluated using 24 well defined mouse single genes to predict the structure of protein coding genes. Our analyses indicated that different methods often produce different and sometimes contradictory-results. In the nucleotide level, the highest Correlation Coefficient (0.87) and Approximate Correlation (0.86) values, and the lowest Correlation Coefficient (0.67) and Approximate Correlation (0.67) values were detected only for FGENESH+ and GenView 2 programs, respectively. Furthermore, at the exon level, similar results were obtained. In general, our results at either the nucleotide or exon levels showed that FGENESH+ (HMM puls sequence similarity programs), provide a level of improvement over the ab initio gene prediction methods such as Genview 2 and suggested

that, probably, FGENESH+ and Genscan can be more helpful than the others. Moreover, based on these results, we realized that the accuracy of these programs is strongly dependent on GC content. Lastly, on the basis of whole known sequences it was concluded that, predictive accuracy of these programs is low.

Keywords: exon, gene, gene-finding programs, mouse, protein

O-10-142-2

Analysis of transgenic tobacco for transgene stability test and purification of human recombinant t-PA

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Over the past two decades recombinant tissue-type plasminogen activator (rt-PA), the main physiological plasminogen activator, has been produced in different hosts. rt-PA is used as a fibrin-specific thrombolytic agent for the treatment of various thromboembolic diseases. We tried to show the presence of t-PA gene and protein in transgenic tobacco plants produced previously by our group. Because of instability of the gene in host and costly purification process, we had to be sure that our transgenic plants stably and correctly express the transgene. By planting the plant seeds on Murashige-Skoog medium culture containing kanamycin antibiotic only transgenic plants that received plasmid were germinated. PCR, RT-PCR with specific primers for t-PA and internal control gene GAPDH, and SDS-PAGE were used to assay the transgenic plants. PCR and RT-PCR products were observed on agarose gel electrophoresis after ethidium bromide staining. The results showed that the t-PA gene is successfully introduced and is stably expressed in third generation transgenic plant (T3) at the level of mRNA and protein, while no expression was seen in non transgenic control plants. These results also indicate the high stability and efficiency of Agrobacterium-mediated plant transformation. We are going to purify rt-PA from the transgenic tobacco with specific chromatography columns. The extracted protein can be ultimately used as recombinant Alteplase drug after passing functional assay and quality control experiments.

Keywords: transgenic tobacco, t-PA, kanamycin, SDS-PAGE, RT-PCR

P-10-370-1

Efficiency of Taq polymerase increment in preventing inhibition by HA in DNA extracted from ancient bones

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Human DNA quantification has became an essential method for ensuring the quality of PCR based studies performed from low copy and/or highly damaged DNA samples. Theoretically, PCR makes it possible to amplify a DNA sequence from any biological sample. In practice, however, compounds that inhibit PCR may co-purify with the DNA template, making the amplification impossible. Inhibition is an especially significant problem when extracting DNA from old and ancient bones. When the template DNA is extracted directly from soil, humic acid (HA) can inhibit PCR. Also, total DNA extraction from soil always results in co-extraction of other soil components, mainly HA or other humic substances, which negatively interfere with DNA detecting processes. Characteristic features of humic substances are their structural heterogeneity, their property to bind metal ions by complex formation, and their property to interact with a variety of organic compounds. Humic substances are ubiquitous in soil and water and can contaminate any material exposed to those environments, which cause false negative results. Real-time detection is a very fast and accurate technology that does not require post-PCR processing, since detection is done during each PCR cycle. QRT-PCR methods have the ability to quantitative trace amounts of human DNA isolated from old bone samples. To enhance the PCR efficiency in samples containing inhibitors, example HA or other humic substances, by 2-4U extra Taq DNA polymerases were included in the reactions. AmpliTaq Gold® was from Applied Biosystems. Altered amplification plots were observed during the analysis of old bones and teeth to the presence of inhibitors. The addition extra amounts of Taq DNA polymerases has been proven efficient in overcoming the effects of inhibitors.

Keywords: ancient bone, DNA extraction, Taq polymerase, humic acid (HA)

O-10-392-1

A variant of histone H2A, named H2A.Z, can be considered as an epigenetic mark in teratocarcinoma cells

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Embryonic carcinoma (EC) cells, the pluripotent stem cells of teratocarcinomas, show many similarities to embryonic stem (ES) cells. Since EC cells are malignant but their terminally differentiated derivatives are not, understanding the molecular mechanisms that regulate their gene expression profile through differentiation maybe of value for diagnostic and therapeutic purposes in cancer. Through development of higher eukaryotes, multiple primary amino acid variants of histones have been identified, with several functional significances on gene regulation. The histone protein H2A.Z is a highly evolutionarily conserved variant of H2A core histone, with a variety of seemingly unrelated, even contrary functions. In this study we analyzed total histones in the pluripotent human EC cell line, NTERA2, and also after induction of differentiation by retinoic acid (RA). Using gel electrophoresis coupled with western blot analysis, we observed that the expression of one histone variant of H2A has significantly decreased after RA treatment of the cells. The histone variant was further identified by MALDI-TOF mass spectrometry as H2A.Z, and its total expression level in the cells was checked by immunocytochemistry. Using chromatin immunoprecipitation (ChIP) technique coupled with real-time PCR we have also shown that this epigenetic variation had a differential incorporation profile on the regulatory regions of some marker genes involved in stemness and differentiation, respectively. Our finding implies the dynamic interplay of H2A.Z histone variant in regulating gene expression of embryonic

carcinoma cells, and maybe suggests a diagnostic/prognostic value for this epigenetic variation in cancer.

Keywords: carcinoma, embryonic, histone variant, H2A.Z

P-10-393-1

DNA binding study of 2-tert-butylhydroquinone and butylated hydroxyanisole using Circular Dichroism spectroscopy

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Food additive have been extensively applied in recent decades in food industry throughout the world. 2-tert-butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) are a highly effective preservative for unsaturated vegetable oils, many edible animal fats and meat products. At high doses, there have some negative health effects on lab animals, such as precursors to stomach tumors and damage to DNA. Also metabolically, TBHQ is formed from BHA by O-demethylation. In this study, DNA binding properties to TBHQ and BHA in Tric-HCL buffer (PH=7.4), has been monitored as a function of [TBHQ]/[DNA] and [BHA]/[DNA] molar ratio, by Circular dichroism (CD) spectroscopy method. The UV circular dichoric spectrum of CT DNA exhibits a positive band at 275nm due to base stacking and a negative band at 245nm due to helicity of B DNA. The results of CD studies indicated that by increasing of [food additives] / [DNA] ratio, clear changes occurred in the CD spectra of B-DNA. Incubation of DNA with TBHQ leads to stabilizing the right-handed B form of DNA and incubation of DNA with BHA leads to conversion from a more B-like to a more C-like structure within the DNA molecule.

Keywords: CT-DNA, TBHQ, BHA

P-10-396-1

The effect of heavy metals, nickel and cobalt, on chromatin proteins in alveolar macrophages

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Cobalt and nickel are known as human carcinogens, widely distributed in modern industrial process. In the occupational environment, exposure to many metals cause diseases of the lung. Although these metals mimic hypoxic responses in cultured cells including generation of ROS and transcriptional changes of some genes, the molecular mechanism by which their carcinogenicity is preceded is not yet fully understood. The aim of the present study was to investigate the effect of nickel and cobalt chloride on chromatin proteins in alveolar macrophages of rat lung and compare the result with those of intact nuclei. The cells were prepared by lavage exposured to various concentrations of nickel or cobalt for different times of incubation. The proteins were then extracted by either acid or salt and analyzed on SDS polyacryl amide gel electrophoresis. Exposure of macrophages to various concentrations of nickel or cobalt for different times of incubation (6-48 hour) had no considerable effect on the extractability of the proteins especially the histones. But when isolated nuclei were exposed to these metals in the same experimental condition, the proteins pattern on the SDS-PAGE was significantly changed, thus after 1 hour of incubation the extractability of the histones and HMG proteins were decreased suggesting that upon binding of nickel and cobalt to chromatin, not only DNA, but the chromatin proteins may have a fundamental role in the compaction process of DNA.

Keywords: nickel, cobalt, macrophage, HMG proteins, chromatin, histone proteins

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P-10-197-1

Extraction and purification of (1→3)-β-D-glucan from Saccharomyces cerevisiae yeast cell wall and its NMR spectral analysis

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(1→3)-β-D-Glucan is a polysaccharide in microorganisms, fungi and plants. It's available in the cell wall of yeasts, particularly in the baker's and brewer's yeast saccharomysces cervisiae. Glucan extracted from yeast cell walls is a powerful immune-enhancing nutritional supplement. This unique compound stimulates the innate immune system to help the body defend itself against viral and bacterial invaders. (1→3)-β-D-Glucan was extracted and purified from saccharomyces cervisea yeast according to the methods of Jamas. We applied some modifications to Jamas's method to increase its purity. At first, the saccharomyces cervisea yeast was hydrolised by NaoH (10M, 1M) to remove some internal proteins, nucleic acids, mannan, soluble glucans, and polar lipids in soluble form. Then, the extraction was followed by acidic hydrolisis by hydrochloric acid and acetic acid (glacial). In this step pH is very critical, and should not damage the glucosidic bonds. By this method glycogene, chitin, chitosan and some proteins were removed and the remaining solid phase was washed with some solutions such as water, acetone and isopropyl alcohol. The difference with Jamas's method is that in this stage samples were succylated by hexan for a few hours which helps to remove more lipids and proteins and increases the purity of the samples. Finally, the samples were dried in a vacuum oven, analysed with NMR spectroscopy and compared with the standard samples. The structure of the samples and their purity were determined by CNMR and HNMR.

Keywords: insoluble glucan, extraction, purification, Saccharomyces cerevisiae, NMR spectroscopy

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P-10-380-1

Heavy chain antibodies: Immunohistochemistry detection of EGFRvIII receptor in thyroid carcinomas

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The epidermal growth factor receptor (EGFR) vIII, a ligand-independent, constitutively active, mutated form of EGFR has been

shown to play a role in the pathogenesis of some cancers. Consequently, the immunohistochemical detection of EGFRvIII with novel camel antibodies which are valuable for their ability to interact with less antigenic epitopes in contrast to the conventional antibodies, might be worthy in diagnostic techniques of cancers. Hence Molecular targeted therapy in many diseases especially non treatable ones has captured the eyes of the scientists; we examined the presence of this receptor in thyroid cancer tissues. Forty samples of paraffin-embedded tissues of thyroid neoplasm containing: follicular carcinomas, papillary carcinomas, medullary carcinomas, follicular adenomas, and goiter were selected from the archives of the department of pathology. EGFRvIII was then evaluated on each sample by immunohistochemistry with camel heavy chain antibody and conventional antibody. Positive immunostaining of neoplastic tissues with camel antibodies and rabbit polyclonal antibodies, as control, were 81.3% and 39.1%, respectively. No goiter tissue was stained with any of those antibodies. Also, the results showed that the sensitivity of camel heavy chain antibodies (65%) is higher in contrast to conventional rabbit antibodies under the same conditions (39.1%). Considering the results of this study, exploiting the new heavy chain antibodies of camels against EGFRvIII seems promising in the diagnostic procedures of thyroid neoplasms.

Keywords: immunohistochemistry, camel antibody, EGFRvIII, thyroid carcinoma

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O-10-162-1

S100A8/A9: A double-faced protein in tumorgenesis and cancer therapy

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Members of the S100 protein family comprise a multigenic group of non-ubiquitous cytoplasmic Ca²⁺-binding proteins of the EF-hand type, differentially expressed in a wide variety of cell types. Using different cancerous cell lines we showed that S100A8/A9 induced dual effects with different concentrations, cell death, and cell proliferation. S100A8/A9 provokes both apoptosis and autophagy in cancerous cells. Using knock down technology we proved that S100A8/A9 cell death was not mediated via receptor for advanced glycation endproducts. Investigation of cell lines either deficient in, or over-expressing components of the death signaling machinery provided insight into the S100A8/A9-mediated cell death pathway. S100A8/A9 induced Bax and Bak activation with subsequent selective release of Smac/Diablo and Omi/HtrA2 release, and without AIF and Endo G translocation to the nucleus. We also showed that S100A8/A9 inhibited mitochondrial fission machinery via suppression of Drp1 expression. Using immunocytochemical and biochemical methods we also proved that autophagy plays an important role in S100A8/A9 cell death mechanism. S100A8/A9 treatment caused the increase of Beclin 1 expression, LC3-β cleavage, and Atg12-Atg5 formation. Interestingly, S100A8/A9 (<20μg/ml) induced cell proliferation in cancerous cells via RAGE ligation. Both RAGE gene silencing and co-treatment with a RAGE-specific blocking antibody revealed that this activity was mediated via RAGE ligation. We showed that MAP-kinase phosphorylation and NF- k B activation was involved in S100A8/A9 cell proliferation induction. S100A8/A9 caused a significant increase in p38-MAP and p44/42 kinase phosphorylation without SAPK/JNK phosphorylation. All these finding together showed the importance of S100A8/A9 in tumor biology and future new cancer therapy.

Keywords: S100 proteins, cancer, apoptosis, autophagy, RAGE, calprotectin

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P-10-471-1

Synthesis of a new copper (II) complex containing mixed aliphatic and aromatic dinitrogen ligands and binding studies with bovine serum albumin using different instrumental methods

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The new copper (II) complex, [Cu(N-N)(L)(EtOH)](NO3)2.2H2O; in which N-N = 2, 9-dimethyl-1, 10-phenanthroline and L=N,N-dimethyltrimethylenediamine; has been synthesized and characterized by 1H, 13C NMR, absorption spectroscopy and elemental analysis (CHN). The interaction of this copper (II) complex with bovine serum albumin (BSA) was investigated under physiological condition in 0.01 M phosphate buffer solution (pH 7.00) by spectroscopic methods including fluorescence spectroscopy, UV-Vis absorption spectroscopy and circular dichroism (CD). The results of fluorescence titration revealed that the Cu (II) complex strongly quench the intrinsic fluorescence of BSA through a combination of static and dynamic quenching procedures. Binding constants (K), association constants (Ka) and the number of binding sites (n≈1) were calculated using modified Stern-Volmer equations. The distance r0 between donor (BSA) and acceptor (copper complex) was obtained to be 1.9 nm according to Foster's non-radiative energy transfer theory and showed that the energy transfer from BSA to the Cu (II) complex occurs with high probability. The results of UV-Vis spectroscopy and CD data revealed that binding of the Cu (II) complex with BSA can induce the conformational changes in BSA.

Keywords: bovine serum albumin, water soluble Cu (II) complex, circular dichroism, fluorescence quenching

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P-10-468-1

Differentiation of human umbilical vein -derived mesenchymal stem cells into hepatocytes in vitro

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Stem cell-based therapy and transplantation are of potential value in tissue and organ replacement and regeneration approaches. There is much interest in the generation of mature hepatocyte from stem cells. Of particular concern is hepatogenic potential that can be used for liver-directed stem cell therapy and transplantation. In this study, we have investigated whether human umbilical vein (UV)-derived MSCs

are able to differentiate into hepatocyte-like cells. The cells were cultured with hepatocyte growth factor (HGF), and oncostatin M (OSM). Expression of a variety of hepatic lineage markers was analyzed by RT-PCR and immunofluorescence (IF). The functionality of differentiated cells was assessed by Indocyanine green (ICG) cellular uptake. The cells showed the remarkable transition from bipolar fibroblast-like morphology to round or oval shape. The temporal gene expression pattern for a number of hepatocyte-specific genes (Albumin, cytokeratin-18, cytokeratin-8, Hepatocyte nuclear factor 3β), were detected during differentiation. The IF analysis showed that the differentiated cells were stained positively for albumin. The differentiated hepatocyte-like cells were positive for ICG. Based on these observations, we conclude that UVMSC retain hepatogenic potential suitable for cell therapy and transplantation against intractable liver diseases.

Keywords: stem cell, hepatocyte, umbilical vein

P-10-474-1

In vitro differentiation of human bone marrow-derived mesenchymal stem cells into cardiomyocytes

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The inability of adult cardiomyocyte to divide to a significant extent and regenerate the myocardium after injury leads to permanent deficits in the number of functional cells, which can contribute to development and progression of heart failure. Transplantation of stem cells into the injured myocardium is a novel and promising approached in the treatment of cardiac disease and the restoration of myocardial function. In the present study we investigated the potential of human mesenchymal stem cells from adult bone marrow to differentiate into cardiomyocytes. Human Bone Marrow Mesenchymal Stem Cells (hβMSCs) cultured in enriched medium were treated with 10-6M oxytocin for one month. Morphologic characteristics were analyzed by phase contrast microscope. Expression of hα3-actinin and hβMHC (myosin heavy chain beta) was detected by RT-PCR. Protein expression of α-actinin and Troponin I-C was analyzed through immunostaining. hβMSCs were spindle-shaped with irregular processes. Cell treated with oxytocin were connect with adjoining cells forming myotube like structures. Immonostaining of the differentiated cells for α-actinin and Troponin I-C were positive. These results indicate that adult human bone marrow mesenchymal stem cells can differentiate into cardiomyocytes in vitro and can be considered as a source of cells for cellular cardiomyoplasty.

Keywords: human bone marrow mesenchymal stem cells, cardiomyocyte, cell differentiation

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P-10-187-2

The Study of expression of pdx-1 in mouse embryo

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Several transcription factors are known as critical regulators of pancreatic development in mouse. Expression patterns of transcription factors limit the boundaries of developing pancreas and determine the differentiation programs of individual cell lineages. Pdx-1 gene is an important regulator in pancreas development. The importance of pdx-1 gene is its role in early development of pancreas and differentiation of pancreas endocrine cells, especially β cells. In this research morphology of the developing pancreas and expression of pdx-1 were studied. Serial section of formalin fixed, paraffin embedded mouse embryos was prepared. Total RNA of developing pancreas was extracted. RT-PCR with specific primer of pdx-1 was done and expression of pdx-1 was studied. There was pdx-1 excretion in development pancreas.

Keywords: transcription factor, pancreas, Pdx-1 gene, β cell, development

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P-10-476-2

Haplotype analysis of beta thalassemia patients in Western Iran

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β-thalassemia (β-thal) is the most common single gene disorder in Iran. To determine the chromosomal background of beta thalassemia mutations in Western Iran we studied β-globin gene cluster haplotypes in 314 β-thal and 70 βA chromosomes with a Kurd ethnic background from the province of Kermanshah, Iran using PCR-RFLP. β-thal mutations were analyzed using PCR-ARMS, RFLP and direct genomic sequencing. Haplotypes were constructed by analyzing the pattern of seven restriction sites through the β-globin gene cluster. Haplotype I was the most prevalent haplotype (35.7%) among β-thal chromosomes followed by haplotype III (28.6%). βA chromosomes similar to β-thal chromosomes were linked to diverse haplotypes but predominantly with haplotype I (42.9%). The predominant IVSII-1 (G A) mutation in this population (33%) was strongly linked to haplotype III (66.1%) but was also found on chromosomes with haplotypes I, II, V, X and atypical. The second prevalent mutation was CD8/9 +G (13.5%) and showed a strong association with haplotype I (96.4%) and a weak association with haplotype V (3.6%). Haplotype background for Kurdish mutations among our studied population was similar to those among Kurdish Jews and people of Kurdistan of Iran. Identification of the most common mutations on different haplotype backgrounds can be explained by a variety of gene conversion and recombination events.

Keywords: haplotype, β-thalassemia, mutation, XmnI site, Western Iran

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P-10-476-3

Detection of responsible mutations for beta thalassemia in the Kermanshah Province of Iran using PCR-based techniques

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β-Thalassemia (β-thal) has been reported to be a common genetic disorder in Iran. To establish the molecular spectrum of the beta-thalassemias in the Kermanshah province 185 unrelated beta thalassemia patients with Kurdish ethnic background were studied (181 β-thal major and 4 β-thal intermedia). Using polymerase chain reaction-amplification refractory mutation system (PCR-ARMS), restriction fragment length polymorphism (RFLP) and direct genomic sequencing twenty different mutations were identified accounting to the 98.10% of the studied alleles. Around 80.8% of β-thalassemia chromosomes had β0 mutation. The most prevalent mutation was the IVSII-1 (G:A) (32.97%), followed by CD8/9 +G (13.51%), IVSI-110 (C:T) (8.38%), CD 36/37 –T (7.84%), CD8 –AA (5.94%), CD15 (G:A) (4.86%) and IVSI-1 (G:A) (4.59%). All of them together accounted for the 78.09% of studied alleles. The result presented here could be of valuable help in the screening of β-thalassemias in the population of Western Iran.

Keywords: beta thalassemia, molecular analysis, mutation, Western Iran

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P-10-463-1

Differential gene-expression of Metallothionein 1M and 1G in response to zinc in Sertoli cell (TM4) line

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Several studies have suggested that male infertility may be related to zinc and low molecular weight proteins, metallothionein. Our objective was to determine the effect of zinc on metallothionein 1M and 1G gene expression using different timing on testicular Sertoli cells. Cultured TM4 sertoli cells were exposed to zinc in different doses (physiologic 2, pharmacologic 20, 50, 100, and toxic 500 micromoles) at different periods of time (1, 2, 3 and 6 hours and also 18 hours for cells treated with 50 micromoles). MTT assay, flame atomic absorption spectrometry and real time PCR method were performed. Zinc concentration and both gene expression in treated cells were greater than that in untreated cells (p value<0.05). In the treated group, there is no evident differences in zinc concentration and gene expression in low dosage treated cells (2, 20, 50), but in the high dosage treated cells (100, 500), especially 3 hours after treatment, zinc concentration and gene expression of metallothionein 1M and 1G increased, except for metallothionein 1M at 6 hours of treatment which decreased below the initial level, then at 18 hours of treatment the expression of both genes in cells treated with 50 micromoles increased dramatically, but zinc concentration decreased. Cell viability of treated cells decreased in the cells treated with toxic dose. Zinc exposure at physiologic doses did not have a notable effect on metallothioneins gene expression but pharmacologic dose and long term exposure dramatically increased gene expression of both isoforms and toxic dose reduced cell viability.

Keywords: male infertility, zinc, metallothionein

P-10-509-1

Investigation of point mutations at seventh exon of p53 gene in hepatocellular carcinoma patients in Kermanshah province by PCR-RLFP and non-radioactive PCR-SSCP methods

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Hepatocellular carcinoma (HCC) is a major cause of cancer morbidity and mortality in many parts of the world including Asia and Africa. The point mutation in p53 gene, exon 7, codon 249 has the highest frequency in patients affected with HCC. Major risk factors for HCC include chronic infection with HBV or HCV and dietary exposure to AFB1. The aim of this study is to investigate the status and carcinogenic role of p53 gene, exon 7 point mutation in HCC affected patients in Kermanshah. 25 formalin-fixed paraffin-embedded tissues related to HCC patients were collected from pathology centers which were diagnosed using histological methods. Extracted DNA from these cases was amplified by PCR. Furthermore, to analyze point mutation of p53 exon 7, RFLP and SSCP were applied. According to the RFLP results that were cleaved by restriction enzyme, Hae III no point mutations were found and all samples were cleaved by this enzyme. The SSCP results showed two mutations in other codons of exon 7. The results of this study indicate that there are not enough evidences to prove the correlation between AFB1 and HCC index in Kermanshah. However, finding mutation in other codons may suggest the contribution of other risk factors rather than exposure to aflatoxin B1 to the incidence of HCC. By performing statistical studies, no significant correlation was observed between p53 mutation and histological grade, tumor stage, sex, and age. However, there is a direct relationship between these mutations and cirrhosis.

Keywords: HCC, p53, PCR, RFLP, SSCP

P-10-508-1

Detection of hepatitis B virus (HBV) genotypes in hepatitis B surface antigen (HBsAg)-positive carriers in Kermanshah province

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Hepatitis B virus (HBV), a human pathogen, causing the serious liver disease such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma worldwide, has been classified into eight viral genotypes, A-H, which vary in geographic distribution. HBV genotypes might influence severity of liver disease and response to antiviral treatment.

A total of one hundred hepatitis B surface antigen (HBsAg) positive serum samples were collected from HBV carriers in Kermanshah province, and used to determine the HBV genotypes. HBV DNA was extracted from 200 µl of each serum samples by using a nucleic acid extraction Kit. In this study, nested-PCR strategy combined with restriction fragment length polymorphism (RFLP) technique was used to detection of HBV genotypes. The nested-PCR was done with 4 primers that were universal primers for all HBV genotypes. The enzymes used for RFLP, were MboI, StyI, HpaII, CfrI and BsrI. Genotype D was predominant genotype in our province (85 of 100 samples). We recognized genotype B only in one sample, while no product was obtained in other 14 samples. The results of this study show that genptype D, similar to other region of Mediterranean areas and Middle East is the main and most frequent HBV genotype in Kermanshah province.

Keywords: genotypes, hepatitis B virus, Kermanshah province

O-10-326-2

Neutrophil Gelatinase Associated Lipocalin modulates expression of antioxidants

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Neutrophil Gelatinase Associated Lipocalin (Lcn2, NGAL) is a member of the lipocalin superfamily whose precise function has not been fully known yet. Much interest has recently been focused on the physiological/pathological role of the Lcn2/NGAL. Protective factor against oxidative stress is one of the novel roles of Lcn2. On the other hand antioxidant enzymes play an important role for removing of toxic oxygen byproducts and a state of oxidative stress in cells is associated with an increased expression and activity of antioxidant genes/proteins. For more clarify the role of NGAL/Lcn2 in oxidative stress, the current study was conducted to test the effects of Lipocalin2 on the expression of antioxidants such as Heme oxygenase (1,2), superoxide dismutase (1,2), Metallothionein (MT-1), glutathione S-transferase (A1, M1). Lcn2 was isolated by using specific primers and cloned to pcDNA3.1 plasmid by using genetic engineering methods. The recombinant vector was transfected to CHO and HEK293T to establish stable cells ectopically expressing recombinant Lcn2 and the expression of antioxidant genes/ proteins were compared with appropriate controls by RT-PCR and western blot analysis. The results showed that the expression of antioxidant genes/proteins were higher in cells expressing recombinant NGAL/Lcn2 compared with the control cells. In other words, Lcn2/ NGAL modulate expression of other antioxidants. Our results suggest that part of antioxidant property of NGAL/Lcn2 could be attributed to the induction of antioxidants.

