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Applications of Proteomics in Cell and Molecular Biology

P-10-121-1

Multiple alignment of adipokines protein sequences

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Adipose tissue has been recognized as the quantitatively most important energy store of the human body for many years, in addition to its functions as mechanical and thermal insulator. During the last 10 years, adipose tissue has come into focus as an endocrine organ important for development of many diseases related to obesity including insulin resistance, type 2 diabetes, dyslipidemia, hypertension and cardiovascular disease. Adipose tissue secretes a variety of bioactive peptides that play important roles in insulin action, energy homeostasis, inflammation, and cell growth. These secretory proteins from the adipose organ are named adipokines and have many physiological effects on different organs including the brain, bone, reproductive organs, liver, skeletal muscles, immune cells and blood vessels. Adipokines may locally regulate fat mass by modulating adipocyte size/number or angiogenesis and inversely increased fat mass leads to dysregulation of adipocyte functions. In this research we consider to analyze the sequences of different adipokines and multiple alignments of protein sequences in order to identify conserved sequence regions, possible similarities and differences and understanding of biological and evolutionary relationship between adipokins. In this regard the protein sequence of nine adipokines which are believed to be involved in fatty acid metabolism and obesity were extracted from UnipratKB/Swissprot entries in a FASTA format and the data were analyzed by employing Multiple Sequence Alignment using ClustalW program. Results revealed that leptin and visfatine have the most and RBP-4 and IL-6 have the least homology in sequence. According to the phylogeny tree the proteins with the minimum distance are leptin and visfatin. These results suggest that visfatin not only interacts with leptin but also with other adipokines investigated in this research.

Keywords: adipokines, multiple alignments

P-10-158-1

Elucidation of the 3D structure of growth hormone-growth hormone receptor complex by using of bioinformatics softwares

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Growth hormone receptor (GHR) gene encodes a protein that is a transmembrane receptor for growth hormone. Binding of growth hormone to the receptor leads to receptor dimerization and the activation of an intra- and intercellular signal transduction pathway leading to growth. This receptor has 4 isoforms. Isoform 1 (GHRfl) has 638 amino acids. Isoforms 2 (GHRtr) and 3 consist of amino acids 1-279, 1-277 respectively. Isoform 4 (GHRd3) has 616 amino acids. Structural analysis of the 1:1 growth hormone-receptor complex reveals the molecular basis for receptor affinity. Therefore through bioinformatics literatures and websites we evaluated 3D structure of the 1:1 growth hormone-receptor complex. General information, description and origin, references of the hGHR could be found In the site (http://srs.ebi.ac.uk/srsbin/cgi-bin/wgetz?[swissprot-ID:GHR_HUMAN]+-e). The protein (hGHR) has 4 isoforms and 4 domains, belongs to the type I cytokine receptor family and contains 1 fibronectin type-III domain. We have extracted the graphical structural features of hGHR in the site (http://expasy.org/cgi-bin/aligner?P10912). We have found feature aligner of the receptor (sequences of domains and motifs) by use of a Sequence Element Veiwer Version 2.0b. Crystallographic structure of the 1:1 complex between G120R mutants of human growth hormone (hGH) and the receptor extra cellular domain (hGHbp) have been determined. By using of PDB Protein workshop 1.50 (powered by the MBT), veiwerlite and PyMOL viewer softwares, we have elucidated the 3D picture of 1:1 complex of human growth hormone with hGHR extra cellular domain. We could not find growth hormone receptor complete crystallographic structure. We could only elucidate the 1:1 complex of human growth hormone with hGHR extra cellular domain and hGHR extra cellular domain by itself. In this paper we collected different views of the protein structure by using of the softwares.

