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Study on the ultrafine structure of tumor cell with atomic force microscopy

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Multiple pathways are involved in maintaining the genetic integrity of a cell after its exposure to physical environments. Although repair mechanisms such as homologous recombination and non-homologous end-joining are important mammalian responses to double-strand DNA damage, cell cycle regulation is perhaps the most important determinant of physical environments sensitivity. A common cellular response to DNA-damaging agents is the activation of cell cycle checkpoints. The DNA damage induced by physical environments initiates signals that can ultimately activate either temporary checkpoints that permit time for genetic repair or irreversible growth arrest that results in cell death. Such checkpoint activation constitutes an integrated response that involves sensor, transducer, and effectors genes. The cell cycle phase also determines a cell's relative environmental sensitivity, with cells being most environmental sensitive in the G2-M phase, less sensitive in the G1 phase, and least sensitive during the latter part of the S phase. But the structure always can show the inner function, vice versa. Besides the method of inside-to-outside, we can realize the detail of the tumor cell by detecting the ultrafine surface structure. The advancement of scanning probe microscopy has introduced a powerful method of detecting biological structural features. It is now generally accepted that contact mode atomic force microscopy (AFM) does not lead to significant cellular damage under appropriate imaging conditions. The power of AFM methods in resolving the structure and dynamic operations of cellular systems is just being recognized. In the latest tapping mode AFM, the cantilever is oscillated at high frequency; 3D topography of the surface can be generated under milder scanning conditions. For the other way, we can utilize the nanomanipulation of the AFM to see the interaction between inner molecules, and realize the relationship of macromolecules. Aim: Through compared the different detecting condition, get the best method of all and found the proper parameters to describe the physical and chemical properties in the AFM study on tumor cell in synchronal，thereby find the suitable experimental process an narration way. Results and Discussion：We interdict the cell into four different cycles with colchicum i.e. G1, G2, S and M phase. From the clear and beautiful AFM topographies, we can easily get the differences among the four different phases, in which we can see the different character in the protrusion distribution and number. Particularly in the nanometer scale, there are some prominent domains like any field distribution; maybe, it's the particular structure in the nano-space which related to the biologic magnetic or electromagnetic fields. According to the prominent distinguishment character in the AFM image, we found the credible method to discriminate cells in different cycle or living condition with the AFM and the proper techniques.

***Keywords*:** ultrafine structure; tumor cell; atomic force microscopy; synchronization; cell cycle

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The effect of solanine on DNA and RNA in the tumor cells

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Commonly called "nightshade", *Solanum nigrum* L. is an annual plant belonging to the Solanaceae family. It is found throughout China, but is more abundant in the Northeast and North China regions. Its main contents are alkaloids, but it also contains vitamins and many trace elements. There have been reports of the plant being used in folk medicine to treat tumors with good effect. In earlier experiment, we found that solanine can significantly prolong the survival time of H22 tumor-bearing mice. In the present experiment, we further observed the effect of solanine on the DNA and RNA in tumor cells of S180 and H22 mice. S180 and H22 mice were divided into solanine (37.50, 18.75, and 9.37 mg/kg) groups, negative control group, and Cytoxan (30 mg/kg) group, each of which was given drug through sc. Levels of RNA and DNA in tumor cells in each group were measured using a laser scanning confocal microscope (LSCM). Confocal images obtained by scanning the sample with a laser beam not only reveal the distributions of DNA and RNA on different sections and changes in the RNA/DNA ratio, but can show intuitively changes in DNA and RNA after the cell is damaged in a state more clearly approaching the real life situation. The results demonstrated in the 37.50 and 18.75 mg/kg solanine groups, RNA/DNA ratio in the tumor cell of both S180 and H22 mice was significantly lowered. According to

results, we could concluded that Solanine could lower the RNA/DNA ratio in the tumor cells of S180 and H22 mice, which may be one of the mechanisms for the antitumor effect of solanine.

***Keywords*:** solanine; tumor cell; DNA/RNA

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Study on the apoptosis effect induced by isothiocyanates in broccoli on human gastric adenoma cells SGC-7901 and its mechanism

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To investigate the pro-apoptosis effect of isothiocyanates (ITCS) on human gastric adenoma cells SGC-7901, and its mechanism, SGC-7901 cells were treated with different concentrations of ITCS. MTT assay was used to evaluate the influence of ITCS on cell proliferation. Flow cytometry was used to test ROS levels, intracellular mitochondrial transmembrane potential (Δψm), and hypodiploid apoptosis peak in SGC-7901 cells. The results showed that ITCS obviously inhibited proliferation of SGC-7901 cells. When treated with 0, 15, 30, 60, 120, 240 μg/mL of ITCS for 24 h, ROS levels were (1.6±0.5)%, (2.0±0.3)%, (5.5±0.4)%, (25.8±1.4)%, (83.7±1.2)% and (97.4±4.2)%, respectively; and Δψm were (98.6±4.3)%, (98.4±4.8)%, (95.7±5.4)%, (92.6±4.0)%, (74.0±5.6)% and (63.7±4.0)%, respectively; when treated with 0, 60, 120, 240 μg /mL of ITCS for 48 h, cell apoptotic rates were (4.3±1.6)%, (9.1±3.8)%, (20.1±4.2)% and (55.4±4.9)%, respectively. From the results above, we could draw the conclusion that ITCS generates ROS in gastric cancer SGC-7901 cells, which causes mitochondrial membrane permeabilization and Δψm decrease, therefore, leads to apoptosis of SGC-7901 cells.

***Keywords*:** broccoli; isothiocyanates; apoptosis; reactive oxygen speciesmitochondrial; mitochondrial transmembrane potential

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Study on the induction of tumor cell apoptosis by *Sargassum fusiforme* polysaccharide

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Research has shown that *Sargassum fusiforme* polysaccharide (SFPS), the effective element in *Sargassum fusiforme* which is a brown alga of the Sargasaceae family, has some inhibitory effects on tumor cells. In this study, we further study the induction of the apoptosis of the human gastric carcinoma cell SGC-7901 by SFPS and its effect on intracelluar concentration of Ca^{2+}($[Ca^{2+}]i$), so as to uncover the mechanism for its anti-tumor effect. The effect of *Sargassum fusiforme* polysaccharide on the cell cycle and apoptosis of tumor cells is observed using the flow of cytometry (FCM). Intracellular Ca^{2+} is marked with Fluo-3/AM and $[Ca^{2+}]i$ is measured by using laser scanner confocal microscopy (LSCM). We find that SFPS can inhibit the passage of the human gastric carcinoma cell (SGC-7901) from the G0/G1 stage to the S stage of the cell cycle and increase the index of apoptosis (APO %). The results show that before the treatment of SFPS, $[Ca^{2+}]i$ in SGC-7901 cells is fairly constant. After treatment, $[Ca^{2+}]i$ rapidly rises before it declines, but rises again after $CaCl_2$ is added. Finally we can conclude SFPS can induce apoptosis in tumor cells by increasing $[Ca^{2+}]i$ in the tumor cells. The rise in $[Ca^{2+}]i$ is because Ca^{2+} ions are released from intracellular calcium stores.

***Keywords*:** *Sargassum fusiforme* polysaccharide; SGC-7901; apoptosis; anti-tumor activity

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Effects of solanine on *Km* and *Vmax* of NATase in HepG2 cells

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To explore the effects of solanine on *Km* and *Vmax* of NATase of HepG2. METHODS: employing HPLC, using 2-AF as substrate, taking concentration of 2-AF as concentration of substrate, in intact HepG2 cells and their cytoplasm, taking the speed of 2-AF being acetylated to 2-AFF by NATase as the rate of NATase, using double reciprocal plot, taking 1/S(the reciprocal of concentration of 2-AF) and

1/V(reaction rate of NATase) as coordinates, got regression equation, calculated *Km* and *Vmax*. RESULTS: Study on enzyme kinetics demonstrated, as for intact HepG2 cells, *Km* and *Vmax* of control group were $2.37\times10^{-3}\pm8.37\times10^{-5}$ mM, $9.16\times10^{-4}\pm7.54\times10^{-5}$ nmol/10^6 cells, *Km* and *Vmax* of the solanine group were $2.22\times10^{-3}\pm9.05\times10^{-5}$ mM, $5.14\times10^{-4}\pm3.72\times10^{-5}$ nmol/10^6 cells. As for the cytoplasm of HepG2 cells, *Km* and *Vmax* of control group were $8.95\times10^{-3}\pm2.61\times10^{-4}$ mM and $2.55\times10^{-6}\pm1.92\times10^{-8}$ nmol/min.mgprotein, *Km* and *Vmax* of the solanine group were $9.48\times10^{-3}\pm3.63\times10^{-4}$ mM and $2.43\times10^{-6}\pm1.32\times10^{-8}$ nmol/nmol/min.mgprotein, statistically, as for intact HepG2 cells and their cytoplasm, there was no difference between the *Km* of control group and that of solanine group, but there was remarkable difference between *Vmax* of control group and that of solanine group, $P<0.001$ for intact cell and $P<0.05$ for cytoplasm.

***Keywords*:** solanine; NATase; HepG2 cells

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Effect of solanine on the contents of Caspase-3 and Bcl-2 in HepG2

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To observe the effect of solanine on the contents of caspase-3 and bcl-2 in HepG2,and to explicate the mechanism by which solanine induces the apoptosis of tumor cells. METHOD: Laser confocal scanning microscopy and Western blot are used to measure the contents of caspase-3 and bcl-2, and LCSM is used to determine their locations in the cell. RESULTS: Solanine can markedly increase the content of caspase-3 while decreasing that of bcl-2 in HepG2, both in a dosage-dependent way. Both caspase-3 and bcl-2 are unevenly distributed in the cytoplasm, but are not found in the nucleus. Solanine does not affect their distribution. CONCLUSION: By suppressing the activity of Bcl-2, solanine activating the caspase-3 protease family and thus inducing cell apoptosis.

***Keywords*:** solanine; caspase-3; Bcl-2; anti-tumor mechanism

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Knockdown of STAT3 expression by RNAi inhibits the induction of breast tumors in immunocompetent mice

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Constitutively activated STAT3 is involved in the formation of multiple types of tumors including breast cancer. We examined the effects of Stat3 protein knockdown by RNAi using a di-cistronic lentivirus shRNA delivery system on the growth of mammary tumors in BALB/c mice induced by the 4T1 cell line. A single exposure of 4T1 cells to shRNA/STAT3 lentivirus transduced 75% of the cells with GFP within 96 h. In cells selected for GFP expression, neither Stat3 protein nor phosphotyrosine Stat3 was detected. Tumor formation induced by injecting 4T1 cells into the mammary fat pad was blocked by expression of the shRNA for STAT3 whereas all mice injected with 4T1 cells expressing only GFP efficiently formed tumors. C-Myc expression was reduced 75% in cells expressing greatly reduced levels of Stat3 compared to the GFP control. Of interest, the level of activated Src, which is known to activate Stat-3, was virtually eliminated but the level of the Src protein itself remained the same. Importantly, expression of Twist protein, a metastatic regulator, was eliminated in STAT3 knockdown cells. Invasion activity of STAT3 knockdown cells was strongly inhibited. However, the proliferation rate of cells in Stat3 knockdown cells was similar to that of the GFP control; the cell cycle was also not affected. We conclude from these studies that activated Stat3 protein plays a critical role in the induction of breast tumors induced by 4T1 cells by enhancing the expression of several important genes including c-Myc and the metastatic regulator Twist. These studies suggest that stable expression of siRNA for STAT3 has potential as a therapeutic strategy for breast cancer.

***Keywords*:** STAT3; breast cancer; RNAi; lentivirus

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Survivin is involved in the mechanism of drug resistance in MCF-7/ADR cell

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Survivin is a member of the inhibitor of apoptosis (IAP)

family,which is highly expressed in during G_2/M phase. It is also constitutively overexpressed in most cancer cells where it may play a role in chemotherapeutic resistance. The development of resistance to multiple chemotherapeutic drugs occurs frequently during the treatment of advanced carcinoma of the breast. The purpose of this research is to investigate the expression of Survivin in multidrug-resistant breast carcinoma cell line (MCF-7/ADR), explore the relationship between expression of Survivin and multidrug resistance in MCF-7/ADR cell. Using gradually increased concentration of adriamycin in culture, an adriamycin-resistant breast cancer cell line (MCF-7/ADR) was established in vitro. The survivin mRNA of MCF-7/ADR cell was higher than the sensitive cell assessed by RT-PCR. In order to investigate the function of survivin in MCF-7 and MCF-7/ADR cell, a recombinant survivin plasmid vector (pEGFP-survivin) was designed and constructed. The survivin sequence-specific shRNA was also designed according to the designation principle of shRNA. Two pairs of oligos for hairpin RNA expression which targeted survivin gene were chemically synthesized and annealed. pCMV5 vector was linearized with *Apa*I and *Hind* III. The annealed oligos were inserted into the vector to construct RNAi plasmid (psh1-survivin). After transfection of pEGFP-survivin into drug sensitive MCF-7 cell, the drug resistance measured by MTT and P-glycoprotein mRNA expression which play important role in multidrug resistance measured by RT-PCR were increased markedly. At the same time, the drug resistance and P-glycoprotein expression were decreased distinctly when the psh1-survivin was transfected into drug resistance MCF-7/ADR cell. But the P-glycoprotein inhibitor couldn't suppress survivin expression in MCF-7/ADR cell. The results showed that survivin played crucial role in multidrug resistance in cancer cell, which maybe directly or indirectly modulate P-glycoprotein or other MDR-related gene, but the reversed modulation way haven't been found in our experiment. It suggest that the survivin gene was responsible for adriamycin-resistant of MCF-7/ADR cell, which made the cell to avoid being damaged induced by chemical drugs through stimulating P-glycoprotein overexpression.