Keywords: NGAL/LCN2, antioxidant genes, SOD1, SOD2, oxidative stresses

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P-10-465-1

Soluble carcinoembryonic antigen blocks mouse skeletal muscle C2C12 myogenic differentiation

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The GPI-linked carcinoembryonic antigen (CEA) is upregulated in many different tumors including colorectal, breast, and lung. Previous studies have proposed that CEA over-expression and cell surface homotypic binding could block various types of differentiation programs including rat L6 and mouse C2C12 myogenic differentiation. CEA proteins are secreted or shed from the cell surface spontaneously. In this work we have investigated whether the soluble, secretory form of CEA protein could also block myogenic differentiation. For this purpose, C2C12 myoblasts were plated at a density of 70×103 cells/ml in DMEM containing 10% FBS supplemented with penicillin, and streptomycin in 6-cm culture plates. Differentiation into myotubes was induced after 4 days by switching 95-100% confluent cells to DMEM supplemented with 2% horse serum. To investigate the effects of soluble form of CEA we added media from the cultured human colon carcinoma cell line LS-180, containing CEA protein at various concentrations, to the differentiation media of cultured C2C12 cells. The media of cultured CHO cells were also used as controls. After 5 days treatment with differentiation and conditioned media, C2C12 cells were stained with Hematoxylin and Eosin. Our data showed that myotube formation of C2C12 was efficiently blocked by 0.02-10 ng/ml concentration of soluble CEA in comparison with controls. This work has established that differentiation blocking is an inherent feature of CEA and the cell surface attachment by the GPI-anchor is not important in this process, except for facilitating the protein to be more readily released into the medium.

Keywords: CEA, GPI, differentiation

P-10-526-1

Encapsulation of curcumin into dendrosomes, significantly improves efficacy of drug treatment in the human gastric cancer cell line

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Gastric cancer is a major cause of mortalities with a large number of newly diagnosed cases per annum worldwide. Current modalities for gastric cancer include surgery and chemotherapy, but local recurrence and severe side effects are still unresolved issues in this regard, indicating the necessity of development of safer and more effective therapeutics. Curcumin is a well known herbal compound with inhibitory effects on cell proliferation as shown by pre-clinical studies in a variety of human cancer cell lines. Apoptosis induction, is a proposed mechanism of action for curcumin, however, its exact mechanism remains largely unknown. In the present study, effects of curcumin treatment, along with Dendrosome-encapsulated drug were assessed on the proliferation of the human Adenocarcinoma AGS cells. FACS analysis was performed to elucidate the effect of the drug on cell cycle and apoptosis status of cells. Curcumin treatment resulted in a decrease in the G1 population and a significant increase in the number

of the apoptotic cells compared to untreated cells. Further, encapsulation of curcumin into dendrosomes, a novel family of biodegradable nanocarriers previously used by our group for transfection and therapy, significantly enhanced the number of apoptotic cells and the G1-population decrease as compared to curcumin treatment alone. Moreover, treatment of cells with dendrosomes alone caused no significant effect on the normal cell cycle distribution. Our results indicate that curcumin, may be a promising chemotherapeutic for inhibition of tumor growth and cell proliferation and that dendrosome encapsulation might enhance the efficacy of the drug treatment while administrating lower amounts of the drug.

Keywords: gastric cancer, curcumin, dendrosomes encapsulation, chemotherapy

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P-10-508-2

Comparison of three methods for Hepatitis B virus DNA extraction from infected serum samples

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The presence of hepatitis B virus (HBV) DNA in serum is a reliable marker of viral replication and infectivity, and PCR, one of the molecular biology techniques, is becoming the preferred method for its detection. 50 hepatitis B surface antigen (HBsAg)-positive samples were collected and used for DNA extraction by using three methods, including QIAamp MinElute Virus Spin Kit, DNPTM Kit and phenol-chloroform extraction procedure. Nested-PCR was done to determine of presence of PCR inhibitors, and to evaluate of three different methods of HBV DNA extraction from serum samples. 43 of 50 (86%) samples that were extracted by QIAamp MinElute Virus Spin Kit were positive for PCR. The result was 31 of 50 (62%) samples for DNPTM Kit and 20 of 50 (40%) for phenol-chloroform extraction procedure. Our results reveal that QIAamp MinElute Virus Spin Kit is the most efficient method for HBV DNA extraction of serum samples, and its PCR inhibitors are fewer than the other two methods.

Keywords: DNA extraction, PCR, HBV

P-10-291-3

Elevation of intracellular calcium by treatment with ATP

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ATP is present in milimolar concentrations in the cytosol of all cell types and there is minimal permeation of ATP or MgATP across lipid bilayer. However a significant extracellular concentration can occur if cytosolic ATP (~3-5 mm) is released on sudden breakage of intact cell, as might occur during rupture of blood vessel or other type of tissue trauma. We used the ATP as a mean of extracellular activation of elevating the intracellular Ca2+ concentration on MG-63 cell line. MTG-63 cells were grown on coverslipes for 3 days. On day 3, they were refed with DMEM +10% FCS and incubated for further 2-3 days. Prior to loading with Fura-2 changes in the calcium level were induced by perfusing the cell layer with A23187, from 10 second after the start of the incubation. Cells were then monitored using the magical system. Measurements of intra cellular calcium in MG-63 cells cultured was performed using the calcium sensitive fluoresim probe fura-2.In unstimulated Cells the level of intracellular calcium was approximately 50nM. In stimulated cells the [Ca2+] was increased to 4oo nm after 50 seconds after addition of Ionomycin. The osteosarcoma cell line (MG-63) express P2_purinorceptors. Activation of these receptors by extra cellular ATP cause a transit increases in Ca2+ levels.

Keywords: osteosacoma cell, ATP, Ca2+, MG-63

O-11-639-1

Expression of an enterotoxigenic Escherichia Coli fusion protein, colonization factor B-heat labile toxin B subunit (CfaB-LTB), in transgenic plant as edible vaccine candidate

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Enterotoxigenic Escherichia coli (ETEC) are the main cause of diarrhea in fewer than 5 year old children living in developing countries and travelers. The prevalence of the disease is about 400,000,000 annually among which 400,000 to 800,000 lead to deaths. Vaccination against the disease is one of the significant objectives of World Health Organization. After intestinal colonization, heat labile and stable toxins are released which lead to diarrhea. Cfa B and LT molecules are the most important virulent factors of ETEC. The critical role of these proteins in ETEC pathogenesis, they are candidate for vaccine development. Mucosal immunity is necessary for efficient vaccination. The transgenic plants are able to administrate vaccines at mucosal surfaces and induce local as well as systemic immunity. An optimized synthetic gene, which encodes CfaB-LTB fusion protein was designed and synthesized. After construction of a plant expression vector, the gene was introduced into the nuclear genome of tobacco model plant; by using agrobacter tumefaciens mediated transformation method. Several independent transgenic lines were regenerated via somatic embryogenesis and transferred to a greenhouse. The presence of CFaB-LTB fusion gene in the genomic DNA of transgenic plants was detected by PCR method. Southern blot and RT-PCR analyses confirmed integration and transcription of synthetic construct. Western blot analysis showed that the cells of transgenic plant synthesize CFaB-LTB fusion protein. Results showed that fusion protein (CfaB-LTB) could be expressed in tobacco leaf tissues. Analysis of immunogenicity of protein in mice model as an oral vaccine is in progress.

Keywords: ETEC, vaccine, transgenic plant

P-10-539-1

The effect of tryptophan 323 mutation on binding SMAC peptide to Xiap-Bir3

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The ability of the wild type Xiap-Bir3 domain as well as its Trp 323 mutation to ser on binding to AVPFVASLPN, SMAC, peptide was investigated. Xiap, X-linked inhibitor of Apoptosis Protein, prevents cell death, mainly through inhibition of caspases. The activity of XIAP on

the other hand is antagonized by a protein called SMAC. According to X-ray crystallography data, Trp323 from Bir3 domain of XIAP protein forms a hydrogen bond with the proline in P3 position of the SMAC peptide. Thus W323 residue was mutated to ser using overlapping PCR technique. Following expression and purification of the wild type as well as the mutant Bir3, SMAC peptide binding was investigated by fluorescence spectroscopy. Addition of the peptide to Xiap-Bir3 caused quenching of the emission spectra at 340 nm with 50 % quenching at 1.5 μM, while for the mutant 50 % quenching occurred at 26.5 μM. These data indicate that Trp323 plays a very important role in binding to SMAC.

Keywords: apoptosis, caspase-9, Xiap–Bir3, SMAC

P-10-540-1

Study of biochemical factors in hepatocyte derived from rat bone marrow mesenchymal stem cells

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Bone marrow mesenchymal stem cells (MSCs) are multipotent and capable to differentiate to specified tissues. The aim of this study was to investigate the hepatocytic differentiation of rat bone marrow MSCs by induction of fibroblast growth factor-2 (bFGF), oncostatin M (OSM) and hepatocyte growth factor (HGF) in order to find suitable resource of hepatocytes for bioartificial liver or liver transplantation. In this research MSCs were obtained from the rat's bone marrow and cultured at 37C in humid air with 5% CO2 in Dulbecco's Modified Eagle Medium-Low Glucose (DMEM-LG) supplemented with 15% fetal bovine serum (FBS). These cells were treated with differentiate medium supplemented with HGF, bFGF and OSM for 24 days. Morphological assay, RT-PCR, ELISA and biochemical assays were used to identify differentiated cells. MSCs exhibited round in shape after differentiation, instead of fibroblast-like morphology before differentiation. Albumin, urea and Alpha-fetoprotein (AFP) were detected positively in differentiated cells. The enzyme activity of alkaline phosphatase was detected positively by biochemical tests. The mRNA expression of CK-18 and tyrosine amino transferase (TAT) was demonstrated by RT-PCR. It can be concluded that rat MSCs from bone marrow can differentiate hepatocytic-like cells in the presence of HGF, bFGF and OSM in vitro. This method is easy to operate. Bone marrow derived MSCs are a new source of cell types for cell transplantation therapy of hepatic diseases.

Keywords: cell differentiation, hepatocytes, mesenchymal stem cells (MSCs), stem cells

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P-10-257-1

Effect of selenium on telomerase gene expression breast cancer cell line

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Telomerase is a RNA dependent DNA polymerase which synthesizes telomeric repeats and in most cases are molecular base of unlimited proliferation. Telomerase gene expression occurs in most tumor cells rather than normal cells. The aim of this study was to evaluate the effect of selenium on telomerase gene expression in breast cancer T47D cell line. Time and dose dependent manner treatment of T47D breast cancer cell lines were carried out by adding selenium in tissue culture. Relative telomerase activity (RTA) of T47D cell line was measured by Telomere Repeat Amplification Protocol (TRAP) assay. After being treated with 10, 30 μM /L selenium-L- methuonin, telomerase activity were markedly inhibited. Telomerase activity of T47D cells for 24 hours were 0.93, 0.60 and for 48 hours were 0.76, 0.12 respectively (control 49.2%). Based on obtained results, it is speculated that pharmacologic doses of Se, Zn and Cu could have an inhibitory effects on telomerase gene expression and it could be used as a target therapy in tumor cells.

Keywords: breast Cancer, selenium, telomerase

P-10-503-1

Expression analysis of five new chitin synthase genes in the biotrophic rust fungus Puccinia triticin

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Leaf rust, caused by Puccinia triticina, is the most common rust disease of wheat. The fungus is an obligate parasite capable of producing infectious urediniospores as long as infected leaf tissue remains alive. Rust cell walls are important structures for the integrity of the cell and potentially are involve in pathogenicity and resistance. One of the major structural components of most fungal cell walls is the chitin polymer which is synthesized by a family of enzymes called chitin synthases. In a library of 50,000 ESTs belonging to Puccinia triticina, five ESTs were found with high homology to Puccinia graminis chitin synthase genes, whose whole genome sequence is already known. Accordingly, primers were designed and the developmental expression profiles of these chitin synthase genes (chsII, chsIIIa, chsIIIb, chsIV, and chsV) were studied in two different stages of germination and housteria using their respective cDNA. Result showed different expression of chs genes in this stage.

Keywords: EST, Puccinia triticina, housteria cDNA

P-10-561-1

Optimization of proinsulin digestion with carboxypeptidase B

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Human insulin can be produced by different methods. In this study, production based on fermentation and utilizing recombinant DNA technology has been considered. In this research, we tried to optimize the most important step of insulin production, digestion with carboxypeptidase B. Insulin gene was cloned in E.coli and cultured in fermentor and expressed using control T7 promoter. To separate biomass from extracellular impurities and other undesirable components, centrifugation at 13000 x g was used. A high pressure homogenizer was applied to break the cells and release the inclusion bodies. At sulfitolysis step, DTT was added to unfold proinsulin molecules by breaking all disulfide bonds. Then the sample was

precipitated under acidic condition (pH4.5). The precipitation was chromatographically purified in an anion exchange resin (DEAE) column, Amersham Pharmacia Biotech AB. At refolding step, disulfide bond formed and proinsulin folded. The removal of the C peptide from human proinsulin was carried out enzymatically using trypsin and carboxypeptidase B in the best reaction condition. We optimized different parameters of the digestion reaction such as enzyme concentration, time of reaction, ratio of enzyme to substrate, temperature, and buffer condition. Then the best carboxypeptidase B digestion resulted using Tris-HCl 50mM (pH 7.5) buffer condition, 1:10 enzyme to substrate ratio. The reaction took place at 37˚C for 5 hr, reaching the highest conversion yield. In this research, we tried to optimize converting Arg-insulin to insulin with carboxypeptidase B. It was practical method and hydrolysis of the sample was achieved efficiently.

Keywords: carboxypeptidase B, digestion, recombinant insulin

P-10-403-1

Serum paraoxonase activities in an Iranian population

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Human serum paraoxonase (PON1) is an HDL-associated esterase that protects against organophosphate neurotoxicity, and plays an important role in the prevention of LDL and HDL oxidation damage, which is proposed to have an antiatherogenic action. Paraoxonase activity is under genetic and environmental regulation and varies widely among individuals and populations. Basal, salt-stimulated paraoxonase and arylesterase activities of 150 healthy individuals (61 male and 89 female, aged 5-75 years) in Khorramabad were measured spectrophotometrically using paraoxon and phenylacetate as substrates. Ratio of salt-stimulated paraoxonase vs. arylesterase activities was used for definition of phenotypes (double substrate method). The mean of basal, salt-stimulated paraoxonase and arylesterase activities were 88±50.5 U/ml, 172±110.2 U/ml and 53.2±16.1 kU/L, respectively. Age and sex had no effect on enzyme activities. The double substrate method showed a trimodal repartition in population. According to this method, 55.3% individuals classified as AA (low activity), 33.4% as AB (intermediate activity) and 11.3% as BB phenotype (high activity). In conclusion, the phenotype distribution was similar to that of other Iranian populations; the majority was low homozygous (AA) type with enzyme activity lower than European populations.

Keywords: activity, arylesterase, paroxonase, phenotype

P-10-594-1

Effect of vitrification on quality of in vitro produced bovine blastocysts

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Cryopreservation of oocytes and embryos is a widespread practice. An alternative form of cryopreservation is vitrification which is a process in which glasslike solidification of a solution occurs without the formation of ice crystal inside living cells within the solution during cooling and warming. The aim of this study is the assessment of the effect of vitrification on bovine blastocystes quality produced in vitro. After in vitro maturation and fertilization of immature bovine oocytes, presumptive zygotes were cultured for 8 days (39C %5 CO2). Then, 80 blastocystes were selected and vitrified (40% ethylene glycol, 18% ficol, 0.3 mol sucrose). After thawing the blastocyte were cultured in SOF2+ vero cell medium for 48h, re-expended blastocysts were selected, inner cell mass (ICM) for them and control group were recovered by immunosurgery and stained with Hoechst. The 80% of thawed blastocysts re-expanded and the mean total number of cells in control group and vitrified group were 85 and 73, respectively. The mean number of ICM in control group and vitrified group were 19±3 and 15±2 respectively. There was significant difference between the total and ICM cell number in the control group and vitrified group. Many factors like type of materials, toxicity, and speed of freeze-thawing and cultured medium after vitrification can reduce viability and quality of in vitro produced Embryos. In this study the number of ICM in vitrified group was lower than the control group so, the vitrification method reduced the quality of in vitro produced blastocysts.

Keywords: vitrification, bovine embryo, ICM, in vitro

O-10-275-1

The relationship between a deletion polymorphism in the gene encoding angiotensin I-converting enzyme and diabetic retinopathy in type 2 diabetes

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The relationship between diabetic retinopathy (DR) and an insertion (I) deletion (D) polymorphism in intron 16 of the angiotensin–converting enzyme (ACE) gene is still under debate, which might be due to ethnic and geographical variations. The aim of this study was to evaluate the possible association between I/D polymorphism and retinopathy in the Iranian type 2 diabetic patient population. A total of 178 consecutive patients with type 2 diabetes and retinopathy (Group A) and 206 patients with type 2 diabetes but without retinopathy (Group B) were included. The following variables were determined for each patient: age, sex, body mass index (BMI), diabetes duration and the drugs used, history of coronary artery disease and its complications, blood pressure (systolic and diastolic), fasting blood sugar (FBS), haemoglobin A1c (HbA1c), total cholesterol (Chol), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides (TG), plasma creatinine (Crt) and 24 h urine albumin excretion. Polymerase chain reaction (PCR) was used to detect the I/D alleles. Univariate (chi-squared and t-test) and multivariate (multivariate binary logistic regression with adjusted odds ratios) analyses were applied to determine the association between I/D polymorphism (with genotype II as reference) and retinopathy. In univariate analysis, the groups were statistically similar in all variables except for ACE activity (71.53±23.17 in Group A vs. 59.47±21.56 in Group B; P<0.001), and the frequency of DD genotype (58 cases in Group A vs. 40 cases in

Group B; P=0.008). Multivariate logistic regression (using age, sex and albuminuria as clinically significant variables and, ACE activity, and genotype as statistically significant variables) was then used to determine independent associations and adjusted odds ratios (OR). The DD genotype was the independent predictor of retinopathy [P=0.031, OR=1.789, 95% confidence interval (CI)=1.055-3.005], followed by log (ACE activity) (P<0.001, OR=1.026, 95% CI=1.015-1.037). The DD polymorphism in the ACE gene was independently associated with retinopathy in the diabetic population.

Keywords: angiotensin-converting enzyme, polymorphism, diabetic retinopathy, albuminuria

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P-10-593-1

EVALUATION OF LACTATE DEHYDROGENASE ACTIVITY IN DIFFERENT CANCERS

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Cancer is large class of diverse disease characterized by excessive, uncontrolled growth of abnormal cells, which invade and destroy other tissues. Lactate dehydrogenase is an enzyme found in the cells of many body tissues, including the heart, red blood cells and lung, and catalyses a critical step in the glycolysis pathway, the reversible transformation between pyruvate and lactate. LDH test is used to detect tissue alteration. Cellular damage causes an elevation of total serum LDH. The serum LDH was measured using LDH (P-L) kit which follows IFCC method of estimation. Rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is directly proportional to LDH activity in the sample. Pyruvate+ NADH+ H→lactate+ NAD. Lactate dehydrogenase (LDH) is expected to increase in neoplastic tissues therefore LDH was proposed as a good biochemical marker in diagnosis of different categories of cancers. LDH was chosen and evaluated in serum of patients having different cancers. A total of 23 patients showed a mean value of 295.58U/L. This was highly significant when compare with controls with values of 241.56 indicating its importance in the pathogenesis of the malignant disease. The assessment of LDH for different categories of cancers when dine it was found that gastro intestinal tumors, carcinoma cervix and breast cancers showed a highly significant value with controls. LDH levels when analyzed according to sex, males or females, the LDH values did not differ statistically indicating that LDH enzyme activity does not differ by the effect of hormones during the disease stage.

Keywords: cancer, LDH, neoplastic malignancy

P-10-651-1

Extraction of total RNA in the developing chicken forebrain

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Fertilized eggs from Ross breed (Gallus gallus) were incubated at 38°C and 60% relative humidity in a forced-draft incubator and were turned every 3 hours. After 3, 7, 14, 20 days of incubation, eggs were cooled on ice to induce deep anesthesia. Then whole brains were dissected out. As brains could not be excised in a reproducible way from earlier embryos (embryonic days 4 and 6), whole heads were collected. Chicken embryos between day 7-20 and one day after birth were decapitated, and their brains removed. Samples were immediately inserted into RLT buffer and stored at -70°C. Total RNA was isolated using the RNeasy mini kit according to the manufacturer's recommendations (Qiagen, USA). Any contaminating genomic DNA was digested by DNase treatment (DNA-free, Ambion, Austin, USA), according to the manufacturer's recommendations. RNA quality was checked using gel electrophoresis. We obtained 52µg/ml to 745µg/ml with A260/280 1.7-2.2. Only high quality RNA, with no signs of degradation, was used for further experiments.

Keywords: RNA, developing embryo, chicken, forebrain

P-10-326-6

Cloning and expression of lipocalin 2 trough gate way technology in insect cell line

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Lipocalins constitute a broad but evolutionally conserved family of small proteins; however, the functions of many lipocalins remain unclear to date. Neutrophil gelatin aseassociated lipocalin (NGAL; also known as lipocalin 2 or human neutrophil lipocalin) is a25-kDa glycoprotein that was initially purified from neutrophil granules. NGAL exists as a 25-kDa monomer, as a 46-kDa homodimer, and in a covalent complex with neutrophil gelatinase, also known as matrix metalloproteinase 9. Under various pathophysiological conditions such as infection, cancer, inflammation, kidney injury, cardiovascular disease, burn injury, and intoxication, expression of NGAL is induced. Isolation, cloning and expression of recombinant Lcn-2 (NGAL) by gateway technology was the aim of this study. Lcn-2 gene was isolated from HepG2 cell line by RT-PCR and PCR, and then it was cloned to prokaryotic TOPO vector by TOPO cloning reaction. Recombinant vector was transformed into E.coli. Recombinant colonies were screened by kanamycin. The construct was ligated to gateway adapted Baculovirus DNA by LR recombination reaction. The recombinant virus was transfected into insect cell line, Sf9. High level expression of recombinant Lcn-2 was detected by SDS-PAGE, ELISA, and Western-blot analysis (3µg/ml). Biological activity of expressed protein was determined by inhibition of bacterial growth.

Keywords: NGAL, gateway, TOPO cloning, Baculovirus, Sf9

P-10-327-3

Expression and purification of recombinant human coagulation Factor VII fused to His-Tag through gateway technology

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Factor VII is a plasma glycoprotein that participates in the coagulation process leading to generation of fibrin. Construction, expression and purification of recombinant FVII fused to poly histidine tag through gateway technology were aimed in this study. To construct entry clone, blunt-end FVII cDNA and subsequent PCR product isolated from HepG2 cell line was TOPO cloned into pENTR TOPO vector. To construct expression clone, LR recombination reaction was carried out between entry clone and destination vector, pDEST26. CHO cells were transfected with 1µg of DNA of PDEST26–FVII using the FuGENE HD transfection reagent. Two cell lines that permanently expressed recombinant factor VII were established. The expression of recombinant FVII was confirmed by RT-PCR and ELISA. Culture medium containing his-FVII was added to the nickel-nitrilotriacetic acid resin column and bound protein was eluted. The purified protein was detected by SDS-PAGE and western blot analysis. Biological activity of the recombinant factor VII was determined by prothrombin time assay using factor FVII-depleted plasma. The results showed that human recombinant FVII successfully was cloned and accuracy of the nucleotide sequence of the gene and its frame in the vector were confirmed by DNA sequencing. Stable clones were transfected with the construct expressed FVII mRNA and related protein but any expression was not detected in the CHO cells containing empty vector. A protein of about 52KD was detected in SDS-PAGE and was further confirmed by western blot analysis. A three-fold decrease in clotting time was observed by using this rFVII. As we are aware, this is the first report of expression of recombinant FVII fused with his-tag trough gateway technology. The next steps including large scale expression, purification, activation and stabilization are underway.

Keywords: gateway technology, hemophilia, recombinant FVII, His-Tag, purification

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O-10-662-1

Random mutagenesis for protein breeding of firefly luciferase

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Firefly luciferase catalyzes Ca2+es the oxidation of a benzothiazole substrate (beetle Luciferin) in the presence of magnesium ions, ATP and molecular oxygen. This enzyme efficiently converts chemical energy into light with a quantum yield of 0.42. There has been intense commercial interest directed for developing bioluminescence-based techniques; however, several factors limit further application and development of this technology, including the low stability of the enzyme both in vitro and in vivo, a low turn over number and high Km for the substrate, ATP. Directed evolution attempts to mimic the evolutionary process on a greatly accelerated time-scale. Evolutionary adaptation takes millions of years, but directed evolution may evolve molecules, usually proteins, with modified or novel functions, in weeks. In this study we have attempted to improve the usefulness of this enzyme using an approach based on random mutagenesis. A large numbers of heterogeneous mutant molecule were produced, the mutated gene library is then cloned into an appropriate expression vector and screened for the appropriate mutants.

Keywords: protein breeding, firefly luciferase, random mutagenesis, directed evolution

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P-11-109-2

Isolation of membrane peroxins complex with pull down assay approach

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Pex3p, Pex16p, Pex19p are peroxins required for maintenance of peroxisomal membranes. These three factors are required components for integrity of peroxisome membranes in which any defect in these factors will lead to the destroying the whole peroxisomes with severe pathologic characteristics in man. As we had already isolated a CHO cell mutant cell line defective in PEX16 gene (ZPEG301). In order to have a detail view in molecular mechanism of Pex16p, PEX16 gene was stabley transfected into ZPEG301 cells called301p16 cells. Normal peroxisomes were restored in this cell 301p16 showed the functional complementing activity of PEX16 gene. A tertiary complex, composed of Pex3p, Pex16p, Pex19, was identified by blue native analysis in 301p16 lysated cells while it was absent in ZPEG301 lysated cells. Moreover, pull down experiment using specific antibodies against Pex19p and Pex3p which were fused with various tags was performed on lysates of 301p16 cells that was already cotransfected with PEX19 and PEX3. However we were not able to define such data in ZPEG301 lysated cell. Data indicated Pex16p is responsible for Pex3p and Pex19p docking at the membrane of peroxisomes, which supported the formation of the complex. It is concluded that Pex16p plays a major role in stability of peroxisome membranes which can form a complex with Pex19p and Pex3p.

Keywords: peroxisome biogenesis, peroxisomes, PEX Genes, pull down assay

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P-10-443-1

Non-radioactive labeled probe preparation for detection of hu gene

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HU family proteins are abundant histone-like proteins that exist in all bacteria and have at least 20% amino acid sequence similarity. HU protein sequence even in various strains of one species could be different. Among some *Bacillus* species, a protein highly homologous to HU, classified HB, has been isolated and characterized; its conservation is more than 80% and coded by *hbs* gene. According to recent studies,

the sequence of *hbs* gene just in one strain of *Bacillus subtilis* exists in gene bank (ATCC 23857). In this study, DNA from *Bacillus subtilis* with ATCC 6633 was extracted and investigated by PCR. PCR product was sequenced and shown that just one nucleotide differs from *B. subtilis* ATCC 23857, but there is no change in codon. So, it was chosen as reference and it was used for non-radioactive labeled probe preparation. Thus, DNA from *B.subtilis* ATCC 6633 was extracted and *hbs* gene amplified by PCR. The PCR product was labeled by using a non-radioactive DIG-labeled nucleotides and optimized conditions of probe preparation and hybridization. Then for checking, it was used for detection of *hbs* gene in three strains of *Bacillus subtilis* with ATCC 12711, 6051 and 6633 by dot and southern blotting.