Keywords: 3D structure, growth hormone, growth hormone receptor

P-10-173-1

Multiple alignment of estrogen receptor β single nucleotides polymorphism

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During menopause and the years thereafter, the loss of female sex steroid hormones causes profound changes in the female body. Across different stages of life, the absence of endogenous estrogens in women affects cardiovascular function and these effects may be closely related to different stages in the progression of atherosclerosis. When endogenous estrogen concentrations subside after menopause the gender-based differences in men and women continue to disappear. This strongly suggests that endogenous estrogens attenuate the progression of cardiovascular disease. However, In the Women's Health Initiative trial, women who received hormone replacement therapy were more likely to experience cardiovascular events. The increased risk for CHD observed in clinical trials could be explained by a women's subgroup at risk for adverse cardiovascular effects of HRT, by virtue of genetic variation in genes in pathways of female steroid hormones. Estrogens exert the majority of their actions with two specific estrogen receptors (ESR1 and ESR2). Because the risks and benefits of hormone replacement therapy are, at least in part, mediated by the metabolic individuality of women and their genetic variation in estrogen receptors β, it is important to evaluate potential associations between SNP variants of this receptor in human and cardiovascular disease. Multiple alignments of the SNP with Mega Blast (blast of very small size sequences) of ER2 gene can give us some information about most dangerous polymorphisms by comparison the most similarity between them. Blastn compares a nucleotide query sequence against a nucleotide sequence database. So bioinformatics data can give us some opportunity for predicting the estrogen receptor β function with new reported SNPs by comparing it with previous SNPs. In this study we can see that the identity of ESR2 SNPs is 99% in all cases. The expected value is zero in all SNPs, which means a high similarity between them or the high bit score.

Keywords: estrogen receptor β, nucleotides polymorphism, menopause

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P-10-93-3

Methodology of assessment of serum glucokinase enzyme activity injection of halothane

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In this research project, effect of halothane on activity of glucokinase enzyme in serum and hepatic tissue using two different procedures of assessing glucose-6- phosphate dehydrogenase and coenzyme NAD+ Vs NADP+ were compared. Twenty-four hours following inhalation of halothane, activity of glucokinase enzyme vs. control group showed a significant difference ($p<0.5$). The enzyme activity produced by glucose -6-phosphates dehydrogenase obtained from fungus leuconostoe mesenteries and coenzyme NAD+ shows a greater difference compared with controls. Therefore, it can be concluded that methodology of assessing glucokinase enzyme and level of precision and sensitivity of employed procedure can affect determination of the halothane effect on hepatic tissue following increased activity of glucokinase enzyme. Therefore, conduction of all studies and investigations of human and the on male subjects, and use of precise proposed procedure for assessment of this enzyme show that such studies could be conducted on other enzyme. On the other hand, comparison of these two methods can provides an appropriate model for minimum drug allergy in particular to halothane in man or animals.

Keywords: serum, glucokinase, enzyme

P-10-99-1

Study of the human serum proteome of psoriasis disease: a preliminary report

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Psoriasis is a chronic inflammatory skin disease characterized by anthological skin lesions due to various exogenous and endogenous factors and associated with a number of biochemical and immunological disturbances. Serum proteins undergo some quality and quantity changes when the human body suffers pathological damage and differential profile of serum proteins can illustrate changes due to development disease. Clinical proteomics widely uses to identify differentially displayed proteins in blood to understand the molecular and cellular events leading to diseases. The aim of the present study was to identify the differentially displayed proteins in psoriasis patients as compared with healthy subjects. After sample preparation with acetone and TCA/acetone precipitation of serum we used two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). Comparative 2-D PAGE electrophoresis of psoriasis patients with case controls allowed us to identify differential expression of proteins. Relative differences in the proteins were observed in pH range of about 4-6 and molecular weight of 32-40KDa, respectively. Selected proteins will be further verified by mass spectrometry. This study shows how the proteomic approach might be useful for the identification of proteins in complex mixtures

Keywords: proteomic, psoriasis disease, serum

O-10-319-3

Proteome analysis of Persian sturgeon (Acipenser persicus) ova

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The Persian sturgeon ova are a key material both for inevitable artificial propagation and for caviar production. In this study, the proteome profile of Persian sturgeon ova was analyzed using 2-DE and MALDI-TOF/TOF in order to determine its protein composition. Out of 192 spots analyzed with MALDI-TOF/TOF, 135 spots corresponding to

