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Influence of various concentrations of substrate and temperatures on the production of hexadecadienoic acid from unsaturated fatty acid auxotrophic mutant of *Hansenula polymorpha*

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The incorporation of exogenously supplied fatty acid, palmitoleic acid, was examined in an unsaturated fatty acid auxotrophic mutant of *Hansenula polymorpha* M2 at different growth temperatures and various concentration of substrates. The proportion of C16:2 delta 9, 12-cis-hexadecadienoic acid, which has not been synthesized in *Hansenula polymorpha*, was found to increase in the mutant with the specific growth conditions. The differential production of C16:2 in *H. polymorpha* M2 cells demonstrates that regulation of unsaturated fatty acid levels, possibly by control of the delta 12 desaturase, is an important control point in membrane composition in response to physiology features such as temperature and concentration of substrate in this species. The results of the optimal conditions such as temperature and concentration of C16:1 gained from the mutant was used to develop the hypothetical pathway of unsaturated fatty acid, C18:2, which used used C18:1 as a substrate. The increasing production of C18:2 has led to the improvement of essential unsaturated fatty acid, gamma linolenic acid (C18:3), production from this yeast.

***Keywords*:** fatty acid auxotrophic mutant; Hansenula polymorpha; temperature; hexadecadienoic acid; desaturase

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The changes in activase of RuBPcase and Rubisco, PsbP protein induced by the PS II inhibiting herbicide Atrazine in *Triticum aestivum*

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Response of wheat and corn to the photosystem II inhibiting herbicide Atrazine was studied by monitoring changes in the contents of chlorophyll a, b, carotenoid, activase of RuBPcase and Rubisco, PsbP protein. 10mg/L PS II inhibiting herbicide Atrazine caused a significant decrease in the contents of chlorophyll a, b and carotenoid in wheat which was thought an Atrazine-susceptible plant during a 10 days treatment, while that of corn, an Atrazine-resistant plant, was not affected. Electrophoresis analysis showed that 2 activase isoforms of RuBPcase (Rubisco beta form and precursor) and a 23 kDa oxygen evolving protein of photosystem II (PsbP protein) disappeared compared with control. Atrazine-treatment affected the synthesis of chloroplast protein in wheat, however, has no adverse effect on the synthesis of chloroplast proteins in corn. The experiment results in this study further support the hypothesis that Atrazine is a photosystem II inhibiting herbicide, but in previous reports only 32 kDa D1 protein was thought to be a binding protein of PS II inhibiting herbicide. In this paper, however, Atrazine inhibited synthesis of activase isoform of RuBPcase, activase beta form precursor of Rubisco and PsbP protein in wheat. It was the first observed Atrazine effect on the synthesis of novel chloroplast proteins. It will undoubtedly enhance our understanding of Atrazine action mechanism.

***Keywords*:** atrazine; chloroplast protein; PsbP protein; photosystem II; MALDI-TOF-MASS

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A high-throughput and large-scale silkworm cDNA expression in silkworm using BmNPV expression system

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Challenges and competitions are involved in the high-throughput and large-scale human and eukaryotic cDNA cloning and expression in the post genomic era. The high-throughput and large-scale protein expression and purification are considered as the key and basic technology in Proteomics research. The silkworm genome draft draw published on Science starts the hot research on silkworm post-genomics and proteomics. And it is necessary to develop the high-throughput and large-scale expression system suitable to silkworm proteomics research. Baculovirus Expression System (BES) was widely used for expressing recombinant proteins at high levels from cultured cells since Smith et al. expressed human β-interferon in insect cells infected with AcMNPV. On the basis of site-specific trans-

position of an expression cassette into a baculovirus shuttle vector (Bacmid), which propagated in Escherichia coli, the Bac-to-Bac system provides a rapid and efficient method to generate recombinant baculoviruses and is widely used for high level expression of heterologous proteins. We first constructed the Bac-to-Bac system based on BmNPV. The result recombinant Bm-Bacmid can infect susceptible Bm5 cells. The marker gene *egfp* was highly expressed in both Bm5 cell and silkworm larvae using the recombinant Bm-Bacmid-egfp. But the typical Bac-to-Bac system is not suitable for high-throughput expressing foreign protein because the positive recombinant Bm-Bacmid clony is only 10 %. For large scale construction of recombinant BmNPV baculovirus, a new method is introduced to construct recombinant virus quickly. We developed a novel and efficient system to generate recombinant BmNPV based on the mating-assisted genetically integrated cloning (MAGIC). MAGIC uses bacterial mating to catalyze the transfer of a DNA fragment between different *E.coli* to bring together two different plasmids. Recombination between these plasmids can be forced by inducing I-SceI and the red-gam recombinase. We constructed a novel donor transfer vector, in which the fragment of interest is flanked by two different 50 bp homology regions, H3 and H4, which in turn are flanked with linked I-Sce I sites. The novel recipient vector Bm-Bacmid propagated in genetically modifiedn *E.coli* also contains two I-Sce I-linked H3 and H4 sites. Using this method, it is rapid to generate a recombinant BmNPV in 3-5 d and it ensures 99% positive construction. This method can be used as a novel manner to high-throughput creation of recombinant bacloviruses and large scale expression of silkworm cDNA. 20 silkworm cDNA were expressed in BmN cells using recombinant BmNPV and 80% of the target genes could be highly expressed in the model expression system.

***Keywords*:** cDNA expression; BmNPV expression system; BmN; *Bomyx mori*; MAGIC

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Proteomic identification and functional characterization of a novel ARF6 GTPase-activating protein ACAP4

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ARF6 GTPase is a conserved regulator of membrane trafficking and actin-based cytoskeleton dynamics at the leading edge of migrating cells. A key determinant of ARF6 function is the lifetime of the GTP-bound active state, which is orchestrated by GTPase-activating protein (GAP) and GTP-GDP exchanging factor. However, very little is known about the molecular mechanisms underlying ARF6-mediated cell migration. To systematically analyze proteins that regulate ARF6 activity during cell migration, we perform a proteomic analysis of proteins selectively bound to active ARF6 using mass spectrometry and identify a novel ARF6-specific GAP, ACAP4. ACAP4 encodes 903 amino acids and contains two coiled coils, one PH domain, one GAP motif, and two ankyrin repeats. Our biochemical characterization demonstrates that ACAP4 has a PIP2-dependent GAP activity specific for ARF6. The co-localization of ACAP4 with ARF6 occurs in ruffling membranes formed upon AIF4 and EGF stimulation. ACAP4 overexpression limits the recruitment of ARF6 to the membrane ruffles in the absence of EGF stimulation. Expression of GTP-hydrolysis resistant ARF6Q67L results in accumulations of ACAP4 and ARF6 in the cytoplasmic membrane, suggesting that GTP hydrolysis is required for the ARF6-dependent membrane remodeling. Significantly, the depletion of ACAP4 by siRNA or inhibition of ARF6 GTP hydrolysis by overexpressing GAP-deficient ACAP4 suppresses ARF6-dependent cell migration in wound-healing, demonstrating the importance of ACAP4 in cell migration. Thus, our study sheds new light on the biological function of ARF6-mediated cell migration. Importantly, ACAP4 is overexpressed in certain cancer with high metastatic potential. Our current activity aims to evaluate the possible involvement of ACAP4 in tumor cell spreading in animals.