Keywords: Bacillus subtilis, histone-like proteins, hu gene, non-radioactive labeled DNA probe, southern blotting, dot blotting

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P-10-667-1

A novel primer annealing-ligation-extension approach to produce variable fragment length of CAG repeat

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Expansion of Cytosine Adenine Guanine (CAG) trinucleotide repeat within open reading frame is a characteristics feature of at least 10 known genetic disorders. CAG encodes amino acid glutamine and expansion of polyglutamine (polyQ) tracts in the protein will lead to insolubility of the protein followed by aggregation. Huntington's disease (HD) is one the well known disorders in this group which is a neurodegenerative disorder and inherited in an autosomal dominant fashion. There is a range between normal and abnormal size of the CAG repeat in the huntingtin gene. While the CAG number below 35 is considered normal, above 35 repeat will lead to aggregation and accumulation of the toxic protein in the cell. Also there is a relation between HD severity and age-at-onset of symptoms with the number of CAG repeat. In order to produce model cells with variable length of CAG repeat for these disorders, here we have used a novel approach to produce variable fragment length of CAG repeat. Using this approach which we called primer annealing-ligation-extension, we were able to produce a wide range of CAG repeat from thirty to thousands of repeats.

Keywords: CAG repeat, Huntington's disease, polyglutamine

P-10-569-1

The anti-aging effect of SNP on hematopoietic stem cells from umbilical cord blood

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Hematopoietic stem cells have low telomerase but with high activity. Evidence show their telomere length shorten despite of their high activity telomerase in the period of life span. The activity of telomerase decreases in the period of life span. In this work we have studied the anti-aging effects of NO donors in hematopoietic stem cells. For this purpose, we have isolated HSC from healthy umbilical cord blood by immune magnet coated bead affinity chromatography. The HSC were treated with SNP (sodium nitro proside) as a NO donor in different dose and then measured nitrate and nitrite concentration by Griess method, and the telomerase activity was assayed by TRAP-PCR-ELISA. Cell proliferation was assayed by MTT, BrdU and hemocytometer cell count slide. Our results indicated that there hasn't been any significant increase in cell proliferation and telomerase activity that is dependent to the concentration of SNP.

Keywords: hematopoietic stem cells, nitric oxide, SNP, telomerase

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O-10-109-3

Efficient transfection of mouse stem cells with a new constructed plasmid containing attachment site (attB) sequence related to ΦC31 integrase and Oct-4 Promoter by Phage integration system

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In order to study the Oct-4 regulation and its function in the differentiation process, we have designated to clone its related promoter upstream of EGFP as a gene marker. Oct-4 is a transcription factor of the POU family. This protein is critically involved in the self-renewal of differentiated embryonic stem cells and also is a transcription factor used to create induced pluripotent stem cells, demonstrating its capacity to induce an embryonic stem cell-like state. As such, it is used as a marker for undifferentiated cells. In this study, after mouse genome preparation, a part of mouse genome in the size of 2177 bp containing the mouse Oct-4 promoter was amplified by specific designated primers for a SOE-PCR method in two separated steps and the final product was cloned in a T-vector for analysis. Next, promoter was placed in an expression vector, upstream of EGFP cDNA near to attachment recognizable sites, for integrase activity of PhiC31 phage (attB sequence). The recombinant vector was verified by sequencing. Co-transfecting of this plasmid and a vector expressing integrase cDNA, into the mouse stem cell line (Royan B1), we obtained numerous transformant cell colonies expressing EGFP under regulation of this promoter. According to the ability of the integrase for stable integration, into the specific sites in the genome of the transfected cells, we expect that these cells can express EGFP stably and are a good source for studying the molecular mechanisms of stemness and cellular differentiation.

Keywords: attachment site (attB), integrase, oct-4 promoter, PhiC31 phage, stem cell

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P-10-314-1

Response of protein unfolding to a non-equilibrium temperature change

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In the last two decades, derivations of fluctuation theorems have provided a breakthrough in the field of non-equilibrium statistical mechanics. Demonstration of the first relationship in 1993 by Evans et al., and the derivation of the Evans-Searles Transient Fluctuation Theorem and the Gallavotti-Cohen Fluctuation Theorem motivated new research in the field. Since their inception, Fluctuation Theorems have been developed to investigate non-equilibrium states in nanophysics and biophysics where the energies involved are typically small and thermal agitation cannot be neglected. Considering protein unfolding as an endothermic reaction we have used the Evans-Searles Transient Fluctuation theorem to quantify predictions of the classical Le Chatelier's principle. Le Chatelier's principle for a system that is in equilibrium and is subject to a temperature increase states that the equilibrium will adjust so that the internal energy (constant volume) or enthalpy (constant pressure) increases. We have shown that this can be predicted from statistical mechanics and the molecular level dynamics of the system. Furthermore we show how the principle is adapted for small systems monitored over short periods. Preliminary results on some protein unfolding simulations will be presented to demonstrate the results.

Keywords: non-equilibrium statistical mechanics, fluctuation theorem, molecular dynamics, protein unfolding, Le Chatelier's principle

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P-10-708-1

The effect of aluminum on the activity and gene expression of catalase in lisianthus (Eustoma grandiflurum L.)

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Under stress conditions (either biotic or abiotic stress), defense responses are activated. In recent studies on tea, both at cellular level as well as in intact plants, tobacco, broad bean and lisianthus, it has been shown that heavy metals e.g., manganese, nickel, and aluminum toxicity is linked with oxidative stress. To detoxify reactive oxygen species produced during oxidative stress, the plants have two major non-enzymatic and enzymatic antioxidant defense systems. It has been supposed that among the latter, catalase plays a key role in antioxidant system. Sensitivity of catalase and increase of its activity has been reported to be induced by stresses such as light, temperature, UV-B, O3, SO2, pathogens, salt, etc. It has been also reported that the expression of catalase gene is sensitive to various environmental signals as well. In the present research, rooted cuttings of lisianthus (Eustoma grandiflurum L.) were treated with or without Al in a modified Hoagland solution containing 880 µM Al, for 24 h. The activity of catalase was measured by spectrophotometer. The relationship between activity of catalase and expression of its gene was examined using semi-quantitative RT-PCR technique. The results showed the activity and the gene expression of catalase was increased in Al-treated group in comparison to control group. The results implied that under oxidative stresses, plants increase their defense mechanism by increasing the activity of catalase, in expression and also protein level, as a major scavenger for reactive oxygen species.

Keywords: antioxidant system, catalase, gene expression, lisianthus, RT-PCR

O-10-690-1

Cloning and nucleotide sequence of a lipase gene from a soil isolate

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The present study was aimed to isolate and characterize the lipolytic enzyme producing bacteria from soil samples collected from regions around Zayande-rood river of Isfahan, Iran. In this study, samples were collected using 15 cm depth of soil surface and suspended in a sodium chloride solution and serially diluted. Samples from different dilutions were spread onto standard plate agar. Based on morphology, distinct colonies were isolated and purified through streak culture of each soil sample. Isolated colonies were examined for lipase activity through plate assay using egg-yolk agar medium. Total of 15 isolates were found to developed clear zones around their growth area and was considered as lipase positive. Preliminary identification of lipolytic active isolates revealed them to be gram positive, rod-shaped, endospore-forming and catalase positive bacteria, characteristic indicative of the genus bacillus. The gene coding for an extracellular lipase was cloned using PCR techniques. The gene was identified to be 639 bp in length and encoded a peptide of 212 amino acids with calculated molecular mass of 19353.96.Da, and pI 9.28. The DNA sequence and deduced amino acid sequence of the hypothetical lipase shows striking similarities to lipases from Bacillus subtilis strains. The cloned gene in this study may be of research and biotechnological significance.

Keywords: B. subtilis, lipase sequence, Isfahan soil

P-10-724-1

Effect of NBS1 gene polymorphism on the risk of cervix carcinoma

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Cervical cancer is one of the most common neoplastic diseases affecting women, with a worldwide incidence of almost half a million cases. A history of smoking and use of oral contraceptives have been confirmed to be risk factors for cervical cancer. Genetic susceptibility and immune response, especially impaired cellular immune response may well be related to the development of cervical cancer. NBS1 is one

of the key proteins participating in the recognition and repair of double-strand breaks that may lead to genomic instability and cancer if unrepaired. The objective of the present study was therefore to investigate NBS1 Glu185Gln gene polymorphisms and the risk of cervix cancer in a northern Indian population. We found that passive smokers having particular NBS1 genotypes (Glu/Gln, Gln/Gln or Glu/Gln + Gln/Gln) have an increased risk of developing cervix cancer (OR 5.21, p≤0.001; OR 4.60, p=0.001; OR 5.10, p≤0.001; respectively).The risk was increased 2.4-fold in oral contraceptive users with a Glu/Gln genotype. We conclude that the risk of cervical cancer is increased in passive smokers and in users of oral contraceptives with certain NBS1 genotypes.

Keywords: cervical cancer, NBS1, polymorphism, oral contraceptives, smoking

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P-10-705-3

Thermostability improvement of enzymes by error-prone and site directed mutagenesis

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Xylan, the main hemicellulosic component of graminaceous plants and hardwoods, is hydrolysed by the cooperative activity of several enzymes, among which the xylanases (EC 3.2.1.8) are the most important. These enzymes are produced by several microorganisms found in natural environments such as soil and the rumen of animals, and hydrolyze xylan to short xylo-oligosacharides. Thermostable xylanases may be exploited for xylan digestion processes at elevated temperatures. Many methods have been exploited to increase the enzyme thermostability and here we looked at two different methods, error-prone PCR and site-directed mutagenesis by changing specifically threonine to proline at 274 position of xylanase A gene from Bacillus halodurans (xylA). The enzyme activity of different mutants at higher temperature was checked and the results will be presented here.

Keywords: error-prone PCR, site directed mutagenesis, enzyme, xylanase, thermostability

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P-11-693-1

Effect of 50 Hz EMF on Gstt1 gene expression of rats

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The aim of this study was to evaluate the possible effects of electromagnetic fields (EMF) on the glutathione s-transferase T1 (Gstt1) gene expression in the liver of male Wistar rats. Animals were exposed to EMF (50 Hz, 500 μT) and control condition thereafter sacrificed after 30 days. Livers were removed and processed for Gstt1 mRNA levels (RT-PCR). No significant difference was observed in Gstt1 gene expression due to EMF. In conclusion the result of this study indicates exposures to 50 Hz and 500 μT EMF in this duration and intensity don't affect Gstt1 gene expression. Further research on the effects of EMF exposure on free radical levels and other GSTs isoenzymes are warranted.

Keywords: electromagnetic field, Gstt1, gene expression

P-11-730-2

Application of an inducible suicide gene system based on human caspase 8

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The efficacy of adoptive T-cell therapy as treatment for malignancies may be enhanced by genetic modification of infused cells. However, oncogenic events due to vector/transgene integration, and toxicities due to the infused cells themselves have tempered enthusiasm. We describe a "safety switch" that can be stably and efficiently expressed in human T-cell without impairing phenotype, function or antigen specificity. This construct is a human caspase 8 fused to a mutated human FK506 binding protein (FKBP') to allow conditional dimerization using a small pharmaceutical molecule to induce apoptosis in transfected cells. We transfected FKBP'-caspase 8 construct in Jurkat T cells containing VHH-Hinge-CD28-OX40-CD3ζ construct, so apoptosis could be induced by Ap20187 drug. As a result, apoptosis will be induced when Jurkat T cells containing VHH-Hinge-CD28-OX40-CD3ζ construct are transfected with FKBP'-caspase 8 construct and apoptosis will be induced after treatment of cells with Ap20187 drug. This system has several advantages over currently available suicide genes. First, it consists of human gene products with low potential immunogenicity. Second, administration of dimerizer drug has no effects other than the selective elimination of transduced T-cells.

Keywords: apoptosis, caspase 8, FKBP', safety switch

P-10-705-4

Use of computer software to created virtual mutants in order to reduce the cost of lab mutagenesis; application to determine thermostability of mutants

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Mutagenesis plays a major role in shaping the present and future outlook of biotechnology techniques. Different methods such as site-directed mutagenesis and random mutagenesis have already been employed in biotech labs to create new favorite genes, but are time and cost consuming and prevent checking all possible mutants in lab. Here we employed and developed computer application to create all possible mutants in virtual mode which give us more flexibility to check their features. Xylanase (Ec 3.2.1.8) enzyme hydrolyzes xylan, the main hemicellulosic component of graminaceous plants and hardwoods, and need for thermostable xylanases in many industries have been elaborated. DNA sequence of xylanase enzyme from Bacillus halodurans with 370 amino acids (active at 70˚C) was taken, and 7030 possible one-hop mutants (single point mutation) of it were virtually created in computer environment by substituting every amino acid with the other 19 possible ones (about 50000 mutants). More than 70 protein features of those mutants were determined and neural networks used to find the relationship between enzymes features and thermostability. The results showed some mutants had up to 10ºC increases in thermal stability.

Keywords: mutagenesis, virtual mutants, enzyme, xylanase, thermostability

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P-10-734-1

A specific vector for cancer gene thrapy

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One of the main goals for all cancer therapies is selective targeting and killing of tumor cells. Cancer gene therapy has presumably this advantage and also it has suitable strategies that can be applied to target the therapeutic transgene directly to tumor cells. An example of this kind of strategies is selective expression of pro-apoptotic genes in tumors by the regulatory regions of genes that are preferentially activated in tumors. To follow this strategy, a tumor targeting construct was built in this study. The regulatory region of midkine (MK) gene which is a heparin binding growth factor and has limited expression in normal adult tissues but is frequently upregulated in a huge number of tumors could activate a fused pro-apoptotic gene in a variety of tumors. Truncated Bid (Bid is an important pro-apoptosis Bcl-2 family protein) was used as pro-apoptotic gene in our construct. tBid which is active form of the Bid protein can then induce mitochondrial pathway of apoptosis. tBid gene which lacks the N-terminal part (177 bps) of Bid gene was amplified by reverse transcripted PCR from Bid mRNA and cloned into multiple cloning site of pcDNA3.1 vector. A 0.6 kb of MK promoter was PCR-amplified and replaced with the CMV promoter to produce pMK-tBid construct. Since tBid is a crucial gene in induction of apoptosis and MK promoter is a tumor specific promoter, the pMK-tBid as a tumor targeting construct is expected to induce apoptosis in cancerous cells preferentially.

Keywords: apoptosis, MK promoter, tBid gene

P-10-746-1

Construction of mammalian expression vectors expressing Nurr1 and Pitx3 transcription factors that promote development of dopaminergic neurons

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Dopaminergic neurons supply brain dopamine and degenerate in the course of Parkinson disease. Cell developmental studies have implicated Nurr1 and Pitx3 as two most important transcription factors that determine fate of dopamine precursor cells. This study is aimed at construction of mammalian expression vectors carrying cDNA sequences that encode these two factors. Nurr1 cDNA was excised from its original vector gifted by an overseas laboratory and inserted into pIRES2-EGFP, a mammalian expression vector commercially available. Our lab also has developed pIRES2-Jred a second expression vector in which Jred substitutes for EGFP. This time the cDNA for Pitx3 was digested from its gifted vector and subcloned into pIRES2-Jred. Both EGFP and Jred are detectable under fluorescent microscope. Transfection of HEK-293 cells by each expression construct resulted in robust expression of reporter genes as we observed under fluorescent microscope. Next, we carried out RT-PCR experiments which at mRNA level detected expression of both transcription factors. We are currently applying these two expression vectors to dopaminergic precursor cells for neurogenesis studies in vitro and in vivo.

Keywords: dopaminergic, Nurr1, Pitx3, expression, neurogenesis

P-10-802-1

Methylation status of p21waf1/cip1 gene in T47D and MDA-MB-468 breast cancer cell lines

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Hypermethylation of CpG islands (CGIs) existing in promoter regions are being revealed as one of the most frequent mechanisms of loss of tumor suppressor genes (TSG) function and its Transcriptional silencing. Therefore, changes in DNA methylation have been recognized as one of the most common molecular alterations in human neoplasia. In this study, we have analyzed the methylation status of the cyclin-dependent kinase inhibitor (CKI) p21Waf1/Cip1 by the methylation-specific polymerase chain reaction (MSP). The MSP was performed using designed primers (methylated, M-MSP and unmethylated, U-MSP) for the known alleles of p21 gene in the breast cancer T47D (ER positive) and MDA-MB-468 (ER negative) cell lines. Based on our result, we found that promoter of p21 gene was consistently unmethylated in both T47D and MDA-MB-468 cell lines. Therefore, methylation inactivation of the p21 in the two mentioned breast cancer cell lines is infrequent.

Keywords: breast cancer, CpG islands, p21Waf1/Cip1, MSP, MDA-MB-468, T47D

O-10-739-1

Molecular cloning and characterization of SOS genes homologous from Aeluropus lagopides

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Five percent of the cultivated lands are affected by salinity. Salinity has different effects on crops and limits their productivity. Halophytes tolerate high salt conditions. Aeluropus lagopides is a stoloniferous prennial halophytic grass with C4 photosyntesis from poacea family. This grass can grow in high salt conditions without any symptoms of salt stress. In this study we isolated and cloned Salt Overly Sensitive genes (SOS1, SOS2, SOS3) from A. lagopides shoots and roots by specific primer of wheat which is designed for their conserved regions. Expression analysis has been done for shoots and roots of A. lagopides using Semi Quantitative PCR in contrast to B-Tubuline, a housekeeping gene, under different conditions. 21 days seedling treated with NaCl (600mM), ABA (50 mM), Ca2SO4 (5mM), (NaCl-Ca2SO4), (NaCl-ABA) and (Ca2SO4-ABA) in hydroponic medium supplemented by ½ strength MS for 10 days. SOS1 expression did not change in shoots but it was upregulated in roots in response to NaCl and NaCl-Ca2SO4 treatment. SOS2 expression was up-regulated in response to single treatment of NaCl and Ca2SO4 in roots and shoots; but in roots, all treatments containing NaCl were effective on enhancement of expression. SOS3 expression was up-regulated in shoots and roots in response to only NaCl-Ca2SO4 treatment. These expression patterns suggest that A. Lagopides most likely excretions Na+ from root to soil

and transfers excess Na+ to shoot for salt tolerance. Also SOS2 regulates Ca2+ homeostasis by modulating the activation of Ca2+/H+ vacuolar antiporter.

Keywords: Aeluropus lagopides, SOS gene, salt tolerance, semi Q RT-PCR

P-10-755-1

Recombinant lentivirus-mediated expression of glial cell line-derived neurotrophic factor protects dopaminergic neurons from parkinsonian toxicity

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Neurotrophic factors have been implicated in biological activities vital for brain function. We have generated recombinant lentivirus vector carrying cDNA cassettes encoding mouse glial cell line-derived neurotrophic factor (GDNF) and a Jred reporter protein in IRES. We applied this vector for co-transfection of virus-producing HEK-293T cell line along with packaging and envelope vectors. Jred expression was detected 24hrs post-transfection under fluorescent microscope. Forty-eight hrs post-transfection, cell supernatant containing viral particles was collected and concentrated using Amicon filter columns and used for infection of fresh HEK-293T cells as well as human astrocytes. Twenty four hours post-infection Jred expression began emerging from both transduced cell lines. Total RNA from transduced 293T cells was extracted and reverse transcribed into cDNA. The RT-PCR experiments detected expression of the murine GDNF in the human cell line. Once increased GDNF expression in and secretion from transduced astrocytes was demonstrated by ELISA assays, these cells can then be used for neuroprotection and neurogenesis studies both in co-culture conditions and conditioned medium addition.

Keywords: GDNF, lentiviruses, transduction, astrocytes

O-10-589-1

Development and characterization of an aequorin-based bacterial biosensor for detection of toluene

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A photoprotein aequorin-based E.Coli strain DH5α biosensor was constructed and characterized for its potential to detect toluene in aqueous solutions. The biosensor is based on a plasmid PGL2 that carried the lower pathway promoter (Pu) of the xyl operon of Pseudomonas putida mt-2, which was incorporated with the transcriptional activator xylR and fused to aequorin cDNA. The xylR protein binds to a subset of toluene-like compounds and activates transcription at Pu promoter. Then expression of aequorin was controlled by xylR and Pu. According to sensitivity and thermostability of aequorin and increased of signal to noise, this reporter enzyme has the higher advantage than other common reporter's genes such as luciferase and green flueresence protein (GFP). Our results demonstrate that this bioluminescence response was specific for toluene-like molecules and so this biosensor cells were able to accurately detect toluene derivative contamination in environmental samples.

Keywords: aequorin, biosensor, toluene, xylR

P-10-758-1

Overexpression of human glutathione peroxidase-1 gene protein protects dopaminergic neurons against parkinsonian toxicity

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Degeneration of dopaminergic neurons is a hallmark of Parkinson disease. A proposed strategy to protect these neurons from toxicity-induced cell death is to enhance their anti-oxidant defense system. In this study, we transfected PC12 dopaminergic neurons and retineal pigmentous epithelium (RPE) cells with an expression vector carrying a gene cassette that expresses anti-oxidant enzyme human glutathione peroxidase-1 (hGPX1) under CMV promoter. A GFP reporter gene-carrying expression vector was applied to measure transfection rate in both cell lines. We achieved a rate of up to 45% and 30% in PC12 and RPE cells, respectively. RT-PCR analysis revealed over expression of hGPX1 enzyme in both transfected cell lines. Next, we treated transfectants with a range of 50 to 150uM parkinsonian toxin 6-OHDA and measured cell survival 12 hrs after treatment. Up to 40% increase in surviving neurons against 6-OHDA toxicity was detected compared to controls. We conclude from this work that hGPX1 acts as an anti-oxidant enzyme protecting dopaminergic neurons from degeneration.

Keywords: glutathione peroxidase, dopaminergic, 6-OHDA, neuroprotection

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P-10-595-1

Berberine induces apoptosis in K562 cell line

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Berberine, an isoquinoline alkaloid, the major constituent of coptidis rhizome has multiple pharmacological activities including anti-inflammatory, promotion of apoptosis and anticancer effect. The apoptotic effect of berberine is mediated by mitochondrial pathway and observed in various tumor cell including, prostate, colon, leukemia, and liver cancer cell lines. The mechanism by which berberine initiates apoptosis is not fully understood. In the present work the effect of different concentrations (25-100 μM/ml) of berberine on promotion of apoptosis in K562 cells have been studied. Cell growth has been detected by using MTT assay, DNA laddering and staining with hoscht dye were performed to confirm apoptosis and the alterations in Bcl-2 and Bax content has been evaluated by using western blotting. Treatment of K562 cells with 75μM berberine resulted in decreasing cell growth 72 hours after treatment. Morphological evidence of apoptosis, including DNA fragmentation were observed in cells treated with 75μM for 72 h. Western blotting analysis have shown that the level of Bcl-2 were decreased after 72h treatment with berberin (75μM), whereas, the expression of Bax were increased. The results indicate that berberine induces apoptosis of k562 cells in dose and time dependent manner and suggest that it can be used as a

chemotherapeutic agent for the treatment of chronic myeloid leukemia (CML).

Keywords: berberine, apoptosis, K562

P-10-571-1

Studying the protein profile of Artemia Urmia embryos

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Encysted embryos of Artemia species are arguably the most stress resistant of all animal life history stages. It does not have ecological limitation control by temperature, salinity, oxygen, food and other factors; it produces several macromolecules that can aid it to tolerate different ecological condition. Previous research indicated that other species of Artemia exist, such as Artemia Franciscka; in this work we have studied the protein profile of Artemia Urmia from Urmia Lake. For this purpose first, the encysted embryos of Artemia Urmia were hatched in Artificial sea water for 21 hour at 30°c on shaking condition, then the hatched embryos were collected and the embryos proteins were extracted by different methods such as sonication and homogenizer. The proteins were purified by DE52 anion exchange chromatography and analyzed by SDS-PAGE. Our result indicated that the hatching efficiency was 80% in those conditions. The content of extracted protein was about 2.0 mg/gr of wet hatched embryos mass. The DE52 anion exchange chromatography and SDS-PAGE analysis indicated some different protein pattern compared to the last reported ones.

Keywords: Artemia Urmia, protein pattern, chromatography, purification

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O-10-722-1

Integration of chimeric receptor gene into genome of T cell using PhiC31 integrase

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The specific activation of the immune system to control cancer growth has been a long-lasting goal in cancer immunotherapy. Employment of T cells for tumor therapy is an attractive approach. In order to redirect and enhance the ability of the patient's own immune cells to fight cancer, the chimeric antigen receptor (CAR) approach that endows lymphocytes with antibody specificity was developed. The CAR consists of scFv , linked through an extracellular linker to transmembrane and cytoplasmic domains of lymphocyte triggering moieties. Part of the humoral immune response of camels and lamas is based on heavy-chain antibodies, where the light chain is totally absent. The variable part of their heavy chain is called nanobody or VHH and is the smallest fragment with high affinity toward its antigen. In previous studies in our lab nanobody gene containing CARs were designed and transfected into Jurkat T cell line. In order to improve CARs efficiency we tried to integrate CAR gene in the T cell genome. For having stable and high expression of CAR, PhiC31 integrase system was chosen for gene integration. In this research work three plasmids were constructed:1) pCDNA-INT which include PhiC31 integrase gene 2) pCDNA-1 hinge CAR-attB 3) pCDNA-2 hinge CAR-attB. Jurkat cell line was co-transfected by these three plasmids and CARs expression was quantified 1 and 30 days after transfection by semi-quantitative RT-PCR method. Our results confirmed that using PhiC31 integrase resulted in stable and high expression of the constructs on day 30 after cotransfection.

Keywords: chimeric receptor, nanobody, PhiC31 integrase

O-10-744-1

Surface proteins and adhesion ability of probiotic Lactobacillus strains

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The Lactobacillus cell-surface proteins are intensively studied due to their importance for bacterial colonization in the gastro-intestinal tract. With this aim four Bulgarian Lactobacillus strains, selected as probiotics were characterized. The presence of surface layer proteins and their role in the adhesion to HeLa cell line were estimated. Whole-cell protein profiles obtained by treatment with 1% SDS, showed a significant band of apparent molecular mass between 41- 44 kDa as measured by SDS–PAGE for three out of the strains. Quantitative adhesion tests of the probiotic strains proved clear correlation between the adhesion and the presence of surface proteins. In addition, PCR analyses of genetic determinants responsible for the production of specific adhesion promoting proteins were performed. Visualization of Mub gene coding the mucus binding protein, MapA gene/ mucus adhesion protein and EF-Tu/ elongation factor allowed comparing the molecular mechanism of adhesion of food and human originated lactobacilli. Our results provide a good base for in vivo characterization of new strains for further development of probiotic formulas.