***Keywords*:** survivin; multidrug resistance; MCF-7; RNAi

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Regulation of microtubule-dependent protein transport by the TSC2/mTOR pathway

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Protein transport plays a critical role in the interaction of the cell with its environment. Recent studies have identified TSC1 and TSC2, two tumor suppressor genes involved in tuberous sclerosis complex (TSC), as regulators of the mTOR pathway. Cells deficient in TSC1 or TSC2 possess high levels of Rheb-GTP resulting in constitutive mTOR activation. We have previously shown that the TSC1/TSC2 complex is involved in post-Golgi transport of VSVG and caveolin-1 in mammalian cells. Here, we show that modulation of mTOR activity affects caveolin-1 localization, and that this effect is independent of p70S6K. Tsc1- and Tsc2-null cells exhibit abnormal caveolin-1 localization that is accompanied by disorganized microtubules (MT) in the subcortical region. Analyses of GFP-tagged EB1 and tubulin in live mutant cells suggest a failure of the plus-ends to sense cortical signals and to halt MT growth. Down-regulation of CLIP-170, a putative mTOR substrate with MT-binding properties, rescued the abnormal MT arrangement and caveolin-1 localization in the Tsc2-/- cells. Together, these findings highlight a novel role of the TSC2/mTOR pathway in regulating MT-dependent protein transport.

***Keywords*:** trafficking; Rheb; CLIP-170; Caveolin-1; rapamycin

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The effects of anti-human VEGF hairpin ribozyme on angiogenesisand growth of hepatocarcinoma cells *in vitro* and Xenografted tumor *in vivo*

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Angiogenesis, the development and proliferation of new blood vessels, is critical for the growth of tumors. Among numerous angiogenesis factors, vascular endothelial growth factor (VEGF) plays an important role. Based on high expression of VEGF in hepatocarcinoma, we designed hairpin ribozyme aiming at the third exon of human VEGF to explore the effect of anti-human VEGF hairpin ribozyme on biological characteristics of hepatocarcinoma cell line SMMC-7721 and xenografted tumor of nude mice. The

hairpin ribozyme were synthesized and subcloned into an eukaryotic express vector pcDNA3.1+. After transfected into hepatocarcinoma cells (abbreviated as pcDNA3.1+/RZ cells) by limpofectamine, the pcDNA3.1+/RZ cells were screened by G418 and identified by RT-PCR. ELISA and MTT assay were carried respectively to examine the expression of VEGF and cell proliferation, then the cell cycle phases and apoptosis were analyzed by flow cytometry. SMMC-7721/RZ cells and SMMC-7721/PC cells (SMMC-7721 cells transfected with empty vector) were inoculated into nude rice, to observe the changes of tumor volume and weight. Microvessel density and VEGF expression were conducted by immunochemistry. The results indicated that the recombinant eukaryotic express plasmids pcDNA3.1+/RZ and the transgenic cell line SMMC-7721/RZ were successfully obtained. The level of VEGF mRNA and protein decreased dramatically in SMMC-7721/RZ cells when compared with SMMC-7721/PC cells or SMMC-7721 cells. The expression level of VEGF protein in SMMC-7721/RZ cells was 363.64±19.68 ng/L, whereas, in SMMC-7721 cells and SMMC-7721/PC cells were 1358.69±49.81 ng/L and 1369.57±32.61 ng/L respectively. The growth rate of SMMC-7721/RZ cells was obviously lower than that of SMMC-7721/PC cells and the SMMC-7721 cells. The apoptosis rate in SMMC-7721/RZ cells attained to 11.0%. However, in control groups, no apoptosis peak was found. Furthermore, VEGF expression and the microvessel density of the xenografted tumor were obviously declined by the ribozyme. The formation and growth speed of xenografted tumor decreased. Compared with SMMC-7721/PC group(8.6±3.3d), the formation time of xenografted tumor in SMMC-7721/RZ group was 17.2±5.7d. At the fifth week after cancer cells were inoculated into nude rice, the volume of tumor in SMMC-7721/RZ group and SMMC-7721/PC were 0.19±0.0085m^3 and 0.59±0.019cm^3, the weight were 0.26±0.076g and 0.74±0.050g respectively. We conclude that in hepatocarcinoma, anti-VEGF hairpin ribozyme can inhibit VEGF expression and cell proliferation, induce cell apoptosis, reduce angiogeneisis, and slowed down growth speed of xenografted tumor. This study provided an experimental evidence for hepatocarcinoma gene therapy.

Keywords**:** VEGF; hairpin ribozyme; hepatocarcinoma; apoptosis

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LAPTM4B plays critical roles in tumerigenesis of human cells by activating several signaling pathways

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Lysosomal-associated protein transmembrane-4 beta (LAPTM4B) is a novel gene that we cloned (AC: AY057051) and characterized. LAPTM4B is remarkably up-regulated in hepatocellular carcinoma (HCC) with very high frequency (87.3%) and the altitudes of upregulation are proportional to the pathological grades of HCC at either mRNA or protein level. Subsequently, It has been found by other laboratories that LAPTM4B is also over-expressed in various cancers, including lung cancer (88%), adrenocorticotrophin (ACTH)-secreting adenoma and non-functional pituitary adenoma (NFPAs), gallbladder cancer and breast cancer etc. Here we report our recent progress on functions of LAPTM4B. Over-expression by transfection or knockdown by RNAi of LAPTM4B exerts promotive or suppressive effects, respectively, on cell proliferation, migration and invasion; and also enhances or reduces drug efflux from cancer cells. Moreover, expressions of some oncogenes and cyclins are up-regulated or down-regulated, and some tumor suppressor genes are down-regulated or up-regulated by LAPTM4B transfection or RNAi, respectively; Meanwhile some signaling pathways, including Ras and PI3K etc, are activated or attenuated, respectively, by transfection or RNAi. Importantly, the LAPTM4B over-expressed L02 cells by infection of Ad-LAPTM4B showed potential ability in colony formation in soft agar and cancerogenesis when inoculated in nude mice. In conclusion, LAPTM4B plays critical roles in tumerigenesis of human cells by activating several signaling pathways and may be applied as a target in therapeutic intervention in cancer.

Keywords**:** LAPTM4B; tumorigenesis; signaling; oncogene; tumor suppressor gene

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Morphological experimental research of R-Phycoerythrin β subunit as photosensitizer on mouse cancer treatment

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Photodynamic therapy (PDT) is a non-invasive therapeutic modality for the treatment of superficially localized tumor. A photosensitizer, in most case hemotoporphrin derivatives, is mixture of unknown components with multiple absorption peaks in the visible light, strong skin phototoxicity, and no specific target to tumor. Phycobiliprotein

R-phycoerythrin (R-PE), a sun light harvesting protein, is prepared from sea alga, it consists of 3 subunits: α, β and γ subunits. β-subunit has not only better PDT effect but also fluorescence activity, there are only two absorption peaks of R-PE β subunit in visible light area, the higher are located in 498 nm and 550 nm. Whereas, there are multi-absorption peaks in photofrin II between 250 nm ~ 650 nm, the higher absorption peaks are located in 250 nm ~ 450 nm. *In vitro* test, when treated with photofrin II and R-PE β subunit in S180 cell and mouse marrow cell, irradiated with iodine-tungsten lamp as light source, the survival rate of the cells were analyzed with MTT method, photofrin II showed stronger phototoxicity than β subunit in this condition. Therefore, we choose β-subunit as photosensitizer in follow experiment. To study the effects and mechanisms of PDT of R-PE β subunit on mouse cancer treatment. The S180 tumor bearing mice were firstly treated with 100 $\mu g \cdot ml^{-1}$ β subunit, irradiated with different dose of Argon laser, than the tumor cells inhibited by PDT were morphological observed with β subunit penetrability of its fluorescence, acridine orange staining and TEM. The PDT effect of β subunit related to irradiation dose of laser and the volume of tumor, the complete remission dose of irradiation is 200 $J \cdot cm^{-2}$. It can be clearly seen that β subunit posses good penetrability in tumor cells than in normal tissues. Otherwise, cell apoptosis is a typical property both in acridine orange staining and TEM observation. PDT using R-PE β subunit is a promising treatment for S180 tumor cell *in vivo*. Tumor death is a combined result of direct cell killing, injured tumor blood vessels, anti-inflammation reaction of leucocyte and inducing of cell programmed death in nuclei.

***Keywords*:** PE-β subunit; fluorescence; PDT; TEM; cell apoptosis

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Identification of transthyretin acted as a potential serous biomarker for cholangiocarcinoma

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As SELDI-TOF-MS (Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry) has been broadly used to screen biomarkers for variety of diseases, the identification and validation of the revealed biomarkers focused more attention. In this paper, the serum samples from 60 cholangiocarcinoma, 49 benign diseases of hepatobiliary and 53 normal individuals were analyzed by SELDI-TOF-MS. Among a set of differential proteins automatically selected as specific biomarkers by Biomarker Wizard software, three protein peaks, with the molecular weight 13.71, 13.83 and 13.99kDa, were found significantly decreased in cholangiocarcinoma samples. The candidate proteins were carefully picked up from the 1-D gels by matching the molecular weight (MW) of the corresponding obvious differential bands to the MW ranges where the SELDI peaks were presented. The bands were cut and subjected to the ESI-MS/MS analysis. Three proteins, native transthyretin (TTR), cysteinylated transthyretin (cysTTR) and glutathionylated transthyretin (glutTTR) were thus revealed to be responsible for these three peaks respectively. These preliminary results were further proven by Western blot and immunoprecipitation using commercial TTR antibodies. This allowed us to re-measure the TTR levels in all the groups more simply by ELISA assay. It showed a firm consistency between ELISA and SELDI analysis. In addition, while TTR level in cholangiocarcinoma were found lower than that in normal healthy control, TTR level in benign diseases of the hepatobiliary system were found higher than that in healthy control. Therefore, TTR could be a biomarker that better discriminates cholangiocarcinoma patients from the benign diseases compared to other biomarkers presently available.

***Keywords*:** cholangiocarcinoma; TTR; serum biomarkers; SELDI-TOF-MS

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Expression and regulation of G protein-coupled receptor 56 in human pancreatic cancer cells

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Pancreatic cancer represents a serious human health problem. The etiology of this disease, however, is barely understood. Although numerous studies have demonstrated that G protein-coupled receptors (GPCRs) regulate the development of various tumors, only few reports have related GPCRs to human pancreatic cancer. G protein coupled receptor 56 (GPR56) is a newly discovered orphan GPCR.

Based on its unusually long extracellular region, GPR56 has been speculated to play a role in cell adhesion. It has been reported that GPR56 was overexpressed in human gliomas, and down-regulated in human melanoma. Here, we report the expression and regulation of GPR56 in PANC-1 human pancreatic cancer cells. Unlike conventional localization of GPCRs on cell plasma membrane, the majority of GPR56 was localized in the cytoplasm of PANC-1 cells and presented in a pattern of scattered spots. When PANC-1 cells were treated with protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA), significant level of GPR56 was translocated to the plasma membrane and co-localized with the actin microfilaments and vinculins on the cell edge, suggesting that cytosolic GPR56 may be directed to newly formed focal adhesion sites to support cell migration. Further analysis indicated that the total level of the GPR56 protein was not changed after PMA treatment, suggesting that activation of PKC signaling induced the redistribution of GPR56 from the cytoplasm to the plasma membrane. Taken together, our results suggest that GPR56 may play a role in the migration of pancreatic cancer cells, and this function is regulated by the PKC signaling pathway.