***Keywords*:** proteomics; ARF6; ACAP4; cell migration; cytoskeleton

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SELDI-TOF-MS profiling of serum for detection of HBV-induced hepatocellular carcinoma

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For a long time, liver cancer has been the second leading cause of cancer death in China, just behind lung cancer. The incidence of hepatocellular carcinoma (HCC) is the highest among primary liver cancer. HBV and HBV-induced liver cirrhosis may lead to HCC. At present, it is difficult to diagnose HCC at early stage or to differentiate HCC patients, especially those patients without any clinical

symptom from healthy people by using the conventional methods without a biopsy. Therefore it is urgent to explore and develop a simple, rapid diagnostic method, which has higher sensitivity and specificity for HCC at early stage. Surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF-MS) is a novel non-electrophoresis-based proteomic technology. In this system, proteins are retained on a solid-phase chromatographic surface and are subsequently ionized and detected by TOF-MS. SELDI offers the advantages of rapid and simple examination as well as high specificity and sensitivity. New proteins specific to some diseases and characterization of these proteins can be discovered and captured by comparative analysis of the mass spectra of the samples from patients and normal controls. To our knowledge，there has been little study reported using SELDI time-of-flight MS (SELDI-TOF-MS) technology to investigate HCC. OBJECTIVE: to discovery biomarkers using SELDI-TOF-MS in the sera from HCC patients and healthy people. METHODS: 25 cases of the patients with HCC without receiving any therapy, 25 cases of the patients with HCC receiving the interpose chemotherapy and 50 cases of the healthy people were tested by Weak cationic exchange (WCX2) protein chip and SELDI-TOF-MS of Ciphergen Inc. The differentially expressed proteins were analyzed by BioMarker Wizard. RESULTS: At the different M/Z value range, seven proteins were obviously different among the groups of the patients with HCC without receiving any therapy, the patients with HCC receiving the interpose chemotherapy and the healthy people. Analyzed by BioMarker Wizard software, four proteins including 6489.48Da, 6662.34Da, 8593.75Da and 8720.23Da were up-regulated in healthy controls, two including 7777.27Da and 9250.00Da were up-regulated in the patients with HCC without receiving any therapy and one (16200.17Da) was up-regulated in the patients with HCC receiving the interpose chemotherapy. By means of Biomarker Pattern, two protein profiles (7777.27Da, 9250.00Da) that can separate patients with HCC without receiving any therapy from normal controls have been developed. It gives the much-improved sensitivity of 92% and the specificity of 100%. Through searching database, these seven proteins were found to be Galanin related peptide, Pro-neuregulin-4 protein, small inducible cytokine A15 precursor, 9 kDa protein, CSL-zincfinger protein 1, mitochondrial hinge protein, actin related protein, respectively. CONCLUSION: SELDI-TOF-MS is an efficient technology to find novel potential tumor markers from HCC. The proteins identified could be candidates for developing biomarkers of HCC in the serum and drug targets for treating HCC.

Keywords**:** hepatocellular carcinoma ; proteomics; serum protein; SELDI-TOF-MS

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Bos tauraus and _Bos indicus_: the main ancestress of cattle breeds in China revealed by mtDNA diversity

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The mitochondrial cyt b genes for 136 Chinese native cattle and D-loop regions for 84 individuals were completely sequenced, which included 105 and 102 variable sites, respectively. The overall nucleotide diversity (0.00960) and haplotype diversity (0.804±0.031) of cyt b genes for Chinese cattle are quite high, so it was with nucleotide diversity (0.02599) and haplotype diversity (0.919±0.027) of D-loop regions. Phylogenetic analysis revealed that all the cattle breeds fell into two distinct lineages: *Bos taurus* and *Bos indicus*. Nearly all the Northern cattle breeds were clustered in the clade of *Bos taurus* in maternal lineage, while most Southern breeds were found to be included in *Bos indicus* lineage. The significant genetic divergence between Northern and Southern breeds was resulted from the dominant geographical segregation of Qinling Moutains. The cattle breeds in Central Area were also proved to originate from the two progenitors: *Bos taurus* and *Bos indicus*, meanwhile, the influence of *Bos taurus* to Qinchuan cattle and Jinnan cattle was greater than that to the other breeds of Central Area, whereas, Luxi cattle, Jiaxian Red cattle and Nanyang cattle were more greatly influenced by *Bos indicus*. Jinnan region of Shanxi province and Northwest region of Shandong province were suggested to be the geographically converging area of *Bos taurus* and *Bos indicus*.

Keywords**:** cattle breeds; mtDNA diversity; origin; phylogenetic relationship

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Mitochondrial proteomes in T cytoplasmic male-sterile corn (_Zea mays_)

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In this study, to identify the difference of mitochondrial proteome between T cytoplasmic male sterile line and maintainer line of corn (*Zea mays*). We used mitochondria which were isolated from leaf, root, plumular axis of yellow seedlings as experimental materials by two-dimensional gel electrophoresis and mass spectrometry. The result indicated that more than 250 mitochondrial protein spots, including about 100 protein spots in leaf, 70 protein spots in root and about 80 protein spots in plumular axis have been found in the mitochondrial proteomes of T cytoplasmic male-sterile line. Three kinds of mitochondrial proteins were identified in leaf, root and plumular axis of male-sterile line. (1) A set of protein spots were identified as soluble proteins (2) a second of protein spots were identified as peripheral and integral membrane proteins (3) additional protein spots could not be easily classified as a group. By comparison, we found that there are 12 mitochondrial protein spots, including 8 protein spots (36 kDa/pI 7.3, 28 kDa/pI 6.7, 46 kDa/pI 7.5, 97 kDa/pI 5.8, 88 kDa/pI 5.8, 80 kDa/pI 5.6, 80 kDa/pI 5.2, 80 kDa/pI 6.0) in leaf, 3 protein spots (60 kDa/pI 8.2, 50 kDa/pI 7.9, 50 kDa/pI 7.7) in root and 1 protein spot (45 kDa/pI 7.8) in plumular axis were present in T cytoplasmic male-sterile line, but they are absent in maintainer line. We think these 12 mitochondrial proteins may associate with cytoplasmic male-sterile trait.

***Keywords*:** cytoplasmic male-sterile; mitochondrial proteome; *Zea mays*; 2D-PAGE; mass spectrometry

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The changes in chloroplast proteome caused by different nuclear background in cytoplasmic male-sterile (CMS) of wheat

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Comparative studies of chloroplast proteome on different developmental stage (seeding, tillering, shooting, booting stage) of leaves have been made in isoplamic allonuclear male-sterile lines Nongda 3237A, Xiaoyan No.6A and their maintainer lines by 2D-PAGE. The results indicated that no obvious differences were found in chloroplast proteome between Nongda 3237A, Nongda 3237B, and Xiaoyan No.6A, Xiaoyan No.6B at different developmental stages of leaves. Obvious differences, however, were observed in chloroplast proteome between isoplasmic allonuclear male-sterile lines Nongda 3237A and Xiaoyan No.6A. For instance, 2 protein spots (pI 5.4/34 kDa, pI 5.4/32 kDa) at seedling stage, 6 protein spots (pI 5.4/80 kDa, pI 5.4/65 kDa, pI 5.4/60 kDa, pI 5.4/48 kDa, pI 5.4/40 kDa, pI 5.4/35 kDa) at booting stage were present in Xiaoyan No.6A and absent in Nongda 3237A. pI 6.3/18 kDa was present at seedling stage in Nongda 3237A and absent in Xiaoyan No.6A. pI 6.8/28 kDa protein spot revealed the developmental changes. It was present in leaves at seedling, tillering, shooting stages and absent at flowering stage in Xongda 3237A. No development changes of the protein spot were observed in Xiaoyan No.6A.These experiment results demonstrated that it was possible chloroplast proteome wasn't associated with the cytoplasmic male-sterile characteristics. But nuclear background in male-sterile lines can obviously affect chloroplast protein composition. Distant relative on nuclear-cytoplasmic has large differences on chloroplast proteome than close relative on nuclear-cytoplasmic in CMS.