Keywords: adhesion, cell-surface proteins, Lactobacillus, probiotics

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P-10-773-1

Evaluation of some oxidative stress parameters in blood exposed to vitamin C

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Vitamin C is water soluble which is a potent antioxidant in organisms. But, it also has per-oxidant effects. In this study, blood of healthy animals was incubated with different concentration of vitamin C at 4C for 24 hours. The lipid per-oxidation and protein damage were investigated. The incubation of samples with high dose showed significant change in the level of lipid per-oxidation and protein carbonyl. These results supported that oxidative stress may play a role in mechanism of toxic action of vitamin C.

Keywords: vitamin C, lipid peroxidation, protein carbonyl

P-10-238-1

Differentiating and growth inhibitory effects of boric acid in human leukemia K562 cell line

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Recently, it has been reported that Boron as a form of acescent Boric acid induced apoptosis and differentiation in prostate and breast cancer cell lines, suggesting its efficiency as a new anti-cancer agent. However, its effects on other cancers remain to be disclosed. In order to test anti-cancer property of boric acid in human leukemia, this study was designed and effects of boric acid on human leukemia investigated using K562 cell line as an experimental model. K562 cell were cultured in suspension in RPMI 1640 medium supplemented with 10% fetal bovine serum and treated with boric acid in 100 µM to 10 mM concentrations for different time intervals. Growth inhibition and cell viability were assessed using trypan blue test and MTT assay, respectively. The results showed that boric acid inhibited growth of K562 cells in a time- and dose-dependent manner. For example, 10 mM boric acid inhibited growth of K562 cells after long treatment times (>72 h). However, no significant decrease in viability was observed even after longer treatment times, point to cytostatic effects of this compound. Careful examination of treated cells showed that after drug treatment a portion of cells were adhered to the culture plates and showed characteristics of differentiated cells such as cytoplasmic protrusions and long pseudopodia. These treated cells did not show benzidine staining capability, indicating differentiation toward dendritic or macrophage lineages but not erythroid cells. Attain to this fact that universal efforts have focused on finding differentiatiation-inducing agents for cancer treatment, thus our results may pave utility of boric acid as a new compound for differentiation therapy of human leukemia.

Keywords: apoptosis, boric acid, chronic myelogenic leukemia, differentiation, K562 cells

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P-10-238-2

Adenosine 5'-triphosphate induces apoptosis and down-regulates stem cell marker nucleostemin in K562 cell line

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Adenosine 5'-triphosphate (ATP) not only is the current energy source in all cell types but also involves in pivotal intracellular cell signaling such as differentiation and apoptosis. Recently, several group reported anti-cancer efficiency of this naturally-occurring nucleotide in various cancerous cell lines. However, its mechanism of action and the effects of ATP on other cancer cell lines (especially in leukemia cells) have been elusive. In order to investigate effects of ATP on human leukemia with a mechanistic approach, we focused on nucleostemin (NS) which is a new stem cell marker related to self-renewal of both normal and cancer stem cells. In this study, effects of ATP on K562 cell line as a well-known model of leukemia cancer stem cell were studied and expression of NS gene have been studied. K562 cell were treated with different concentrations of ATP (10 to 1000µM) for various time intervals. Viability and growth inhibition were assessed using MTT assay and trypan blue test, respectively. Apoptosis was studied by florescent microscope and DNA fragmentation assay. Expression level of NS was studied by semiquantitative reverse transcriptase PCR. The results shown, in the presence of more than 10 µM ATP, cell proliferation of K562 cells were reduced by 20% (10 µM) or 60% (500 µM). Under this condition, Ao/EtBr double staining, detected 20% and 50% apoptotic K562 cells after 72 h treatment with 10 µM and 500 µM, respectively. ATP-induced apoptosis was further confirmed by DNA fragmentation. These results indicate that ATP at low incubation times (24 h) inhibited growth which is linked to apoptosis after longer treatment times (72 h). Moreover, ATP down-regulated NS expression in a time- and dose-dependent manner so that up to 90% gene expression inhibition was observed after 72 h treatment K562 cells with 500 µM ATP. In addition to introduce of ATP as a potent agent in chemotherapy of stem cell leukemia K562 cells, our results may highlight some novel pathways involved in its mechanism(s) of action.

Keywords: apoptosis, CML, extracellular ATP, K562, nucleostemin

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P-10-788-1

Cold and heat stresses induce Lcn2 in heart, liver and kidney of mouse

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Expression of lipocalin 2 has been implicated under various pathophysiological conditions. Since oxidative status, inducer of Lcn2, has been reported in thermal stress, this study was conducted to determine whether cold and heat stresses can induce Lcn2 expression. Mice were exposed to the stress and expression of Lcn2 was performed by RT-PCR, Western Blot and immunohistochemistry. The highest level of expression of Lcn2 was observed after 1 hour post cold stress- exposed recovery in liver and kidney and after wards declined and reached to normal level after 3 h. Highest level of expression of Lcn2 in heart was observed after 2 hour post cold stress- exposed recovery and reached to normal level after 4 hours. In accordance to expression of Lcn2 mRNA, induction of Lcn2 protein was also observed as determined by western blot and immuno histochemistry. In kidney, Cold stress induces Lcn2 expression notably in glomeruli. Up regulation of Lcn2 also was detected in intra tubular spaces. Induction of Lcn2 in kidney after exposure to heat stress was lower than cold exposure and glomerul cells expressed low level of Lcn2 expression. Augmentation of Lcn2 expression in liver was observed in both thermal stresses. Cold stress induces Lcn2 expression notably in sinusoid and around of central vein. In heart, endothelial cells notably up regulated lcn2 expression after heat and cold stresses treatment. As we are aware, here for first time. We report that thermal stress induces Lcn2 expression and could be suggested as a new cold and heat stress protein for reestablishment of homeostasis.

Keywords: oxidative status, thermal stress, Lcn 2, immunohistochemistry

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P-10-791-1

Comparison of Microcystis and Oscillatoria strains of the Mazandran Province rivers with whole-cell protein profiles

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Cyanobacteria are autotrophic bacteria that comprise a heterogenous set of photosynthetic prokaryotes having an extraordinary biosynthetic potential. Many genuses of them play remarkable roles in the variety of biotechnological purpose including: biofertilizer, human food, animal feed, pharmaceuticals and etc. Isolation, identification and classification of the cyanobacteria genuses are difficult and very important for investigators. In this paper, cyanobacteria samples were collected from Mazandaran province's rivers. 5 *Microcystis* and 7 *Oscillatoria* morphotypes were isolated by different subcultures on TYG medium and BG-11 medium supplemented with 0.05 mg/ml cycloheximide, 1mg/ml cycloserine. *Microcystis* and *Oscillatoria* strains cells were homogenized in a buffer (50 mM Tris-Cl pH7.8; 0.25M Sucrose; 25mM KCl; 10 mM MgCl2; 1 mM PMSF; 0.1 mM EDTA; 0.1 b-mercaptoetanol; 0.5% v/v Triton-100) and total protein were precipitated by 50% TCA. Bradford assay, SDS-PAGE, Coomassie brilliant blue R-250 staining and detection of the protein molecular weights were performed by general methods. Whole-Cell proteins were separated in 10% resolving polyacrylamide gel. Analysis of SDS-PAGE bands showed significant difference in protein bands between two genuses. Therefore, total cell protein pattern is useful technique for classification and clarifying phylogenetic relationships in cyanobacterial genuses.

Keywords: cyanobacteria, Microcystis, Oscillatoria, SDS-PAGE, whole-cell proteins

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P-10-789-1

Molecular construction of tenecteplase coding sequence and expression of its protein as a thrombolytic agent in CHO cell line

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In this project, we have employed a rapid and efficient method to introduce three sets of mutation into defined positions in human tissue plasminogen activator cDNA sequence. A site-directed mutagenesis approach was done based on megaprimer PCR procedure to produce tenecteplase coding sequence. Tenecteplase is a variant of TPA with better pharmacokinetic properties and more selective thrombolytic activity. The final PCR-product was confirmed by agarose gel electrophoresis, restriction digestion and sequencing. At the next step, a fragment containing this sequence was cloned into pTZ57R/T by T/A cloning method and propagated in One Shot TOP10 chemically competent E. coli and the positive clones were selected by blue/white screening procedure. After isolation and purification of recombinant plasmid and its digestion by suitable restriction enzymes, tenecteplase coding sequence was purified and subcloned into an appropriate vector. The recombinant vector was propagated in One Shot TOP10 chemically competent E. coli and finally, recombinant plasmid was isolated and verified by restriction digestion and sequence analysis to confirm that the insert has been cloned in the proper orientation and contained the appropriate features required for expression and secretion into the culture medium. At the next step, recombinant vector which contained a consecutive promoter was transfected and integrated in the genome of a CHO cell line. Generated stable cell line secreted recombinant protein into the culture medium that was verified by SDS-PAGE and Western blotting. Moreover catalytic activity of tenecteplase was assessed by a specific substrate, indicating that active tenecteplase was produced by this approach.

Keywords: integration, site directed mutagenesis, tenecteplase, thrombolytic, tissue plasminogen activator, CHO

A-10-788-2

Lipocalin 2 as a cytoprotective factor against cytotoxicity and apoptosis induced by cold stress

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Lipocalin 2 (Lcn2, NGAL) is a 25-kDa glycoprotein that was initially purified from neutrophil granules. Induction of Lcn2/NGAL expression under oxidative stress condition has been reported. On the other hand, oxidative states have been reported in cold stress. In this study, we have investigated the ability of Lcn2 in cell survival after cold stress. Stable clones expressing Lcn2 were generated. Lcn2 gene in A549 cell line was also downregulated with the siRNA. The cells were treated with cold stress followed by incubation in 37 C at different time intervals. Apoptotic cells were detected by flow cytometry and western blot. MTT assay showed that Cell proliferation in HEK293T cell expressing Lcn2 was higher compared to control cells and in A549 cells transfected with siRNA was lower than the control. The number of apoptotic CHO and HEK293T cells expressing Lcn2 and proapoptotic proteins such as p53, Bim, Bad, Bax, Caspase 6 were lower and higher in siRNA transfected A549 cells compared to the controls. All together, these results indicate Lcn2 protects cell from cold stress and indicate potential application of Lcn2 against cold stress injury such as organ transplantation

Keywords: Lcn2/NGAL, cold stress, proapoptotic proteins

A-10-788-3

Dual role of Lcn2 after exposure to heat stress

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Lipocalins constitute a broad but evolutionally conserved family of small proteins; however, the functions of many lipocalins remain unclear to date. Exploring function of lipocalin 2 was aimed at in this study. Stable CHO and HEK293T cells expressing Lcn2 were

established. Lcn2 also was down regulated with siRNA in A549 cells. The cells were exposed to heat stress and return to 37 C at different time intervals; cytotoxicity and apoptosis were followed. Cell proliferation was higher in engineered CHO and HEK293T and lower in siRNA transfected A549 cells compared to control cells. Administration of exogenous recombinant Lcn2 to the cells exacerbates toxicity of heat stress and was more effective when expression of Lcn2 was down regulated. The number of apoptotic CHO and HEK 293T cells expressing Lcn2 and expression of pro-apoptotic proteins were higher than empty vector transfected cells but number of apoptotic cells in the A549 Lcn2 downregulated cells and expression of pro-apoptotic proteins were higher than control siRNA transfected cells, and administration of exogenous lcn2 to the cells increased apoptotic cells. However, treatment of the cells with recombinant Lcn2 after heat stress exposure, just before incubating at 37C, protects cells from heat stress- induced apoptosis. All together, these results indicate dual role of Lcn2 in heat stress. In future, this funding might be applicable to treatment of some cancer cells by hyperthermia.

Keywords: lipocalin 2, siRNA, hyperthermia, dual role

O-10-797-1

High frequency of SEN virus infection on healthy blood donors in Guilan state, north of Iran

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SEN virus (SENV) is a blood-borne, ssDNA virus circular, ~3800 nucleotides in length and about 26 nm in size that is non-enveloped and possesses at least 3 ORFs. Among SENV genotypes, SENV-D and SENV-H genotypes are comparatively more prevalent in the patients with unknown (None-A to E) hepatitis. The frequency of SENV-D and SENV-H genotypes was studied in 120 sera of healthy blood donors of Guilan state, North of Iran. SENV ORF-1 nucleic acids were screened with Nested-Polymerase Chain Reaction (Nested-PCR). SENV-D and SENV-H viremia were detected in 73/120 (60.8%) (95% Confidence Interval, 52.0-69.6) and 103/120 (85.8%) (95% CI, 79.5-92.1) respectively. SENV-D and SENV-H co-infection was detected in 67/120 (55.8%, CI, 46.8-64.8). SENV (SENV-D or SENV-H) viremia was identified in 109/120 (90.8%) (95% CI, 85.6-96.0).These results advocate that infection with one SENV genotype most likely does not protect against another SENV genotype. High frequency of SENV infection in Guilan state in comparison with other areas in the world can probably nominate this virus as an endemic virus in Guilan state. As a result frequency of SEN virus infection needs to be studied in the other states in Iran for comparison with our results with the same method and primers. The results of our studies also suggest that the other transmission routs of SENV, including parenteral and fecal-oral transmission routs, may be involved in addition to the main route of blood transfusion. Clinical impact of SEN virus infection needs to be investigated.

Keywords: frequency, infection, Nested-PCR, SENV

O-10-788-4

Establishment of a cell line expressing recombinant factor VII and activation to FVIIa through hepsin by genetic engineering method

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Factor VII is a plasma glycoprotein that participates in the coagulation process leading to generation of fibrin. Factor VII is converted to factor VIIa which plays an important role in the coagulation cascade. The aim of this study was isolating and cloning the genes of human factor VII and Hepsin and co-transfecting the constructs to CHO cell line to express rFVIIa. FVII and Hepsin cDNA were isolated from HepG2 cell line and cloned to pcDNA3.1 (+) vector. The constructs were co-trasfected to CHO cell line. A cell line that permanently expressed recombinant factor VII and hepsin was established. The expression of recombinant FVII was determined by RT-PCR, ELISA, immunoprecipitation and western blot analysis. Biological activity of recombinant factor VII was evaluated by prothrombin time assay in factor FVII-depleted plasma. The results showed that human recombinant FVII and Hepsin successfully cloned and expressed. Stable CHO co-transfected with pcNDA3.1-FVII and Hepsin expressed FVII and Hepsin mRNA but there was no expression in the CHO cells transfected with insert free pcDNA3.1. FVIIa protein was also secreted to medium of CHO cells co-transfected with pcNDA3.1- FVII and Hepsin. The expected band of rFVII was detected in western blot analysis. A three to four fold decrease of the specific coagulant activity of rFVII was observed when human FVII-depleted plasma was used in combination with human thromboplastin indicating rFVII was biologically active. As we are aware, this is the first report of establishing a cell line expressing FVIIa using genetic engineering methods.

Keywords: recombinant FVIIa, hepsin, genetic engineering, co-transfection, CHO

P-10-798-1

TTV-Like mini virus infection in hepatitis B and C infected individuals

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TTV-like mini virus (TLMV) is a small DNA virus with single-stranded, closed circular, negative sense genome. TLMV is transmissible by transfusion. Viral hepatitis infection is an increasing problem with millions of affected cases all over the world. TLMV is an intermediate between the remotely related TTV of the floating genus Aanellovirus and Chicken Anemia Virus (CAV) of the family circoviridae. Recently, some new viruses have been identified for their association with hepatitis which TLMV is among them. There has been no study about TLMV infection in Isfahan. The aim of this study was to determine the frequency of TLMV in hepatitis B and C infected individuals in Isfahan. A total of 25 human serum samples from hepatitis B and 25 from hepatitis C infected patients were collected from the Mahdieh Laboratory in Isfahan. Viral DNA was extracted and Nested-Polymerase Chain Reaction (Nested-PCR) was used to detect a conserved region in ORF2 gene of the virus. TLMV DNA was detected in 20% (5/25) and

48% (12/25) of human serum samples from hepatitis B and C infected individuals, respectively. TLMV was detected in both hepatitis B and C infected individuals, but the rate of infection was higher in hepatitis C infected ones. The etiology of the higher infection in hepatitis C individuals needs to be determined.

Keywords: hepatitis B, hepatitis C, Nested-PCR, TLMV

P-10-749-1

Detection of human papillomavirus in cervical cervicitis in Isfahan

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Cervicitis is very common, affecting more than half of all women at some point during their adult life. The most common cause is human papillomavirus (HPV) infection which may lead to cervical cancer. Recent studies show that HPV DNA is present in all cervicitises, with some geographical variation in viral subtypes. The HPV group comprises over 70 different epitheliotropic genotypes of which more than 30 are mucosotropic. Approximately one-third of these mucosotropic HPV genotypes have been either isolated from or associated with cervical samples, thus determination of the presence of HPV in general population of each region can help to reveal the role of these viruses in cervicitis reported. This study aimed to estimate the frequency of infection with HPV in cervicitis samples in Isfahan population. Seventy formalin fixed paraffin embedded tissue samples of cervicitis already examined for histopathological changes were collected and then the blocks were cut and 10 four micrometer thick slices were collected and subjected to Nested PCR using consensus primers of MY09/MY11 and GP5+/GP6+ designed for amplification of conserved region of genome coding for L1 protein. Data about histopathological changes were collected by the reexamination of hematoxylin and eosin stained sections. Eighty five percent of the tested samples were positive for HPV. The high level of infection in cervicitis samples necessitates more attention to the role of human papillomaviruses in the induction of cervicitis in the area studied.

Keywords: cervicitis, HPV, Nested-PCR

O-10-652-1

siRNA-mediated IGF-IR silencing inhibits growth and enhances chemo-radiosensitivity in SW480 cells

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Colon cancer is the second leading cause of cancer death worldwide. Elevated expression of IGF-IR (insulin-like growth factor1 receptor) is a frequent genetic abnormality seen in this malignancy. For a better understanding of its role in maintaining the malignant phenotype, we used RNA interference (RNAi) directed against IGF-IR in our study. RNAi provides a new, reliable method to investigate gene function and has the potential for gene therapy. The aim of this study was to examine the anti-growth and chemo-radiosensitization effects elicited by a decrease in the expression level of IGF-IR by RNAi in SW480 cancer cells. A plasmid-based polymerase III promoter system was used to deliver and express short interfering RNA (siRNA) targeting IGF-IR to reduce its expression in SW480 cells. The effects of IGF-IR silencing on cancer cell growth, 5-FU and ionizing radiation induced cell deaths were investigated by cell growth curves. Transfection of expression vector pkD containing IGF-IR shRNA was shown to reduce IGF-IR mRNA levels by 95%. SW480 cells transfected with pkD-shRNA-IGF-IR-V2 significantly decreased cell growth and rendered cells more sensitive to chemo-radiotherapy. The highest growth inhibitory rate was 53±2% and chemo-radiation enhancement ratios were 1.78±0.2 and 2.02±0.3, respectively. These data indicated that IGF-IR gene played a definite role in the development and aggressiveness of human colon carcinoma. It also could be a therapeutic target in colorectal carcinoma. The synergistic activation of RNAi and cell toxicity agents indicated that the combination of chemo-radiotherapy and gene therapy will be a promising approach in the future.

Keywords: colon cancer, RNA interference, IGF-IR, chemo-radiotherapy, combination of chemo-radiotherapy and gene therapy

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O-10-809-1

Study of anti cancer property of Scrophularia striata on astrocytoma cell line (1321)

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There are considerable efforts to identify naturally occurring substances as new drugs in cancer therapy. Many components medicinal plants have been identified that possess substantial anticancerous properties. Nonetheless, there are many plants yet to be studied for anticancer components. Therefore, the extraction of Scrophularia striata (S. striata), an Iranian species of family of Scrophulariace, was investigated in the growth of astrocyte cancer 1321 cell line. The 1321 cell line were seeded in 96-well culture plates in the presence and absence of various concentrations of either leaf and seed extract (filtrated and infiltrated) of Scrophularia striata to determine their anticancer effects in comparison to etoposide using MTT assay. The mechanism of extract effect on the cell line was investigated by flow cytometry. Filtrated leaf extract of S.striata showed strong anticancer effect on 1321 cell line when compared to control and etoposide. The cytotoxicity of the filtrated leaf extract was greater than infiltrated leaf extract of S. striata. In spite of leaf ability for cell growth inhibition, the seed extract has activated cell proliferation in all experiments. This is interesting that single herb induced contradictory effects as simultaneously. Flow cytometry findings indicated that apoptosis is the effective mechanism of extract on the inhibition of cell proliferation. The results indicate that anticancer reagent has a micro dimension but dimension of cell growth enhancer molecule is larger than 0.22µm. Findings indicate that there are both anti cancer and cell growth enhancer factors in the leaf and seed of S.striata simultaneously. Here the two factors are introduced

as separated molecules. The herb extract induces its cell growth inhibition via apoptosis.

Keywords: cancer therapy, drug, medicinal plants

O-10-811-1

Improvement of autodegredation resistance in a zinc-metalloprotease by site-directed mutagenesis

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Thermostabilizing of an enzyme while improving its activity may be difficult regarding general trade off relation between stability and function. Zinc-metalloproteases are members of a family of homologous proteases which differ in their resistance to thermally induced unfolding and subsequent autolytic degradation. We designed surface located mutations (Ala39→ Pro) and (Thr63→ Phe) on a novel zinc-metalloprotease from Salinivibrio proteolyticus. Two new mutations were introduced by site-directed mutagenesis techniques, and then biochemical and kinetic parameters, thermal stability and resistance to autodegradation of the enzyme variants were assessed. The variants were cloned in pQE-80L as expression vector. Maximum expression was reached at 30°C, 1 mM IPTG in LB medium. After purification of the enzyme with Q-Sepharose column chromatography, the variants were characterized for their resistance to temperature and autodegradation. Intrinsic fluorescence and far-UV CD spectroscopy as secondary and tertiary conformational probes were used to monitor the effect of mutations on the structure of proteins. The mutants possessed higher autodegradation resistance, while their thermostability and kinetic parameters did not change significantly relative to that of wild type enzyme.

Keywords: protein engineering, autodegredation, metalloproteases, spectroscopy

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P-10-827-1

Disruption of signal peptidase type I gene of Leishmania major by homologous recombination

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Leishmania comprises different parasites that are agents of leishmaniasis. In Iran, both cutaneous and visceral leishmaniasis is endemic. There are no effective drug or vaccination tools for controlling the disease. Most of secretory proteins are synthesized as precursor protein with a signal peptide (SP). Signal Peptidases (SPase) are a family of proteases that cleave SP from exported proteins within the cell. Most bacteria have a single copy gene of SPase type I that is essential for their cell viability. The result of topology prediction by computational analysis suggested that there are two transmembrane domains located in N and C terminal of SPase. To determine the role of SPase in L. major, we attempted to do target disruption of the SPase locus by Homologous Recombination (HR). To allow a precise replacement of SPase by the drug resistance genes, 5' and 3' flanking region of SPase were isolated from Leishmania major genome and cloned in both sides of Neomycin and Hygromycin genes in pX63-Neo and pX63-Hyg plasmids. To generate a heterozygote mutant, wild type strain was transfected with pX63-5'F-Neo-3'F or pX63-5'F-Hyg-3'F. To confirm the HR event, genotypic analysis of mutants was determined by PCR and southern blot. The morphological analysis by light, TEM and SEM microscope has been shown some difference in size of mutants in comparison with wild type. Further phenotypic analysis between wild and mutants L. major showed that SPase can play a crucial role in macrophage infection.

Keywords: homologuous recombination, Leishmania, signal peptidases

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P-10-438-1

Protein engineering of catalase from the yeast S. cerevisiae

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Catalase is a tetrameric enzyme which dismutase H2O2 into water and oxygen. The aim of this study was to exchange of the tyrosine 355 to cysteine lead to a product with catalytic activity similar to cytochrome P-450. The plasmid YEp355E containing the gene coding for the wild type catalase isolated from E. coli. This plasmid DNA was mutated using site-directed mutagenesis. The products of PCR transformed into supercompetent E.coli cells. The plasmid DNA isolated from E.coli transformed into competent cells of the yeast strain #578(CTA-CTT-). The transformation mixture was plated on SC^{-ura} plates and colonies transfer in YPD-medium. The cells were harvested and disrupted by homogenizer and the crude extracts were tested for catalase activity. (In parallele, the plasmid DNA was sequenced). The other steps of enzyme purification included: purification of the crude extract with EHOH/CHCL3, ammonium sulfate and affinity chromatography. Analysis of the various stages of the protein isolation by SDS-PAGE and western blotting demonstrated the presence of protein in all stages of purification, but a rather low yield of the final two more specific affinity chromatography steps. We also tested the purified protein for the "new" enzymatic activities and found some low activity in compared with wild type (285u/mg vs.5140u/mg). This low activity is due to a partial deficiency of the enzyme with respect to heme. Obviously, the protein structure is to some degree destabilized by the introduced tyrosine to cysteine exchange, leading to a tendency to lose heme upon standing.

Keywords: catalase A, protein engineering, CYP-450, S.cerevisiae

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P-10-834-1

Molecular phylogeny of the tribe Hedysareae based on chloroplast trnL-F sequence

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We here report phylogenetic analysis of the tribe Hedysareae using chloroplast trnL-F sequences. The tribe Hedysareae is one of the 28 tribes of Fabaceae-Faboideae distributing mainly in Eurasia. A total of 57 accessions representing 56 taxa including 40 species of the tribe Hedysareae and 16 species from Galegeae plus 3 species of Trifolieae as outgroups were included in the analysis. The sequence data was obtained through PCR amplification of the trnL-F fragment with appropriate primers and using the cycle sequencing reaction run in an automated DNA sequences. The resulting sequence data was aligned using Clustal W. Maximum parsimony approach as implemented in PAUP* using heuristic search strategy was performed. The length of the data matrix was 961 nucleotide sites, of which 268 were parsimony informative. The analysis showed the all ten hedysaroid genera but Alhagi was formed a clade. Within this clade, Stracheya tibetica, an endmic to Tibet, was sister to a well supported subclade comprising the 8 remaining genera, from Hedysarum through Eversmannia. Our data revealed that both Onobrychis and in particular Hedysarum are non- monophyletic, whereas, both Ebenus Taverniera and Sulla are monophyletic. Eversmannia and Corethrodendron, each represented herein by a single species, are closely related taxa and loosely allied with Onobrychis. The monotypic genus Sartoria, endemic to Turkey, was nested within Eurasian Hedysarum. Astragalean clade including Astragalus and its related genera, were loosely allied with Hedysaroid clade. This data also indicated that Caragana and Halimodendron are not related to this clade.