Keywords**:** GPR56; pancreatic cancer; PKC; migration; GPCR

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Human F-LANa, a member of the Derlin protein family, promotes NIH3T3 cells transformation through regulating the expression and activities of cancer-related genes in Wnt pathway

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Human F-lana, a member of the Derlin protein family and initially cloned by our group, was previously shown to be up-regulated in human liver cancer and had a role in growth control of hepatoma cells. In this study, we find that F-lana functions as an oncogene in transformed NIH3T3 cells. Our results show that the F-LANa expressing vector transfected NIH3T3 cells grow 20% quicker than parent NIH3T3 cells. In addition, F-LANa expression in NIH3T3 cells promotes their anchorage-independent growth in soft agar and tumorigenesis in nude mice. These results indicate that F-lana is a putatitve proto-oncogene and has a direct role in oncogenic transformation. To study the mechanism of F-LANa-induced tumorigenesis, we compared the gene expression profiles of parent NIH3T3 cells with NIH3T3 cells that over-expressed F-LANa. The gene expression analysis using cDNA microarray indicated that 252 genes were up-regulated and 354 genes were down-regulated in F-LANa over-expressed NIH3T3 cells. Among these differentially expressed genes, twenty genes were classified as oncogenes/proto-oncogenes including Wnt and the members of its signaling pathway, while thirteen genes were classified as tumor-suppressor genes/putative tumor suppressors including Rb and relatives. In conclusion, the data suggest the transformation potential of F-LANa both in vitro and in vivo. The up-regulated expression and activity of cancer-related genes in Wnt pathway in F-LANa over-expressed NIH3T3 cells may contribute to the oncogenic effect of F-LANa.

Keywords**:** F-LANa; transformation; oncogene/proto-oncogene; tumor suppressor; mouse oligo cDNA expression assay

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Chromosomal imbalances in lung squamous cell carcinomas detected by comparative genomic hybridization

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Chromosomal abnormalities were detected by comparative genomic hybridization (CGH) in 32 cases of lung squamous cell cacinoma (SqCas) and their correlations with clinical and pathologic staging were analyzed. We also focus on the chromosome11q13 which show high DNA copy number gain. We investigated two candidate genes CCND1 and EMS1 on chromosome 11q13 by differential PCR, their expression examined by RT-PCR and IHC. Nonrandom chromosome DNA copy number changes were similar between gains and losses in 32 tumor samples, which were 5.75±2.20 and 5.22±2.35, respectively. Frequent chromosomal amplification was 3q(23/32), 11q13(12/32), 5p(11/32), 12p (9/32) and 8q(8/32). Chromosomal deletions were most prevalent at 1p(17/32), 3p(15/32), 17p(13/32), 9p(13/32), 21q(10/32) and19(9/32). 12 cases showed both loss of the entire 3p and gain of almost the whole 3q.CCND1 amplification occurred in 8 out of 32(25%)cases and 6 involved 11q13 amplification. EMS1 amplification was identified in 7 out of 32(21.9%) cases, 5 with 11q13 amplification. Coamplification of both genes occurred in 4 of all cases (4/32, 12.5%). Overexpression of CCND1 was observed in 6 out of 32 cases by RT-PCR and 7 cases by

IHC, no overexpression of EMS1 was observed.Genomic imbalances especially gains of 3q, 11q13q, 5p, 12p, 8q; and deletions of 1p32-ter, 3p, 17p, 9p and 19 were a characteristic feature of lung squamous cell carcinoma, which provided candidate regions for potential oncogenes and tumor suppressor genes related to SqCas, to which further molecular studies should be addressed. The amplification and overexpression of CCND1 may contribute to the gain of chromsome11q13.

***Keywords*:** lung squamous cell cacinoma;chromosomal alterations;comparative genomic hybridization; CCND; EMS1

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Inhibiting cell growth and integrin β4 expression in A549 lung cancer cells by (6-tert-butyl-3, 4-dihydro-2H-benzo[b][1, 4]oxazin-3-yl) methanol (TBM)

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Previously, we found that integrin β4 was strongly expressed in A549 cells. But, it does not express in the normal type II secretory lung cells. In this study, our purpose is to depress the expression of this integrin by using small molecule so as to understand the possible role of integrin β4 in controlling A549 cell growth. Our previous study showed that （6-tert-butyl-3,4-dihydro-2H-benzo[b][1,4] oxazin-3-yl）methanol (TBM) inhibited A549 lung cancer cell growth. However, whether it affects the level of integrin β4 is not known. Here, we further investigated the effects of TBM on the cell cycle distribution and the expressions of integrin β4 and P53. The results showed that TBM could suppress integrin β4 expression, elevate P53 level and make the cells partly arrest at G1 phase. The data suggested that TBM might perform its proliferation inhibitory effect on A549 cell growth via down regulating integrin β4, up regulating P53. Our data indicated that integrin β4 might be an important factor in controlling A549 lung cancer cell growth.

***Keywords*:** A549 cells; integrin β4; P53; TBM; proliferation inhibition

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Cyclin D1 repression of NRF-1 integrates nuclear DNA synthesis and mitochondrial function

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The cyclin D1 gene encodes a regulatory subunit of the holoenzyme that phosphorylates and inactivates the pRb tumor suppressor protein to promote nuclear DNA synthesis. Cyclin D1 is overexpressed in human breast cancers and is sufficient for the development of murine mammary tumors. In cyclin $D1^{-/-}$ mice, both mitochondial size and activity were increased. This result was validated using siRNA to cyclin D1 *in vitro* and cyclin D1 anti-sense transgenic mice. Global gene expression profiling and functional analysis of mammary epithelial cell-targeted cyclin D1 anti-sense transgenics demonstrated cyclin D1 inhibits mitochondrial activity, and aerobic glycolysis *in vivo*. Mitochondrial transcriptional factor A (mtTFA) and mitochondrial nuclear respiratory factor 1 (NRF-1) are key regulators of mitochondrial DNA synthesis and function. Cyclin D1 repressed expression of mtTFA, NRF-1 and cyclin D1 inhibited D-loop transcriptional activity. NRF-1, which induces nuclear-encoded mitochondrial genes, was transcriptional repressed and the activity was inhibited by cyclin D1. Cyclin D1 levels and NRF-1 expression were inversely correlated during cell cycle progression. In addition, NRF-1- and cyclin D1-regulated genes were inversely correlated by microarray expression profiling. Cyclin D1 associated with NRF-1 *in vivo* by immunoprecipitation and in mammalian two-hybrid assays. Screening for the potential phosphorylation site of NRF-1 demonstrated that cyclin D1-dependent kinase phosphorylated NRF-1 at S47. Cyclin D1 abundance thus coordinates nuclear DNA synthesis and mitochondrial function.

***Keywords*:** cyclin D1; Mitochondria; nuclear respiratory factor 1

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Induction of differentiation and histone acetylation of HL-60 cells by Diallyl disulfide in SCID mice

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In previous studies, diallyl disulfide(DADS) induced differ-

entiation in HL-60 cell *in vitro*. In the present work, induction of differentiation and the level of histone acetylation of HL-60 cell by DADS was investigated *in vivo*. Methods: Differentiation was Studied by pathological examination and cycle of HL-60 cells in SCID distribution was monitored by flow cytometric. The level of histone H3, H4 and the expression of p21WAF1 were measured by Western blot. Results: HL-60 cells from mice treated with DADS were blocked G1 phase, from 25.4% to 63.4%(P 0.05), which was used as positive controls. Conclusion: DADS could induce the differentiation of HL-60 cells in SCID mice by increased accumulation of acetylated histone H3 and H4 and the expression of p21WAF1.

***Keywords*:** diallyl disulfide; HL-60/SCID mice models; histone atylation; p21WAF1

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Functional investigation of prostate specific G-protein coupled olfactory receptor

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Olfactory receptors (ORs) build the greatest superfamily of G-protein coupled receptors. They are expressed individually in the neurons in the olfactory epithelium where they detect volatile substances. Besides their expression in the olfactory epithelium, ORs are shown to be expressed in other tissues, such as testis where they may play an important role in sperm development, chemotaxis and oocyte-sperm interaction. We cloned and functionally expressed a human OR, which was named prostate specific G-protein coupled receptor (PSGR) due to its reported expression in prostate epithelial cells. We employed Ca^{2+} imaging in HEK293 cells to identify ligands for the recombinant expressed receptor and characterized the molecular receptive field. As PSGR was found to be overexpressed in prostate cancer, we investigated whether the identified ligands also induce Ca^{2+} influx in a prostate cancer epithelial cell line (LNCaP). In addition to ligand stimulated Ca^{2+} increase, treatment of LNCaP cells resulted in a time dependent activation of members of the MAPK family. The ligand was also found to be a potent inhibitor of cell proliferation and an inducer of apoptosis. Similar results were obtained using primary prostate cancer cells from resection specimens. Together these results suggest that ligands for PSGR could be developed as novel therapeutic agents for the treatment of prostate cancer. In our study we will further try to find out which signal transduction mechanisms are involved in OR mediated Ca^{2+} influx in prostate epithelial cells.

***Keywords*:** olfactory receptor; prostate cancer; proliferation; apoptosis

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Epitope mapping of an anti-ErbB2 antibody A21 and its tumor inhibitory mechanism

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Anti-ErbB2 antibodies with distinct epitopes have different biological functions. Our previous studies have developed a tumor-inhibitory anti-ErbB2 monoclonal antibody A21. Its engineered chimeric antibody showed the potentials for therapy of ErbB2-overexpressing tumors. Here we reported a precise epitope mapping of A21 by combinatorial utilization of phage display, domain expression and mutagenesis scanning. The results show that A21 recognizes a conformational epitope comprising a large region mostly from ErbB2 extracellular subdomain I, which is quite different from the other two antibodies, Herceptin and 2C4 using in the clinic. It suggested that the A21 epitope should be another valuable target for designing new anti-ErbB2 antibody therapeutics. Furthermore the determination of the X-ray crystal structure for the A21 single-chain antibody (scFv) fragment at 2.1 Å and molecular docking of the A21 scFv-ErbB2 complex provided more critical contact residues involved in the binding interface. Investigation of A21 inhibit activities on tumor cell growth by ErbB2 internalization, phosphorylation and expression of downstream signal proteins also demonstrated its unique functional properties. The anti-proliferative activity of A21 may be associated with its ability to promote the formation of inactive ErbB2 homodimers and thus downregulate ErbB2 signals. These data provide insight into molecular mechanism of development the engineering A21 antibody for therapeutic applications.

***Keywords*:** epitope mapping; ErbB2; antibody; structure; mechanism

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Validation of ovarian cancer biomarkers in the MAPK signaling pathway

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Biomarkers discovered through high-throughput proteomic profiling of clinical samples require further validation. Establishment of direct involvement of such biomarkers in known cancer-related signaling pathways could provide evidence for their role in the disease process and directions for future hypothesis-driven research. We have previously reported the discovery and identification of a number of potential biomarkers for the detection of ovarian cancer and followed with large-scale independent validation studies using multi-center clinical samples. More recently, we used immunoprecipitation pull-down followed by mass spectrometry (IP/MS) to analyze changes in specific subproteomes of interest. Activation of mitogen-activated protein kinase (MAPK) occurs in response to various growth stimulating signals and as a result of activating mutations of the upstream regulators, KRAS and BRAF, which can be found in many types of human cancer. There is also evidence that an activated MAPK pathway is critical in tumor growth and survival of ovarian tumors with KRAS or BRAF mutations. In the current experiment, we selected a few proteins from our previously reported biomarkers and applied IP/MS to monitor their expression levels in ovarian tumor cells with known mutations in either KRAS or BRAF. More specifically, we monitored the changes in expressions of these proteins over a time course in the cells with and without being treated by CI-1040, a compound that specifically inhibits MEK, an upstream regulator of MAPK and thus prevents MAPK activation. We discovered that one of the biomarkers was noticeably expressed in the ovarian tumor cells harboring an activating mutation in BRAF and its expression was almost completely suppressed with MAPK deactivation by CI-1040. In conclusion, we have established tentative evidence that links one of the biomarkers that we discovered though proteomic profiling of clinical samples to changes in the MAPK signaling pathway. In presentation, we will provide the identity of the biomarker and additional experiment results.