97 different proteins were identified. The identified proteins were classified into 11 groups with regard to their main known function involving cell structure (21.64%), translation and transcription (15.50%), metabolism and energy production (12.37%), protein synthesis (11.34%), membrane protein receptors or cell signaling (7.21%), cell defense (5.15%), transport (6.18%), cell division (7.21%), vitellogenin (1.03%), unclassified (7.21%) and unknown function (5.15%). The results of this study provide a valuable resource for molecular analysis of normal and abnormal conditions affecting female reproduction. Moreover, it may help to better understand factors affecting caviar quality during refrigerated storage.

Keywords: Persian sturgeon ova, Acipenser persicus, proteomics

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P-10-319-4

Proteome modifications of juvenile beluga (Huso huso) brain as an effect of dietary methylmercury

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In this work we used a proteomics analysis to determine the changes in the brain proteome of juvenile beluga (Huso huso) exposed to dietary MeHg. The juvenile beluga were fed the diet containing 0.8 ppm MeHg for 70 days. Proteins of the brain tissue were analyzed using two-dimensional electrophoresis and MALDI-TOF/TOF mass spectrometry. We found eight proteins with significantly altered expression level in the fish brain exposed to MeHg. These proteins are involved in different cell functions including cell metabolism, protein folding, cell division, and signal transduction. Our results support the idea that MeHg exerts its toxicity through oxidative stress induction and apoptotic effects. They also suggest that chronic MeHg exposure would induce an important metabolic deficiency in the brain. These findings provide basic information to understand possible mechanisms of MeHg toxicity in aquatic ecosystems.

Keywords: methylmercury (MeHg), proteomics, brain, beluga

P-10-390-1

Expression of nuclear factor κ-B1 in human bronchial wall who suffer from mustard gas lesions

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Sulfur mustard (SM) has been known as an effective chemical agent and was used in 1915 during World War I for the first time. At the present time there are more than 40000 people suffering from pulmonary lesions due to mustard gas in Iran. Though much is known about the gross pathology of SM damage, the molecular and cellular basis for this pathology is not clear. A group of most important proteins involved in inflammatory responses is NF-kappaB proteins. They are members of DNA-binding protein factors that are necessary for transcription of many proinflammatory molecules, such as adhesion molecules, cytokines, enzymes, and chemokines. NF-kappaB activation seems to be a major early event in a variety of cell and animal model systems developed to reveal the pathobiology of lung diseases. In our research, we studied NF-kappaB1 which has a key role in induction of cytokines and lung inflammation. We investigated five normal individuals and fifteen SM induced patients. Expression of NF-κB1 in healthy and the patients' samples was measured by semiquantitative RT-PCR. Although the expression of NF-κB1in Normal control samples was the same, expression levels of NF-κB1 were upregulated about 4 folds in the patients in comparison with normal samples, which point to the inducing effect of SM on NF-κB1 expression. To our knoeledge, this is the first finding of induction of NF-κB1 in patients exposed to SM. NF-κB1 may play a major role in inflammation induced by mustard gas in bronchial wall of patients.

Keywords: nuclear factor κ-B1, Sulfur mustard, inflammation

P-10-387-1

The study of the effect of oral morphine dependency on expression of NMDA subunit NR1 of hippocampus in male rats

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NMDA (n-methyl D-Aspartate) receptors play a pivotal role in the development of tolerance and physical dependence to opiates. Activation of NMDA receptors is one of the most important mechanisms involved in hippocampus dependent spatial learning and memory. Exposure to morphine in the form of chronic oral administration augments long-term potentiation (LTP) in CA1 hippocampal area in rats. The present study was designed to examine the change in NR1 subunit of NMDA receptor proteins in the hippocampus after repeated treatment with oral morphine using western blotting. Animals were divided into two groups in simple randomized manner (control and morphine dependent groups). Dependent animals received morphine sulfate in drinking water for 21-30 consecutive days. Hippocampus protein of two group's animals was extracted by buffer contains Tris-HCl, anti proteases and SDS. The proteins of total extract (evaluated by SDS-PAGE) were transferred to PVDF membrane by tank blotting and NR1 subunit of NMDA receptor reaction with their specific antibody analyzed by Immunoblotting. Results provide both biochemical and statistical evidence to suggest that NMDA receptor function in the hippocampus, at least with the increase expression of NR1 subunit protein in morphine-dependent rats. Taken together, these data support several studies in the literature indicating that NMDA receptors in the hippocampus are involved in the process of opiate dependence and augments the LTP.