***Keywords*:** isoplasmic allonuclear; 2D-PAGE; chloroplast proteome; cytoplasmic male-sterile; wheat

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Neuroprotective effect and mechanism of *Panax notoginseng* saponins on cortical neuron cell injured by hypoxia/reoxygenation

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Neuronic apoptosis is the main mechanism of tardive death of neuron postischemia, which increases impairment of cerebral ischemia. Therefore, inhibiting postischemia neuronic apoptosis is important for preventing ischemic cerebral impairment. We studied the protective effect of panax notoginseng saponins (PNS) on cultured rat cortical neuronal cell (CNC) which were challenged by hypoxia/reoxgenation (H/R). Our result showed that PNS increased cell viability and reduced the releasing of LDH with H/R group without PNS treatment.

***Keywords*:** *Panax notoginseng* saponins; cortical neuronal cell; hypoxia/reoxgenation; apoptosis; mitochondrial membrane potential

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Differential proteomic expression analysis of colonic cancer SW480 cells induced by Diallyl disulfide

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This study was designed to explore the differential proteomic expression induced by diallyl disulfide (DADS) in human colonic cancer SW480 cells and its related molecular mechanisms. Methods: A series of methods, including immobilized pH gradient-two dimensional polyacrylamide gel electrophoresis, peptide mass fingerpringting based on matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) ,database searching and so on, were used to separate and identify the differential proteomic expressions inducing effect of DADS on SW480 cells. Cells were processed for two-dimensional (2-D) electrophoresis and proteins were separated by isoelectric focusing and visualized by silver staining and Coomassie brilliant blue staining. After the pattern of polypeptide expression was evaluated by computer-assisted image analysis (PDQUEST), the different gel spots were excised and digested in situ and identified by MALDI-TOF-MS, and database searching. Results: The results showed that the good 2-DE pattern including high resolution and reproducibility was obtained. After silver staining, the 2-DE image analysis by PDQuest 2-DE software detected average (492±36) spots in SW480, and (523±24) spots in DADS treated SW480. And the average matching rate was 92% and 98% respectively. There were 167 spots that exhibited detectable quantitative changes between control and DADS-treated cells, 98 of them realized up-regulation while 69 of them realized down-regulation. Twenty of which were identified by mass spectrometry unambiguously. The expression of cytokeratin-18, IgE-dependent histamine-releasing factor, Cellular retinol-binding protein I, cytokeratin-1, myosin light chain, ubiquitin, H+-transporting ATPase, heat-shock protein-64, typothetical protein, guanylate cyclase-acting protein(GCAPs), oxytocinase/insulin-responsive aminopeptidase and sequence 9 from patent WO0164904 was increased in DADS-treated cells, whereas the expression of cytokeratin-19, actin-depolymerizing factor, V-1 protein, fructose bisphosphate aldolase, FK506-binding protein, capping protein, thioredoxin peroxidase, transformation-sensitive protein was down-regulated. These proteins were associated with cell differentiation, cell apoptosis, cell cycle, cell metastasis, transcription and cytoskeleton etc. DADS can inhibit the SW480 cells' ability of proliferation and induce the differentiation of SW480 cells by those proteins. Conclusion: Up-regulated expression of guanylate cyclase-acting protein and myosin light chain or down-regulated of fructose bisphosphate aldolase and FK506-binding protein by DADS can inhibit the SW480 cells' ability of proliferation.Ubiquitin was involved in the effect of DADS on G2/M arrest in SW480 cells. DADS may induce the differentiation of SW480 cells via up-regulated expression of Cellular retinol-binding protein I, increase the expression of cytokeratin-18 and inhibit the expression of thioredoxin peroxidase induced SW480 cells apoptosis. DADS can inhibit the metastasis of colonic cnacer cells via down-regulated the expression of cytokeratin-19, capping protein and actin-depolymerizing factor.

***Keywords*:** diallyl disulfide; colonic cancer; SW480 cells; differential proteome

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Identification of tumor-associated proteins in human gastric cancer

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Gastric carcinogenesis is generally believed to be a multistep progression from normal gastric mucosa to premalignant lesions and ultimately cancer and multiple genetic and molecular alterations were involved in this progression. The study of gene expression profiles in different stages of this progression may bring new insight into the process of carcigenesis. While gene expression profiling of this progression has been performed, no comparable protein analysis has been reported. Encoded proteins carry out most biological functions, and to understand how cells work, we must study which proteins are present, how they interact with each other and what they do. Proteomics research offers new tools for studying proteins, and is poised to boost our understanding of systems-level cellular behavior. Comparative proteomics was applied to identify cancer-associated proteins in human gastric carcinoma. Methods: The total proteins of normal gastric mucosa, dysplasia and gastric cancinoma were separated by two-dimensional electrophoresis (2-DE). After a modified Neuhoff's colloidal coomassie blue G-250 staining, image scanning and PDquest software analysis, the differentially expressed protein spots were incised from gels and digested by trypsin in gel. Then matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) were used for peptides mass analysis. The acquired peptide mass fingerprints (PMFs) were used to search for matches in SWISS-PROT

and NCBI databases with Mascot software. The functions and subcellular locations of identified proteins were further analyzed according to bioinformatic resources. Results: Through comparative proteomic analysis of gastric mucosa among normal, dysplasia and carcinoma tissue, we obtained 43 differential proteins, 11 of which were identified by MS. The identified proteins could be divided into five groups based on their functions: redox-status regulatory proteins, Aflatoxin B1 aldehyde reductase member 3, Peroxiredoxin 5, Acyl-CoA dehydrogenase (short-chain specific), Cytochrome c oxidase polypeptide Va, Cytochrome coxidase subunit VIb isoform 1, chaperones, Hsp27, Ph-Hsp27, proteins related to immune, Galectin-1, proteins related to cellular structure, Cofilin-1, and calcium binding proteins, Annexin A1, S100C protein. Conclusion: There are different proteins in normal gastric mucosa, dysplasia and gastric cancinoma. 11 of them were identified. The results indicated that changing of cell skeletal, declining of antioxidant capability and immune tolerance may be important reasons for the development of human gastric carcinoma.

***Keywords*:** human gastric carcinoma; premalignant lesions; proteomics; mass spectrometry; 2-DE

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Molecular cloning and characterization of phosphatidylethanolamine-binding protein 4 from porcine seminal plasma

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Phosphatidylethanolamine-binding protein 4 (PEBP4), a new member of the phosphatidylethanolamine-binding protein family has purified from porcine seminal plasma. The parent molecular weight of protein is 23 kDa on SDS PAGE. We have determined. The cDNA is composed of 669 bp of nucleotides in length and encodes 223 amino acids in the coding region. The overall homology of amino acid sequence between porcine, dog and human is 73.6 to 89.7%. We have demonstrated that PEBP 4 inhibits several serine proteases including thrombin, neuropsin, and chymotrypsin, but has no inhibitory effect on trypsin, tissue type plasminogen activator, and elastase. Since PEBP does not share significant homology with other serine protease inhibitors, our results define it as the prototype of a novel class of serine protease inhibitors.