***Keywords*:** biomarker; ovarian cancer; MAPK pathway; proteomics; tumor marker

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Glioma cancer stem cells promote tumor angiogenesis through vascular endothelial growth factor

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Malignant gliomas are highly lethal cancers dependent on angiogenesis. Critical tumor subpopulations within gliomas share many characteristics with neural stem cells. We examined the potential of Stem Cell-Like Glioma Cells (SCLGC) to support tumor angiogenesis. SCLGC isolated from human glioblastoma biopsy specimens and xenografts potently generated tumors when implanted into the brains of immunocompromised mice, whereas non-SCLGC tumor cells isolated from only a few tumors formed secondary tumors when xenotransplanted. Tumors derived from SCLGC were morphologically distinguishable from non-SCLGC tumor populations by widespread tumor angiogenesis, necrosis and hemorrhage. To determine a potential molecular mechanism for SCLGC in angiogenesis, we measured the expression levels of a panel of angiogenic factors secreted by SCLGC through angiogenesis antibody array. In comparison to the majority of tumor cells, SCLGC consistently secreted markedly elevated levels of vascular endothelial growth factor (VEGF), which were further induced by hypoxia. In an *in vitro* model of angiogenesis, conditioned medium from SCLGC population significantly increased endothelial cell migration and tube formation compared with non-SCLGC tumor cell-conditioned medium. The pro-angiogenic effects of glioma SCLGC on endothelial cells were specifically abolished by an anti-VEGF neutralizing antibody bevacizumab, which is in clinical use for cancer therapy. Furthermore, bevacizumab displayed potent anti-angiogenic efficacy *in vivo* and suppressed growth of xenografts derived from SCLGC but limited efficacy against xenografts derived from a matched non-SCLGC population. Together these data indicate that stem cell-like tumor cells can be a crucial source of key angiogenic factors in cancers and that targeting pro-angiogenic factors from stem cell-like tumor populations may be critical for patient therapy.

***Keywords*:** cancer stem cell; angiogenesis; VEGF; glioma; brain tumor

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Experimental studies on As4S4 induced Hela cell apoptosis and molecular mechanism

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While arsenic has long been known as a poison and environmental carcinogen, its dramatic effect in the treatment of acute promyelocytic leukemia (APL) has made its mechanism of action a topic of intense interest. Realgar is one kind of arsenics, and Tetra-arsenic tetra-sulfide (As_4S_4) is a major component in realgar. In recent years, a series of studies had been undertaken *in vitro* and *in vivo*, and demonstrated that As_4S_4 is effective in the treatment of cancer such as Acute Promyelocytic Leukemia (APL). But it is not reported in cervical cancer. Cyclooxygenase-2 (COX-2) has been identified to have a close relation with tumor genesis, there is abundant documented evidence of elevated expression of COX-2 in colon tumors and a variety of other malignancies. The resultant high level Prostaglandin E(2) (PGE-2) production may play an important role in cell proliferation, modulation of apoptosis, angiogenesis, inflammation and immune surveillance. This study was designed to investigate the Hela cells growth inhibition and apoptosis induced by As4S4 and its relationship with COX-2 and PGE2. Methods: Hela cells were treated with various concentrations (7.5, 15, 30, 60 mmol/L) of As4S4 at different hours (12h, 24h, 36h, 48h, 72h). Cell growth was measured by MTT, apoptosis was detected by double staining flow cytometry (FCM). Levels of PGE2 was measured by Radioimmunoassay. The expression of COX-2 protein was also examined by Western blot analysis. Results: After treated with different concentrations (7.5, 15, 30, 60 mmol/L) of As4S4 at different hours (12h, 24h, 36h, 48h, 72h), the Hela cell growth was suppressed significantly with dose-and time-dependent manner. The IC50 of 24 h was 30 mmol/L (P<0.01). The As4S4 could inhibit the activity and expression of COX-2 in Hela cells. Conclusions: As4S4 could inhibit the proliferation and increase apoptosis of human Hela cell lines in dose-and time-dependent manner. These effects may depend on the inhibition of the expression of COX-2 and the inhibition of level of PGE2 by As_4S_4.

***Keywords*:** As_4S_4; Cyclooxygenase-2; PGE-2; Hela cell; apoptosis

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Rotating clinostat simulated microgravity inhibits breast cancer cell line MCF-7 invasion, adhesion and gelatinase production

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To investigate the effects of clinostat-simulated microgravity on tumor cell invasion, adhesion and gelatinase production. Breast cancer cell line MCF-7 was rotated in horizontal plane as a model of simulated microgravity. MCF-7 cells were grown for 24h and then rotated for 24, 48 and 72h, respectively. After being rotated, the abilities of MCF-7 cells adhesion to ECM proteins such as fibronectin (FN), laminin (LN) and collagen IV, the invasive abilities of MCF-7 cells and gelatinase production were detected. The invasive and adhesive abilities of MCF-7 cells decreased significantly after being rotated for 48h and 72h. MMP-2 production descended significantly, meanwhile, CD147, FAK and vinculin expression also showed a digressive tendency after being rotated for 48h. The results indicate that rotating clinostat simulated microgravity inhibits MCF-7 cells invasion, adhesion and MMP-2 production. These results also strongly support the utility of the rotation technology as an effective ground-based model for identifying key steps in tumor cell metastasis.

***Keywords*:** simulated micrgravith; breast cancer; invasion; adhesion; gelatinase

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Effect of GHGKHKNK on suppression of cancer metastasis both *in vitro* and *in vivo*

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High molecular weight kininogen (HK) is well known to be a cofactor in the intrinsic pathway of blood coagulation cascade. HK consists of six domains in which domain 5 (D5H) has cell-binding sites and a negatively charged surface-binding site. It suggests that D5H or peptide fragments in D5H have relationship with cell adhesion and invasion. Method: (1) D5H recombinant protein is expressed and P-5 (HKHGHGHGKHKNKGK), P-5m (GHGKHKNK) and P-5n (KHGHGHGK) are synthesized. (2) The effects of peptides on mice B16-F10 malignant melanoma cells metastasis and inhibition are assayed. And it is identified

that peptides inhibit VN-mediated haptotaxis and haptoinvasion of MG-63 cells. Results: Cell adhesion activity is expressed as percent of the control level. D5H, P-5, P-5n and P-5m inhibit cell adhesion by 57, 48, 31, and 40% of the control level at concentrations of 2 μM D5H and 400 μM peptides. And they inhibit cell invasion by 66, 54, 40, and 50% of the control level, respectively. While 500μg of each peptides and 2×10^5 B16-F10 cells are co-injected into the tail vein of each mouse. After 14 days, the presence of P-5m results in a reduction of colony formation on the surface of the lung. Conclusion: From results above, it shows P-5m might be responsible for inhibition of metastasis and invasion of tumor. But the mechanism should be elucidated further.

Keywords**:** GHGKHKNK; suppression; cancer; metastasis

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A pathway of heneration of aneuploid cells

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Most cancers are aneuploid, but how these aneuploid cells generated remained unknown. Although mutations in cell cycle regulators or spindle proteins can perturb chromosome segregation, the causes and consequences of spontaneous mitotic chromosome nondisjunction in human cells are not well understood. Here we show that chromosome nondisjunction is tightly coupled to regulation of cytokinesis in human cell lines, such that nondisjunction results in the formation of a binucleated rather than two mononucleated aneuploid cells. We observed that spontaneously-arising binucleated cells exhibited chromosome nondisjunction rates up to 166-fold higher than the overall mitotic population. Long-term imaging experiments indicated that most binucleated cells arose through a bipolar mitosis followed by regression of the cleavage furrow hours later. Nondisjunction occurred with high frequency in cells that became binucleated by furrow regression, but not in cells that completed cytokinesis to form two mononucleated cells. More than half (54.8%) of mitoses showing chromosomal bridges during ana-telophase completed cytokinesis, which indicates bridging chromosomes are not sufficient for the regression of cleavage furrow. In p53 functional binucleated cells, 40% entered mitosis with 35% of which underwent multipolar mitosis. In p53 deficient binucleated cells, 90% entered mitosis with 94% of them undergoing multipolar mitosis. FISH (fluorescence in situ hybridization) studies demonstrated that almost all the daughter cells of multipolar mitosis are aneuploid cells. Our findings indicate that nondisjunction does not directly yield aneuploid cells, but rather tetraploid cells which may subsequently become aneuploid through further division. The coupling of spontaneous segregation errors to furrow regression provides a potential explanation for the prevalence of hyperdiploid chromosome number and centrosome amplification observed in many cancers.

Keywords**:** aneuploidy; tetraploidy; nondisjunction; cytokinesis; real time imaging

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Assesment the frequncy of N-RAS gene mutations in codons 12 , 13 and 61 in patients with acute myeloid leukemia

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The pathogenesis of acute myeloid leukemia (AML) involves the cooperation of mutations promoting proliferation/survival and those impairing differentiation. Activation of the RAS pathway plays a major role in hematological malignancies such as AML and MDS. We have screened 60 *do novo* AML patients before starting chemotherapy from Tehran Shariati hospital, predominantly younger than 40 years using PCR- restriction fragment length polymorphism (RFLP) analysis for the presence or absence of N-RAS gene mutations in codons12, 13 and 61. The mean age was 30 years (14 to 57 years). 58% of the patients were male (35/60) and 42% were female (25/60). N-RAS mutations were confirmed in 20% of patients (12/60). Seven of the patients in whom the mutation was found were male and 5 were females. Eight patients with a mutation had more than 40 years and 4 patients were less than 40 years. The frequency for mutations were in N12 (15%), N13 (11.6%) and N61 (5%). In 5 patients a mutation was coexisted in codons 12 and13, and 2 patients simultaneously showed a mutation in codons 12 and 61. 33.3% mutations were found to happen in AML-M4, 25% in each AML-M2 and AML-M5 and 16.7% in AML-M3 FAB subtypes. We could not detect any mutation in AML-M0, M1, M6 and M7. In general, the frequency of the mutations we found is in agreement with the results of other studies. However, we suggest a study with more patients and a wider age range.

Keywords**:** acute myeloid leukemia; N-RAS gene; PCR-RFLP; mutations; frequency

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Dynamics of cancer-correlative P^{53}-MDM2 feedback loop regulated by P^{19}ARF

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The p53 tumor suppressor protein plays a key role in preventing the development of cancer and is inactivated in many human malignancies. Mutations in the p53 tumor suppressor gene occur in about 50% of human tumors. In response to genomic stress, p53 activation may elicit cell-cycle arrest or apoptotic cell death, as well as contribute to DNA repair processes. Because some of the cellular effects of activated p53 can be irreversible, keeping p53 function under tight control in normal cells is critical. A key player in the regulation of p53 is the Mdm2 protein. This duality defines a negative feedback loop, which is widely recognized. P19ARF is another important new tumor suppressor protein ,it has its own independent promoter .The P19ARF protein can increase the level of p53 by neutralizing Mdm2 which destabilize p53,ultimately play a role in suppressing cancer. During the years, several models addressing p53 in the context of statistical theories of multistage tumor igenesis have been proposed. We now present a dynamical model of the p53-Mdm2 feedback loop regulated by P19ARF both in individual cell and in population of cells. In our attempt to capture the gross mechanisms of p53-Mdm2 interactions regulated by P19ARF, we have investigated numerically how different parameters can shape the types of behavior that the system can exhibit. In particular, we show that specific assumptions characterizing the interactions between p53 and Mdm2 regulated by P19ARF lead to an oscillatory behavior of p53, Mdm2 and p19 protein levels after a sufficiently strong damage signal. Such oscillation may enable the more effective execution of a reversible p53 response. In agreement with this prediction, the levels of three proteins are proved to satisfactorily fit experimental results reported in lung cancer cells. The dynamical model of cancer correlative P53-MDM2 feedback loop regulated by P19ARF and its numerical results will help to understand the origin of cancer and oscillatory behavior of p53, Mdm2 and P19ARF more deeply and rationally.

***Keywords*:** cancer; p53; MDM2; P19ARF; dynamics

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Functional genomic analysis of breast cancer metastasis

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Metastasis represents the most devastating stage of cancer progression. We applied a multidisciplinary approach to analyze the molecular basis of breast cancer metastasis, combining functional genomics tools with animal models and advanced *in vivo* imaging technologies. Human MDA-MB-231 breast cancer cells form typical osteolytic bone metastases when inoculated into the arterial circulation of athymic mice. Highly bone metastatic subpopulations were isolated using *in vivo* selection to generate differential gene expression profiles. We identified a gene set whose expression pattern is associated with, and functionally promotes the formation of metastasis to bone but not other tissues. Many genes in this group encode secretory or cell surface proteins implicated in cell homing to bone, angiogenesis, invasion and osteoclast recruitment, thus influencing the tumor microenvironment in favor of metastasis. The clinical significance of some of these genes in breast cancer bone metastasis was validated using tissue or serum specimens from breast cancer patients. Using similar strategy, metastasis genes associated with other tissue tropisms, such as lung and liver, are currently been identified and investigated in our lab and others. Although genomic studies of metastasis have identified a series of gene expression signatures that are associated with metastasis phenotypes, the global regulatory network underlying these tissue-specific metastasis gene profiles remained largely unexplored. We combined a series of novel computational approaches with *in vivo* and *in vitro* functional studies to identify and analyze the metastasis regulatory programs (MRPs) that link the emergence of metastasis gene expression profiles with altered activities of transcriptional factors and other regulators of cellular physiology. We used biclustering analysis to identify synexpression gene groups according to their expression concordance in 46 variants of MDA-MB-231 breast cancer cell line. The co-expressed genes that play significant roles in metastasis profiles are further identified by gene set enrichment analysis. Subsequently, transcription factor binding site enrichment analysis of promoter sequences was performed to identify the master regulator of metastasis-related synexpression groups. The function of putative MRPs in metastasis are currently been validated by functional studies of genetically modified human cells in mouse models of cancer metastasis and by clinical correlation assessment in human breast cancer samples. Results from this study will not only enrich our

understanding about the molecular mechanism of metastasis, but also provide key targets for developing novel therapeutic and diagnostic approaches.