Keywords: chloroplast trnL-F, Fabaceae, Hedysareae, molecular phylogeny

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O-10-838-1

Efficient transfection of mouse stem cells with a new constructed plasmid containing attachment site (attB) sequence related to ΦC31 integrase and Oct-4 Promoter by phage integration system

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In order to study the Oct-4 regulation and its function in the differentiation process, we have designated to clone its related promoter upstream of EGFP as a gene marker. Oct-4 is a transcription factor of the POU family. This protein is critically involved in the self-renewal of differentiated embryonic stem cells and also is a transcription factor used to create induced pluripotent stem cells, demonstrating its capacity to induce an embryonic stem cell-like state. As such, it is used as a marker for undifferentiated cells. In this study, after mouse genome preparation, a part of mouse genome in the size of 2177 bp containing the mouse Oct-4 promoter was amplified by specific designated primers for a SOE-PCR method in two separated steps and the final product was cloned in a T-vector for analysis. Next, promoter was placed in an expression vector, upstream of EGFP cDNA near to attachment recognizable sites, for integrase activity of PhiC31 phage (attB sequence). The recombinant vector was verified by sequencing. Co-transfecting of this plasmid and a vector expressing integrase cDNA, into the mouse stem cell line (Royan B1), we obtained numerous transformant cell colonies expressing EGFP under regulation of this promoter. According to the ability of the integrase for stable integration, into the specific sites in the genome of the trasfected cells, we expect that these cells can express EGFP stably and are a good source for studying the molecular mechanisms of stemness and cellular differentiation.

Keywords: attachment site (attB), integrase, Oct-4 Promoter, PhiC31 phage, stem cell

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P-10-836-2

Down regulation of catalytic subunit of telomerase in HepG2 cells by the benzophenanthridine alkaloid chelidonine

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This study focused mainly on the effect of chelidonine, the main alkaloid of Chelidonium majus, on telomerase activity and its regulation in HepG2 cells. Cytotoxicity of chelidonine for HepG2 cells was determined by neutral red assay. A modified PCR- based telomerase repeat amplification protocol (TRAP) was used to estimate relative telomerase activity in chelidonine-treated cells in comparison with the untreated control cells. Relative expression level of the catalytic subunit of telomerase (hTERT) gene was estimated using semi-quantitative real-time RT-PCR. Telomerase activity was reduced considerably after administration of very low doses of chelidonine, whereas the higher concentrations of chelidonine did not remarkably enhance the effect. Real-time RT-PCR experiments indicated a drastically decrease in expression level of hTERT subunit of telomerase under treatment with chelidonine. Reduction of telomerase activity under chelidonine treatment is probably caused by down-regulation of the expression of the catalytic subunit of the enzyme.

Keywords: chelidonine, telomerase, inhibition, HepG2, Chelidonium majus

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O-10-848-1

Cloning and expression of aerolysin and protease genes from Aeromonas hydrophila in E. coli and Lactococcus Lactis and effects of recombinant cells on Tilapia Fish

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Aeromonas hydrophila is a Gram-negative bacterium that infects a wide range of hosts including amphibians, reptiles, avians and mammals such as cows and man, but it is most well known as a fish

pathogen. In this study, the pathogenic A. hydrophila strain was isolated from infected fish. Primary identification of pathogenic A. hydrophila AHMP was carried out by selective medium, biochemical tests, and API 20NE kit, while subsequent identification was conducted using Biolog system. The effects of A. hydrophila AHMP was detected on Tilapia fish and results showed that the concentrations of 108 CFU/g pellet diet caused 43% mortality after 15 days. In the second stage, full length aerolysin and protease virulent genes of A. hydrophila were amplified and cloned in TOPO cloning vector. The cloning was verified by RE digestion, and DNA sequencing. The aerolysin gene was then cloned in pRSETC plasmid prior to expression in E. coli BL21 DE3 PLysS under the control of T7 promoter. SDS-PAGE analysis showed a very strong aerolysin gene expression with molecular mass of around 53 kDa. PCR results also confirmed the presence of aerolysin and protease virulent genes in A. hydrophila. In addition, it was shown that E. coli BL21 is a potential host for the expression of virulent genes such as aerolysin. In the third experiment, several virulent DNA fragments, domain 1 and domain 4 of aerolysin and protease genes from pathogenic A. hydrophila were amplified, cloned and electrotransformed into Lactococcus lactis NZ9000. RE digestion, PCR and DNA sequencing verified the presence of the genes of interest. Results showed that the recombinant plasmids were stable in genetically engineered L. lactis up to 250 generations. In addition, SDS-PAGE analysis demonstrated a 27 kDa protease band which was in agreement with the reported size. At last, survival rates and weight loss of Tilapia fish exposed to non-recombinant and three different genetically engineered L. lactis were evaluated. After 15 days of vaccination, fish were then challenged with pathogenic A. hydrophilia. Results showed that the mean of mortality rates in three recombinant L. lactis was 22.4%, whereas it was 43% in control groups. In contrast to mortality rates, no significant differences were found in weight loss between treated and control fish groups. Based on the results of this study, it was found that the Biolog detection kit is an accurate, easy, and precise technique for identification of A. hydrophila. Results also showed that both Gram-negative and Gram-positive (E. coli and L. lactis) systems are potentially useful for cloning and expression of some virulent genes such as aerolysin and protease. The results of immunization and challenge experiments indicated that genetically engineered L. lactis strains carrying virulent DNA fragments of A. hydrophila could be used as novel vaccines against A. hydrophila infections.

Keywords: A. hydrophila, aerolysin, E. coli, pRSET, protease, Lactococuss lactis

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P-10-848-2

Cloning of Aeromonas hydrophila virulent protease gene in Lactococcus lactis and its use as a vaccine delivery system against Aeromonas hydrophila infection

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Proteases produced by A. hydrophila cause tissue damage and aid in establishing an infection by overcoming host defenses and by providing nutrients for cell proliferation. Gene replacement protease deficient mutants of A. hydrophila indicated that mutant caseinolytic and elastolytic activities were lower up to 90% than wild type. Fish injected with the A. hydrophila parental strain die more rapidly than those are injected with isogenic and Tn5-induced protease deficient mutants. Research has also shown that proteases can play an important role in pathogenicity and are also major antigenic components of a vaccine against hemorrhagic septicaemia in fish. Bacterial-based systems as live vectors for the delivery of heterologous antigens offer a number of advantages as a vaccination strategy. Using of molecular biology, genetic and recombinant DNA techniques has allowed the insertion of genes encoding the antigens to be delivered, into non-pathogenic carrier for expression. Among the Gram-positive bacteria, Lactococcus are designed, GRAS, generally recognized as safe, probiotic organisms. They are non-pathogenic, non-invasive, and non-colonizing and they bare no treat to human and animal health. They also have the capacity to secret proteins allowing surface expression or extracellular production of heterologous enzymes or proteins. Developments in genetic engineering have given these Gram-positive lactic acid bacteria (LAB) the advantage to be used as a host expression system for antigen delivery to induce immune response. A fragment containing the full length of protease gene was amplified by PCR with the ProForward and ProReverse primers, using Aeromonas hydrophila AHMP strains genomic DNA as template. The amplified 1038-bps fragment was digested by PstI and HindIII, followed by ligation into the corresponding sites on pCR®-Blunt II-TOPO and pNZ8048 plasmids. The ligated DNA was transformed into Escherichia coli TOP10 and Lactococuss lactis NZ9000 cells by the heat-shock and electroporation methods respectively. Verification of cloning was done using of RE digestion, PCR and DNA sequencing. SDS-PAGE analysis also detected the expression of the recombinant protease protein. Western blot analysis using antigen specific antibodies can be utilized to more confirm the presence or absence of a protein. This can be carried out in future by Western blot or ELISA studies. In the present work, cloning of eprA 1 gene, a temperature-stable metalloprotease, of fish isolated Aeromonas hydrophila into E. coli and expression in probiotic Lactococcus lactis system were successfully done. This can develop a useful and safe system to control microbial infections in different living things.

Keywords: Aeromonas hydrophila, protease, Lactococuss lactis, pNZ8048, E. coli

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P-10-781-1

Construction of a recombinant lentivirus carrying murine glial cell line-derived neurotrophic factor and a reporter gene protein

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This study has taken preparative steps for transfer and expression of murine cDNA of glial cell line-derived neurotrophic factor (GDNF) that is a physiologically important and clinically applicable growth factor for neurodegerative conditions including Parkison's disease. We first used total mouse RNA and amplified GDNF encoding cDNA that was confirmed by gel electrophoresis and automated sequencing. A Kozak sequence was accommodated within the forward primer and NheI-XhoI restriction sites were introduced into the 5′ and 3′ oligos, respectively. The PCR fragment was then isolated, extracted and inserted into our lentivirus transfer vector to make pLV-mGDNF. In order to co-express GDNF with a reporter gene, these two steps were taken: 1) an IRES element was inserted at the beginning of Jred reporter in pLEX-Jred vector, 2) the IRES-Jred cassette generated in step (1) was digested using XhoI restriction enzyme and inserted into the 3′ end of GDNF in pLV-mGDNF. The final product was termed pGDNFred. Pending to safe virus packaging and GDNF-IRES-Jred integrity, this transfer vector can then be used to generate

recombinant lentiviruses simultaneously expressing GDNF and reporter gene.

Keywords: GDNF, Jred, Lentivirus vectors, RT-PCR

P-10-738-2

Lentiviruses: Versatile vectors for gene delivery to mammalian cells

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Transfer of exogenous genes is an important step in genetic manipulation of host cells. Lentivirus vectors have been developed based on HIV-1 and related non-primate family members that display significant capabilities for mammalian cell transformations. Recombinant lentiviruses (rLVs) harboring transgenes of interest can be readily generated and concentrated to enhance their transducing capacity. rLVs enjoy several advantages compared to their well-known cousin retroviruses. rLVs can infect both dividing and non-dividing cells, resist host cell-induced gene silencing and guarantee longer term transgene expression. They can also accommodate inducible expression systems as well as tissue specific promoters to produce spatio-temporal patterns of gene expression. Finally, improved efficacy and safety profile of rLVs has paved their way to gene therapy trials in clinic. In this review, I will summarize technical issues with respect to rLV generation, host cell transduction and their potential applications in cell/molecular biology studies and clinical trials. Experiences and some rLV data produced in my lab will also be shared with audience.

Keywords: Lentiviruse, gene delivery, vector, mammalian

P-10-841-2

Anti-inflammatory effect of Rose bengal in LPS-activated macrophages in dark

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Rose bengal is a water-soluble, anionic xanthin dye and used as a safe compound and an anti-cancer agent. For study its anti-inflammatory effect, J774A.1 macrophages were treated with Rose bengal with or without lipopolysaccharide (LPS) in dark. Griess reagent was used for determination of nitric oxide production. Western blotting was evaluated the expression of inducible nitric oxide. The amount of nitrite oxide concentration by different concentrations of Rose Bengal (1, 10, 50, 100, 200 and 300 micromolar) were 17.1±0.56, 14.3±0.17, 13.25±0.04, 8.9±0.91, 6.17±0.09 and 0 micromolar and 21±0.2 micromolar by LPS-activated macrophages. It decreased the expression of iNOS in a dose dependent manner. Obtained results showed that Rose bengal decreased nitric oxide production and iNOS expression in inflammatory macrophages without significant decreasing in viability of macrophages. In conclusion, anti-inflammatory effect of Rose bengal is mediated by inhibition of iNOS expression and could be a new anti-inflammatory agent for study in vivo.

Keywords: Rose bengal, inflammation, nitric oxide, inducible nitric oxide synthase

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P-10-265-1

Bioconjugation of bovin seum albumin (BSA) to fluorescent isothiocyanate (FITC) and it's binding studies by optical spectroscopy

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In the therapy of various diseases, parenterally administered protein drugs are of steadily rising importance. In order to reduce the application frequency, these proteins can be incorporated into drug delivery systems. To evaluate the characteristics of these vehicles, fluorescent labelled proteins like FITC-BSA may be used as model drugs to allow the visualizing of the protein localization within the microparticle and the detection of microparticles in cell cultures or tissues. Fluorescence Detected Cirecular dichroism (FDCD) has been shown as a new method to monitor conformational changes in protein structure. In this study, a simple method for bioconjugation of BSA with FITC was described. The experimental work includes two parts: i) labeling BSA with FITC, ii) analyzing the results of absorption and fluorescence spectra. The labeling was performed by FITC addition to the buffer solution (0.1 M bicarbonate buffer, pH 8.8) containing BSA. After bioconjugation process, dialysis was performed for removal 25 of the free FITC. The fluorescence intensity of FITC-BSA at 1mg/mL was determined using spectrofluorometry at 490 nm. The FITC-concentration was calculated using the following ε494 nm=77000 cm-1M-1 for FITC. BSA concentration after labeling was calculated using ε278 nm=44890 cm-1M-1. We used spectrofluorimetery and FDCD to investigate BSA to FITC biocongugation. The maximal excitation for FITC at 490 nm and emission wavelength at 520 nm were observed, and FDCD signal was observed at 520 nm.

Keywords: FITC, FDCD, BSA, bioconjugation

P-10-841-3

Leishmanicidal activity of some extracts from Artemisia species of Khorassan Province

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Ccutuneous leishmaniasis infects many people and its drugs have unpleasant side-effects or are not effective. So, development of effective leishmanicidal agents is urgently needed. Eleven species of Artemisia were collected from Khorasan Provinces and their ethanol, ethylacetate, dichloromethan and hexan extracts were prepared. Leishmania major promastigotes were cultured in vitro. Leishmanicidal effects of these extracts were evaluated by MTT assay and reported as 50% inhibition concentration (IC50). All extracts inhibited proliferation of promastigotes in a dose-dependent manner. Ethanol extracts had

the strongest effect and hexan extracts (except for A.fragrans) had the weakest effect. Ethanol extracts of A.kulbadica (IC50:0.025), A.ciniformis (IC50:0.025) and A.santolina (IC50:0.080) had the most leishmanicidal activity. All ethylacetate extracts (except for A.fragrans and A.turanica) were stronger than dichloromethan extracts. Our results demonstrated that Artemisia spp. from Khorasan Province could be good candidates for the investigation of leishmanicidal activity in vivo. So, isolation of effective compounds and elucidation of their structures will be essential.

Keywords: Artemisia, Leishmanicidal activity, Leishmania major, Promastigote

P-10-443-2

Assaying the presence of some histone- like proteins (Hu) in Bcillus subtilis by ammonium sulfate precipitation

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Histone like protein (HLPs) in bacteria are small basic proteins that contribute to the control of gene expiration, recombination and DNA replication, they are also important factors in compressing the bacterial DNA in the nucleoid. Among the HLPs, Hu protein as a dimer is attracted to DNA containing structural aberration such as 4 way junctions or single stranded lesions. The protein plays an important role in banding as a dimmer and bending of the DNA replication. In this study Bacillus subtilis (ATCC 1023) was grown on TSB medium. Biomass was collected in the mid log then Hu protein was extracted from the bacterium by using ammonium sulfate (65-90% W/V) precipitation. After extracting was done for overnight and the extracted protein was run on SDS-PAGE (15%) it was seen as a band with 10KDa molecular weight. Hu protein has 10KDa molecular weight therefore Hu protein occurred in Bacillus subtilis Bacterium.

Keywords: histone–like proteins, Hu, Bacillis subtilis, ammonium sulfate precipitation

O-10-841-4

Association of risk of prostate cancer with m1 & m2 alleles of cytochrome p450 (CYP1A1)

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Genetic susceptibility for prostate cancer is an important research area in the prostate-specific antigen (PSA) era. CYP1A1 is involved in xenobiotic metabolism and the m1 variant has a T to C mutation in the 30 noncoding region, which has been associated with elevated enzyme activity. The m2 variant has an A to G transition in exon 7, and leads to an amino acid substitution of Val for Ile in the heme-binding region. Fifty patients affected by adenocarcinoma of prostate and 80 persons of benign prostatic hyperpolysia (control group) were studied. Genomic DNA samples were extracted using proteinase K digestion. PCR reactions were performed using specific primers. PCR products were determined by 2% agarose-gel electrophoresis followed by ethidiumbromide staining. In the present study, we investigated the prevalence of two single nucleotide polymorphisms in the CYP1A1 gene. Mutated m1 allele frequency was significantly higher in cases (prostate cancer patients) compared with controls (benign prostatic hyperplasia). Mutated m2 allele frequency was higher in controls compared with cases but not significantly. Obtained results showed that m1 allele of CYP1A1 gene is associated with the frequency of adenocarcinoma of prostate.

Keywords: CYP1A1 gene, adenocarcinoma of prostate, benign prostatic hyperplasia

P-10-862-1

cDNA cloning and sequence analysis of a luciferin-regenerating enzyme from firefly Lampryris turkestanicus

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Bioluminescence is the conversion of chemical energy into light in living organisms. In beetles, luciferin is the substrate of a bioluminescence enzyme, luciferase, and after emitting light as a result of luciferase reaction, is converted to oxyluciferin. ATP measurement methods using luciferase are widely used in the fields of medical science and food hygiene. However, luciferin which is used as a substrate is expensive and the luciferase reaction is inhibited by oxyluciferin produced after reaction. Thus, removal of oxyluciferin or regeneration to luciferin will enable further development of the ATP measurement methods using luciferase. The luciferin-regenerating enzyme (LRE) plays an important role in the recycling of oxyluciferin into luciferin. In this study the cDNA cloning and sequence analysis of a LRE from firefly Lampryris turkestanicus will be reported.

Keywords: firefly luciferase, luciferin-regenerating enzyme (LRE), Lampryris turkestanicus, oxyluciferin

O-10-870-1

Effect of different fatty acid on the ABCA1 gene expression in THP-1 macrophage

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ATP Binding Cassette 1 (ABCA1) is a key mediator of cholesterol efflux to apoA-I in cholesterol loaded macrophages, a first step of RCT in vivo. EPA can inhibit cholesterol efflux from macrophages by increasing degradation of ABCA1. However, the detailed mechanisms of ABCA1 regulation by unsaturated fatty acids are not fully understood. In the present study, we investigated the effects of EPA on ABCA1 expression and ABCA1-dependent cholesterol efflux inTHP-1 macrophage-derived foam cells. Results showed that Incubation of linolenic acid (LA), conjugated linolenic acid (CLA), or eicosapentaenoic acid (EPA), ABCA1 mRNA levels 1.7, 2.48-fold respectly THP-1 but EPA decreased its mRNA levels (0.72) in THP-1. THP-1 human monocyte were cultured in

RPMI1640 medium and treated with PMA for 72 h before experiment for differentiation to macrophage. Macrophages were transformed into foam cells by incubation with the presence of 50 mg/ml Ac-LDL in serum-free medium for 48 h and then macrophages were stained with Red O Oil. Total RNA was extracted from RNA expression was quantified by real-time PCR (RT-PCR), using SYBR Green detection chemistry. These results provide evidence that CLA compare with other fatty acids may have protective effects on cardiovascular disease

Keywords: ABCA1 , linolenic acid, ecosapantaenoic acid, THP-1

P-10-776-1

Cloning of the allergen Che a 1 from Chenopodium album in Escherichia coli

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Chenopodium album (Salmeh in Persian) is a fast-growing weedy annual plant in the genus Chenopodium. Chenopodium album pollen represents a predominant allergens source in Iran. The main Chenopodium album allergens have been described as Che a 1, Che a 2 and Che a 3. The aim of this work was to clone the Che a 1 in Escherichia coli (E. coli) to be a launch for over producing the recombinant allergen. The cloning, production and purification of recombinant allergen in E .coli is an economical may provide sufficient amount of highly purified proteins for diagnostics and therapeutics. In order to clone this allergen, the pollens were subjected to RNA extraction. A full-length fragment encoding Che a 1 was prepared by polymerase chain reaction amplification of the first strand cDNA synthesized from Chenopodium album extracted pollen total RNA. Cloning was carried out by inserting the cDNA into the pET 101/D vector, and transformed into E. coli /Top10. PCR was conducted to proof the cloning system efficiency. For further analysis, the constructed plasmid containing Che a 1 was subjected to sequencing. The result of PCR confirmed the existence of Che a 1 in E. coli /Top10 included pET 101/D vector. The obtained sequence showed high similarity to the deposited sequence in NCBI GenBank. In conclusion the cDNA for the major allergen of the Chenopodium album pollen, Che a 1, was successfully cloned. This study is the first report of using the E. coli as a prokaryotic system for Che a 1 cloning.

Keywords: allergen, Che a 1, Chenopodium album, cloning

P-10-393-2

Investigation of DNA interaction with butylated hydroxyanisole using fluorimetery

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Synthetic antioxidants, such as butylated hydroxyanisole (BHA) are very commonly used antioxidants in the food industry. In fact BHA has been used to suppress the formation of free radicals and prevent lipid oxidation and food spoilage. BHA may also be a tumor initiator or a tumor promoter in some tissues of animals. Steady state fluorescence spectroscopic experiments of DNA interaction with BHA in Tris HCL buffer were carried out at 10.0, 20.0, 30.0 and 37.0 °C. 2 mLs of BHA was placed in a 1-cm thermostated quartz fluorescence cuvette and was titrated with 20µ aliquots of 325 µmol of DNA with continuous stirring. After each titration, the solution was mixed thoroughly and was allowed to equilibrate thermally for 3 min prior to the fluorescence measurements. Fluorescence quenching data was fitted to Stern–Volmer equation (Equ I): $F0 / F = 1 + Ksv [DNA]$ (I) By means of this equation we calculated the Ksv (Stern–Volmer quenching constant), which is a measure of the efficiency of fluorescence quenching by DNA, at 4 temperatures. This study shows that the Ksv is increased by rising the temperature, indicating that DNA quenches the fluorescence of BHA in a dynamic way.

Keywords: CT-DNA, BHA

P-10-874-1

Cloning, expression and purification of entrotoxigenic Escherichia coli heat-labile enterotoxin B subunit as a component of vaccine candidate

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Entrotoxigenic Escherichia coli (ETEC) are the main cause of diarrhea in children aged less than 5 years in developing countries and travelers. The prevalence of the disease is about 400,000,000 annually among which 400,000 to 800,000 lead to deaths. Vaccination against the disease is one of the significant objectives of World Health Organization (WHO). After intestinal colonization of ETEC, heat labile and heat stable toxins are released leading to diarrhea. Heat labile enterotoxin (LT) is one of the most important virulent factors of ETEC. The critical role of this protein in ETEC pathogenesis, as well as its adjuvancity property, make it a likely candidate it for vaccine development. In this study, LTB gene from gene bank was obtained and primer was designed. After genome extraction, it was used as template for PCR amplification. Initially, PCR product was cloned in pBlue script cloning vector and then subcloned in expression vector (pET28a). This vector has been introduced into BL21DE3 E.coli strain. Expression condition was 1mM IPTG, 5hours, and 37°C. SDS PAGE and Immunoblott Techniques was confirmed the rLTB Eexpression. rLTB protein was purified with Ni-NTAaffinity chromatography. Result showed that protein (LTB) has been expressed. However its usefulness as a component of oral vaccine should be determined. Analysis of immunogenicity of protein in mice model as an oral vaccine is in progress.

Keywords: cloning, expression, rLTB, ETEC vaccine

P-10-393-3

DNA binding study of 2-tert-butylhydroquinone using viscosimetery

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Food additive have been extensively applied in recent decades in food industry throughout the world. 2-tert-Butylhydroquinone (TBHQ) is a highly effective preservative for unsaturated vegetable oils, many edible animal fats and meat products. At high doses, it has some negative health effects on lab animals, such as precursors to stomach tumors and damage to DNA. A number of in vitro studies have shown that TBHQ caused DNA cleavage and the formation of 8-hydroxydeoxyguanosine in calf thymus DNA due to the generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide. In this study, DNA binding properties to TBHQ in Tric-HCL buffer (PH=7.4), has been monitored as a function of TBHQ/DNA molar ratio, by viscosimetry method. The specific viscosity of the DNA sample clearly increases with the addition of the TBHQ. The viscosity increase of DNA is ascribed to the intercalative binding mode of TBHQ molecule due to increase in the helix length contour.

Keywords: CT-DNA, TBHQ

P-10-750-1

Frequency of mutated allele CYP2D6*4 in the breast cancer patients

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Important polymorphisms causing genetic differences in phase I drug metabolism are known and therapeutic failures or adverse drug reactions caused by polymorphic genes can to a great extent be foreseen. Tamoxifen therapy reduces the risk of recurrence and prolongs the survival of oestrogen-receptor-positive patients with breast cancer. The CYP2D6 gene is responsible for the majority of tamoxifen metabolism. The CYP2D6 phenotypes associated with these different alleles include poor, intermediate, extensive, and ultrarapid metabolizers. The frequency of functionally important mutations and alleles of the gene coding for CYP2D6 shows wide ethnic variations. The present study aimed to determine the most common mutated allele CYP2D6*4 gene of 94 Breast censer patients, by using RFLP-PCR. CYP2D6*4 allele was not detected in 79 subjects (79%). Among the remaining 20 subjects (21.3%), 8 (8.5%) were carriers of two *4 alleles, being homozygous for CYP2D6 and genotyped as CYP2D6*4/*4. 12 subjects (12.8%) were carriers of one *4 allele, being heterozygous for CYP2D6*4. The frequency of allele *4 was 14.9%. These data indicate that 8.5% of the Breast censer patients are carriers of two nonfunctional mutated alleles *4, being homozygous for CYP2D6*4. It is clinically important to be able to identify those individuals who are likely to have altered pharmacokinetics for CYP2D6 substrates in order to avoid adverse drug reactions.

Keywords: CYP2D6 *4, polymorphism, RFLP- PCR

P-10-871-1

Tubulin structure is disordered in an electromagnetic field

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Although some effects of an electromagnetic field (ELF) on macromolecules and proteins have been reported, tubulin assembly and structure in an electromagnetic field has not yet been characterized. Microtubules (MTs) are cytoskeleton polymers made of repeating α B-tubulin dimers that play essential roles in all eukaryotic cells with functions extended from cellular transport to cell mobility and exceptionally memory. in this study MTs prepared from sheep brain by serial centrifuging and MTs were exposed in the (50Hz, 100Hz, 217Hz/0.2mT) ELF for 30 minutes and absorbed at 350 nm, used to monitor polymerization MTs. Then, effects of ELF on structures of tubulin were studied by fluorescence spectroscopy and CD measurements. The results demonstrated that tubulin self-organization and tubulin conformation were affected by the ELF also the images of electron microscopy showed apparent normal MTs with longer length. This distinction suggests that electromagnetic fields affected on function of microtubule in brain cell and may have effects on memory and function of brain.