Keywords: hippocampus, morphine dependency, NMDA receptor, NR1subunit, rat

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O-10-249-1

Proteomic patterns of sulfur mustard exposed lung patients

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Sulfur mustard (bis-2-(chloroethyl) sulfide) is a chemical warfare agent causing extensive lung injury. The mechanisms underlying HD-induced lung damage are not fully elucidated. Main late respiratory complications are chronic obstructive pulmonary disease, bronchiectasis, asthma, and bronchiolitis obliterans. The aim of the present study was to identify differentially expressed proteins in bronchoalveolar lavage (BAL) fluid of control healthy and SM exposed lung disease patients. The BAL protein profile of ten healthy and thirty exposed patients with mild, moderate and severe condition (10 males in each group) were separated with two dimensional SDS-PAGE and differentially expressed protein spots were successfully identified with MALDI TOF TOF MS. Among the differentially expressed proteins we observed a significant increase in vitamin D binding protein isoforms, haptoglobin isoforms and fibrinogen especially in exposed moderate and severe lung diseases patients ($p<0.01$). Moreover, compared to healthy controls, significant decreases were noted in calcyphosine, surfactant protein A and transthyretin in these patients ($p<0.01$). Apolipoprotein A1 was expressed in all patients but none of the healthy controls. Furthermore, S100 calcium binding protein A8 was only expressed in moderate and severe groups. These finding will be useful to improve current methods of monitoring and helps to identify new therapeutic targets for treatment of this complicated illness.

Keywords: apolipoprotein A1, bronchiolitis obliterans, Surfactant protein A, S100 calcium binding protein A8

P-11-123-2

Amyloid fibril formation of α-lactalbumin in crowded system and its preventing by α-casein

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α-Lactalbumin is a milk protein containing four disulphide bonds, which adopts a partially folded conformation under denaturing conditions. It can form fibrils under the conditions of low pH or by partial disulfide reduction at neutral pH. At these conditions, α-lactalbumin forms the so-called 'A' state with characteristics of a molten globule state (i.e. most of the secondary structure is in place but little tertiary structure is present), which makes it prone to amyloid fibril formation. In this study, we have compared the kinetics of the fibril formation of destabilized α-lactalbumin and its prevention by molecular chaperone, α-casein, in the presence and absence of dextran (68 kDa) as a macromolecular crowding agent by thioflavin T binding assay, visible absorption and near CD spectroscopy. An increase in the thioflavin T fluorescence intensity upon the addition of dextran reveals that the rate and extent of amyloid formation were significantly increased. Dextran caused structural change in α -lactalbumin, which was supported by CD spectroscopy. However, the effect of α-casein in preventing fibril formation was significant, although reduced in comparison with the absence of crowding. Thus, kinetics factor may be determinants in the reduced efficiency of α-casein in crowding conditions.

Keywords: α-casein, amyloid, crowding agent

P-10-681-2

Dog blood plasma preparation for two-dimensional gel electrophoresis

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Dog plasma consists of mainly large proteins, which vary in terms of both composition and concentration with the physiological state of the individual. Alterations in protein concentrations reflect the current state of the individual's health and thus may be utilized as valuable biomarkers for a specific biological process or disease. Two-dimensional gel electrophoresis (2-DE) has proven to be a valuable method for the separation and comparison of complex protein mixtures, for example, from disease and healthy states, as this method provides information regarding the variation, relative quantities, and structures of the intact proteins. The procedures utilized for the preparation of samples for 2-DE are critical to the acquisition of high-quality results for the discovery of biomarkers. The objective of this study was to review the preparation methods of plasma for 2-DE, particularly those designed to improve the detection of proteins in low abundance in plasma on 2-DE. The use of anticoagulants and protease inhibitors during the collection of blood, the removal of abundant proteins using multicomponent immunodepletion system, and desalting procedure allow us to compile profiles of proteins occurring in low concentrations in the plasma and to improve the pattern generated during 2-DE.