***Keywords*:** mouse model; metastasis; genomics; systems biology

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Induction of apoptosis by Buckwheat trypsin inhibitor in HL-60 cells

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In recent years, the incidence of various tumors and hematopathies has remained high, whereas the number of anti-tumor drugs is still comparatively low. To find new and effective drugs for treatment of various tumors, much research has been carried out. Recent interest has been focused on protease inhibitors due to their unique role as anti-apoptosis and they are now being developed into targeted anti-tumor polymeric agents. Buckwheat is an ancient and specialty grain in China. Due to its unique chemical and bio-activity components, buckwheat has been found to have many uses in food products and medicine. However, very little is known about the toxicity of protease inhibitors from buckwheat. Previously, a high-purity recombinant buckwheat trypsin inhibitor was obtained by cloning, expression and one-step affinity purification, and the analysis of inhibitory activity showed that the recombinant buckwheat trypsin inhibitor could strongly inhibit trypsin in specific activity assays. Here, the possible effects of a recombinant buckwheat trypsin inhibitor on the induction of apoptosis of the human HL-60 cell line were investigated. Apoptosis of the HL-60 cell line induced by inhibitor was identified by MTT (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assays, DNA fragmentation and electrophoresis, flow cytometric analysis and morphological observation of nuclei. The results showed that the inhibitor could specifically inhibit the growth of human chronic myeloid leukemia Hl-60 cells at a low concentration (12.5–100 μg/ml) of target protein in a dose-dependent manner, but there were minimal effects on normal human peripheral blood mononuclear cells. The results indicated that recombinant buckwheat trypsin inhibitor can induce apoptosis of HL-60 cells and that it might be a potential protein drug of the trypsin inhibitor family. This indicated that recombinant buckwheat trypsin inhibitor is highly toxic to Hl-60 cells. This may have potential applications in the prevention or treatment of certain tumors.

***Keywords*:** trypsin inhibitor; apoptosis; Buckwheat; HL-60 cells

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Transcriptional activity and specificity of the human telomerase catalytic subunit (hTERT) gene promoter could be increased by the SV40 enhancer

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Telomerase is a ribonucleoprotein complex of which the function is to add telomeric repeats to chromosomal ends. Telomerase consists of two essential components, the telomerase RNA template (hTR) and the catalytic subunit (hTERT). hTERT is expressed only in cells and tissues positive for telomerase activity, i.e., tumor and fetal cells. Objective: To construct the tumor-specific and high efficient expression vector driven by hTERT promoter modified by SV40 enhancer in various telomerase positive cancer cells. Methods: The hTERT promoter of 260bp was amplified with polymerase chain reaction (PCR) method from the genomic DNA. After DNA sequencing with correct result, the hTERT core promoter was inserted into luciferase reporter vectors (pGL3Basic and pGL3Enhancer) to reconstruct the recombinant plasmids named pGL3hTP and pGL3hTP-SV40. Then the pGL3hTP, pGL3hTP-SV40, pGL3-Control and pGL3-Basic separately with pRL-TK were transiently transfected into cancer cell lines HT-29, SW-620, MGC-803 and human fibroblast cell line MRC5. Among them pGL3-Basic taken as negative control, pGL3-Control taken as positive control and pRL-TK taken as inner reference control. The transcriptional activities of hTERT promoter and hTERT-SV40 promoter system in various cells were determined by measuring the luciferase activities after 48 hours of transfection. The value of pGL3-control/pRL-TK is taken as 100% and the other values are relative percent of it. Results: Electrophoresis demonstrated that cloned hTERT promoter was about 260bp, and DNA sequencing showed a same sequence as registered in GenBank. The recombinant plasmids pGL3hTP and pGL3hTP-SV40 were confirmed by double restricted enzemy digestion and PCR method with correct results. The activity of different promoters(CMV, hTP, hTP-SV40)were assessed in tumor cell lines and one normal cell line by luciferase assays. However, the hTERT-SV40 promoter activity was about 2-3 fold higher than the hTERT promoter in tumors

cell lines and remained very weakly active in normal cell line. Conclusions: These results show that the hTERT promoter and the SV40 enhancer might be used for targeted cancer gene therapy. The hTERT–SV40 promoter-enhancer may drive tumor targeting gene therapy as a potential tumor specific promoter system.

***Keywords*:** hTERT promoter; SV40 enhancer; transcriptional activity; luciferase

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Role of histone acetylation in the differentiation induced by Diallyl disulfide in human gastric cancer MGC803 cells

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To investigate the change of histone acetylation level in the differentiation induced by diallyl disulfide (DADS) in human gastric cancer MGC803 cells. Methods: Morphologic observation, bearing tumor experiment and Western Blot which measured the histone acetylation and related protein p21WAF1 were used to elucidate the possible mechanism of DADS-induced MGC803 cells differentiation in vitro. Results: morphologic observation indicated morphology changed and atypia significantly decreased when treated with DADS in MGC803 cells. The experiment of bearing tumor on Blab/C nude mice proved the formation of xenograft tumor declines obviously after MGC803 cells were treated with DADS in vitro. By western blot, the acetylation level of histone H3 was elevated after treated by DADS at 6h, and the maximum effect was higher 38 % than untreated cells at 24h. Meanwhile, the expression of p21WAF1 also increased, but the acetylation level of histone H4 did not changed in MGC803 cells. Conclusion: DADS-induced differentiation of gastric cancer MGC803 cells possibly involved in the increase of histone H3 acetylation and the expression of p21WAF1.

***Keywords*:** diallyl disulfide; gastric cancer MGC-803 cells; histone acetylation; p21WAF1

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Establishment and evaluation of human leukemia HL-60 cells model in SCID mice

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To establish human acute promyelocytic leukemia HL-60 cells Model in severe combined immunodeficient (SCID) mice and to study the biological behaviors of HL-60 cells in SCID mice. Methods: SCID mice were transplanted by intraperitoneal route(ip) and tail vein (iv) injection with 5×10^6 HL-60 cells respectively. Leukemic cells were detected by chromosome karyotype analysis and histopathologic methods. Results: HL-60 cells could perform tumors in the SCID mice via both ip and iv route. Tumors were grow fastly in those animals through ip injection than iv injection and those animals through ip injection had a longer survives. Conclusion: The degree of proliferation and infiltration of leukemia in SCID mice model could reflect its basic biologic feature. The HL-60/SCID mice model is a useful model for tumor reseach *in vivo*.

***Keywords*:** leukemia; HL-60 cells; SCID mice; animal models

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Establishment of the model of apoptosis initiation phase in human leukemia HL-60 cells induced by Diallyl disulfide

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To establish the apoptosis initiation model in human leukemia HL-60 cells induced by diallyl disulfide (DADS) and investigate its molecular mechanism regulating the apoptosis. Methods: After incubation of HL-60 cells with 3.6 $mg\cdot L^{-1}$ DADS, The effects of DADS in HL-60 cell growth inhibition were estimated by growth curve, flow cytometry method was used to determine the induction of apoptosis and the expression actived caspase-3, DNA agarose electrophoresis was used to determine the induction of apoptosis, and protein activity of caspase-3, Bcl-2 was tested by western-blotting. Results: Growth curve show the growth of HL-60 cells could not be inhibited at d 1, but remarkably reduced the growth rate at d 2 to d 6 (24.1%, 36.5%, 44.2%, 52%, 53.6%), Flow cytometry analysis showed that the apoptotic rate of DADS treated cells at d 1 and d 2 was 3.1%, 4.3%， with no difference from control cells (3.0%), while from d 3 to d 5, the apoptotic rate was 8.5%, 15.2%, 27.4% respectively. Have great difference from control cells. the DADS-withdrawal group from d 2 to d 5 the apoptotic rate was 7.9%, 12.4%,16.5%,18.8% respectively, significantly increased than the control

group and the 1 day with DADS withdrawal (P<0.05). the expression of actived caspase-3 from d 2 to d 5 was 6.3%,10.0%,10.4%,14.9%,17.3% respectively, was higher than control group and DADS treated 1 day (P<0.05), DNA agarose electrophoresis was showed the DADS treated group from d 4 and the DADS-withdrawal group from d 2 had DNA ladder, and western-blotting showed caspase-3 was upregulated and Bcl-2 was downregulated from d 2.

***Keywords*:** DADS; HL-60 cells; initiation; apoptosis; model

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Proliferation inhibition of Diallyl disulfide on human colon cancer SW480 cells Xenografts in nude mouse

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To investigate the effect of human colon cancer SW480 cells proliferation capability *in vivo* and its oncogenicity by diallyl disulfide (DADS), and to explore the preventive and therapeutic effects of DADS on colon cancer xenografts in nude mice and its mechanism. Methods: 20 nude mice were randomly divided into 4 groups: control group, therapeutic group, prevention group and treatment group in vitro. Treated 48 h by DADS, SW480 cells were collected and inoculated into nude mice. The second day, 5 mice at random as prevention group, and medicated through abdominal cavity, the rest 10 mice was distributed into control group and therapeutic group in same method. Nude mice's body weight and transplant tumor's growth were observed; transplant tumor's morphologic change was analyzed by optical microscope and electron microscope. Distribution of cell cycle was analyzed by flow cytometry. Immunocytochemical staining and morphometric quantitative analysis detected the expression of PCNA, p53, p21WAF1, Bcl-2 and Bax. Results: SW480 cell xenograft tumor modle was successfully established. After treated by DADS *in vitro*, the rate of tumor formation was about 40%, prevention group was 60%, whereas the rate of control group was 100%, the volum and weight of transplant tumor were significantly less than that of control group (P<0.05). Conclusion: DADS markedly decreased the oncogenicity of human colon cancer SW480 cells, and has preventive and therapeutic effect. DADS has significant proliferation inhibition of human colon cancer SW480 cells *in vivo*. DADS can induce SW480 cells blocked in G2/M, and induce it apoptosis. Its mechanism may relate to downregulated expressions of p53, PCNA, Bcl-2 and upregulated expression of p21WAF1 and Bax.

***Keywords*:** diallyl disulfide; colon cancer SW480 cells; proliferation inhibition; cell cycle; nude mice transplantable tumor

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Traditional Chinese medicinal empirical formula Wenxiafang might increase chemotherapeutic sensitivity of A549/DDP cell by down-regulating expression levels of P-gp, LRP and MRP

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The decrease of chemotherapeutic sensitivity of cancer cells brought many difficulties for cancer treatment. Our previous mouse model studies proved that the traditional Chinese herbal medicine wenxiafang compound, composed of *Panax ginseng* C.A.Mey, RheumpalmatumL, *Aconitum carmichaeli* Debx, obviously inhibited the proliferation of malignant S180 and H22 tumor tissues, and significantly increased the tumor-bearing mouse' sensitivity to chemotherapeutic agent cyclophosphoacylamine. To further investigate underlying mechanism, we studied the effect of this medicine on A549/DDP cells *in vitro* with serum pharmacological method. A549/DDP cells were incubated with normal mouse serum or different concentrations of wenxifang medicated serum, (serum isolated from normal mouse fed with wenxiafang) added DDP, respectively. Cell viability was assayed by MTT, and levels of lung resistance protein (LRP), multidrug resistance associated protein (MRP) and P-glycoprotein (P-gp) in cells were evaluated by immunofluorescence technique, Laser Scanning Confocal Microscope and flow cytometry. Results of this study showed that wenxifang medicated serum decreased the viability of A549/DDP cells stimulated by DDP 50 % ~ 30 % in a dose-dependent way, at the same time, significantly suppressed expression levels of P-gp, LRP and MRP in the cells in the same way. Our results demonstrated that wenxiafang might increase chemotherapeutic sensitivity of A549/DDP cell by down-regulating expression levels of P-gp, LRP and MRP.