***Keywords*:** phosphatidylethanolamine-binding protein 4; porcine; seminal plasma; purification; cloning

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MLN51 and GM-CSF involvement in fibroblast-like synoviocytes (FLS) proliferation in the pathogenesis of rheumatoid arthritis

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Rheumatoid arthritis (RA) is an inflammatory autoimmune disease of unclear etiology. This study was conducted to identify critical factors involved in the mechanisms by which synovial hyperplasia occurs in RA. We applied cDNA microarray analysis to profile the gene expressions of RA fibroblast-like synoviocytes (FLS) from RA patients and bone marrow-derived dendritic cells (BmDC) from DBA/1 mice. We found that the MLN51 (Metastatic Lymph Node 51) gene identified in breast cancer is remarkably upregulated in the FLS of RA patients. In addition, GM-CSF in all synovial fluids (SFs) obtained from RA patients (n= 6) existed at a significant level, compared to the inflammatory cytokines, IL-1β and TNF-α. Most FLSs in passage 10 or more recovered from growth retardation when cultured in the presence of 10-fold-diluted SF. The SF-mediated growth recovery was markedly blocked by anti-GM-CSF neutralizing antibody. Growth-retarded RA-FLS recovered their proliferative capacity by the addition of GM-CSF in culture at a concentration of 100 ng/ml. These results imply that the GM-CSF in SF plays an important role in the proliferation of RA FLS. We found that the MLN51 gene was highly expressed in the hyperactive RA FLS in low passage or RA FLS cultured in the presence of SF or GM-CSF in the microarray analysis and semi-quantitative RT-PCR. Moreover, MLN51-gene expression was significantly enhanced by GM-CSF, and FLS proliferation was improved by GM-CSF treatment in a dose-dependent manner. These data with GM-CSF treatment were further demonstrated in the MLN51 protein level by Western blot analysis. MLN51 knock-down by siRNA completely blocked the GM-CSF/SF-mediated RA FLS proliferation. These results strongly suggest that the MLN51 gene is very likely involved in the chronic synovitis of RA patients in line with GM-CSF signaling.

***Keywords*:** MLN51; GM-CSF; rheumatoid arthritis; synoviocyte; hyperproliferation

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Increasing of alkaline protease activity in *Bacillus cereus* and *Bacillus polymixa* as an economical and biotechnological product simultaneously with starting sporulation phase as a defend mechanism

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Bacterial spores are amongst the most resistant of all microbial forms to inactivation by chemical or physical agents. These resistant forms are created in extreme conditions such as famine, water deficiency and dryness, presence of harmful agents for instance chemicals, antibiotics, radioactive and ultraviolet rays and so on. Among all spore forming bacteria the genus *Bacillus* is very outstanding and considerable due to its various physiological abilities and production of valuable biological products. The existence of resistant endospores in the *Bacillus* spp. have been resulted in extensive distribution of these organisms through the nature and various ecosystems. One of the significant biological substances that are produced by genus *Bacillus* are alkaline proteases with several important applications in medicine, pharmaceutical industries, sanitary and cosmetic industries, food industries and other routine and economical industries. One of the most important applications of alkaline proteases is in detergent and sanitizers industries. The presence of endospores in *Bacillus* spp. is very effective and efficient in isolation, passage, preserving and ultimately production of valuable biological products. In this research, the growth curve, the curve of sporulation phase and the alkaline protease activity in the *Bacillus cereus* and *Bacillus polymixa* were comprised and examined simultaneously. These examinations showed that the production of alkaline protease started with beginning of log phase in growth curve simultaneously and after a small stopping in according to stationary phase increased and then with starting of death phase increased rapidly again. The maximum of alkaline protease activities in *Bacillus polymixa* and *Bacillus cereus* were measured at the death phase of growth curve and with starting of the sporulation phase of these two microorganisms. The mentioned experiments were made in Glucose synthetic medium [Glucose (6 g/l), $(NH_4)_2SO_4$ (10 g/l), Na_2HPO_4 (8 g/l), KH_2PO_4 (4 g/l), $MgSO_4$ $7H_2O$ (0.5 g/l), $CaCl_2$ (0.02 g/l)] at 30 degree centigrade with 100 rpm during 144 h. The maximum of alkaline protease activity in *Bacillus cereus* and *Bacillus polymixa* were occurred after 72 h simultaneously with starting the sporulation phase of two bacteria and were measured 383 (μ/ml) and 418 (μ/ml) respectively. Probably the increase of alkaline protease activity in these two spp. with starting of sporulation phase is considered as a defend mechanism in creation of endospore for destroying of obtrusive and harmful proteins of sporulation procedures.

***Keywords*:** sporulation phase; alkaline protease; *Bacillus cereus*; *Bacillus polymixa*; enzyme activity

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Differential proteomic expression induced by Diallyl disulfide in human gastric cancer MGC803 cells

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This study was designed to explore the differential proteomic expression inducing effect of diallyl disulfide in human gastric cancer MGC803 cells and its related molecular mechanisms. Methods: A series of methods, including immobilized pH gradient-two dimensional polyacrylamide gel electrophoresis, silver staining, PDQuest 2-DE software analysis, peptide mass fingerpringting based on matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and SWISS-PROT database searching, were used to separate and identify the differential proteomic expressions inducing effect of diallyl disulfide (DADS) on human gastric cancer MGC803 cells. Results: The results showed that the good 2-DE pattern including high resolution and reproducibility was obtained. After silver staining, the 2-DE image analysis by PDQuest 2-DE software detected average (576±14) spots in MGC803, and (583±4) spots in DADS treated MGC803. And the average matching rate was76% and 70% respectively. Using the differential proteomic expression analysis, we found that there were 421 spots matched and 200 spots unmatched between MGC803 and DADS treated MGC803 maps. The spots on treated group, whose quantity of expressed proteins was above two or ten times compared with the control, were 291 and 61. Part of the differential spots were cut off from silver staining gel at random, digested in gel with TPCK-trypsin, measured with MAIDI-TOF-MS and searched in related database with MS-FIT software. Twenty-four proteins were preliminarily identified. These proteins were related to cell differentiation, cell metastasis, cell apotosis, cell cycle, cell immunity and metabolism, etc. There was a significant difference at protein level between MGC803 and DADS treated MGC803 cells. Conclusion: Up-regulated expression of nM23 protein or down-regulated of uPAR, LIM kinase and CDC2 by DADS can inhibit the ability of metastasis. Inhibiting expression of XIAP by DADS can promote cell apotosis and inhibit the growth of gastric

cancer cells. CDC2 was involved in the effect of DADS on G2/M arrest in human gastric cancer cells. DADS may induce the effect of reducing tumor via up-regulated expression of MHC-I, TCR, Snake venom-like protease and activation of NF-kappaB pathways through Toll/Interleukin-1 receptor-like protein 3. NY-CO-45 antigen may be treated as a potential marker of diagnose and prognosis. DADS may inhibit the proliferation via increasing the expression of AMP, activating malate dehydrogenase precursor, and down-regulating hnRNP F protein, transcobalamin II and phosphodiesterase.