Keywords: tubulin, microtubule, electromagnetic

O-10-277-1

The role of siRNA in molecular biochemistry research

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Mammalian genetic approaches to study gene function have been amply by the lack of tools to generate stable and transient loss-of-function phenotypes efficiently. There is a new system, which directs the synthesis of small interfering RNAs (siRNAs) in mammalian cells. In several organisms, introduction of double stranded RNA has proven to be a powerful tool to suppress gene expression through a process known as RNA interference. We show that siRNA expression mediated causes efficient and specific down-regulation of gene expression, resulting in functional inactivation of the targeted genes. Transient and Stable expression of siRNAs using mediates persistent suppression of gene expression, allowing the analysis of loss-of-function phenotypes. We designed three related siRNA directing the synthesis of the same 19–21 base pair double stranded cortactin (Cor.). Target sequence and compared the ability of these constructs to inhibit Cor. function to that of dominant negative cortactin in a transient expression in CHO cells. Introduction of Cor. siRNA resulted in a reduction of more than 90% of Cor. protein. Importantly, it was able to knockdown Cor expression to

the same extent as was seen with the dominant negative cortactin indicating that the size and nucleotide sequence of the design siRNA is very important. Next, we examined whether siRNA design produce siRNA. Western blot analysis revealed that cells transfected with siRNA Cor. inhibited actin organization, which are similar data in cells transfected with the dominant negative cortactin. These results demonstrate that suppression of gene expression by synthetic siRNA is highly target sequence-specific. To further test we designed a scrambled siRNA that there is no any effect on actin filament organization. Transfection of cortactin siRNA reduced endogenous and overexpressed cortactin protein to very low levels and prevented entirely its induction. In contrast, cells transfected with the scrambled cortactin almost completely lost their dependent arrest. These results indicate that our design can suppress endogenous cortactin expression (knockdown cortactin) to the extent that it completely abrogates its function in the DNA damage response. Therefore, the siRNA is a new and powerful system to analyze gene function in a variety of mammalian cell types.

Keywords: cortactin, siRNA, knockdown, knockout,

P-10-880-1

Performance of two PCR methods targeting different regions of viral genome for the detection of TTV in healthy blood donors

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Torque Teno Virus (TTV) is an unenveloped single-stranded circular DNA virus. TTV was discovered in 1997 in the blood of a Japanese patient with post- transfusion hepatitis of unknown etiology by Representational Difference Analysis. Currently PCR (Polymerase Chain Reaction) is only available means for detection of TTV. Currently PCR (Polymerase Chain Reaction) is only available means for detection of TTV. PCR protocols for the detection of TTV that have been developed to target either N22 region (in ORF1) which is reported to be sensitive to certain viral genotypes or UTR (untranslated) region in order to have increased specificity for more viral genotypes/subtypes compared to N22 region. Although sensitive PCR methods with primers designed for very conserved regions of the viral genome (UTR) indicate high prevalence (>90%) of TTV infection in general population of many countries word-wide, in studies using primers for N22 region (ORF1), lower levels of TTV prevalence have been reported. This study aimed to compare of two PCR methods targeting different regions of viral genome for the detection of TTV in healthy blood donors. Serum samples were collected from 50 healthy blood donors in Blood Transfusion Organization, Isfahan. DNA was extracted and subjected to two different PCR methods, a single round PCR that targets 5´-UTR region and a semi-nested PCR for targeting N22 region in ORF1. Torque Teno Virus (TTV) Torque Teno Virus (TTV) 86% of samples were found to be positive for TTV DNA by 5´-UTR PCR and 14% of samples were found to be positive for TTV DNA by N22 PCR. The results showed a considerable difference between the two PCR methods in identification of the virus. The results were the same as other reports.

Keywords: TTV, single-stranded circular DNA virus, PCR, viral genome

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P-10-784-1

Study of interaction between Gαq and β-catenin in HT29 colon cancer

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Canonical Wnt signaling (Wnt/β-catenin) pathway plays an important role in multiple cellular processes and embryonic development. In addition, inappropriate activation of Wnt/β-catenin signaling occurs in many human cancers including colon and ovarian cancers. These effects are accomplished through the stabilization of β-catenin and its translocation to the nucleus where it activates gene transcription by co-activating the TCF/LEF-1 transcription factors. Wnt signaling will be activated when Wnt molecules bind the Frizzled receptors and LRP co-receptors. The structure of Frizzled receptors resembles heterotrimeric G-protein coupled receptors and therefore, a role of G-protein in the regulation of Wnt signaling has been suggested. We have already shown that Gαq activates β-catenin, in HEK293T cells and that this effect can be blocked by a specific small peptide. The effect of Gαq on β-catenin stability and function in HEK293T cells was mainly examined on exogenous β-catenin, since these cells have a very low amount of cytosolic β-catenin. In this study we have used HT-29 colon carcinoma cells, harboring a truncating APC mutation, and therefore have detectable amount of β-catenin protein in the cytosol. We have performed Western blotting, immunofluorescence microscopy and RT-PCR experiments to examine the role of Gαq on β-catenin expression and function. The role of Gαq has been investigated by expression of a minigene encoding a specific blocking peptide. The result of this study will be presented in this conference.

Keywords: Wnt signaling, β-catenin, Gαq, colon cancer

O-10-865-1

Gaq activation accumulates B-catenin in HEK293T cells

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Wnt/B-catenin signaling pathway plays a decisive role during cell proliferation, cell differentiation and embryonic development. Following activation of Wnt signaling, B-catenin translocates to the nucleus where it makes a complex with TCF/LEF transcription factors to activate their target genes like *FGF20* and *c-myc*. Activation of B-catenin occurs in various human cancers, and therefore B-catenin might be a good target for cancer clinical experiments. Our previous results have suggested that Gaq activation leads to accumulation of B-catenin in the cytoplasm. To further investigate the relationship between these two proteins, we have used a minigene encoding the Gaq inhibitory peptide. A minigene encoding the Gas inhibitory peptide was used as a control. HEK293T cells were transfected with plasmids encoding Gaq (or its active form, GaqQL), B-catenin and Gaq inhibitory peptide and the effect of Gaq protein on B-catenin expression and function was examined by immunofluorescence, western blotting and RT-PCR experiments. When we exogenously expressed B-catenin, some cells accumulated the protein. *c-myc* and *FGF20* gene transcription was enhanced by accumulated response to B-catenin and This response was completely blocked by expression of the Gaq minigene. In the presence of GaqQL,

the cellular accumulation of b-catenin was remarkably increased at the protein level (but not at RNA level). Interestingly the exogenous Gaq-mediated B-catenin accumulation was specifically blocked by the Gaq inhibitory peptide. However, this effect was dependent on cellular growth conditions. We conclude that Gaq positively regulates B-catenin cellular accumulation and function in 293T cells.

Keywords: B-catenin, Gaq, Gaq inhibitory peptide

P-10-892-1

Viral and non-viral gene transfer methods for genetic modification of Human Large Cell Lung Cancer Cell Line

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The future of gene therapy relies on the development of efficient and safe systems for gene delivery. The aim of this study was to compare the efficiency of adenoviral infection and five non-viral methods for transfection of Mehr-80 as the target cell. Mehr-80 is a Human Large Cell Lung Cancer Cell Line that has been established in Shiraz University of Medical Science by purification from peritoneal effusion of a patient with large cell undifferentiated carcinoma of the lung. To evaluate the capacity of the cells to become transfected, vector expressing enhanced green fluorescent protein (pEGFP) were transferred to the cells by different non-viral methods including Calcium phosphate (Ca/Ph), DEAE, Superfect, Electroporation and Lipofectamine 2000. For the viral method Mehr-80 was infected with recombinant Adenoviral vector encoding GFP (rAd-GFP). Gene transfer efficiency was compared on the basis of GFP expression assessed by fluorescence microscopy and flow cytometry. According to the results, cell transfection with Ca/Ph, DEAE, Superfect, Electroporation and Lipofection methods reported as 9%, 18%, 16%, 32% and 44%, respectively. More than 90% of the cells expressed GFP when 300 virus particles per cell (VP/cell) were used for the cell infection. The non-viral gene transfer methods applied were less efficient compared to the viral method tested. However, due to advantages with respect to safety issues and ease of handling, improvement of non-viral gene delivery deserves further attention.

Keywords: Mehr-80, transfection, transduction, cancer, gene therapy

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P-10-896-1

Evaluating SW480 as a cell system to investigate the interaction between Gαq and β-catenin

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Wnt/β-catenin signaling is the most important pathway that controls animal development. Deregulation of this pathway is a major factor during human carcinogenesis. Wnt ligands send signals through the Frizzled receptors which have structural similarity to G-protein receptors (GPCRs). Therefore a question has been raised whether G-proteins regulate Wnt signaling. Our previous experiments in HEK293T cells have shown that activation of the Gαq class of G-proteins stabilizes β-catenin protein. The Gαq-mediated stabilization of β-catenin was blocked by expression of a minigene encoding the Gαq blocking peptide. As a different cell system in this study we have used SW480 colon cancer cells which intrinsically have high levels of cytoplasmic β-catenin protein. β-catenin expression and function are going to be examined in these cells in the presence and absence of the Gαq inhibitory peptide. The results will be presented in this conference.

Keywords: Wnt signaling, β-catenin, Gαq, colon cancer

P-10-897-1

Y-chromosome short tandem repeats (Y-STRs) variation in a Kurdish group of Iranian population

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Y-chromosomal polymorphic markers, locating in nonrecombinant region of the chromosome, have been extensively investigated in forensic medicine for male identification and paternity testing, as well as in evolutionary and population genetics studies. These markers are characterized by a moderate number of polymorphic loci, passing down as distinctive haplotypes from father to son. In this study we use the multiplex and monoplex polymerase chain reaction (PCR) system for analysis of polymorphism of 20 loci Y-STRs DYS390, DYS389I/II, DYS385a/b, DYS19, DYS391, DYS393, DYS392, DYS426, DYS448, DYS437, DYS447, DYS439, H4,YCAIIa/b, DYS438, DYS460(A7.1), DYS388, in a random sample of 80 Kurdish male Group. Blood samples obtained from 80 unrelated males from Kurdistan province of Iran and DNA was extracted using a modified salt/chloroform method. The Y STR PCR analysis includes a series one 3plex, six 2plex and three 1plex amplification reactions. Allele and haplotype frequencies were estimated through the gene counting method. Gene and haplotype diversity was calculated according to Nei and Roychoudhury. For the Y chromosome, haplotype diversity is numerically identical to two parameters used in forensic genetics, i.e. the power of discrimination (PD) and chance of exclusion (CE). Furthermore, we want to follow comparison of allelic distribution for different populations (Lurish, Kurdish and Turkmen populations and a group of males randomly selected among city of Tehran residents) of Iran, and determine the phylogenetic map of these populations according to Y-STRs polymorphism.

Keywords: Y-STR, multiplex PCR, Tehran, Iranian kurds

P-10-771-2

Y-chromosomal STR haplotypes in Iranian Torkaman population

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Y-chromosomal short tandem repeats (Y-STRs) are useful markers in forensic medicine and paternity testing as well as in population and lineage studies. The Y chromosome differs from the autosomes in that it is transmitted only from father to son, and escapes recombination over most of its length, so consists largely of male specific markers individuals Y-STRs used for forensic purposes. Due to the lack of recombination, a son carries the same Y-STR haplotype as his father except when mutations occur. The aim of this study was to assess the distribution of 20 Y-STR markers, which are DYS390, DYS389I/II, DYS385a/b, DYS19, DYS391, DYS393, DYS392, DYS426, DYS448, DYS437, DYS447, DYS439, H4, YCAIIa/b, DYS438, DYS460 (A7.1), DYS388, and haplotype diversity in a random sample of males in Iranian Torkaman population. DNA was extracted from peripheral blood of 100 males with salting-out method. PCR was carried out in mastercycler gradient thermocycler. The products were separated electrophorectically on 2% agarose gel, followed by polyacrylamide gel electrophoresis for further resolution. PCR amplification was performed in a series of two triplex and six duplex reactions. In Torkaman population respective loci showed between 2 and 8 different alleles. Gene diversity ranged between 0.2719 (DYS388) and 0.8996 (DYS385). A total of 87 different haplotypes were found and the haplotype diversity was 0.9992. Nine haplotypes occurred more than once, while 78 haplotypes were unique among individuals studied.

Keywords: multiplex PCR, STR, Y chromosome

P-10-659-2

Y-chromosomal STR haplotypes in Tehran population

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Y-chromosomal short tandem repeats (Y-STRs) are useful markers in forensic medicine and paternity testing as well as in population and lineage studies. The Y chromosome differs from the autosomes in that it is transmitted only from father to son, and escapes recombination over most of its length, so consists largely of male specific markers individuals Y-STRs used for forensic purposes. Due to the lack of recombination, a son carries the same Y-STR haplotype as his father except when mutations occur. The aim of this study was to assess the distribution of 20 Y-STR markers, which are DYS390, DYS389I/II, DYS385a/b, DYS19, DYS391, DYS393, DYS392, DYS426, DYS448, DYS437, DYS447, DYS439, H4, YCAIIa/b, DYS438, DYS460 (A7.1), DYS388, and haplotype diversity in a random sample of males in the Iranian Torkaman population. DNA was extracted from peripheral blood of 100 males with salting-out method. PCR was carried out in mastercycler Gradient thermocycler. The products were separated electrophorectically on 2% agarose gel, followed by polyacrylamid gel electrophoresis for further resolution. PCR amplification was performed in a series of two triplex and six duplex reactions. In Torkaman population respective loci showed between 2 and 8 different alleles. Gene diversity ranged between 0.2719 (DYS388) and 0.8996 (DYS385). A total of 87 different haplotypes were found and the haplotype diversity was 0.9992. Nine haplotypes occurred more than once, while 78 haplotypes were unique among individuals studied.

Keywords: multiplex PCR, STR, Y chromosome

P-10-238-3

Co-expression of Nucleostemin and Oct-4 genes in human chronic myelogenous leukemia cells

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Chronic myeloeid leukemia (CML) is a clonal bone marrow disorder witch arises from genetic changes in pluripotent hematopoietic stem cells. Despite improved therapeutic strategies for this disease, CML still remains an unsolved challenge in medical treatments. Indeed, most primitive leukemia cells, defined CD34+, are drug resistant and current chemotherapeutic drugs fail to eradicate these leukemic stem cells. This means that elucidation of molecular effector mechanisms involved in leukemic stem cell proliferation and self-renewal may improve therapeutic options in CML. In this content, two pivotal stem cell markers related to self-renewal are Nucleostemin (NS) and octamer binding transcription factor-4 (Oct-4). Ns is a novel p53 binding protein and plays a pivotal role in stem cells proliferation. Oct-4 plays critical role in maintaining self-renewing stage of stem cells. Here, we aimed evaluating the expression of these two new stem cell genes in K562 cells as a well-known experimental stem cell model of CML. To do this, K562 cells were cultured in optimum conditions and total RNA was extracted in exponentially phase of cell growth. Expression of Oct-4 and NS were evaluated by semiquantitative RT-PCR and normalized by β2-microgolobin as an internal control. Data showed that both NS and Oct-4 were simultaneously expressed in CML cells but with different levels. In fact, the expression level of NS was higher than Oct-4, so that relative expression of NS and Oct-4 were about 2.3 and 0.16, respectively. These results, for the first time, demonstrated the co-expression of NS and Oct-4 in K562 cells; suggest that these genes are closely related to the origination, pathogenesis and development of CML. Therefore, these findings may pave some novel therapeutical approaches through suppressing of expression of Oct-4 and NS genes in order to control leukemic stem cells in CML.

Keywords: Chronic myeloeid leukemia, Nucleostemin, Oct4, Self-renewal, Stem cell leukemia.

O-10-892-2

Combination suicide gene therapy using E.coli nitroreductase and p53 therapy by adenovirus vector

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E.coli nitroreductase together with CB1954 is one of the enzyme/prodrug systems that can be applied for directed enzyme/prodrug viral gene therapy. This enzyme is able to convert the prodrug CB1954 to a cytotoxic derivative that is able to kill tumor cells. Activated CB1954 leads to the formation of interstrand DNA cross-links

and apoptosis start in dividing and non dividing cells. At present developed usage of p53 therapy in cancer and recombinant human adenoviral p53 injection (Gendicine) is the first approved gene therapy product. In this study, we investigated the effects of the p53 therapy in combined with the suicide gene therapy, NTR/CB 1954, on different cancer cell lines. We constructed a recombinant adenovirus carrying wild-type E.coli nitroreductase and human wild-type p53. This consists of the CMV immediate early promotor driving expression of NTR and p53. In 1μM and 10μM concentration of CB1954, IC50 of adenovirus encoding NTR was 100 & 1000 VP/cell (virus particle per cell), respectively. By using adenovirus encoding wild-type p53, IC50 was 300 VP/cell. Results demonstrated that combination of suicide gene therapy using NTR/CB1954, and p53 therapy gives synergistically decreased IC50 compared either modality alone. Therefore, the combination of NTR/CB1954 and p53 can significantly improve cancer gene therapy.

Keywords: cancer, E.coli nitroreductase, p53, adenovirus vector, genetherapy

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P-10-910-1

Designing and set up of a STR typing method and investigation of the allele frequencies of 12 STR loci in Iranian population

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Nowadays, Short Tandem Repeat (STR) sequences are of major importance in the field of human identification in forensic cases, genomic mapping and linkage analysis. Most of the STR sequences show polymorphic alleles that differ in length. The difference in length is caused by a variation in number of times that a core sequence is repeated. The STR core sequence length is normally 2-6 bp. STRs can be readily amplified by PCR and is very perfect marker where the samples are degraded. STR typing technique which is based on PCR-amplification of STR markers is a very useful and sensitive technique compared with others, such as (Restriction Fragment Length Polymorphism) RFLP. To select STRs with high polymorphism, many known STRs were evaluated in this study and 12 STR loci were selected for investigation of their allele frequencies in Iranian population. The 12 STR loci are: THO1, TPOX, CSF1PO, D16S539, D7S820, D13S317, D18S51, D8S1179, D5S818, FGA, D2S1338, and D3S1358. In order to save cost and time, the PCRs of the STR Typing were designed as four Triplex. The genomic DNA of 120 blood samples (collected with different ethnicities) was extracted and PCR-amplified. The resulting PCR products were separated by 10% polyacrylamide gel electrophoresis. The size of PCR products were analyzed using size markers with the help of Total lab software and related alleles assigned manually. All selected loci were highly polymorphic except TPOX.

Keywords: triplex PCR, STR, RFLP, DNA profile

P-10-277-2

Bradykinin-stimulated GLUT4 translocation is independent actin stress fiber formation

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Bradykinin is a nonapeptide involved in multiple biological processes as vasodilatation, increase in capillary permeability, smooth muscle relaxation/contraction, and inflammation. Moreover, bradykinin seems to be involved in the modulation of glucose metabolism in peripheral tissues. In this study, we demonstrated the role of cortactin, a cortical actin binding protein, on bradykinin-trrigerd translocation of GLUT4 in Chinese hamster ovary (CHO-GLUT4myc). Bradykinin-induced GLUT4 translocation is not blocked by wortmannin, an inhibitor of PI3-K and as well as PP1, Src-family protein tyrosine kinase inhibitors. Activation of receptor-coupled Gq by bradykinin could still induce GLUT4myc translocation in the presence of PI3-K inhibitor, without increase in remodeling of actin filament. In the cells with cortactin mutant, however, actin rearrangement and GLUT4myc translocation stimulated by bradykinin were completely inhibited. The results suggest that bradykinin pathway was independent on cortactin-actin remodeling, although basal actin arrangement was necessary for this pathway. These findings indicate that cortactin binding site(s), especially Src homology 3 (SH3) domain, play a key role in the signaling pathway of insulin-triggered GLUT4myc translocation.

Keywords: insulin, bradykinin, GLUT4, cortactin, cytoskeleton

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O-10-925-1

Hyperoxia induced protection against rat's renal

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Pre-exposure to hyperoxic gas (≥95%) has been shown to protect the heart and central nervous system from ischemia-reperfusion injury. In the present study we investigated whether oxygen pretreatment induces delayed renal protection in rats. The possible role of some renal antioxidant agents was also investigated. Adult male Wistar rats were kept in a hyperoxic (HO) (≥95%O2) environment for 0.5h, 1h, 2h, 3h, 6h, 2 h/day for 3 consecutive days and 4 h/day for 6 consecutive days and in control group (IR) animals were kept in the cage with no HO; one day before subjecting their kidney to 40 minutes of ischemia and 24h of reperfusion. Renal function was assessed by comparing plasma creatinine (Cr), blood urea nitrogen (BUN), creatinine clearance (CLCr) and fractional excretion of sodium

(FENa%). Histopathological injury score was also determined according to Jablonski method. To examine the antioxidant system induction by hyperoxia we measured renal catalase and superoxide dismutase activity and renal glutathione and malondialdehyde content. Our data demonstrated that only in 4 h/day HO for 6 consecutive days the renal function tests (Cr, CLCr, BUN and FENa%) and Jablonski histological injury were better than control group($p<0.05$). The beneficial effect of oxygen pretreatment in this group was associated with increased renal catalase activity compared with those obtained from control group ($p<0.05$). The present study demonstrates that repeated exposure to hyperoxic (≥95% O2) environment can reduce subsequent rat's renal ischemia-reperfusion damage. Induction of endogenous antioxidant system may partially explain this beneficial effect of hyperoxic preconditioning.

Keywords: antioxidant system, hyperoxia, IR injury, preconditioning

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P-10-926-1

Effects of knockdown P75 receptor using a small interfering RNA in primary Schwann cell on signaling pathway

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The low affinity neurotrophin receptor (p75NTR) is a multifunctional receptor with different roles in neurotrophin signaling and axon outgrowth and it is best known for mediating neural cell death during development as well as in the adult following injury and later making it a target for treatment of neurodegenerative disease. P75NTR is expressed at high level on Schwann cells. We used siRNA for p75NTR and demonstrated that it mediated silencing of components of the inhibitory silencing cascade including P75NTR followed by RhoA and relevant protein; while changes in levels of protein and cellular immunoreactively were detected with scramble (siRNA control sequences). Importantly after 24h using siRNA in Schwann cell culture medium, siRNA mediated knockdown of p75NTR and after 48h the level of P75NTR mRNA upregulated higher than 24h and level of mRNA of RhoA decreased compaired to control (scramble). This result suggested that P75NTR knockdown by siRNA might be effective in RhoA signaling pathway and RhoA might be a target for disinhibition strategy to promote CNS axon growth and regeneration.

Keywords: p75NTR, siRNA, RhoA, Schwann cell

P-10-747-1

Transplantation of spermatogonial stem cell suspension through rete testis of mice after chemotherapy

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Spermatogenesis is a biological process that results in the conversion of the relatively undifferentiated germ cell into the highly differentiated spermatozoa. The loss of spermatogonia following chemo-or radiotherapy leading to temporary or permanent infertility of the patient is a well known and unwanted site effect of many oncological therapies. Testis cell transplantation from a mouse into a recipient mouse rete testis results in donor-cell-derived spermatogenesis in nearly all the hosts' testes. This requires the transfer of cells from a donor animal into the testis of a recipient animal, in which the spermatogonial stem cells will colonize and initiate donor-derived spermatogenesis. To isolate germ cells, a two to 4-day old mouse testis cells were dissociated enzymatically. Busulfan treatment is used to eliminate proliferating cells in the testes of recipient mice. The donor cells, suspended in DMEM, were introduced into the rete testis of recipient mice via microinjection method .To distinguish the progeny of the transplanted donor stem cells from endogenous germ cells, BrdU-labeled cells were used. In addition, reverse-transcriptase polymerase chain reaction (RT-PCR) was performed to determine levels of c-kit and cyclin B1 expression in spermatogonial stem cells after transplantation. Transplantation of stem cells into rete testis of the recipients left testis was done and cryosection of the transplanted testis showed a significant increase in the number of spermatozoa in the left epididymal lumen compared with that of the control (right testis). It is interesting that cells that had been cultured on feeder layers were able to colonize busulphan-emptied recipient testes after spermatogonial stem cell transplantation. Spermatogonial stem cell was not alkaline phosphatase activities but C-kit gene was expressed in spermatogonial stem cells. Cyclin B1 expression was reduced after busulfan treatment compared with untreated mouse. The expresson of this gene was however increased after germ cell transplantation. Elimination of differentiating germ cells is believed to provide the necessary environment for donor spermatogonial stem cells to migrate to the basement membrane and establish a stem cell niche. Brdu-labeled testis cells were successfully transplanted into recipient mouse rete testis. These cells remained in all recipient testes up to two months after transplantation. Culture of spermatogonial stem cells before transplantation helps proliferation and improves stem cell transplantation efficiently. The present study confirms that regeneration after cytotoxic treatment is based on morphological criteria. We demonstrate the increase in stem cell numbers during regeneration and after transplantation. The results of this study provided functional data in support of stem cell self-renewal, and demonstrated the increase in the number of stem cell during regeneration upon transplantation. Transplantation of spermatogonial stem cell suspension through rete testis of Azoospermic mice considerably enhances the efficiency of the rete testis injection in this species.

Keywords: spermatogonial stem cell, Busulfan, transplantation, cloning, fertility

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P-10-935-1

Lentivirus vectors combined with TetON-inducible gene expression system regulates transgene expression in mammalian cell lines

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Efficient transfer and regulated expression of transgenes have been two major achievements in molecular manipulation of target cells and tissues in recent years. In this study, we have combined the transduction power of recombinant lentivirus vectors with tetracycline (tet)-mediated inducible gene expression to express a reporter gene in hardly transfectable mammalian cell lines in a controlled manner. This

combined system consisted of two lentivirus transfer vectors: pLV-rtTA in which tet-transactivator rtTA-M2 was expressed under constitutive CMV promoter, and pLV-GFPTRE in which the reporter GFP gene cassette is under control of minimal promoter induced by tet response elements (TRE). We generated recombinant viruses by co-transfection of HEK-293T cell line with each transfer vector plus packaging and envelope vectors. These two viruses were concentrated and used for co-transduction of human astrocytes, human retinal pigmentous epithelium cells and rat dopaminergic PC12 cell line. In the absence of antibiotic, no or very minimal induction of GFP expression was detected; however, when doxycycline, tetracycline analog, was added to cell medium it began inducing expression of GFP. Our initial assessment shows several fold induction of transgene expression in a dose-dependent manner in each cell line. These findings indicate that firstly efficient transfer to and expression of exogenous genes in various mammalian cells is possible using lentiviral vectors and secondly this expression can be regulated by combining the virus vectors with an inducible system such as tetracycline-based system applied in this study.