***Keywords*:** traditional Chinese medicine wenxiafang; chemotherapeutic sensitivity; lung resistance protein (LRP); multidrug resistance associated protein (MRP); P-glycoprotein

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Biochemical characterization and therapeutic significance of a recombinant chlorotoxin-like neurotoxin from the Chinese scorpion to the human gliomas cancer

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The nucleotide sequence of a type of chlorotoxin-like peptide, an inhibitor of small-conductance Cl- channels, from the scorpion, Buthus martensii Karsch, was synthesized (named rBmK CTa) according to the sequence optimized for codon usage in *E. coli*. It was over-expressed in *E. coli* BL21 (DE3) using a pExSecI expression system. According to the characteristics of the pExSecI expression system, the IgG-binding domain-ZZ of protein A is fused to the N-terminal of rBmK CTa. The fusion protein, ZZ-rBmK CTa, was expressed in soluble form (7.8 mg/l) and was purified to give a single band on SDS-PAGE. The domain-ZZ of fusion protein ZZ-rBmK CTa was removed by cleavage of an Asn-Gly peptide bond with hydroxylamine. The rBmK CTa was separated from the IgG-binding moiety by a second passage through the IgG affinity column. Acute toxicity assay in mice demonstrated that the rBmK CTa had a LD50 value of 4.3 mg/kg. Human glioma cells (SHG-44) were used for examining the cytotoxicity of rBmK CTa. The results of MTT assay showed that rBmK CTa inhibits the survival of glioma cells in a dose-dependent manner, and the IC50 value was approximately 0.28 μM while as high as 8 μM on normal astrocytes, which demonstrated that rBmK CTa had specific toxicity against glioma cells but not astrocytes. Polycolonal antibodies to the purified protein were raised in rats. The titer of this antibody, estimated by ELISA assay, was about 1:7000. Overlay assay and pull-down assay showed that this toxin specially binds to two proteins in the glioma cells (SHG-44) with corresponding molecular weights of about 80 kDa and 35 kDa. They may serve as candidate receptors or alternative cellular component for interaction with rBmK CTa. Whole-cell patch-clamp recording showed chloride current in SHG-44 was inhibited by rBmK CTa in a voltage-dependent manner and percent inhibitions for the blocking action of rBmK CTa (0.07 μM and 0.14 μM) on ICl was 17.64±3.06% and 55.86±2.83%, respectively. But this inhibition was not presented in kalium current and sodium current, which was identical with the predicted function based on its sequence homology with chlorotoxin. Histological analysis showed that brain, leg muscle and cardiac muscle were the target organs of this toxin. The uptakes of rBmK CTa in muscle was most likely due to the up-regulation of votage-gated chloride channels (CLC family) in these tissues. In conclusion, the findings presented in this study are essential for the further exploration of this peptide. It represents an approach for developing a novel therapeutic agent and a potential clinical treatment of human gliomas cancer.

***Keywords*:** chlorotoxin-like peptide; human glioma cells (SHG-44); chloride current; target organs; therapeutic agent

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Loss of Runx3 expression in gastric carcinoma

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Runx3 is a new tumor suppressor gene on chromosome 1p36.1. To investigate the role of the *Runx3* gene in the development of gastric carcinoma, we examined a series of stomach cancer for the expression of *Runx3*. A total of 45 primary gastric cancers were analyzed. Loss of *Runx3* protein expression was found in 48.87% of gastric carcinoma specimens. The expression of *Runx3* was obviously lower in gastric carcinoma than in normal stomach. In addition, we found a high correlation between promoter hypermethylation of *Runx3* and Runx3 protein expression. Thus, the main mechanism that made *Runx3* loss expression was promoter hypermethylation. Our study suggested that a lack of *Runx3* function was causally related to the genesis and progression of human gastric cancer. *Runx3* gene may be involved quite frequently in gastric tumorigenesis. Our data could be used to develop diagnostic and targeted therapy of stomach cancer in future studies.

***Keywords*:** gastric cancer; *Runx3*; mutation; methylation

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Expression of PIG11 protein in human tissue and corresponding tumor tissue

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The aim of this study was to investigate the expression of PIG11 (p53-induced gene 11) protein in human body normal tissue and corresponding tumor tissue, discussing the distributing characteristic of PIG11 protein in human

body normal tissue and its function in the tumorigenesis and development. Methods: The expression of PIG11 protein in 324 cases of human body normal tissues and corresponding tumor tissues was determined by immunohistochemical peroxidase-conjugated streptavidin (SP) method. Results: It was positive expression of PIG11 protein in human gastric mucosa, intestine mucosa, liver, galactophore, and squamous epithelium tissue; however, its expression was negative in human fibre, fat tissue, smooth muscle, lymph tissue and brain tissue. Expression of PIG11 protein was descent in liver cancer, poorly differentiated stomach adenocarcinoma, poorly differentiated intestine adenocarcinoma, breast cancer, comparing with those of corresponding normal tissues (P<0.01); and its expression was low in squamous carcinoma too, comparing with normal squamous epithelium (P<0.05). It was lower that expression of PIG11 protein in cervical part of normal gastric mucosa gland than its caudomedial part and bottom. Conclusion: PIG11 protein is mainly existed in human gastric mucosa, intestine mucosa, liver, galactophore, and squamous epithelium tissue; however, it has no positive expression significantly in human fibre, fat tissue, smooth muscle, lymph tissue and brain tissue. Expression of PIG11 protein was down regulation significantly in epidermic malignant tumors tissues.

Keywords**:** PIG11; immunohistochemistry; human normal tissue; tumor

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Roles of caveolin-1 in murine hepatocarcinoma cells lymphatic metastatic potential *in vivo*

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Caveolin-1 is the major protein component of caveolae, flask-shaped invaginations found in a number of different cell types. Previous results indicated that caveolin-1 is a growth-inhibitory protein that may act as a tumor-suppressor. However, recent research showed expression of caveolin-1 is positively correlated with some tumor cells grade and progression stage. Using an model of spontaneous murine hepatocarcinoma lymphatic metastasis, caveolin-1 expression levels were higher in Hca-F cells than Hca-P and Hepa1-6 cells which have character of high lymphatic metastatic potential, low lymphatic metastatic potential and no lymphatic metastasis potential respectively. And caveolin-1 expression was not detectable in Hepa1-6 cells. We analysized the role of caveolin-1 in murine hepatocarcinoma cell lymphatic metastatic potential through overexpression of caveolin-1 in Hepa1-6 cells and RNAi expression of caveolin-1 in Hca-F cells. Results indicated overexpression of caveolin-1 in Hepa1-6 cells increased cells growth and transformation ability, suppression of caveolin-1 expression in Hca-F cells led to cells transformation and lymphatic metastatic ability decreased *in vitro* and *in vivo*. This study provides the first functional evidence that caveolin-1 regulates primary hepatocarcinoma growth and spontaneous metastasis of hepatocarcinoma.

Keywords**:** caveolin-1; mouse; hepatocacinoma cells; stable expression; metastasis

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Cyclin D1 repression of NRF-1 integrates nuclear DNA synthesis and mitochondrial function

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The cyclin D1 gene encodes a regulatory subunit of the holoenzyme that phosphorylates and inactivates the pRb tumor suppressor protein to promote nuclear DNA synthesis. Cyclin D1 is overexpressed in human breast cancers and is sufficient for the development of murine mammary tumors. In cyclin $D1^{-/-}$ mice, both mitochondial size and activity were increased. This result was validated using siRNA to cyclin D1 *in vitro* and cyclin D1 anti-sense transgenic mice. Global gene expression profiling and functional analysis of mammary epithelial cell-targeted cyclin D1 anti-sense transgenics demonstrated cyclin D1 inhibits mitochondrial activity, and aerobic glycolysis *in vivo*. Mitochondrial transcriptional factor A (mtTFA) and mitochondrial nuclear respiratory factor 1 (NRF-1) are key regulators of mitochondrial DNA synthesis and function. Cyclin D1 repressed expression of mtTFA, NRF-1 and cyclin D1 inhibited D-loop transcriptional activity. NRF-1, which induces nuclear-encoded mitochondrial genes, was transcriptional repressed and the activity was inhibited by cyclin D1. Cyclin D1 levels and NRF-1 expression were inversely correlated during cell cycle progression. In addition, NRF^{-1-} and cyclin D1-regulated genes were inversely correlated by microarray expression profiling. Cyclin D1 associated with NRF-1 *in vivo* by immunoprecipitation and in mammalian two-hybrid assays. Screening for the potential phosphorylation site of NRF-1 demonstrated that cyclin D1-dependent kinase phosphorylated NRF-1 at S47. Cyclin D1 abundance thus coordinates nuclear DNA synthesis and mitochondrial function.

***Keywords*:** Cyclin D1; mitochondria; nuclear respiratory factor 1

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An experimental study on the antineoplastic activity and induce-cell apoptosis activity of the fraction from cortex periplocae and periplocin

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Recently, the antineoplastic effects by extracting effective components from Chinese herbs is becoming hot spot. We extracted four different fractions by different technology from Cortex Periploca, and isolated one periplocin with a purity of more than 98% from fraction C (PSBF-C). The purpose of this research is to identify the active fraction, primarily study the active fraction and investigate the antineoplastic mechanisms of the active fraction and periplocin. Methods: Human hepatocellular carcinoma SSMC-7721 cells were cultured in regular condition. MTT assay was used to evaluate the antineoplastic activity of different fractions of Cortex Periplocae. Using the morphologic method, antineoplastic activity fraction of Cortex Periplocae was detected and the morphology change of SSMC-7721 after the use of PSBF-C and periplocin was observed. Flow cytometry Annexin V-FITC / PI was used to analyze the apoptotic effects of PSBF-C and periplocin on SSMC-7721 cells. ELISA was applied to investigate the releasing level of cytochrome C from mitochondria and Caspase-3 chluorometric assay was performed to test the activity of Caspase-3. Results: MTT results showed that PSBF-C had antineoplastic activity apparently. PSBF-C can inhibit the proliferation of MDA-MB-231, B16 and LA795 cell lines with 50% inhibition (IC50) value 45.26±6.48, 117.65±27.60, 125.60±58.63 (μg/ml) respectively. And PSBF-D also had some antineoplastic activity, but the effect of PSBF-A and PSBF-B were not obvious. After the used of PSBF-C and peripocin, the cell change greatly: under the inverted microscope, after medication, the tumor cell membrane was broken, some of the cell became round, cell membrane shrinkage. PSBF-C and periplocin can inhibit SSMC-7721 *in vitro* time or dose dependently. The IC50 of PSBF-C, periplocins were 18.86±5.45, 0.69±0.33 (μg/ml) respectively (relevant coefficient r>0.9). Flow cytometry results showed that apoptosis rate was increased when the concentration of PSBF-C and periplocin increased or when the action time increased. PSBF-C or periplocin can time-dependently promoted the release of cyt.C from mitochondria into cytosol, especially at 6h. The activity of caspase-3 was increased especially at 12h. Conclusions: The active fraction that has antineoplastic activity of Cortex Periplocae is PSBF-C. PSBF-C and periplocin both can inhibit proliferation of SSMC-7721 cells through the apoptosis induction in a dose and time dependent manner. Breakdowning mitochondrial membrane stability, promoting the release of cyt.C and the succedent activation of caspase-3 may be considered to be one of reasons that PSBF-C and periplocin exert an inductive effect to the apoptosis of SSMC-7721 cells.

***Keywords*:** cortex periplocae activate fractions; periplocin; antineoplastic activity; cell apoptosis

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Study on anti-cancer long-term effect of cytokine-based varrier frug

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To investigate the long-term inhibitory effect of co-immobilized cytokines on the growth of Hela cells, IFN-γ and TNF-α were co-immobilized on cell culture polystyrene using photoimmobilization with concentration of 20 ng /well. At the same time, cells were incubated between 1 and 6 d with serum-free medium in the presence or absence of IFN-γ and TNF-α. Cell counting and flow cytometry were used to determine the inhibitory rate of co-immobilized cytokines on HeLa cells. We found that co-immobilized cytokines could inhibit HeLa cells growth in a time-dependant manner, reaching 92% inhibitory rate at d 5. However, the inhibitory rate reached the maximum at d 3 after treatment with free cytokines, and decreased to 41% at d 6. The chromatin of Hoechst33258 stained cells and the immunohistochemistry for Bax, Bcl-2 and P53 appeared typical apoptosis in a time-dependent manner, indicating that co-immobilized IFN-γ and TNF-α exert continuously apoptosis-inducing effect on HeLa cells. The changes of caspase-3 activity indicated that the macromolecule drug-induced apoptosis of HeLa cells was not the same as free-drug induced apoptosis, not only via caspase pathway. Cell cycles studies also indicated that the apoptosis mechanism between the co-immobilized cytokines and the free ones may be different. Our study show that co-immobilized IFN-γ and TNF-α have significant activity of inducing HeLa cells apoptosis, and stable duration of drug activity.