***Keywords*:** diallyl disulfide; human gastric cancer cells; differentiation and proteome; two dimensional electrophoresis; MALDI-TOF mass spectrometry

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Extraction, separation and measuration of the thermal hysteresis activity of antifreeze proteins from *Ammopiptanthus nanus*

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The Antifreeze proteins (AFPs) are capable of protecting organisms from damage in freezing or sub-freezing conditions by lowering the freezing points of the organism's extracellular matrix and body fluids non-colligatively whilst leaving the melting point unchanged. The separation of the melting and freezing temperature is usually referred to as thermal hysteresis activity (THA). As a kind of evergreen broadleaved shrub in the northwestern China desert, the rare endangered species *Ammopiptanthus nanus* is a rather good plant with strong resistant capability and forms typical xerophytic and cold resistant structure due to its long growth in an adverse environment. In this study, the DE-52 ion-exchange cellulose and gel filtration were used to extract and separate the antifreeze proteins from cold-acclimated *A. nanus* leaves which have grown in Turpan Eremophytes Botanic Garden for about 10 years. Differential scanning calorimetry (DSC) was used to measure the thermal hysteresis activity. Data from this study indicates that when the concentration of the protein is 20 mg/ml, the THA of tube 14 is 0.46 ºC and the content of ice crystal is only 0.36 %. The content of ice crystal is decreasing while the THA is increasing. It appears that there are AFPs in the tube 14. So we report here that there are AFPs in *A. nanus* leaves and they have the ability of preventing growth of ice crystal increases substantially when the content of the crystal is diminished. And there is a curve regression relationship between the THA and the content of ice crystal, and hence, the content of crystal has to be taken into consideration when the THA is to be quantified. The AFPs have a great potential to protect the *A. nanus* during winter.

***Keywords*:** *Ammopiptanthus nanus*; AFPs; DSC; THA

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Pravastatin reduces infarct size induced by coronary artery ischemia/reperfusion in anesthetized rats with elevation of eNOS expression

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We have reported that chronically administered pravastatin prevented reperfusion-induced lethal ventricular fibrillation (VF) in anesthetized rats without lowering the serum cholesterol level. The present study was undertaken to evaluate whether pravastatin decreases myocardial infarct size in association with changes of eNOS (endothelial Nitric Oxide Synthase) expression in myocardial tissues. Anesthetized rats were subjected to 30 min ischemia and 60 min reperfusion after chronic administration of pravastatin (0.02 mg/kg, 0.2 mg/kg and 2 mg/kg), fluvastatin (2 mg/kg and 4 mg/kg), or vehicle for 22 days, orally, once daily. ECG and blood pressure were continually recorded; myocardial infarct size was measured by TTC-staining; eNOS was measured by western blotting. Pravastatin and fluvastatin significantly (P<0.05) reduced myocardial infarct size. There were no significant differences in the areas at risk (as a percentage of the total left ventricular areas) among all groups, i.e. 52 ± 1% in control, 48 ± 2%, 56 ± 4% and 51 ± 3% in pravastatin 0.02 mg/kg , 0.2 mg/kg and 2 mg/kg, respectively; 48 ± 4% and 52 ± 2% in fluvastatin 2 mg/kg and 2 mg/kg groups. However, there were significant reductions of infarct areas (shown as percentages of the areas at risk and total left ventricular areas) in three pravastatin groups and one fluvastatin group when compared with control group, i.e. 66 ± 4% and 34 ± 2% in control, 48 ± 3% and 23 ± 2% in pravastatin 0.02 mg/kg, 31 ± 5% and 17 ± 4% in pravastatin 0.2 mg/kg, 50 ± 5% and 25 ± 2% in pravastatin 2 mg/kg; 61 ± 2% (no significant) and 29 ± 2% (no significant) in fluvastatin 2 mg/kg, and 48 ± 5% and 25 ± 2% in fluvastatin 2 mg/kg groups. Both pravastatin and

fluvastatin significantly elevated eNOS protein expression in ischemic and nonischemic tissues as compared with controls. Our results prove further that pravastatin contributes its benefits to decreasing cardiovascular mortality beyond its cholesterol-lowering effect. Pravastatin is more potent than fluvastatin in reduction of infarct size. These effects may be associated with the increase of eNOS expression.

***Keywords*:** pravastatin; ischemia; reperfusion; infarct size; eNOS

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Extraction, antisera preparation and immunolocalization of sperm membrane proteins in *Eriocheir sinensis*

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Mature sperms have many kinds of proteins in plasma membrane. These sperm membrane proteins (SMPs) play important roles in the maintenance of sperm's shape and structure, the metabolism of sperms and the reproduction, especially in the fertilization. Studies presented here were designed to characterize the SMPs of Eriocheir sinensis and to further prepare their antisera and test whether or not the proteins we extracted located at the plasma membrane in mature sperms. Extraction buffers with 1% TritonX-100 and low concentration of sodium dodecyl sulfate (SDS) was applied to dissolve membrane proteins off *E. sinensis*'s sperms. The membrane protein composition was analyzed by SDS- polyacrylamide gel electropheresis (SDS-PAGE). As SDS-PAGE maps showed, there were at least 12 kinds of SMPs in *E. sinensis*, whose molecular weights were between 20 ku and 71 ku, isoelectric point (pI) ranged from 4.4-6.2, and all of them were glycoproteins. The composition of SMPs was obviously different from that of the demembranated sperms. By applying traditional immunity way and taking the extracted SMPs as antigen to inject rabbit, the antisera was obtained. Immunogold labelling electronmicroscopy technique was performed to investigate immunolocalization of the SMPs of *E. sinensis* using the polyclonal antisera. In the control group, the ultrastructure of sperm in *E. sinensis* was clear, intact but without gold granules distribution. In contrast with the control group, the gold granules in the experiment group were found on plasma membrane in mature sperms. In addition, they were observed to be on plasma membrane, acrosomal membrane and nucleus respectively in immature sperms. We speculated that some sperm membrane proteins may be transferred and be concerned with the sperm maturation.

***Keywords*:** *Eriocheir sinensis*; SDS-PAGE; antisera; immunolocalization; electron microscopy

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Isolation, bioactivity assay of CHH /GIH and proteomic analysis of the optic ganglia in *Eriocheir sinensis*

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As other Deoapoda Crustaceans, the optic ganglia in *Eriocheir sinensis*'s eyestalks also have X-organ-sinus gland (XO-SG) complex, which is an important neurosecretory organ and secrets several kinds of neuropeptidergic hormones controlling complex physiological processes. Crustacean hyperglycemic hormone (CHH) peptide family contained in the SG comprises mainly the CHH, molt-inhibiting hormone (MIH), gonad-inhibiting hormone (GIH), and mandibular organ-inhibiting hormone (MOIH). In this paper, CHH and GIH from *E. sinensis* were isolated; at the same time, by means of two-dimensional electrophoresis (2-DE), the proteins expression of mature and precocity *E. sinensis*'s optic ganglia were compared. (1) The crude hormones extract of crab's optic ganglia was isolated by reverse-phase high-performance liquid chromatography (RP-HPLC). And then, 13 samples were obtained. According to the chromatogram, all the samples were divided into 3 groups. Each group protein was injected into different crab's body respectively to detect their hyperglycosemia bioactivity, the result showed that the crabs injected the 2nd group have the highest blood glucose concentration in the 3 groups. And in the 2nd group, the 9th sample was significant in the hyperglycemic activity bioassay. So the 9th sample was estimated to be CHH of *E. sinensis*. (2) From the crustacean hyperglycemic hormone (CHH) peptide family that we determined, the further research was undertaken. To collect the expected samples that had eluted in the district of CHH peptide family, 7 single samples were obtained. The biology activity assay of each fraction was got through the method of injection *in vivo*. As a statistic result that we surveyed the diameter of crab's oocytes, the 7th sample has the greatest biological activity to inhibit the development of the oocytes. So the sample was presumed to be the GIH of E. sinensis. (3) Compared the protein expression of mature and precocity *E. sinensis*'s

optic ganglia by 2-DE. From the 2-DE maps, 115 protein points of mature crab and 108 of precocity crab were found. The result indicated that there were 24 obviously different protein points, and more acidic protein in precocity crab's ganglia. The difference proteins were considered to be the special proteins concerned with the development of crab's gonad. In a word, this research will be a elementary data for the function of the hormone and the solution of the crab's precocity.