Keywords: dog blood plasma, two-dimensional gel electrophoresis (2-DE), proteomics

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P-10-640-1

Prefractionation in proteomics analysis of knock-out and wild cells for ANXC4 gene

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Aspergillus fumigatus is a fungus of the genus Aspergillus, and is one of the most common Aspergillus species to cause disease in immuno-compromised individuals. The annexins are a family of calcium- and phospholipid-binding proteins that have been widely studied in animals and fungi. Investigation of annexins in the fungus Aspergillus fumigatus identified a novel annexin-like gene called ANXC4. In this study we have investigated the effect of knockout gene of ANXC4 in the proteom of Aspergillus fumigatus. Tow groups of cells (mutant and wild) were cultured and lysed and their proteins were extracted. Then the extracted proteins of each group prefractionated by RP-HPLC and the proteins in fractions 4, 5 and 6 were separated by 2 dimensional gel electrophoresis (2-DE) in 7 cm gels with IPG strips of pH 4-7. After staining gels with silver staining, protein spots in each group were analyzed by Image Master 2D Platinum software. Among detected protein spots, some of them were newly expressed, some were

disappeared, some were over expressed and some were under expressed. The results showed that the expression of ANXC4 can affect the expression of some of proteins participating in different functions in Aspergillus fumigatus. Utilization of mass spectrometry can reveal the exact role of each protein in important cell functions.

Keywords: Aspergillus fumigatus, ANXC4, RP-HPLC, 2-DE

P-10-732-1

A fast, inexpensive and highly effective method of serum albumin isolation from blood samples

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Human blood is composed of different kinds of salts, lipids, amino acids, sugars as well as proteins ranging from 60 up to 80 mg/ml. The major protein components of plasma include albumin, immunoglobulins, transferrin, haptoglobulins and lipoproteins. It was estimated that up to 10000 different protein are present in serum most of them at very low concentrations. Conversely human serum albumin with a concentration ranging from 30 up to 50 mg/ml composes almost 60% of total human serum protein component. Being at such a high concentration, the presence of albumin has always constrained investigations of low abundant proteins of serum, especially in proteomic analysis of serum proteins by application of two-dimensional electrophoresis. In addition, the process of immunoglobulin purification is mostly perturbed by the presence of albumin. In present report we introduce a method of specific removal of albumin while the other serum proteins to remain intact. In order to achieve a fast and cost effective method, we introduced a method that basically uses ethanol and ammonium sulfate for removal of albumin from the rest of serum proteins. The method is based on selective precipitation of albumin in one phase and the rest of proteins in the other phase. In order to selectively remove albumin, different organic solvents (methanol, ethanol as well as isopropanol) and a range of pH (based on pI of albumin) were used to obtain the best condition for albumin precipitation in conjunction with application of ammonium sulfate as chaotropic agent. The present method is an optimized protocol which partitions albumin in one and the rest of serum proteins in another phase. Analysis and application of proteins obtained by this method indicates the high applicability of the method, especially in proteomics based studies.

Keywords: serum albumin, extraction, two-dimensional electrophoresis

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O-10-792-1

Study and identification of induced protein profile under hyper-saline concentrations in halo-tolerant bacterium separated from salt mine by proteomics techniques