***Keywords*:** TNF-α; IFN-γ; Photo-immobilization; HeLa cells; caspase-3

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Molecular detection and biological significance of human B-cell lymphomas induced by EB virus in Hu-PBL/SCID mice

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The Epstein-Barr virus (EBV) is involved in the carcinogenesis of several human cancers such as nasopharyngeal carcinoma and lymphomas. Mechanisms of EBV-associated tumor development are incompletely understood. We have successfully induced human B-cell lymphomas in the body of hu-PBL/SCID chimeras by EBV. The aim of this study was to investigate molecular characterization and biological significance of EBV-induced lymphomas. Human peripheral blood lymphocytes were isolated from healthy volunteer donors and were transplanted intraperitoneally into SCID mice, and then hu-PBL/SCID mice were infected with EB virus. IgG concentrations in the serums of SCID mice were measured by unidirectional immunodiffusion assay. EBER-1 in tumor tissues was detected with *in situ* hybridization. Immunohistochemical staining was used to examine EBV gene (LMP1, EBNA2, BZLF1) products and cellular oncoproteins (p53, C-myc, Bcl-2 and Bax). Mutation of p53 gene exons 5-8 in EBV-induced lymphomas was analyzed by PCR-SSCP. The present experiment showed that 24 cases of tumors developed in 34 mice. Observation of histopathology and immunohistochemistry demonstrated that all of the induced tumors in SCID mice were malignant lymphomas derived from human B-lymphocytes. There was DNA of EB virus in tumor tissues by PCR amplication. *In situ* hybridization exhibited resultant tumor cells had EBV encoded small RNA-1 (i.e. EBER-1). Immunohistochemisty showed positive staining of LMP1, EBNA2 and ZEBRA in small number of tumor cells. Human IgG could be found in the serum of SCID mice on the 15th day after hu-PBL engraftment, and then increased with time and with the development of induced tumors in 6 mice. Immunohistochemically detectably p53 protein expression is common (83.3%), but p53 gene mutations have been identified in only four cases (16.7%) of those EBV-induced human lymphomas using PCR-SSCP. Positive rates of C-myc, Bcl-2 and Bax expressions were 100%, 95.8% and 91.7% respectively in 24 cases of the EBV-induced lymphomas. The results indicate that molecular lesions associated with the induced B-cell lymphoma involved EBV infection, expression of oncogenic viral genes, and overexpression of cellular oncogenes in human xenografts. P53 gene mutations are uncommon but p53 is commonly expressed in EBV-induced lymphomas. Human IgG level in the serum of hu-PBL/SCID mice can be considered as a useful index of prediction for oncogenesis and tumor development.

***Keywords*:** Epstein-Barr virus (EBV); induced lymphoma; human IgG; oncogene; hu-PBL/SCID mouse

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Detection and location of *Helicobacter pylori* in human gastric carcinomas

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The epidemiologic and clinical studies have shown that *Helicobacter pylori* infection is closely associated with human chronic gastritis and gastric cancer. The aim of this study is to define the infection status of H pylori in 109 patients with gastric cancers and *H. pylori* localization in gastric carcinoma tissues in South china. The incidence of *H. pylori* infection in gastric carcinomas was estimated by polymerase chain reaction (PCR); simultaneously, both morphological features and the localization of *H. pylori* in gastric carcinomas were demonstrated by Warthin-Starry (WS) staining. The relationships between *H. pylori* infection and the clinical-pathologic factors of gastric carcinomas were analyzed by software SPSS10.0. The results showed that *H. pylori* was found in 42 (39.03%) and 58 (53.21%) cases of 109 patients with gastric carcinomas by PCR and WS, respectively. *H. pylori* infection rate detected in gastric carcinomas by WS was higher than that by PCR ($P<0.01$). WS stain showed that *H. pylori* existed in the gastric antrum mucus, mucosal gland of normal tissues adjacent to gastric carcinomas, and the gland, mucus pool of cancer tissues. The positive rate of *H. pylori* in normal tissues adjacent to carcinomas was higher than that in cancer tissues ($P<0.01$). No significant differences in age, sex, site, histological types and lymph node metastasis were found between *H. pylori*-positive gastric carcinomas and *H. pylori*-negative cases by both methods, but there were statistically significant differences of *H. pylori* positive rate between early and advanced stage of gastric carcinomas ($P=0.033$ or $0.015<0.05$). These findings suggested that *H. pylori* infection might play a certain role in the early stage of carcinogenesis of human gastric mucosa epithelia.

***Keywords*:** gastric cancer; *Helicobacter pylori*

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Loss of heterozygosity on chromosome 7q31.1 in microdissected human gastric carcinoma and precancerous lesions

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Through analyzing Loss of heterozygosity(LOH) on chromosome 7q31.1 in human gastric carcinoma and precancerous lesions with microdissection， to construct allelic loss mappings and define the minimally lost regions(MLR) on chromosome 7q31.1, further to explore the molecular genetics alteration during the malignant progression of human gastric mucosa and analyze the relationships between LOH and clinicopathological parameters. Methods The gastric carcinoma and precancerous lesion cells were microdissected from paraffin sections. Sufficient and qualified DNA of microdissected cells and corresponding paired normal tissues were extracted by Chelex-100 method. Seven high dense microsatellite markers were used, combined with polymerase chain reaction (PCR) and other molecular biological techniques, to detect the frequencies of LOH of every selected microsatellite site on chromosome 7q31.1 in gastric carcinoma and precancerous lesions, then to map detailed alleic losses and define the minimally lost regions on chromosome 7q31.1. Results The positive rate for LOH study of tumor can be raised by the microdissection method, and Chelex-100 method is one more reliable and effective way for extracting DNA from paraffin embedded tissues. Frequency of LOH on chromosome 7q31.1 in gastric carcinoma tissues achieves 70%(21/30); seven microsatellite markers' frequencies of LOH are D7S2459 10.0%, D7S523 6.7%, D7S2502 23.3%, D7S486 43.3%, D7S480 26.7%, D7S650 26.7%, D7S2486 20.0% respectively. Through analyzing allelic loss mapping on chromosome 7q31.1, we have found that the MLR is between D7S2502 and D7S480. However there is no statistically significant correlation between the LOH at these loci and the clinical parameters such as age, sex, early or advanced stage, cell differentiation's degree, primary lesion's position and lymph nod metastasis respectively (P>0.05). In the gastric precancerous lesions, the 7q31.1 LOH is confirmed to be existed in the intestinal metaplasia of gastric mucosa, but the frequency of LOH is very low. In the atypical hyperplasia of gastric mucosa, frequency of LOH reaches 36.7% (13/30), and the microsatellite marker with the highest loss frequency is D7S480 (23.3%, 7/30). Frequencies of 7q31.1 LOH are significantly different bearing on different degree of atypical hyperplasia of gastric mucosa (P<0.01). Conclusions The MLR on chromosome 7q31.1 in gastric carcinoma is at the region from D7S2502 to D7S480, which suggest that the region probably harbor.

Keywords**:** gastric carcinoma; microdissection; chromosome 7q31.1; loss of heterozygosity; microsatellite marker

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Phosphorylation of Pirh2 by calmodulin-dependent kinase II leads to an increase in p53 stability

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Although the recently identified Pirh2 protein is known as a p53-induced ubiquitin-protein E3 ligase, which negatively regulates p53, the detailed mechanism underlying the regulation of Pirh2 remains largely unknown. Here, we demonstrate that while Pirh2 is mostly detected in the phosphorylated form in normal tissues, it is predominantly present in the unphosphorylated form in majority of tumor cell lines and tissues examined. The phosphorylated Pirh2 is far more unstable than its unphosphorylated form. We further identified Calmodulin as a potential regulator of Pirh2 and showed that Calmodulin-dependent kinase II (CaMK II) is involved in phosphorylation of Pirh2 on residues Thr-154 and Ser-155. Concurrent with CaMK II, which displays high kinase activity in G2/M phase, phosphorylation of Pirh2 was also found to reach its peak at this stage. Phosphorylational status of Pirh2 is found closely associated with p53 stability. Treatment with W-7, a specific Calmodulin antagonist, reduces phosphorylation of Pirh2 and increases polyubiquitination of p53. Together, our data suggest that ratio of phosphorylation/dephosphorylation of Pirh2 may act as a fine turning to maintain the balance of p53-Pirh2 auto-regulatory feedback loop, which facilitates the tight regulation of p53 stability and tumor suppression.

Keywords**:** p53; Pirh2; CaMK II; polyubiquitination; phosphorylation/dephosphorylation

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Inhibition effects of various siRNA on expression of somatostatin gene in gastric cancer cell line BGC-823 and SGC-7901

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To study the inhibition effects of various siRNA on expression of somatostatin(SST) in gastric cancer cell line BGC-823 and SGC-7901. Methods : Four nucleotide sequences of siRNA corresponding to four sites of somatostatin gene were designed. siRNAs were synthesized by in vitro T7RiboMAXTM Expression RNAi System and were transfected into gastric cancer cell line BGC-823 and SGC-7901. Cells were cultured in 24-well plate and harvested at 24h, 48h, 72h respectively. The expression of SST mRNA were detected by in situ hybridization and RT-PCR while the expression of SST protein were detected by immunocytochemistry and western blot. The inhibitory effects of siRNA on tumor cell growth and cell cycle were detected by MTT and FCM respectively. SGC-7901 transfected with siRNA were innoculated into nude mice to build a transplantable animal model which suppress the expression of SST gene and to observe the growth of tumor. siRNA and SST analogue octreotide were injected into local tumor to observe the pathological change. Results: (1) The expression of SST gene were suppressed at mRNA and protein levels with different extent showing both time and concentration dependent manners. The inhibitory effect of siRNA1 was significantly. The best time and concentration may be 80 nmol/l and 72h respectively. (2) DNA gel electrophoresis showed DNA ladder can't be seen after siRNA transfected into gastric cancer cells, which indicated the suppression of SST gene couldn't induce cell apoptosis. However, DNA ladder can be observed in the treatment group with SST analogue octreotide which indicated octreotide could inhibit the multiplication of tumor cells by inducing cells apoptosis. (3) The results in MTT showed that the silencing of SST enhanced tumor cell growth. The growth of tumor cells was inhibited in treatment group with SST analogue octreotide. (4) Cell cycle by flow cytometry indicated the expression of SST gene was suppressed which made cells in G2 phase and S phase increase and cells in G0/G1 phase decrease, which indicated SST suppressed proliferation of tumor cells by inhibiting DNA synthesis and mitosis. Tumor cells apoptosis was enhanced treated with SST analogue octreotide. (5) SGC-7901 cells tansfected with siRNA were implanted into nude mice to build animal model with SST expression suppressed. Its oncogenicity was increased compared with un-transfected group. Conclusion: (1) Four siRNAs synthesized *in vitro* suppressed the expression of SST gene with different extent at mRNA and protein levels, showing both time and concentration dependent manners. siRNA1 for the first target was probably the best. The optimal time and concentration was probably 72h and 80 nmol/l respectively. (2) The silencing of SST gene increased gastric cancer cells growth , suggesting SST gene could inhibit the proliferation of tumor cells by inhibiting DNA synthesis and mitosis of tumor cells. (3) The nude mice experiment *in vivo* showed that the silencing of SST gene increased the oncogenicity of SGC-7901 cells.

***Keywords*:** somatostatin; RNA interference; siRNA

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The reversal effect on MDR1 gene-mediated multidrug resistance in gastric cancer SGC7901/VCR cells by siRNA

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To investigate the reversal effect on mdr1 gene mediated multidrug resistance(MDR) in gastric cancer SGC7901/VCR cells by small interfering RNA (siRNA). Methods: The SGC7901 and SGC7901/VCR cells were cultured in RPMI-1640 medium routinely. The SGC7901/VCR cells were cocultured with 0.5 μg/ml—1.0 μg/ml of vincristine (VCR) for maintaining MDR phenotype followed by cultivation with no VCR for two weeks before use. Four different siRNAs (mdr1si326, mdr1si1513, mdr1si2631 and mdr1si3071) were designed to be homologous to mdr1 cDNA consensus sequence (GenBank accession number NM-000927.3) and synthesized by transcription in vitro. The siRNA duplexes were used to transfect into the human gastric cancer SGC7901/VCR cells. The expression level of mdr1 mRNA and glycoprotein(P-gp) were detected by RT-PCR and Western blotting respectively. The accumulation of intracellular adriamycin (ADR) was examined by flowcytometry and the cell sensitivity to ADR was demonstrated by MTT. Results: Among the SGC7901/VCR cells treated by siRNAs for 48h, the depression of mdr1 mRNA expression in mdr1si326 or mdr1si2631 group cells was larger than that of mdr1si1513 or mdr1si3071 group cells (P<0.05). The expression level of P-gp in mdr1si326 group cells was the lowest among the SGC7901/VCR cells treated by siRNAs for 72h. The targeting sequence of mdr1si326 with best and mdr1si2631 with better efficiency encoded the cross membrane region of P-gp, while those of mdr1si3071 with lower and mdr1si1513 with lowest efficacy encoded intracellular region of P-gp. The mdr1si3071 with lower efficacy had both stalk and loop in itself. Besides, the targeting sequences of mdr1si326 with best and mdr1si2631 with better efficiency had less numbers of base matched pairs or hydrogen bonds in the targeting sites and their surrounding sites. The expression level of mdr1 mRNA become lower in the SGC7901/VCR cells treated with

mdr1si326 at final concentration of 1nmol/L and the lowest at final concentration of 20 nmol/L.There was insignificant difference in effects between 20 nmol/L and 40 nmol/L mdr1si326. Conclusion: The siRNA targeting mdr1 can reverse mdr1 gene mediated MDR in the gastric cancer SGC7901/VCR cells. The reversal effects are associated with sequence-specific, time-dependent and dose-dependent. The efficacy of siRNA to mdr1 gene in human gastric cancer SGC7901/VCR cells had close relationship with local RNA target structure.