***Keywords*:** *Eriocheir sinensis*; CHH; GIH; HPLC; two-dimensional electrophoresis

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Study on the basic proteins and acid phosphatase during spermiogenesis in some species of *Decapoda crustacean*

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In the thesis, the distributions of basic proteins and acid phosphatase were observed during the spermiogenesis of several species of *Decapoda crustacean*: *Fenneropenaeus chinensis*, *Macrobrachium nipponense*, *Procambarus clarkii*, *Scylla serrata* and *Eriocheir sinensis*. (1)Using transmission electron microscopy (TEM) and ammoniacal silver reaction (ASR), the basic proteins' distributions were researched: There were basic proteins in the sperm nucleus of *F. chinensis* and *M. nipponense*, but not in the sperm nucleus of *Pro. clarkii*, *S. serrata* and *E. sinensis*. The basic proteins were also found in the acrosomal complex of mature sperms of the four species, but the location of basic proteins was species-specific. It could be presumed that there was species-specificity in the transfering and replacement of the nuclear basic proteins binding DNA during sperm formation in *Decapoda crustacean*. Otherwise, specific stain for lysine and arginine were used in the reseach of about the testis histology of *E. sinensis*. The result showed these amino acids variation on basic protein during the period of spermiogenesis: the content of lysine reduced from spermatocyte to mature sperm. Spermatocyte is stained in every region. In the mature sperm, lysine was mainly presented in acrosome, not in nucleus. But there was no arginine in spermatocyte or mature sperm. (2) Ultrastructural cytochemical techniques and electron microscopy were used in the research of the localization of acid phosphatase activity during spermiogenesis in *F. chinensis* and *E. sinensis*. The results showed that: ①Acid phosphatase was found in the sperm of *F. chinensis*. The reaction product was seen in association with the membrane of endoplasmic reticulum, proacrosomal granule and some vesics in the cytoplasm, and also in the anlage of the spinous process in the spermatids. In the middle spermatids, the reaction product was obsvered in the heteropycnotic chromatin, nor in the nucleoli. In the late spermatids and mature sperms, the reaction products were seen in the outer-membrane of the acrosome complex, also in the membrane of some vesics in the cytoplasm band. ②Acid phosphatase was found in the sperm of *E. sinensis*. The reaction product was seen in association with endoplasmic reticulum during the early spermatids. However, no reaction product was observed in the proacrosomal granule. On the intermediated phase of spermiogenesis the product was uniformly distributed nuclear acrosome tube and acrosomal membrane. In the final stages, the reaction product was observed mainly on the region of acrosome.

***Keywords*:** decapoda; sperm; basic protein; acid phosphatase

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Dynamic study of lipid droplet/adiposome activity by comprehensive proteomics and lipidomics

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Lipid droplets/adiposomes are cellular organelles with a neutral lipid core surrounded by a phospholipid monolayer and coated with structural as well as functional proteins. The determination of these proteins and lipids especially their functional regulations and dynamic movement on and off droplets holds a key to resolving the biological functions of the cellular organelle and understanding several major metabolic diseases such as obesity, diabetes, and atherosclerosis. In order to address this, we carried out a comprehensive proteomic study that includes a complete proteomic, a phosphoprotein proteomic, and a comparative proteomic analysis using purified adiposomes and mass spectrometry techniques. The complete proteome identified 125 proteins in which 79 proteins were first located to droplets. In phosphoprotein proteomic analysis 7 functional adiposome proteins were determined to be phosphorylated including adipose differentiation related protein (ADRP), two Rab proteins, and four lipid me-

tabolism enzymes, especially adipose triglyceride lipase (ATGL). To understand the dynamics of lipid droplets, the GTP-dependent protein recruitment was analyzed by a comparative proteomics. Arf1 and some of its coatomers, three other Arfs, several other small G-proteins including a couple of Rabs, and several lipid synthetic enzymes were found to be recruited from cytosol to purified droplets significantly. We also report here a comprehensive analysis of the lipid composition of droplets purified from various types of cultured cells. We have used a combination of NMR spectroscopy and mass spectrometric approaches (including high-throughput, direct infusion electrospray ionization and tandem mass spectrometry, ESI-MS/MS) to characterize the neutral lipid and phospholipid composition of isolated droplets. We found that these droplets are not only rich in triacylglycerol and cholesterol esters, but also are enriched in the ether lipid, monoalkyl diacylglycerol. Further, even though the phospholipid composition from adiposomes represented just 1-2% of the total lipid, it was represented by diverse molecular species of PC, PE, PI, ether-linked PC (ePC) and ePE but contained very little phosphatidylserine (PS) or sphingomyelin (SM). We identified and quantified more than 160 phospholipid molecular species, which suggests the simple phospholipid monolayer surrounding each droplet has an amazingly complex lipid composition. Based on the prevalence of ether neutral lipids and phospholipids in lipid droplets, we propose that the adiposomal compartment may play an important role in ether lipid metabolism. Together, the present study suggests that adiposome/lipid droplet is an active and dynamic cellular organelle that governs lipid homeostasis, metabolism, and intracellular trafficking through protein phosphorylation as well as protein translocation.

***Keywords*:** lipid droplets; adiposomes; proteomics; lipidomics; ether lipids

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Identification of prostate cancer biomarkers by quantitative proteomics technology

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The high incidence of prostate cancer and the lack of therapies in late stage make its early detection extremely important. The prostate-specific antigen (PSA) has been used clinically as a diagnosis biomarker; however the imperfect correlation with cancer limited the usefulness of PSA. In order to identify new biomarkers for early diagnosis and new targets for therapeutic strategies, we characterized secreted proteins from a set of cell lines that represent different stage of prostate cancer by a quantitative proteomic approach (ICAT), 16 proteins that differentially expressed in different stage of cancer cells have been identified and quantified. Significance of these changes in the context of prostate cancer progression and especially implications as new clinical diagnostic and therapeutic targets for prostate cancer warrants further investigations.

***Keywords*:** prostate cancer; proteomics; ICAT

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Proteomic characterization of death effector filaments

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A crucial protein domain in the Fas / TNFR mediated pathway is the Death Effector Domain (DED) (1). This domain is present in a number of cellular and viral proteins and over-expression of DED from Caspase 8 and FADD, but not the DED of cFLIP, results in formation of novel cytoplasmic structures termed death effector filaments. To gain molecular insights into protein components of these cytoplasmic filaments, we have developed stable cell lines expressing the DED domains of Caspase 8 (A and B) fused to GFP. In this paper, we describe a purification procedure and proteomic characterization of death effector filaments. We found that these cytoplasmic filaments have well defined structure and recruit two endogenous apoptosis regulating protein, caspase-8 and FADD. Additionally, we identify a total of 79 proteins from highly-enriched death effector filament preparations. The identified proteins that co-purified with death effector filaments suggest the participation of this structure in multiple cell regulatory functions.

***Keywords*:** proteomics; apoptosis; death effector filament

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2-DE patterns establish and differential proteomic analysis in gastric carcinoma tissues

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The mechanism of gastric carcinogenesis is not clear yet. Special molecular marker for early diagnosis and prognosis of stomach carcinoma are lacked either. To disclose their carcinogenic mechanism and find special molecular markers, using Immobilized pH gradient (IPG) two dimensional electrophoresis (2-DE) method, the total proteins of the tissue of gastric carcinoma and paired normal gastric mucosa are separated. After silver staining, using ImageMaster image analysis software, the proteins of differential expression are compared and recognized. Using Matrox-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), acquiring the reciprocal peptide mass fingerprint (PMF), searching the database, the proteins of differential expression are identified. The two-dimensional gel electrophoresis image of five samples of gastric carcinoma tissue and paired normal gastric mucosa are established. Whose average matching spots are 769±45. 81 spots are differential expression proteins. 17 of them are expressed only in gastric carcinoma tissue. 24 spots are expressed only in normal gastric mucosa while lacked in gastric carcinoma tissue. 26 spots are up-regulation and 14 spots are down-regulation in gastric carcinoma. 20 selected sharply marginated differential expression spots are analyzed and identified by peptide mass fingerprint. The two-dimensional gel electrophoresis image of high resolution and reproducibility are established and differential expression proteins are found. Those proteins probably participate in gastric carcinogenesis. It is the base for finding and screening the special molecular markers for gastric carcinoma.