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Drought and salinity are among the two most important osmotic stresses which limit the growth and development of living organisms. Studies on plants and bacteria have shown that they adapt various molecular mechanisms to adjust themselves with the reduced water content of the environment. Among such molecular mechanisms is expression or induction of expression of genes which are related to enzymes involved in production of osmo-protecting metabolites or proteins involved in transportation and osmo-adjustment. In present report, we evaluated the changes in the level of proteins whose expression have been subjected to alteration in a bacterium that was identified and isolated from a salt-mine by proteomics based methods of analysis. The bacterium was found to be able to tolerate hyper saline condition and grow under a very harsh osmotic stress condition. The halo-tolerant bacterium was isolated from solid salt samples obtained from such salt mine, grown and its optimum growth condition was determined. Following exposure to a range of salt containing growth medium composed of 0% up to 15% of salinity; total protein was extracted at logarithmic phase of growth. Subsequently, the extracted proteins were subjected to 2-dimential electrophoresis, analysis with an appropriate software and determination of proteins whose expression level showed degrees of alteration in response to hyper saline environment. Here we report protein profile as well as proteins whose expression was subjected to change in response to salinity. Due to nature of the isolated bacterium which is its salt adaptability, the identified proteins could be suggested and correlated to the true and natural proteins related with bacterial response to hyper-saline environment.

Keywords: drought, osmotic stress, hyper-saline condition, halotolerant bacterium, 2-dimential electrophoresis, osmo adjustment

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P-10-846-1

Mutagenesis of the O-helix region (N666E) of the Taq DNA polymerase for investigation of its role in the enzyme fidelity

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Taq polymerase enzyme is the first thermostable DNA polymerase discovered and routinely used in PCR reactions. O-helix region is important for enzyme's fidelity. Therefore in this study, N666 site in O-helix was mutated (N666E). Appropriate primers were designed. By using nested PCR one mutation (N666E) was made in enzyme Taq polymerase's gene. This mutation created a restriction site for EcoRI enzyme. The PCR product and pET-15b as vector were digested by NheI and BamHI restriction enzymes for 1.5h at 37°C. Desired segments were ligated by T4 DNA ligase and were incubated for 24h at 16°C. This product was transferred to E. coli DH5α competent cells by heat shock method. Plasmids were isolated from transformed colonies and digested by EcoRI, NdeI and BamHI restriction enzymes resulting in the isolation of the recombinant plasmids. After PCR reaction, its product and vector were digested by BamHI and NheI restriction enzymes. A bond of 264 bp for PCR product was observed. After ligation and transformation in E.coli, plasmids were prepared from the obtained colonies. By using EcoRI, NdeI and BamHI restriction enzymes, and new recombinant plasmids was recognized. O-helix is one of the helices of Taq polymerase important for Taq polymerase enzyme's function. The role of Asp-666 in this helix on enzyme's function has not been studied, therefore this site was selected. Changing aspargine to glutamic acid at this site was the goal of our study.

Keywords: mutation, Taq polymerase, fidelity

P-10-859-1

Cloning and optimization the expression of the Taq DNA polymerase gene in E. coli

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Taq DNA Polymerase is an essential enzyme used in molecular biology technique. The aim of this study was to amplify and express the Taq polymerase gene in E. coli. Genomic DNA from Thermus aquaticus strain YT-1 was isolated. Specific primers and suitable PCR cycles were used to amplify the DNA polymerase gene. This fragment was analyzed and then ligated into cloning vector pTZ57R. Colonies were screened and then confirmed by sequencing. The confirmed gene was ligated into pET15b plasmid as an expression vector. Expression was performed using different concentrations of IPTG at 37˚C and different length of induction time. Enzyme was purified and its expression was evaluated by polyacrylamide gel and PCR. A 2500 base pair fragment was prepared by PCR. This 2500 bp PCR product was digested with the Hind III. EcoRI and BamHI, KpnI and HindIII confirmed the colonies after digestion. The resulting sequence of recombinant plasmid showed a high homology with the published polymerase gene. The best condition of Taq DNA Polymerase expression was 2-3h induction time with 1mM IPTG at 370C. The expected 94-kDa proteins were observed by denatured polyacrylamide gel electrophoresis. DNA polymerase gene was amplified and placed into cloning and expression vectors. Cloning the full-length polymerase gene in pTZ57R had the advantage of amplifying Taq polymerase gene without the need of growing the bacteria under unconventional condition. The pET expression system leads to production of large quantities of the desired enzyme.