Keywords: Lentiviruses, tetracycline, inducible, gene expression, mammalian cells

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P-10-942-1

Isolation and molecular identification enterotoxigenic Esherchia coli and cloning, expression of recombinant cfaE gene

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Enterotoxigenic Escherichia coli (ETEC) are the most common of diarrhea in children in developing countries and in travelers to these areas. It has been estimated that ETEC infections are responsible for at least 650 million cases of diarrhea, causing about 800,000 deaths each year in children below the age of 5 years. Colonization factor antigen I (CFA/I) plays critical role in most ETEC colonization to small-intestine epithelial cells. cfa E gene is one of the four genes of CFA/I operon that encodes the minor fimberial subunit of CFA/I. cfaE is required for initiation of CFA/I fimberial assembly and mediates bacterial attachment to host cell surface receptors. For this reason the product of cfaE gene should be investigated as a vaccine candidate. In this study we received stool samples from children with acute diarrhea from children's medical center of Iran. Molecular identification of ETEC was made by amplification of its virulence genes (LTb and ST) by PCR. Two primers were designed for cfaE and amplified by PCR and cloned into T.A cloning vector. The vector was cut by XhoI and EcoRI and subcloned into pET28a as an expression vector. Recombinant vector was transformed into E.coli Bl21DE3 and expressed N-terminal His tagged protein in E.coli when induced by IPTG. The CFA/E protein was detected in the total soluble protein of the E.coli Bl21DE3 by SDS-PAGE. The recombinant protein was purified with nickel chelate affinity chromatography. Our findings indicated that the cloning and expression of CFA/E were successful.

Keywords: ETEC, CFA/I, cfaE, cloning, expression

P-10-841-8

Study the cytotoxic effects of some fractions from Pleurotus florida on different cancer cell lines

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Mushrooms are nutritionally functional food and the most significant medicinal effect of them is their anti-tumor property. Today, different medical approaches are used for the treatment of cancers, but in most cases they are not effective or have unpleasant side-effects. This has forced scientists to study for more effective drugs with less toxicity. This study evaluated the cell cytotoxicity effect of some fractions isolated from Pleurotus florida on cancer cell lines and fibroblast cells. R5, F5, R10, R30 and R100 fractions isolated from Pleurotus florida body extract. The growth inhibitory activity of these fractions was determined for cancer cell lines including gastric adenocarcinoma cell line (AGS), renal adenocarcinoma cell line (ACHN), cervical cancer cell line (Hela), colon adenocarcinoma cell line (HT-29) and fibroblast cell line (L929) using colorimetric MTT assay. The results showed that the isolated fractions tested in this study showed significant inhibitory activity for cancer cell lines in a dose-dependent manner. Some of the fractions such as R100 and R30 exhibited the most inhibitory activity against HT-29. Among the cell lines tested, HT-29 was very sensitive to these fractions. In this study a series of fractions isolated from Pleurotus florida extract had cytotoxicity effects on cancer cell lines. All fractions had the most cytotoxicity effect on human colon cancer cell line (HT-29). Fraction R100 had the most cytotoxicity on it. Further studies are needed to elucidate the mechanisms by which R100 fraction act.

Keywords: Pleurotus florida, cytotoxicity, colon adenocarcinoma cell line (HT-29)

P-10-841-10

Effects of fractions from Pleurotus florida on HT-29 cell viability and apoptosis

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Eedible mushroom, Pleurotus florida has been used by mankind from the ancient because of their nutritional values and medicinal benefits. In present study, R5, R10, R30 and R100 fractions were prepared from P. florida and their cytotoxicity activity were evaluated in HT-29 and HGF cell lines. Also, pattern of cell death was determined. Tumoral (HT-29) and non-tumoral (HGF) cells were treated with various concentrations of isolated fractions. MTT assay was used for the

evaluation of cell viability. Pattern of cell death was determined using Annexin V and propidium iodine staining followed by FACS analyses. R5, R10, R30 and R100 fractions inhibited cell viability of HT-29 cells in a concentration-dependent manner, but had less cytotoxicity on normal fibroblast-like cells (HGF). Their IC50 values were 46%, 46%, 8% and 4%, respectively. These fractions inhibited cell viability mostly via induction of early apoptosis in colon cancer HT-29 cells at 18%, 49%, 64% and 72%. Thus, isolated fractions could be good candidates as chemotherapeutic agents in cancer treatment in future.

Keywords: Pleurotus florida, cytotoxicity, apoptosis, HT-29

P-10-841-11

Cytotoxicity of extracts from Iranian Artemisia species on cancer cell lines

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In this study, aerial parts of eight species of Artemisia were collected, identified and the anti-proliferative effects of methanol, ethylacetate, dichloromethane and hexan extracts on four human cancer cell lines were determined. Different concentrations of extracts were added to cultured cells and incubated for 72 h. Cell survival was evaluated using MTT assay. Dichloromethane extract from A. ciniformis (IC50=35µg/ml), dichloromethane extract from A. diffusa (IC50=42µg/ml) and dichloromethane extract from A. ciniformis (IC 50: 29 µg/ml) showed the most inhibitory effect on AGS, Hela, HT-29 and MCF-7 cell lines, respectively. Among different extracts from studied Artemisia spp., dichloromethan extract from A. ciniformis showed the most inhibitory effect on various cancer cell lines.

Keywords: Artemisia spp., MTT assay, cytotoxicity

O-10-690-2

An alkaline lipase from a newly isolated bacillus subtilis strain; heterologous expression and some properties

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The extracellular lipase from a local soil isolate belonging to bacillus subtilis was cloned previously. In this study the cloned gene was released from the cloning vector using NdeI and BamHI restriction enzymes. The released product corresponding to the structural gene for lipase was ligated into the expression vector pET15b and transformed into the expression host Escherichia coli BL21 (DE3). The expression of the recombinant lipase was induced using 1mM IPTG as inducer. Cells were harvested after 3 hours of induction and resuspended in a lysis solution containing 8 M urea and 20mM Tris-HCl pH 8. Cleared supernatant from crude cell extract was used to determine enzyme activity. The enzyme activity was measured by spectrophotometry at 400nm using p-nitrophenyl-decanoate as substrate. The recombinant lipase showed a molecular weight of approximately 25 kDa by SDS-PAGE. Maximum activity was found at pH 9-10 and 40-50°C. The recombinant lipase also showed a remarkable activity in a broad range of pH values from 6 -11 and in a temperature range of 30-80 °C. Various concentrations of ZnCl2 were examined for their effect on enzyme activity and thermostability. The results showed that ZnCl2 at all concentrations were inhibitory to the enzyme activity. It was also shown that the ZnCl2 at all concentrations used doesn't improve enzyme thermostability. The recombinant lipase from this study showed better activity in the temperature range of 40-50°C compared to lipase LipA from bacillus subtilis (its closest relative with respect to primary structure) while showing similar activity in the alkaline pH.

Keywords: alkaline lipase, bacillus subtilis, heterologous expression, soil

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O-11-512-2

Construction of a new functional α-1 antitrypsin with reduced size

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Alpha 1- antitrypsin (AAT) is a member of serpin superfamily and an inhibitor of neutrophil elastase. AAT has a characteristic secondary structure of three β-sheets, nine α-helices and a reactive central loop (RCL). AAT inhibits target proteases by forming a stable complex in which the cleaved RCL is inserted into β-sheet-A, which accompanies conformational change in ATT. Spontaneous polymerization and instability of AAT are challenges in producing drugs against AAT deficiency diseases. Therefore, the purpose is to produce drugs with lower polymerization degree and higher stability. Moreover reduced size drugs are preferred by drug delivery systems. Therefore, we decided to reduce the size of AAT from N-terminal region. To investigate the influence of the presence of N-terminal segment (residues 1 to 43) on the structural properties of the AAT, at first the loop and α-helix region in N-terminal were removed from the structure. Then 10ns molecular dynamics (MD) simulation was run on the native and new AAT. The structural properties; e.g., structural conformation, root mean square, fluctuation, internal non bonded interactions and total accessible surface area (ASA) were compared into resulted structure from each MD run. These results showed no noticeable differences in these properties in two structures. Therefore these findings were a confident cause to continue this study in experimental field. In the experimental phase we constructed two native and new modified AAT. Each construct integrated in pGAPZα and transformed into X33, separately. With TIC method, the assessment of inhibitory activity showed no significant difference in two constructs.

Keywords: Alpha 1- antitrypsin, molecular dynamics, polymerization, serpin, yeast

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P-10-850-1

Nuclear reprogramming factor and induced pluripotent stem cells

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Nuclear reprogramming describes a switch in gene expression of one kind of cell to that of another unrelated cell type. The procedures included mammalian somatic cell nuclear transfer, cell fusion, transduction of somatic cells with defined factors (Oct4, Sox2, c-myc, and Klf4) can initiate reprogramming to a pluripotent state and direct reprogramming. Adult somatic cells have been reprogrammed recently by retroviral transduction using four transcription factors to induce pluripotent stem cells (iPS). The shortage of organ donors for regenerative medicine has stimulated research on stem cells as a potential resource for cell-based therapy. Stem cells have been used widely for regenerative medicine applications. The development of innovative methods to generate stem cells from different sources suggests that there may be new alternatives for cell-based therapies. iPS cells possess morphological, molecular and developmental features of conventional blastocyst-derived ES cells and have the potential to serve as a source of therapeutic cells for customized tissue repair, gene therapy, drug discovery, toxicological testing and for studying the molecular basis of human disease. Nuclear reprogramming is of great medical interest, as it has the potential to generate a source of patient-specific cells.

Keywords: reprogramming, pluripotent stem cells

P-10-973-1

Comparing mannose binding lectin Genetic diversity in intracellular and extracellular pathogens

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One of the important immunological factors in diseases is mannose binding lectin (MBL). The aim of present study is to determine the distribution of the alleles of mannose-binding lectin gene codon 52, 54, 57 and promoter variants H/L, X/Y, P and Q in confirmed visceral leishmaniasis (VL) patients as an intracellular pathogen while compares with extracellular pathogens (in renal infection) and seek correlation between these variants and intracellular and extracellular infections. Fifty eight confirmed VL patients' blood samples were compared with fifty eight blood samples of patients received renal in results of renal infections. MBL genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Frequency of defective allele B in extracellular pathogens was more than intracellular pathogens (P=0.0001), and on the contrary prevalence of wild type allele A in intracellular pathogens was more than extracellular pathogens (P=0.0001), and in other alleles and variants there was not any significant difference. In conclusion, there was more prevalence of alleles with low mannose binding lectin serum level in extrallelular pathogens which can be considered as a risk factor for these infections. On other hand, prevalence of high concentration alleles in intracellular pathogens indicates the role of mannose binding lectin level for susceptibility to intracellular pathogens. .

Keywords: extracellular, genotype, intracellular, mannose binding lectin, pathogen

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P-10-878-3

Association of genotype and serum levels of adipokines with bone mineral density in type 2 diabetes mellitus patients

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There are conflicting findings about bone density alterations in type 2 patients. Regarding the role of adipokines in glucose metabolism, they may have metabolic effect on bone mineral density (BMD) changing. The aim of this study was to assay the association between circulating visfatin, adiponectin and the visfatin genotype with BMD. Thirty-two patients with T2DM participated in this cross-sectional study. Laboratory measurements included were FBS, HbA1C, lipid Profile, fasting serum Visfatin and adiponectin. Hip and spine BMD were measured using DEXA. Genotyping for visfatin gene SNP (rs2110385) was performed by using the PCR- RFLP method. Mean of age, BMI and HbA1C, circulating visfatin, adiponectin, and spine and hip bone mineral density were 56.81± 9.13 years, 30.44 ± 4.91 kg/m2, 7.58± 2.0%, 14.95± 16.93 ng/ml, 5.83± 2.41, 1.08±0.21 g cm-2 and 0.94±0.14 g cm-2, respectively. The frequencies of the Genotypes of GG, GT and TT were 37.5%, 43.8% and 18.8%, respectively. Prevalence of osteoporosis in patients with GG genotypes was 33.3%, but there was not any patient with osteoporosis in other genotypes. Hip BMD and Z-score were significantly lower in GG genotype. We found significant correlation between circulating visfatin and hip BMD and adiponectin (r=-0.31and -0.32 respectively) independent of BMI and age. Our results suggest that adipokines may contribute to the BMD changes in type 2 diabetes mellitus patients and their gene variations may explain the inconsistent BMD changes among these patients.

Keywords: bone mineral density, adipokines, visfatin genotypes, visfatin, adiponectin

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P-10-1000-4

Protein A mediated multicellular behavior in Staphylococcus aureus

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The capacity of S.aureus to form biofilm on host tissues and implant medical devices is one of the major virulence traits underlying persistent and chronic infections. The matrix in which S. aureus cells are encased in a biofilm often consists of the polysaccharide intercellular adhesion (PIA/PNAG). However, surface proteins capable of promoting biofilm development in the absence of PIA/PNAG expolysaccharide have been described. Here, we used two-dimensional nano-liquid chromatography and mass spectrometry to investigate the

composition of a proteinaceous biofilm matrix, and identified protein A (spa) as an essential component of the biofilm; protein A induced bacterial aggregation in liquid media and biofilm formation instanding and flow conditions. Exogenous addition of synthetic protein A or supernatants containing secreted protein A to growth media induced biofilm development, indicating that protein A can promote biofilm development without being covalently anchored to the cell wall. Protein A-mediated biofilm formatting was completely inhibited in a dose dependent manner by addition of serum, purified IgG or anti-protein A specific antibodies. Our results suggest a novel role for protein A complementary to its known capacity to interact with multiple immunologically important eukaryotic receptors.

Keywords: S.aureus, expolysaccharide, proteinaceous, supernatants

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P-10-1005-1

Simultaneous identification of two MspI polymorphisms of the chicken growth hormone in Iranian native fowls

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The chicken growth hormone (cGH) is a polypeptide hormone which is involved in a wide variety of physiological functions. The aim of this study was simultaneous identification of two MspI polymorphisms of the chicken growth hormone in Iranian native fowls. The blood samples were randomly selected from two native chicken population of Iran: Esfahan and Mazandaran. The chicken genomic DNA was isolated according to the salting out method. PCR products of the expected size (770 bp related to Intron 1 and 1170 bp related to Intron 4) were obtained using two pairs of primers. This indicates that the two sets of primers identified their place clearly. The major problems in the molecular works are time consuming and high cost. The simultaneous test of 2 MspI polymorphisms in the current study allows the analysis of two loci of the cGH gene in parallel, which can reduce time consuming significantly.

Keywords: chicken growth hormone, polymorphism, Iranian native fowls, PCR-RFLP

P-10-919-4

Genome-wide combination profiling of copy number and methylation offers in cancer cells

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Recently, a number of microarray studies have been based on copy number and DNA methylation alterations in order to find clinical biomarkers of carcinogenesis. Restriction landmark genome scanning (RLGS) is a quantitative approach that is uniquely suited for simultaneously assessing the methylation status of thousands of CpG islands. RLGS separates radiolabeled NotI fragments (or other CpG-containing restriction enzyme fragments) in two dimensions and allows distinction of single- copy CpG islands from multicopy CpG-rich sequences. The methylation sensitivity of the endonuclease activity of NotI provides the basis for differential methylation analysis, and NotI sites occur primarily in CpG islands and genes. RLGS is also a useful method for integrating methylation analyses with high-resolution gene copy number analyses.

Keywords: methylation, CpG islands, NotI

P-10-1030-2

Isolation and purification of acid phosphatases in camel liver

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Two forms of high molecular weight acid phosphatase were isolated and purified from the liver of a camel. One form of acid phosphatase was purified by ammonium sulphate precipitation , cation-exchange chromatography on SP-Sephadex -50 and gel filtration on Sephadex G-75 which revealed the separation of high molecular weight acid phosphatase (100 kDa) from low molecular weight acid phosphatase (18 kDa) enzymes . Further sequential chromatography of 100 kDa enzyme on CM-Cellulose, Sephacryl HR 200 and Reactive Blue 4-Agarose columns resulted in a purification of 350 fold with a total yield of 1%. Sodium dodecyl sulphate-polyacrylamide gel electorphoresis (SDS-PAGE) of purified enzyme showed a single protein band of 48-50 kDa indicating the dimeric nature of protein. Another form of high molecular weight acid phosphatase, the 132 kDa enzyme as unbound fractions from SP- Sephadex C-50 column was concentrated by 70% ammonium sulphate precipitation followed by chromatography on Sephadex G-75 and Reactive blue columns. SDS-PAGE showed single band corresponding to 66 kDa which indicates that this enzyme is also dimer with subunit molecular weight of 66 kDa. Low molecular weight acid phosphates were purified to homogeneity. A 500 fold purification was obtained with specific activity of 40 U / mg of total protein. The enzyme was found homogeneous on SDS-PAGE. Molecular weight of 18 kDa was obtained. Three enzymes were characterized with respect to pH and temperature optima, inactivation, inhibition, purine activation, substrate specificity and kinetic parameters such as Km & Vmax.

Keywords: low and high molecular weight acid phosphatases, purification, characterization

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P-10-970-1

The study of the effect of ethyl acetate fraction of Rosa Damascena on wound-healing rapidity in rabbit auricles

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Rosa Damascena plant has been long used for various purposes in different Islamic countries. For example, this plant contains acidic components such as Carbocsilic acid with anti-irritation effect and its other different effects have also been researched and identified by researchers. By knowing the useful components of this plant and by acknowledging skin wounds and the significance of healing them, the researchers of the present study intended to study the effects of Alcoholic Fraction (EA) on the rapidity of its healing the wounds. Eight male rabbits were divided into two groups of control and experimental ones on whose ears 64 holes of 4 mm were punched. The experimental group was groomed by EA Fraction with the application of Soxhlet extractor, and the control one by Normal Saline for a month.

Samples from the healing tissues were taken from day 0 (punching time) to day 50. Moreover, before each sample was taken during the treatment, the diameter of the holes was controlled and studied through the Millimeter Sheet to measure the reconstruction. Having been fixed, first paraffin molds and then 7µ cuts were provided and finally painted with H & E (Hematoksilin-eozin). The criterion used to study and evaluate the wound healing rapidity was the diameter of the holes on the ears of the rabbits in the experimental group. The study of the recovered tissues through microscopic and macroscopic skills in both the groups indicated that the rapidity of wound healing in the experimental group was one week earlier than that of the control one. The alcoholic fraction (EA) extracted from Rosa Damascena enjoys a natural composition with such various effects as anti-viral and bacterial ones. Therefore, the positive effect of the fraction on the wound healing was revealed.

Keywords: Pinna rabbit, ethyl acetate fraction, wound healing, H-E, Rosa Damascena

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P-10-1033-1

High expression of Oct4, an embryonic stem cell marker, in breast cancer

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OCT4 (also known as POU5F1) a member of POU family transcription factors, plays a key role in maintenance of pluripotency and proliferation potential and inhibition of differentiation in embryonic stem cells. Regarding the new cancer stem cell concept, expression of such genes can disorganize the self-renewal and differentiation rate in tissues, so is correlated with tumorigenesis. Although many studies showed overexpression of Oct4 in germ cell tumors and have introduced it as a sensitive molecular marker, but there is little knowledge about its expression in somatic cancers. Because of the high prevalence of breast cancer and lack of appropriate molecular markers for diagnosis of this cancer the aim of this study was to evaluate the expression and also to evaluate the potential usefulness of Oct4 as a molecular marker in breast cancer. Expression of Oct4 in 22 tumoral and 14 non-tumoral adjacent tissues was examined by RT-PCR method and was normalized by the ß2m as an endogenous PCR control. Results showed high expression of Oct4 in tumoral samples versus marginal specimens. In which 77.2% of tumor samples were positive for Oct4 expression, so expression rate of Oct4 was 7.1% of total adjacent normal tissues. In conclusion data of this study demonstrated high expression of Oct4 in tumoral tissues and confirmed the potential usefulness of it as a molecular marker in diagnosis of breast cancer. Also involvement of embryonic stem cell genes in tumorigenesis as a result of this study can be used as a new promising target for diagnosis and treatment of breast cancer. Results indicate that targeting Oct4 may have important clinical applications in cancer therapy.

Keywords: Oct4, cancer stem cells, breast cancer, tumor marker, RT-PCR

P-11-290-1

Cadmium suppresses phosphate level and inhibits the activity of phosphatases in germinating lentil seeds

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The effect of cadmium concentration on the metabolism of total phosphate and the activities of acid phosphatase, alkaline phosphatase and pyrophosphatase were examined in germinating lentil, lens esculenta, seeds. During 0–100 h germination period of lentil seeds 40 µM cadmium caused a 2-3 times increase in phosphate level in embryo axes compared to controls. Cadmium concentration of 400 µM in situ caused about 10-12% and 20-25% decline in acid phosphatase activity in endosperms and embryo axes, respectively during 60-70 h of germination. With 500 µM cadmium treatments in situ about 23-38% inhibition in alkaline phosphatase activity was noticed in endosperms and 35-60% in embryo axes. With 2 mM cadmium salts in vitro about 55-80% inhibition in the activity of acid phosphatase and 70-80% inhibition of alkaline phosphatase was observed in enzyme preparations from embryo axes. However a cadmium level of 50-100 µM caused stimulation in the activity of inorganic pyrophosphatase. Isozyme studies revealed four acid phosphatase isoenzymes in embryo axes with Rf values 0.13, 0.22, 0.33 and 0.45 and three isoenzymes in endosperms with Rf values 0.16, 0.22 and 0.37. The band intestines decreased under cadmium treatment .Results indicate possible suppression in the phosphorylitic activities in germinating lentil seeds under cadmium stress.

Keywords: acid phosphatase, alkaline phosphatase, inorganic pyrophosphatase, cadmium, phosphate, lens esculenta

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P-10-1035-1

Molecular cloning, characterization of cfa/B major subunit of entrotoxigenic Escherichia coli CFA/I fimbriae as a component of vaccine candidate

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Entrotoxigenic Escherichia coli (ETEC) strains are the main etiological agents of travelers diarrhea and a leading cause of infantile death in developing countries. ETEC initiates the pathogenic process via fimbriae mediated attachment to the small intestinal epithelium and then produces the heat stable (ST) and/or heat labile (LT) entrotoxins. A common protype ETEC fimbriae, colonization factor antigen I (CFA/I), consist of the stalk forming major subunit cfaB and a tip localized minor adhesive subunit cfaE, both of which are necessary for fimbrial assembly. Cfa/B subunit is a glycoesphangolipid binding protein and therefore it seems that this subunit has a critical and essential role in attachment of bacterium to epithelial cells of small intestinal and supposed that this protein is an appropriate candidate for vaccine development. In this study, ETEC serotype O20 was received from reference laboratory. Primarily biochemical and molecular test for identification and detection of ETEC were done. Amplification of cfaB encoding gene derived from genomic DNA was carried out with two primers. This primer was designed according to cfa/b gene sequence that was obtained from gene bank. In the next

stage, PCR product was cloned into T.A coloning vector in certain condition. The cfa/b gene was subcoloned into PET28a vector for expression. After expression of Cfa/B protein, its immunogenicity alone and along with LTB and Cfa/E proteins as oral vaccine, will be studied.

Keywords: rCfaB, cloning, ETEC vaccine

P-10-310-1

Evaluation of silibinin in metastasis, invasion and adhesion effect on MDA-MB-231 and MDA-MB-468 breast cancer cell lines

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Brain, bone, lung, and lymph nodes metastasis are common complication in breast cancer. In the present study, we showed inhibition of invasion, motility and cell-matrix adhesion effect of silibinin, a flavonoid antioxidant from milk thistle (silybum marrinum L.) on MDA-MB-231, a highly metastatic breast cancer cells and MDA-MB-468 metastatic breast adenocarcinoma cells. We performed methyl thiazolyl tetrazolium bromide (MTT) assay, cell-matrix adhesion assay, scratch-wound healing assay and Boyden chamber assay, for evaluation of the silibinin in cell cytotoxicity, adhesion, invasion, and migration, respectively, on silibinin–treated MDA-MB-231 and MDA-MB-468 breast cancer cells, respectively. We found that silibinin is a dose- and time-dependent cytotoxicant. The silibinin significantly inhibit in vitro cell invasion in scratch-wound healing assay by photographing after 24h. Migration and cell adhesion of silibinin –treated MDA-MB-231 and MDA-MB-468 breast cancer cells 49%,59% after 24h and 48h treatment up to 200 µM and 100 µM concentration of silibinin, were calculated respectively. This result suggested clinical application of silibinin may provide for suppression of breast cancer growth, invasion and metastasis.

Keywords: silibinin, MDA-MB-231, MDA-MB-468, invasion, cell-matrix adhesion

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P-10-1038-1

Mutagenesis of human coagulation factor VII gene aiming to gene therapy of hemophilia

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Hemophilia is due to mutation in either the factor VIII or IX genes, classified as hemophilia A or B, respectively. The major precept of hemophilia care consists of adequate factor protein so as to prevent or reverse acute bleeding episodes which is accomplished by the administration of clotting factor concentrates. However, the acquisition of inhibitors against FVIII and IX is one of the most challenging problems associated with hemophilia. More recently, recombinant factor VIIa(rFVIIa) represents a significant advance in the management of patients with or without inhibitors. This option would simplify hemophilia treatment to a single product. But, high cost, short half life and ongoing concern about risk of thrombosis with rFVIIa have limited this approach. Ideally, continuous endogenous production by gene transfer of FVII obviates the problems and confers long-term expression. We aim to engineer a novel FVII gene containing a cleavage site for the intracellular protease, furin, by PCR mutagenesis. Human FVII cDNA which was prepared from total RNA from HepG2 cell line, cloned in to pTZ57R. In next step, the sequence encoding heavy and light chains were separately amplified using PCR reaction and subsequently cloned into pTZ57R. Cloning was verified by restriction enzyme analysis and PCR reaction. Joining the mutant form of heavy chain with mutant light chain would lead to the generation of new variant of FVII. This variant can be activated by furin and increase proportion of activated form of FVII.

Keywords: furin, hemophilia, heavy chain, light chain, RFVIIa

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P-10-1041-1

Evaluation of silibinin in metastasis, invasion and adhesion effect on MDA-MB-231 and MDA-MB-468 breast cancer cell lines

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Brain, bone, lung, lymph nodes metastasis are common complication in breast cancer. In the present study, we showed inhibition of invasion, motility and cell-matrix adhesion effect of silibinin, a flavonoid antioxidant from milk thistle (silybum marrinum L.), on MDA-MB-231, a highly metastatic breast cancer cells and MDA-MB-468 metastatic breast adenocarcinoma cells. We performed methyl thiazolyl tetrazolium bromide (MTT) assay, cell-matrix adhesion assay, scratch-wound healing assay and boyden chamber assay, for evaluation of the silibinin in cell cytotoxicity, adhesion, invasion, migration, respectively, on silibinin – treated MDA-MB-231 and MDA-MB-468 breast cancer cells respectively. We found that silibinin is a dose- and time-dependent cytotoxicant. Silibinin significantly inhibited in vitro cell invasion in scratch-wound healing assay by photographing after 24h. Migration and cell adhesion of silibinin –treated MDA-MB-231 and MDA-MB-468 breast cancer cells 49%,59% after 24h and 48h treatment up to 200 µM and 100 µM concentration of silibinin, were calculated respectively. This result suggested clinical application of silibinin may provide for suppression of breast cancer growth, invasion and metastasis.