***Keywords*:** human gastric carcinoma; 2-DE; MALDI-TOF-MS; proteome; differential analysis

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Analysis of a novel NPC-related candidate suppressor gene STGC3 by two-dimensional gel electrophoresis and MALDI-TOF-MS

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STGC3 was a candidate suppressor gene associated with nasopharyngeal carcinoma (GenBank accession No: AY078383). This study was designed to reveal the preliminary function of STGC3 in NPC cell line (CNE2). STGC3 gene was transfected into CNE2 with liposome and a stable cell line over-expressing STGC3 was established. Two-dimensional gel electrophoresis (2-DE) was used to identify proteins that were differently expressed in STGC3 transfected cells. 18 differentially expressed proteins were identified by MALDI-TOF-MS, which involved in cell cycling, transcription regulation, signaling pathway *etc*. pcDNA3.1 (+)/STGC3 /CNE2 were treated with 17-β-Estradiol. After 2-DE, 10 differential proteins were identified by MALDI-TOF-MS. Estrogen may exert its effect in pcDNA3.1 (+)/STGC3 /CNE2 cells by regulating the expression of these proteins.

***Keywords*:** STGC3 gene; two-dimensional gel electrophoresis; MALDI-TOF-MS; 17-β-Estradiol

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The differential proteomic expression analysis of Diallyl disulfide—induced human colonic cancer cells

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This study was designed to explore the differential proteomic expression inducing effect of diallyl disulfide (DADS) on human colonic cancer SW480 cells and its related molecular mechanisms. Methods A series of methods, including immobilized pH gradient-two dimensional polyacrylamide gel electrophoresis, peptide mass fingerpringting based on matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) ,database searching and so on, were used to separate and identify the differential proteomic expressions inducing effect of DADS on human colonic cancer SW480 cells. Results The results showed that the good 2-DE pattern including high resolution and reproducibility was obtained. After silver staining, the 2-DE image analysis by PDQuest 2-DE software. There were 167 spots that exhibited detectable quantitative changes between control and DADS-treated cells, 69 of them realized down-regulation. The expression of cytokeratin-19, actin-depolymerizing factor, V-1 protein, fructose bisphosphate aldolase, FK506-binding protein, capping protein, thioredoxin peroxidase, transformation-sensitive protein were down-regulation in DADS-treated cells. Conclusion There are differential expression proteins between control and DADS-treated cells. To search differential expression proteins between control and DADS-treated cells, which provide insight into the changed global protein patterns of DADS-treated cells and prove useful for further investigating the molecular mechanism of colonic cancer.

***Keywords*:** DADS; colonic cancer; 2-DE; MALDI-TOF-MS; proteome

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Classification of lung cancer by measuring a specific biomarker pattern of host response proteins using SELDI-TOF-MS

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Surface Enhanced Laser Desorption/Ionization (SELDI) technique has an ability to display multiple biomarkers spontaneously in a single assay; discovery a specific biomarker pattern for a disease is paid more attention. In the present study, SELDI technique was used to analyze 276 sera including 116 lung cancer, 65 laryngeal carcinoma, 55 laryngopharyngeal carcinoma, and 40 normal individuals. Five protein peaks with the molecular weight 11.51, 11.68, 13.76, 13.83 and 14.04 kDa were automatically selected as the biomarkers for lung cancer, of particularly interested to us was that these five biomarkers constituted a specific biomarker pattern: while the former two were upregulated in lung cancer, the latter three were downregulated. To identify these biomarkers, the candidate bands were obtained from 1-D SDS gel by matching the molecular weight with peaks on CM10 chips and further identified as serum amyloid A (SAA) protein minus its amino-terminal arginine (SAA-R), original SAA, transthyretin (nativeTTR), cysteinylated transthyretin (cysTTR) and glutathionylated transthyretin (glutTTR) by ESI/MS-MS and database searching. These preliminary results were further proven by Western blot and immunoprecipitation. Based on these results, the differential levels of SAA and TTR were re-measured in the same sets of sera from all groups by ELISA assay. It showed a firm consistency between ELISA and SELDI analysis. It suggested that lung cancer could be better discriminated from the other control groups by this specific biomarker pattern, although these two proteins belong to the abundant host response proteins.

***Keywords*:** lung cancer; SAA; TTR; biomarker pattern; SELDI

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Serum amyloid a protein (SAA): a biomarker which was increased with the clinical stages of lung cancer

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Using Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-TOF-MS) to analyze 218 sera including 175 lung cancer and 43 healthy individuals, the protein profilings were analyzed both by Biomarker Wizard™ and Biomarker Patterns™ software. It shown that a cluster protein peaks with the molecular weight ~11.6 kDa significantly increased in lung cancer. Meanwhile，the levels of this cluster biomarkers were progressively increased with the clinical stages of lung cancer. The candidate biomarkers were then obtained from two-dimensional gel electrophoresis by matching the molecular weight with peaks on WCX2 chips and were identified as serum amyloid A (SAA) protein minus its amino-terminal arginine (SAA-R) and original SAA by ESI/MS-MS and database searching. It was further validated in the same serum samples by immunoprecipitation with commercial SAA antibody. To confirm the SAA differential expression in lung cancer patients, the same set of serum samples was measured by ELISA assay. The result showed that at the cutoff point 0.446 (OD value) on the Receiver Operating Characteristic (ROC) curve, SAA could better discriminate lung cancer from healthy individuals with sensitivity 84.1% and specificity 80%. These findings demonstrated that SAA could be characterized as a biomarker to montoring the development of lung cancer.

***Keywords*:** lung cancer; SAA; SELDI-TOF-MS

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Comparative proteomics analysis of metastasis-associated proteins in non-small cell lung cancer

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The development of metastasis is the leading cause of death and an enormous therapeutic challenge for the non-small cell lung cancer (NSCLC). To better understand the molecular mechanisms underlying the metastasis process

and discover some novel potential markers for NSCLC diagnosis and progression, comparative proteomics analysis of two NSCLC cell lines with different metastatic potentials, non-metastatic CL1-0 and highly metastatic CL1-5, was performed by 2-DE followed by MALDI-TOF-MS and MS/MS. 40 differential expression proteins were unambiguously identified, among which 21 were significantly up-regulated and 19 were down-regulated in highly metastatic CL1-5. Eight of the identified proteins were selected for further validation at mRNA level using real-time quantitative PCR. The results showed that mRNA expressions of five genes were consistent with the 2-DE results, whereas 3 genes were no significant changed between two cell lines. Particularly, S100A11, one of the identified proteins which was overexpressed in CL1-5 cells in both protein and mRNA levels, was selected to further determine its correlation with metastasis of lung cancer. Sixty-eight primary lung cancer tissues and some matched local lymph nodes of NSCLC were used to observe the expression of S100A11 by immunohistochemstry. The results demonstrated that overexpression of S100A11 was statistically significantly associated with TNM stage, but not with the subtypes of lung cancer and the size of cancer, implying that S100A11 might be an important regulation molecule in promoting invasion and metastasis of NSCLC.