Keywords: Taq Polymerase, Thermus aquaticus, cloning, expression

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O-10-964-1

Proteomics analysis of included proteins in esophagus, stomach and colon cancers

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Nowadays, gastrointestinal cancers are the center of attention in case that is high spread. Studies that could represent a paper model, by which the development of cancers are assessed, could be efficient for preventing the affliction of gastro-intestinal cancers. In the present study, it is attempted to investigate the proteomics change in esophagus, stomach and colon cancers. The comparison of proteome of related cancers indicated that the expression of 142, 179 and 225 numbers of proteins have been changed relative to normal state in esophagus, stomach and colon cancer, respectively. The results showed that 15 proteins from total proteins were identical in the all three cancers. By analysis of some properties of these changed similar proteins, a central common core is defined which is involved in the related cancer development. Findings could lead to a novel strategy for detection of individual illness of a person who is in expose to gastrointestinal cancer disease.

Keywords: gastrointestinal cancer, proteomics

P-10-853-1

Effects of extremely low frequency electromagnetic fields on electrophysiological properties of rat brain's selected nucleuses: A proteomics approach

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Many researches carried out to determine the different effects of extremely low frequency electromagnetic fields (ELF/EMF) on living systems, especially laboratory animals. Neural tissue is among the most susceptible tissues and ELF/EMF may interfere with nervous system including its proteome and electrophysiological properties. In this study, the effects of ELF/EMF (3 Hz, 4 mT, sinusoidal, for 2 hour) on firing rate of two rat's brain nucleuses, locus coeruleus (LC) and Globus Pallidus (GP) were investigated by extra cellular recording using single glass electrodes followed by identification of its proteome alterations using proteomics approach. Results showed a significant decrease in firing rate of LC after radiation in comparison with sham group. No significant change observed in GP comparing to sham group. Further proteomics analyses showed some changes in rat's brain proteome pattern. According to these finding, ELF/EMF can alter electric activities in some regions of the rat's brain rather than its proteome and probably change the related function of those areas. It can be suggested that these radiations can change some animals behaviors and functions, make it possible to use ELF/EMF in therapeutic targets, for example for people suffering from distortion in their normal neural functions, such as schizophrenia.

Keywords: ELF/EMF, Proteomics, electrophysiology

P-10-159-1

Production of recombinant peroxisomal protein (PEP) by construction of GST-PEP

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Peroxisomal protein (PEP) is a peroxisomal matrix localizing protein. The expression of this protein is increased upon neural induction of stem cells in unraveled molecular mechanism. In order to identify the interacting partners of this protein in a proteomic experiment we decided to purify this protein in vitro. Thus as the first step PEP cDNA was subcloned in an appropriated bacterial expression vector (pGEX6p2) which was transformed in BL21 cells. In order to get an optimum protein expression we set our conditions based on variable parameters as culture temperature, incubation time and concentration of used IPTG as an inducer. Moreover the efficiency of detergents (Triton X-100, NP-40) and their concentrations were verified. The sonication condition was also set to get bacterial cell lysates. Our data

indicated that the best culture temperature for E.coli cells is 20 degrees of centigrade for about 14-20 hours. The optimized concentration of IPTG was 0.1mM. There was not any significant difference between the used detergents as both of them had the same effect on permeabilization of the bacterial membranes. Finally we detected the recombinant GST-PEP by SDS page analysis with both of Coommasie Brilliant Blue staining (CBB) and western blot using anti GST antibody.