Keywords: silibinin, MDA-MB-231, MDA-MB-468, invasion, cell-matrix adhesion

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P-10-1040-1

Apoptosis induction and cell cycle alterations in human breast MDA-MB-453 carcinoma cells by silibinin

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Breast cancer is one of the most common malignancies among women world wide. Despite extensive researches in cancer initiation, promotion and progression, there are still unknown aspects to be

elucidated. In addition, current cancer therapy fails in some of the breast cancer patients. Therefore, searching for new natural or synthetic compounds with more therapeutic efficacy and less side effects is one of the main research activities in research centers. Silibinin is a flavolignan extracted from milk thistle with cancer chemopreventive activity. As a therapeutic agent it is well tolerated and largely free from adverse effects. In this study, we investigated cellular and molecular aspects of anticancer effects of silibinin on human breast carcinoma MDA-MB-453 cells. The MTT assay was used to determine the IC50 of silibinin. Then cells were exposed to IC50 of Silibinin to determine cell cycle distribution and apoptosis induction by flow cytometry using DAPI and Annexin V-FITC / PI fluorescent reagents, respectively. Results of this study will more clarify mechanisms underlying the anticancer effects of Silibinin to be decided for using in breast cancer therapy.

Keywords: silibinin, MDA-MB-453, breast cancer, cell cycle arrest, apoptosis, flow cytometry

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P-10-970-2

The study of alcoholic n-butanol fraction effect of Rosa Damscena on the reconstruction of rabbit ears epidermis

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Different nations have been applying various medicinal plants as healers, and in spite of synthetic chemical medicines and their development, they [medicinal plants] are widely used in medicine among which Rosa Dmascena is widely well-known that contains various constituents such as Jeranule and Sitranule as well as roles. In this study, 64 wounds of 4 mm were punched on eight male rabbits' auricles. The experimental group was treated by NB Fraction with the application of Soxhlet extractor, and the control one by Normal Saline twice a day for a month. Samples from the healing tissues were taken from day 0 (punching time) to day 50. Moreover, before each sample was taken during the treatment and for five times a day the diameter of the holes was controlled and studied through the Millimeter Sheet to measure the reconstruction and recovery. Having been fixed, first paraffin molds and then 7µ cuts were provided and finally painted with H & E (Hematoksilin-eozin). Thence the cuts were microscopically investigated in terms of tissue changes during the reconstruction of the epidermis. The criterion used to study and evaluate the wound healing rapidity was the diameter of the epidermis and that the holes on the ears of the rabbits in the experimental and control groups was carefully investigated. The study of the recovered tissue through microscopic and macroscopic skills in both groups indicated that the rapidity of wound healing in the experimental group occurred five days earlier than that of the control. The Alcoholic Fraction N-butanol enjoys a natural composition with such various effects as anti-irritation and anti-convulsion ones. Therefore, the positive effect of the fraction on the wound healing and epidermis recovery was revealed. Moreover, it can be concluded that the NB fraction probably affects the cellular reproduction of the wounded area.

Keywords: Pinna rabbit, ethyl acetate fraction, wound healing, H-E, Rosa- Damascena

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O-10-1039-1

Evaluating the expression of Oct4, Nanog, Sox2, Dppa4, ZFX, Bmi, Tbx3, and Ns in colon cancer cell line (Caco2, HT-29)

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Colon carcinoma is the second most common cause of death from cancer. The isolation and characterization of tumorigenic colon cancer cells may help to devise novel diagnostic and theraputic procedures. Cancer stem cells have been shown to be critical in tumor development and should harbor the mutations needed to initiate a tumor. These cells express markers of stemness and are also capable to reproduce the human cancer in mouse models. Uncontrolled self-renewal is recently proposed as an important mechanism in carcinogenesis. Oct4, Nanog, Sox2, Dppa4, Zfx, Bmi, Tbx3 and NS are keys regulators of pluripotency and self-renewal in embryonic stem cells. The expression of the genes has not been fully studied in somatic cancers, such as colon carcinoma. Caco2 and HT-29 human colon cancer cell lines were grown in RPMI medium containing 10%FBS with 1%peniciline and streptomycinen. All cell culture was carried out at 37 in a co2 humidified incubator. Total RNA was isolated by ISOGEN method. RNA integrity was checked by electrophoresis in agarose gel. RNA concentration was estimated by spectrophotometry at 260 nm. We used RT-PCR to examin samples. The expression of Oct4 and Ns at protein level was further determined by immunocytochemistry. RT-PCR results confirmed the expression of self renewal genes (Oct4, Nanog, Sox2 and NS) in the human colon cancer cell line. The expression of B2m was monitored as internal control. Immunoreactivity Signals were obtained for Oct4 and Ns. But they were absent in the negative control. The results of all evaluations confirmed the expression of self renewal gene Oct4, Nanog, Sox2 and NS in human colon cancer cell line (HT-29 & Caco2). Collectively, our data approved the expression of Oct4, Nanog, Sox2, Dppa4, ZFX, Bmi, Tbx3 and Ns in colon cancer cells and suggested that the expression of these genes can be used as a potential tumor marker for diagnosis and/or prognosis of colon tumors.

Keywords: cancer, stem cell, self-renewal gene, colon

O-10-1050-1

Cellular effects of four sulfonamide drugs on a human breast cancer cell line (T47D)

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Regarding the previous reported data it has been suggested that the aromatic sulfonamides are able to decrease the growth rate of the tumor cells. Here, determination of the cytotoxic effects of four aromatic sulfonamide drugs (acetazolamide, sulfabenzamide, sulfacetamide and sulfathiazole) on a breast cancer cell line, T47D, has been considered. Markedly, reduced cell viability in 50% (LC50) was estimated by MTT assay after 48 h treatment of the cells with 26, 10.8, 41 and 6.5 mM of the cited drugs respectively. Responding of the cells to the drugs during the incubation time of 48 h were identified using

fluorescence microscopy (via double staining with Annexin-PI fluorochromes), flow cytometry (using Annexin-V-Flous kit) and western blotting (using the monoclonal antibodies against Bcl-2, Bax, AIF, Cyclin D1, pCdc 2 and p21). Our findings revealed that despite of the registered 50% decrease in the cell count in the medium, decreasing of the cell population in response to the existence of the drugs should not be related to the induction of apoptosis or cell cycle arrest on the cells. So, despite of the previous reported findings about cellular influences of these drugs, they have to induce some other cellular processes to reduce the potency of cell proliferation in the medium.

Keywords: Sulfonamide, apoptosis, cell cycle arrest, breast cancer

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P-10-1057-2

Trying to develop transgenic chicken by sperm Cell

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Transgenic chickens could be an efficient bioreactor producing pharmaceutical proteins. Mammalian systems are the best available expression system for the production of biologically functional protein. We hypothesized that avian DNA transfected sperm by electroporation or a linker such as an antibody would be used to produce transgenic chicken. Two procedures were designed, in one of which the sperm cells were electroporated directly with the vectors containing interest gene (Camel antibody fused GFP) and in the other one Linker Based Sperm Mediated Gene Transfer (LB-SMGT) method. In the latter case, the production of anti-sperm antibody as the linker was necessary which was produced in rabbit. Optimum concentration for interaction between antibody and linearized vectors was obtained. After interaction between sperm cell with antibody- linearized vectors complex (containing interest gene), DNA was extracted and the results of Multiplex-PCR experiments did not come to show the desirable results. Here, we report the possibility of production of transgenic chicken by applying of sperm cell in gene transfer technology. Unfortunately in spite of applying different methods for gene transferring to sperm cells; we don't have any positive report of gene transfer to chicken genome.

Keywords: LB-SMGT, sperm, gene transfer, transgenic animal

P-10-458-1

Isolation, DNA sequence analysis and cloning of the putA gene encoding proline dehydrogenase falvoenzyme from Pseudomonas fluorescens

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The bifunctional PutA (proline utilization A) flavoprotein has two essential functions in proline metabolism and the regulation of put regulon expression. The purpose of this study was to perform molecular cloning and sequencing of the putA gene encoding a proline dehydrogenase form Pseudomonas fluorescens isolated from soli. Using the designed primers based on the homologous nucleotide sequences in NCBI database; we performed the PCR reaction to isolate the target gene. The isolated gene was confirmed by restriction map analysis and sequencing and then cloned and introduced in Escherichia coli BL21 by transformation. DNA sequence analysis revealed the open reading frame (ORF) of ProDH domain is 1266 base pairs in length and encodes 422 amino acids. The putA gene showed 90% homology to the putA gene of enteric bacteria. It seemed likely that in p. fluorescens, both enzymatic steps for proline metabolism are catalyzed by a single polypeptide. Sequencing of the gene and comparison of the primary structure between various FAD-dependent dehydrogenase provided the basis for increasing our understanding of the catalytic structure and the structure of ProDH. Further works are in progress.

Keywords: gene, PutA, Pseudomonas fluorescens, proline dehydrogenase, sequence

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O-10-238-4

Adenosine 5'-triphosphate downregulates expression of survivin in human leukemia K562 cell line

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Leukemia is a heterogeneous malignant disease in which disease progression at the level of CD34 positive cells has a major impact in drug resistance and relapse. The multi-drug resistance gene product, P-glycoprotein is expressed mainly in CD34 positive leukemia cells and inhibitor of apoptosis proteins (IAPs), such as surviving, are expressed simultaneously with several putative drug resistance parameters in these cells. In fact, IAPs over-expression and their anti-apoptotic splice variants are associated with CD34 positivity, poor response to chemotherapy and reduced overall survival in leukemic patients. Recently, adenosine 5'-triphosphate (ATP) has been reported to inhibit proliferation and induce apoptosis in several human myeloid leukaemia CD34 negative cells. The K562 CD34 positive human myeloid leukaemia cell line has the unique feature of expressing significant functional IAPs and other drug-resistance genes. Thus, the efficacy of ATP in overcoming the resistance and expression profile of surviving and its splice variants were examined in K562 (CD34+) cells in the present study. For that reason, K562 cell were cultured in RPMI 1640 medium and treated for various times with different concentrations of ATP (10 to 500μM) to induce apoptosis. Apoptosis was studied by florescent microscope (Ao/EtBr double staining) and DNA fragmentation assay. The expression level of survivin and its splice variants were studied by semiquantative RT-PCR. The results showed that over-expression of survivin and its anti-apoptotic splice variant, 3b splice variant, were decreased after treatment by ATP in a time- and dose-dependent manner. For example, above 36% inhibition of gene expression was observed after 72 h treatment K562 cells with 200 μM ATP. The expression levels of other splice variants (ΔEx3, 2b, 2α and 3α) did not show significant difference between the control and the treated cells. With our knowledge, this study for the first time investigated the expression profiles of all the known survivin splice variants in K562 cells as a CD34+ model of leukemia. The results showed that ATP attenuated expression of survivin and its anti-apoptoic splice variant, meaning that this nucleotide can facilitate apoptosis in drug-resistant leukemia cells. In addition, combination of

ATP with standard chemotherapies may be utilized for inhibition of drug-resistance in leukemia cells.

Keywords: apoptosis, drug-resistance, extracellular ATP, K562, leukemia, survivin

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P-10-1068-1

Lithium induce apoptosis in rat ovarian follicles through Wnt/b-catenin pathway

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It is well known that lithium, a drug used as a mood stabilizer, inhibits glycogen synthase kinase-3β (GSK-3β), a key negative regulator of canonical Wnt signaling pathway. Recently, it has been shown that the components of this pathway are expressed in adult rodent ovary. In this study we use lithium chloride (LiCl) to activate Wnt pathway and the roles of this signaling pathway in ovarian follicular development were investigated in vivo. Immature female 23 day old Wistar rats were injected by PMSG (10 IU), to induce folliculogenesis. Starting at the time of PMSG injection, these rats were given four doses of 250 mg/kg LiCl every 12h. Ovaries were removed 48h after PMSG injection and prepared for routine histology and immunohistochemistry. Our results show that relative ovarian weights and size, folliculogenesis and estradiol synthesis were significantly decreased compared to those of controls. Further analysis showed that in LiCl treated rat ovaries, TUNEL positive nuclei were increased while PCNA (proliferation marker) positive nuclei were decreased significantly in contrast with those of control. To investigate whether lithium acts through inhibition of GSK-3β, the expression pattern of pGSK-3β and β-catenin were examined. Our results showed that in LiCl treated group, expression of pGSK-3β and active β-catenin were increased in contrast with control groups. All together, our results suggest that lithium decrease folliculogenesis by inducing apoptosis and inhibiting proliferation possibly through Wnt/β-catenin pathway.

Keywords: lithium chloride, Wnt/ β-catenin, apoptosis, rat ovary

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P-10-866-2

Induction of protein tyrosine phosphorylation during capacitation in the spermatozoa of normozoospermic men

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In mammals, capacitation is defined as series of molecular and biochemical events that enables spermatozoa to bind the oocyte by undergoing the acrosome reaction in response to the zona pellucida. Capacitation is generally accompanied by increases in phosphorylation of sperm proteins. We were interested in studying the tyrosine phosphorylation pattern during capacitation of sperm proteins isolated from normozoospermic men. We obtained semen samples from normozoospermic men referred to Avicenna Infertility Clinic in Tehran. The samples were divided to an experimental group which were allowed for capacitation and control group without capacitation. The spermatozoa were isolated from semen samples, using percoll gradient centrifugation. Spermatozoa of experimental group were then incubated for 6h at 37˚C with bovine serum albumin supplemented Ham's -F10 for capacitation following a standard protocol. Before and after capacitation incubation, total proteins from spermatozoa were extracted and subjected to SDS-PAGE. To evaluate protein tyrosine phosphorylation pattern, western blotting with specific antibody (PY99) against phosphorylated tyrosine residues was performed. The comparative analysis of results from western blotting experiment showed an increase in protein tyrosine phosphorylation in spermatozoa undergoing capacitation. Specifically, capacitation induced tyrosine phosphorylation in sets of sperm proteins (10-50kDa range). Our results demonstrate that this phosphoproteins are likely important in sperm functions.

Keywords: capacitation, tyrosine phosphorylation, normozoospermic men

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P-10-1046-1

Association between the length of GGC repeat in the eRF3/GSPT1 and risk of breast cancer in Isfahan population

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Breast cancer is the most common cancer among women. The eukaryotic release factor 3 (eRF3) has multifunctional properties in eukaryotic cells. Beside its role in translation termination, this protein was also reported to be involved in cell cycle regulation, mRNA decay, recycle of ribosome and apoptosis. Because of its major role in cell survival, mutations and altered expression of this gene have been associated with cancer development. The aim of this study was to evaluate eRF3/GSPT1 gene as a potential genetic susceptibility associated locus for breast cancer, analyzing a stable GGC expansion in exon 1 encoding a polyglycine tract in the N-terminal domain of the protein. We studied the association of breast cancer with the polymorphic GGC repeat in 120 cases of breast cancer and 120 matched controls from Isfahan city of Iran. We applied a modified PCR protocol using betain and DMSO. After analyzing the PCR products on polyacrylamide gels, five different lengths of the GGC repeat in the range of 8 to 12 were observed. The most common genotype in controls and patients was homozygous with allele length of 10. Our findings demonstrate that women with at least one allele length of 12 repeat are significantly at a higher risk of developing breast cancer at an estimated odds ratio of 5.17. To our knowledge this is the first report about the association of GGC repeat in the eRF3/GSPT1 with the risk of breast cancer.

Keywords: breast cancer, eRF3/GSPT1, GGC repeat

P-10-1072-1

Comparison study of clinical and microscopical diagnosis of Trichomoniasis

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Vaginal trichomoniasis is one of the most common sexually transmitted disease in the world, accounting for approximately 180 million infections annually. Several reports from Iran estimated the prevalence of 1% to 42% from different geographical areas. This disease manifests with different clinical symptoms such as: vaginal discharge, itching, burning sensation or may be asymptomatic. The clinical spectrum of the disease in women has wide inflammatory variation manifesting as urethritis, vulvo-vaginitis, and cervicitis with associated complications. This infection also increases the risk for human immunodeficiency virus transmission. The common way for diagnosis of Trichomoniasis is microscopic study of vaginal discharge sample. In this study a sterile vaginal swab was taken, from the posterior vaginal fornix of each non-pregnant woman in Tehran. Samples were transferred to the laboratory in normal saline solution. Results of microscopic diagnosis were compared with clinical diagnosis according to the clinical symptoms. Unfortunately, clinical manifestations are not reliable criteria for the diagnosis of trichomoniasis; demonstration of the parasite is required for accurate diagnosis of infection. There was a significant difference between clinical diagnosis and microscopic diagnosis of trichomoniasis. Therefore, clinical symptoms are not good criteria for diagnosis of trichomoniasis. By considering just the clinical symptoms, asymptomatic patient will be missed and 40% of the non trichomoniasis patients undergo unnecessary treatment.

Keywords: Trichomonas vaginalis, clinical diagnosis

P-10-521-1

The report of Neospora caninum associated bovin abortion in Mashhad area, Iran

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Neospora caninum is an apicomplexan porotozoan which causes abortion in cattle worldwide. The present study was designed to assess the importance of bovine neosporosis for causing abortion in Iranian cattle. Infection was diagnosed by polymerase chain reaction (PCR). Thirty brains of aborted bovine fetuses were collected from Iranian dairy herd between 2008 and 2009 in Mashhad area. N.caninum was detected by PCR in the brains of 5 aborted fetuses. The results indicated that neosporosis is an important cause of abortion in dairy cattle.

Keywords: Neospora caninum, diagnosis, PCR, cattle

P-10-1070-1

Compare apoptosis in renal cell carcinoma cell line (ACHN) by ethanolic extract of nigella sativa and thymoquinone and cisplatin

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In herbal medicine, Nigella sativa, Thymoquinone (TQ) is used against cancers, infectious and inflammatory diseases. Cisplatin is a drug for cancer. It induces apoptosis in renal cell carcinoma (ACHN). ACHN cells were treated with different concentrations of Nigella sativa extract (0-2000 µg/ml) and Thymoquinone (5-60 µM) and cisplatin (0-3 µg/ml) for 24, 48 and 72h. Also, L929 cells were studied as normal controls. The effects on the cell cycle were determined using flow cytometry. Apoptosis induction was assayed using annexinV and propidium iodine (PI) by flow cytometric analysis. Phosphatidylserine (PS) externalization relatively increased in early process of apoptosis. It has high affinity for binding a protein called annexinV. In this method, PS binds to annexinV conjugated fluorescein isothiocyanate (FITC). PI allows discrimination between necrotic and apoptotic cells. Cytotoxicity effect of TQ nigella sativa and cisplatin were time and dose dependent. TQ (60µM 24h), nigella sativa (1250µg/ml 24h) and cisplatin (1.5µg/ml 24h) induced apoptosis and caused DNA fragmentation in ACHN cells, these concentrations in TQ decreased the number of ACHN cells in S-phase and increased them in G1-phase, indicating cell cycle arrest at G1 but cisplatin did not. This study showed that Nigella sativa extract and TQ induced early and late apoptosis and also necrosis in renal cell carcinoma without cytotoxicity on L929 cells and cisplatin induced late apoptosis with necrosis on L929 cells. These results suggested that the tumor cytotoxic effect of thymoquinone on ACHN cells is mediated by process involving apoptosis, necrosis and cell cycle arrest.

Keywords: apoptosis, cisplatin, flow cytometry, Nigella sativa, thymoquine

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P-10-1076-1

Gene expression of PEX3 during neural differentiation of P19 cells

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The P19 murine embryonal carcinoma (EC) cell line is a valuable in vitro model cell that can be differentiated into neurons by cellular aggregation in presence of the differentiating agent retinoic acid (RA). In this project, a peroxisomal gene such as PEX3 has been selected and its expression profile has been investigated in P19 cells. Expression of peroxisomal gene like PEX3, as peroxisomal membrane protein in comparison with pluripotency markers such as Oct4 and Nanog, neural markers such as Pax6, Ngn-1, Map2 and a house keeping gene such as β-tubulin have been investigated by RT-PCR. Total RNA from P19 cells

were extracted and cDNA was synthesized. Using specific RT-PCR primers was done by bioinformatics and soft wares such as oligo, and their expression were analyzed by semi-quantitative RT-PCR. Data indicated that during neural differentiation, expression of pluripotency markers have been down regulated and expression of neural markers have been up regulated. Gene profile of PEX3 has been up regulated and subsequently has been down regulated during neural differentiation. One of the peroxisome functions is plasmalogen biosynthesis. Plasmalogen is involved in neurogenesis; thus elevated level of PEX3 represent increasing in peroxisome numbers due to increase in plasmalogen biosynthesis which is needed in neurogenesis.

Keywords: neurogenesis, Oct4, PEX3, P19

O-10-1136-1

First report of Ageratum enation virus infecting Sonchus oleraceus and Ageratum conyzoides in Pakistan, its phylogenetic relationships and infectivity

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The geminiviruses are a major constraint to the agricultural productivity throughout tropical and sub-tropical regions of the world. Sonchus oleraceous (SOL) and Ageratum conyzoides (ACL) are common weeds grown throughout Pakistan under different environmental and agro-ecological conditions. Leaf samples showing symptom, yellow veins, typical of begomoviruses and apparently healthy leaf samples were collected from the vicinity of School of Biological Sciences, University of the Punjab Lahore. Full length genomic component and betasatellite were PCR amplified from symptomatic samples but no amplification in healthy samples. The complete genomic sequences of begomovirus isolates SOL and ACL were comprised of 2750bp and 2749bp, respectively and shared a nucleotide sequence identity of 98.9% with each other. The comparison of the DNA A of isolates SOL and ACL revealed that both the isolates shared a high nucleotide identity of 96% with a Nepalese isolate AEV-[Nepal:01]. Betasatellites isolated from ACL and SOL shared 96.8% nucleotide sequence identity within them while shared an identity of 92.3% with Ageratum yellow leaf curl beta (AYLCuB). This is the first report of AEV from Pakistan. The isolates SOL and ACL with their cognate betasatellite were used to produce partial repeat constructs for agroinoculation. These partial repeat constructs were infectious to Nicotiana benthamiana, Nicotiana tabacum, Solanum lycopersicon and Ageratum conyzoides. Koch's postulates for AEV causing disease in Ageratum conyzoides are satisfied. This virus is one of an increasing number of monopartite begomoviruses shown to be associated with a betasatellite.

Keywords: geminivirus, Sonchus oleraceus, Ageratum conyzoides, Pakistan, Ageratum enation virus

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O-10-1130-2

The role of msh gene cluster of bundle forming pili in bacterial attachment and biofilm formation in Aeromonas veronii bv. sobria

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Mesophilic Aeromonas are ubiquitous water-borne bacteria. In humans two major diseases associated with Aeromonas are gastroenteritis and wound infections, with or without bacteremia also they are able to form biofilm. Bundle-forming pilus (Bfp) is thought to be the major adhesin of Aeromonas spp. We hypothesize that Bfp, which is predominant pilus type, expressed on A. veronii bv. sobria, is essential for colonization and for the attachment to abiotic surfaces allowing the organism to form biofilms. Investigation on this hypothesis was done by isolating the genes of this structure, creating a series of isogenic mutants, testing whether they were essential for the formation of the pilus structure and for adherence to various adhesion models. The genes that encode the pilus structural proteins were isolated by PCR using degenerate primers and the sequence of the gene locus was completed. Then isogenic mutants were created by insertion of a Kanamycin cassette within the genes (mshA, mshB, mshC, mshD, mshH and tapD) and allelic exchange. The phenotypic changes of such mutants were investigated by adhesion assays to tissue culture cells and assays of biofilm formation and determining the production of different enzymes. The roles of these genes were confirmed by complementation analysis. Also the activity of the most probable promoters between four putative pilin proteins genes was investigated by transcriptional fusion. We found that the msh locus of Aeromonas veronii bv.sobria is composed of 14 genes four of each appears to encode pilin subunits. To determine if each is essential we created individual isogenic mutants. Also transcriptional fusion shows that the four pilin genes expressed by a single promoter upstream the mshB.

Keywords: Bfp, Aeromonas veronii, biofilm, colonization

O-10-4-4

A role of 9-1-1 checkpoint clamp in immunoglobulin gene conversion

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Chicken DT40 cells deficient in the 9-1-1 checkpoint clamp exhibit hypersensitivity to a variety of DNA damaging agents. Although recent work suggests that, in addition to its role in checkpoint activation, this complex may play a role in homologous recombination and translesion

synthesis, the cause of this hypersensitivity has not been studied thoroughly. The immunoglobulin locus of DT40 cells allows monitoring of homologous recombination and translesion synthesis initiated by activation-induced deaminase (AID)-dependent abasic sites. We show that both the RAD9_/_ and RAD17_/_ mutants exhibit substantially reduced immunoglobulin gene conversion. However, the level of nontemplated immunoglobulin point mutation increased in these mutants, a finding that is reminiscent of the phenotype resulting from the loss of RAD51 paralogs or Brca2. This suggests that the 9-1-1 complex does not play a central role in translesion synthesis in this context. Despite reduced immunoglobulin gene conversion, the RAD9_/_ and RAD17_/_ cells do not exhibit a prominent defect in double-strand break-induced gene conversion or sensitivity to camptothecin. This suggests that the roles of Rad9 and Rad17 may be confined to a subset of homologous recombination reactions initiated by replication-stalling lesions rather than those associated with double-strand break repair.

Keywords: 9-1-1 checkpoint clamp, translesion synthesis, gene conversion

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P-10-6-1

Genetic polymorphisms of estrogen receptors in Iranian diabetic women with coronary artery disease

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Estrogen might play an important role in type 2 diabetes mellitus pathogenesis. The low incidence of diabetes in premenopausal women is related to the protective effects that female hormones, estrogens, exert on inhibition of insulin-dependent diabetes. Estrogens clearly inhibit diabetes in different ways especially by reducing both hyperglycemia and plasma insulin levels. Estrogen exerts its physiological effects mainly through its receptors (ERs); ERα and ERβ. ERs are found in many tissues, which all participate in the pathogenesis of type 2 diabetes. Two common polymorphisms, PvuII and XbaI in ERα gene, are reported to be associated with decreased receptor activity, increased risk of diabetes. The objective of the present study was to investigate the association of ERα polymorphisms with diabetes, where a genetic component probably may be the major risk factors for this disease. One hundred women with diabetes type 2 were compared with one hundred non-diabetic women for PvuII and XbaI polymorphisms of whom 61% of cases and 29% of controls were CAD. They were genotyped for these polymorphisms using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. The genotype distribution and frequency of mutated allele showed no significant differences between diabetic and non-diabetic groups in PvuII ($\chi2=0.981$; $P=0.612$) and XbaI ($\chi2=0.362$; $P=0.83$) polymorphisms. When CAD, as the potential confounding factor, was controlled by logistic regression analysis, it was found that the PvuII and XbaI variants were not related to the type 2 diabetes mellitus ($P=0.60$ and $P=0.99$, respectively). Neither PvuII nor XbaI genotypes was associated with increased susceptibility to the type 2 diabetes mellitus in selected Iranian diabetic women with CAD.

Keywords: estrogen, PvuII, XbaI polymorphism, diabetes, CAD, estrogen receptor

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