Keywords**:** S100A11**;** NSCLC; comparative proteomics; metastasis

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Elevation of serum HSP90α correlated with the clinical stage of lung cancer

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The HSP90α, described expression in cytoplasm, has been detected in serum-free cultured medium (CM) from fibrosarcoma cells and breast adenocarcinoma cells recently. To investigate whether HSP90α could be found in the CM of lung cancer cell lines, and is a sensitive and specific biomarker for the diagnosis and progression of lung cancer, the differential secrtome analysis between two human lung adenocarcinoma cell lines with low and high metastatic potentials, CL1-0 and CL1-5 was performed using one dimension polyacrylamide gel electrophoresis (1D SDS-PAGE) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF- MS). One of the candidate biomarkers, significantly up-regulated in the CM of CL1-5 cells,was identified as HSP90α and further confirmed by Western blot. To investigate the relationship of HSP90α elevation with lung cancer, 224 serum samples including 141 lung cancer, 37 benign pulmonary diseases and 46 healthy individuals were measured using ELISA assay. It was found that the levels of HSP90α were specifically increased in NSCLC sera compared with other groups and were progressively up-regulated with the clinical stage of lung cancer. At the cutoff point 0.535 (OD value) on the Receiver Operating Characteristic (ROC) curve, HSP90α could comparatively discriminate lung cancer from benign lung disease and healthy control groups with sensitivity 0.817, specificity 0.919 and total accuracy 80.14%. Based on these results, it demonstrated that a significant coherence between the levels of HSP90α in blood and the clinical stages of non-small cell lung cancer (NSCLC). Therefore, **s**ecretome could open up a possibility to find, identify, and characterize novel biomarkers associated with the development of cancers. HSP90α may be a potential useful biomarker to discriminate lung cancer from the benign lung diseases.

Keywords**:** HSP90αα; CL1-0; CL1-5; secretome; metastasis

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Identification and Validation of S100A7 Associated with Lung Squamous Cell Carcinoma Metastasis to Brain

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To better understand the mechanisms underlying brain metastasis in non-small cell lung cancer (NSCLC) and to search for potential markers for NSCLC prognosis, comparative proteome analysis on two lung squamous cell carcinoma (SCC) cell lines, NCI-H226 and H226Br (the brain metastatic cell line of NCI-H226), was performed using two-dimensional electrophoresis (2-DE) followed by a tandem mass spectrometer with a matrix-assisted laser desorption/ionization (MALDI) source. Twenty differential proteins were identified, of which 6 proteins were up-regulated in H226Br cell compared to NCI-H226 cells, whereas 14 proteins were down-regulated. S100A7, one of candidate proteins significantly overexpressed in H266Br cell, was selected to verify the liability of the differential

proteins by Western blot. The result was in accordance with 2-D analysis. To further determine whether S100A7 expression is actually associated with the brain metastasis of SCC, S100A7 was examined in brain metastases tissues of NSCLC, the primary lung cancer tissues and matched local regional lymph nodes, the primary brain tumors and normal brain tissues by immunohistochemistry. The positive staining of S100A7 could be found in 3/5 (60 %) brain metastases of SCC and 8/39 (20.5 %) the primary NSCLC tissues. and 20 % (1/5). No positive staining was observed in the brain metastases of adenocarcinoma (n=5), the primary brain tumors (n=5), the local positive lymph nodes of the primary NSCLC (n=20) and normal brain tissues (n=2). Based on these results, it suggested that S100A7 expression was associated with brain metastasis of SCC and may be a potential biomarker for monitoring the development of NSCLC.

Keywords**:** S100A7; brain metastasis; two-dimensional electrophoresis; mass spectrometry; immunohistochemistry

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Role of Pin1, a prolyl isomerase in topographic regulation of neuronal intermediate filament phosphorylation and stabilization

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The perikaryal accumulation of phosphorylated neurofilament-heavy (NF-H) is evident in neurofibrillary tangles of Alzheimer's disease (AD), Lewy bodies of Parkinson's disease (PD) and spinal cord aggregates in amyotrophic lateral sclerosis (ALS). Under normal physiological conditions, NF-H is extensively phosphorylated on its proline-directed serine/threonine residues only in the axonal compartment however, in pathological conditions, phosphorylated NF-H accumulates in neuronal cell bodies causing death of affected neurons. Pin1, a prolyl isomerase binds to phosphorylated proline-directed serine/threonine residues and isomerizes them from cis to trans conformations. Pin1 associates with phosphorylated NF-H and co-localized in ALS affected spinal cord. In ALS and AD affected tissue, an increase in phosphorylated NF-H was evident without any change in Pin1 levels. Using cultured dorsal root ganglion and cortical neurons as cellular paradigms of ALS and AD respectively, increases in levels of and perikaryal accumulations of phosphorylated NF-H caused by glutamate exposure, were reduced upon inhibition of Pin1 activity. Our data suggests that the isomerization process played by Pin1 is critical in controlling neuronal cell body and axonal phosphorylation of NF-H.

Keywords**:** toskeleton; neurodegeneration; neurofilaments; kinases; phosphorylation

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Functional proteomics of membrane proteins of Gram-negative bacteria

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Outer membrane proteins (OMPs) are important to the response to environmental changes in Gram-negative bacteria Membrane proteins of Gram-negative bacteria are key molecules that interface the cells with the environment. Despite recent proteomic identification of a lot of oligomer proteins in *E. coli* cell envelope, the protein complex of *E. coli* membrane proteins and their peripherally associated proteins remains ill defined. In the current study, we systematically analyze the sub-proteome of *E. coli* cell envelope enriched in sarcosine-insoluble fraction (SIF) and sarcosine-soluble fraction (SSF) by using proteomic methodologies. One hundred and four proteins out of 184 spots on 2D electrophoresis gels are identified, which includes 31 outer membrane proteins (OMPs). Importantly, our further proteomic studies reveal a number of previously unrecognized membrane-interacting protein complexes, such as the complex consisting of OmpW and fumarate reductase. This established complete proteomic profile of *E. coli* envelope also sheds new insight into the function(s) of *E. coli* outer envelope. pH environment is crucial to bacterial growth, but pH-dependent outer membrane proteins are ill-defined. In the present study, 2-DE proteomics, Western blotting, gene deletion and complementation were applied for the investigation of pH-dependent Omps and the identification of their function. Thirteen proteins were determined to be differentially expressed in gels, in which OstA, TraK and OmpP were firstly reported here. Furthermore, OmpW, OmpC, OmpF, OmpT, OmpX, LamB, Dps, FadL, Tsx and TolC were confirmed by Western blotting for their expression and by gene deletion and complementation for their function. Iron is an essential element for bacterial growth. The OMPs of Gram-negative bacteria play an important role in overcoming the conditions of poor iron availability in the host and natural environments. We undertook a comparative analysis on a sarcosine-insoluble fraction of E.

coli K-12 cultured in LB medium with or without limited iron by 2-DE proteomic methodologies. Three proteins, OmpW, OmpX and Tsx, related to iron homeostasis first reported here were further investigated. The ompW, ompX and tsx were cloned for antibody preparing and overexpression strains. The up-regulated OmpX and down-regulated OmpW and Tsx in 2-DE gel were confirmed by Western blotting. Importantly, our further proteomic studies reveal a previously unrecognized membrane-interacting protein complex consisting of OmpW with fumarate reductase that related to iron homeostasis.

Keywords: outer membrane proteins; functional proteomics; Gram-negative bacteria

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