Keywords: proteomics, peroxisomal protein (PEP), recombinant protein, bacterial competent cell, glutathione sepharose

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Proteomics study of human fibroblasts exposed to Diabetic conditions in vitro

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Fibroblast cells play a critical role in wound healing. They are the most common cells of connective tissue in animals. Tissue damage stimulates fibrocytes and induces the mitosis of fibroblasts. Wound healing processes in diabetic patients are so longer and sometimes cause to cut damaged tissue. Discovering biomarkers in diabetic fibroblast by proteomics can improve human knowledge about rapid wound hilling. Fibroblasts were isolated from foreskin and cultured as primary cell culture in different concentrations of glucose 0, 5 and 25mM for 72h incubation time. Fibroblast in 0 concentration of glucose assume as control. Proteins were extracted from fibroblast and separated by two-dimensional electrophoresis (2-DE) and protein analysis and identification, mainly did by mass spectrometry. The morphological variation of the cells was monitored microscopically. Cell proliferation of fibroblast in lower concentration of glucose is more than higher one. Fibroblast cells in higher concentration of glucose are longer than lower one morphologically. The proteome of the cells provided by 2DE technique showed different gene expression in diabetic fibroblast and control. The result indicated different molecular mechanisms that happened in diabetic conditions. The result of MALDI mass spectrometry also confirmed the identified spots which were determined by bioinformatics analysis of gels. To sum up, the difference between normal and diabetic proteome refer to different mechanisms and new discovered biomarkers can help to control various processes like wound healing in post translational level.

Keywords: diabetics, proteomics, fibroblast, wound healing

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Similar proteins involve in progress of various skin disorders

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Basal cell carcinoma (BCC) and Seborrheic keratoses (SK) are different kinds of skin disorder where alteration in gene expression can be the important event that happened in these diseases. Detection of this event is available by proteomics techniques. In this study, gene expression was detected in BCC and SK to find alteration gene expression. Normal both tissues were taken from BCC patient. Total proteins were purified by standard methods, and proteins separated by two dimensional electrophoresis (2DE). Mass spectrometry (MS-MALDI method) is a powerful instrument for protein identification. About 90 spots proteins detected in 2DE gels that have different expression in BCC and SK gel images. Results show the alteration of protein expression in SK tissues which almost most of them were down-regulated or not have any expression. By comparing to data base, determine 11 proteins in BCC that were ceruloplasmin, C3b, aldolase C, Fibrinogen gamma chain (FGG), unknown protein, cis peroxide reductase (Prx-cis), Protrombin, voltage-dependent anion channel (VDAC), Transthyretin (TTR), apolipoprotein D (Apo D) and leucine-rich glyco-.protein (LRG) proteins. Protein detected in SK were apolipoprotein E (Apo E), TTR, Immunoglobulin A (IgA), apolipoprotein D (Apo D), Betaactin, Act, Prx-cis and VDAC. The mass results in both tissues were Prx-cis and VDAC. Taken together being 4 similar proteins in SK and BCC may emphasize this truth that probably all different kind of disorder in one tissue can obey similar molecular mechanism. Same mechanism in various diseases is consequently able to design one drug in spite of different one.

Keywords: proteomics, biomarker, skin cancer, seborrheic keratoses, basal cell carcinoma

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Study on diagnostic value of lactate and CRP levels in cerebrospinal fluid for differentiation between bacterial and aseptic meningitis

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Bacterial meningitis is a major cause of morbidity and mortality worldwide. Lactate and C-reactive protein (CRP) in cerebrospinal fluid (CSF) have been proposed as diagnostic biochemical markers. A prospective study was conducted to evaluate and compare the diagnostic value of lactate and CRP levels in CSF with other routinely performed tests for the diagnosis of bacterial meningitis. The study population comprised of 140 patients, both children and adults, suspected of meningitis. Biomarker levels in CSF on admission were measured and bacteriologic studies were performed. For differentiating between bacterial and aseptic meningitis, the areas under the Curve (AUC) of lactate and CRP were superior to those of CSF protein and CSF-to-serum glucose ratio. At a cutoff level of 31.6 mg/dl the sensitivity of lactate was 90.32% and specificity was 82.35%. Considering CRP level >5.5mg/dl as a positive discriminatory factor for bacterial meningitis, the sensitivity and specificity were 100% and 73.52%, respectively. CSF lactate and CRP concentrations may serve as a valuable marker in discriminating between bacterial and aseptic meningitis.

Keywords: bacterial meningitis, diagnosis, biochemical marker, lactate, C-reactive protein, cerebrospinal fluid

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