Keywords**:** siRNA; multidrug resistance; MDR1 gene; gene silencing

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Involvement of Akt survival signaling pathway during ellipticine-induced apoptosis in human non-small-cell lung cancer cells

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Topoisomerase II inhibitor ellipticine and its analogues have shown promising antitumor activities. In this work, we described the proliferation of human non-small-cell-lung-cancer (NSCLC) cells was affected by topoisomerase II inhibitor, ellipticine, which inhibited the growth of A549 cells in dose- and time-dependent manners. The induced cytotoxicity was proved by apoptotic cell death. More experiments indicated that the cell populations exposed to ellipticine were shifted to S- and G2/M-phases that vanished with prolonged drug treatment. On the other hand, cells at sub-G0 phase appeared progressively, whereas Akt inhibitor impeded progression of cells into sub-G0 state and reverted ellipticine-mediated apoptosis. Ellipticine stimulates transient Akt and Erk activation followed by the increased expression of characteristic apoptosis markers including p53, MDM2 and PARP fragmentation, which were reversed by Akt inhibitor, wortmannin, thereby reversing apoptotic phenotype. The overall results demonstrated that ellipticine-mediated apoptosis in A549 cells is mediated through transient activation of Akt survival signaling.

Keywords**:** apoptosis; Akt; lung cancer cells

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Proteomic analysis of the nuclear matrix proteins binding with the upstream sequence of the p16 gene in gastric carcinoma cells

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To investigate the characteristics of the nuclear matrix proteins(NMPs) in gastric cancer cell line, SGC-7901 and GES-1. Materials and Methods: (1) Extraction and electrophoresis of the NMPs: The NMPs were extracted from both SGC-7901 and GES-1 cell lines. SDS-PAGE electrophoresis and the two-dimentional electrophoresis(2-DE) were performed. (2) Labeling and detection of the DNA fragments: The 890bp fragment including the upstream of exon 1α of p16 gene was obtained in 2.5% agarose gel electrophoresis from the p16 promoter-luc-pGL2-Basic plasmid digested with both EcolR I and Hind III enzymes. The DNA was labelled by using DIG High Prime DNA Labeling and Detection Starter Kit I of Roche. The DNA probe sensitivity was detected by DNA dot blot. (3) The repeatability of two-dimensional electrophoresis (2-DE): The 2-DE of GES-7901 was carried out repeatedly under the same condition. Compared the gels with each other by PDQuest software, the result of homologous maps of 2-DE can be obtained. (4) Southwestern blot: 66 kDa zone in 2-DE was transferred onto the nitrocellulose membrane (NCM) for detection of Southwestern blot. (5) MALDI-TOF-MS and datebase search: The corresponding spots also showed in the gel stained with Coomassic blue and were cut for digestion with trypsin in gel and analysis of MALDI-TOF-MS. The peptide mass fingerprint (PMF) was generated by MALDI-TOF and was analyzed using the MS-Fit search program in the NCBInr.2006.02.16 protein data bank. (6) The normal gastric tissues was performed as control. Results and conclusion: (1) Two distinct reaction spots were found in SGC-7901 cells by 2-DE. The PMFs (peptide mass fingerprints) of No. 1 protein spot was identified to belong to the SFPQ (splicing factor proline/glutamine) family by searching with MS Fit program in NCBInr.21.03.2006 data bank. (2) The protein of 66 kDa isolated from gastric carcinoma cells was the characteristics compared with normal gastric tissues that suggest the protein may be involved in gastric cancer.

Keywords**:** gastric carcinoma; gastric carcinoma; southwestern blot

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Inhibition effects of RNAi on alpha-fetoprotein expression in human FU97 gastric carcinoma cell line

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To construct expressing vector of siRNA in order to inhibit alpha-fetoprotein gene(AFP) in human FU97 gastric carcinoma cell line and study the influence on FU97 cell growth and apopotosis after silencing the AFP gene. Methods: The pSIREN-DNR-DsRed-Express Donor-Vector-AFP-siRNA1/2/3 containing three different qualified target sequences of AFP gene were constructed and transfected into FU97. After transfection 48h, the silencing efficiency of AFP gene expression were analyzed by RT-PCR, chemistry luminescence and immunocytochemistry. The influence on FU97 growth and apoptosis after silencing the AFP gene were observed by MTT and flow cytometry. Results :The results of gene sequencing indicated that the pDonor Vector AFP-siRNA1/2/3 were successfully constructed. After the transfection 48h, the delivery efficiency of these plasmids reached 30%. The results of RT-PCR showed these three vectors could silence the AFP gene while the pDonor Vector-AFP-siRNA1 and pDonor Vector-AFP-siRNA 3 were more effective. There was a statistical significance (P<0.01) between the experiment vectors and the control vector on the expression of AFP. The AFP-siRNA plasmid can inhibit FU97 cell proliferation and induce apoptosis. Conclusion: Down of Alpha-fetoprotein (AFP) by siRNA can directly inhibits proliferation of FU97 and induce its apoptosis.

***Keywords*:** alpha-fetoprotein; RNA Interfering; FU97; human gastric carcinoma

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Apoptosis of human gastric cancer cell line AGS induced by arsenic trioxide

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To study the effects of arsenic trioxide (As_2O_3) on gastric carcinoma cell line AGS and investigate the potential mechanisms of the effects of arsenic trioxide on gastric carcinoma cell line AGS *in vitro*. Methods: The cell activity and morphologic changes were studied after being treated with arsenic trioxide (As_2O_3) at the concentration of 1, 5, and 10 μmol/L, respectively, for 24, 48, 72 h. By means of MTT cell proliferation assay, the effect of As_2O_3 on the inhabitation of AGS cells was measured. Changes of apoptosis and cell cycle distribution were analyzed by flow cytometry. The characteristics of apoptosis were further examined by TUNEL and DNA agarose gel electrophoresis. The expressions of VEGF and STAT3 were determined by flow cytometry and immunohistochemistry. Results: (1) MTT showed that cell proliferation was remarkable inhibition. The inhabitation rate of 10 μmol/ L As_2O_3 on AGS was almost 53.49 % in 48 h. (2) A typical sub-diploid peak before G0/ G1 phase was observed by flow cytometry. The apoptotic index was 2.38 % -7.82 % by FACS assay. (3) The effect of As_2O_3 on AGS showed a remarkable cell cycle specificity, which indicates that As_2O_3 mainly acts in G2/ M phase. (4) However, early apoptosis were not found by Annexin V/ PI methods. (5) Agarose gel electrophoresis showed a specific DNA apoptotic ladder. (6) TUNEL detection analysis revealed the DNA fragmentation. As_2O_3 treated cells presented some typical features of apoptosis such as in tact cell membrane, chromate in condensation, nucleic fragmentation and apoptotic body formation. (7) The expressions of VEGF and STAT3 were up-regulated after treatment with As_2O_3. Conclusion: As_2O_3 could significantly inhibit the proliferation of AGS cells and the inhibitory effect was dose-and-time dependent. As_2O_3 can inhibit human gastric carcinoma in AGS cell line, the induction of apoptosis is a very important mechanism of As_2O_3 to treat gastric carcinoma AGS cells. It has a potential value in the treatment of gastric cancer, and was worth further studies.

***Keywords*:** arsenic trioxide; apoptosis; gastric carcinoma; VEGF; STAT3

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Apoptosis of gastric cancer cell FU-97 induced by arsenic trioxide *in vitro*

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To investigate the inhibition and apoptosis induced by arsenic trioxide (As_2O_3) on gastric cancer cell FU-97 and its associated mechanism. Methods: FU-97 cells were treated with As_2O_3 at the concentration of 1, 5, and 10 μmol/L respectively for three successive days. The cytotoxicity of As_2O_3 on FU-97 was determined by MTT assay. The cell cycle alteration and apoptotic rate of the cells were determined by flow cytometry. The positive rates of FU-97 cells with the expression of AFP, VEGF and STAT3 protein were examined by flow cytometry. Apoptosis of FU-97 induced by As_2O_3 was investigated by TUNEL method, and DNA electrophoresis. Immunocytochemistry method was used to detect the expression of VEGF, STAT3 and AFP of FU-97. AFP protein production in cultural supernatants of FU-97cells was measured by chemistry luminescence. Results: As_2O_3 can inhibit significantly the growth of FU-97 cells and had a remarkable cytotoxic effect on FU-97. The cell-killing rate of As_2O_3 on FU-97 cells was significant as revealed by MTT, and was both dose- and time-dependent. The cytotoxic rate of 10 μmol/L As_2O_3 on FU-97 was almost 37.51% in 48 h. The effect of As_2O_3 on FU-97 showed a remarkable cell cycle specificity, which indicates that As_2O_3 mainly acts in G2/M phase. The apoptotic peak (sub-G1 phase) appeared and cell apoptotic rate was 5.11% after being treated by 10 μmol/L As_2O_3 for 48 h. TUNEL also demonstrated strand breaks in DNA of FU-97 cells, while control cells showed negative labeling group. Gel electrophoresis of DNA from cells treated with each concentration of As_2O_3 revealed a "ladder" pattern, indicating preferential DNA degradation at the internucleosomal, linker DNA section. Chemistry luminescence showed As_2O_3 could down-regulate AFP expression in cultural supernatants in FU-97 cells. Immunocytochemistry and flow cytometry showed under the concentration of 1, 5, and 10 μmol/L of As_2O_3 could obvious decrease AFP, VEGF and STAT3 protein production in FU-97 cells. Conclusions: As_2O_3 can inhibit significantly proliferation of gastric cancer cell FU-97 and induce apoptosis. The mechanism was related to the change of AFP, VEGF and STAT3. It has a potential value in the treatment of gastric cancer, and was worth further studies.

***Keywords*:** arsenic trioxide; apoptosis; gastric tumor; FU-97

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Synergic effects of proteasome inhibitor PS-341 and tyrosine kinase inhibitor STI571 on chronic myeloid leukemia cells and BCR-ABL oncoprotein *in vitro*

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STI571/Gleevec/imatinib, a rationally-designed agent that occupies the ATP-binding site of BCR-ABL and stabilizes the protein in its closed, inactive conformation, has been a remarkable success for the treatment of chronic myeloid leukemia (CML). However, a significant proportion of patients chronically treated with STI571 develop resistance because of the acquisition of mutations in the kinase domain of BCR-ABL. Furthermore, the effects of STI571 on CML patients in accelerated phase or blastic crisis are unsatisfactory since many patients relapse after transient remission. Hence, additional drugs or STI571-based combination regimens are desired to circumvent resistance and to improve response rates. Here we reported that PS-341, a proteasome inhibitor which offers great promise to patients with multiple myeloma (MM), significantly enhanced the antileukemia activity of STI571 *in vitro* and *in vivo*. We found a synergy exists between low concentrations of PS-341 (5-10 nM) and STI571 (0.1-0.2 microM) in inhibition of cell growth and induction of apoptosis in K562 cell line and CD34+ leukemic cells isolated from CML patients. In K562 cells, combined use of PS-341 and STI571 accelerated activation of caspase-3, 9, and facilitated cleavage of poly-(ADP-ribose) polymerase (PARP) as compared to those in cells treated with PS-341 or STI571 alone. Moreover, PS-341/STI571 combination resulted in potentiated degradation of BCR-ABL and downregulation of phosphorylated BCR-ABL as compared to those in mono treatment. In nude mice inoculated subcutaneously with K562 cells, treatment with PS-341 (injected intraperitoneally, ip) alone (at doses of 0.05, 0.5, 1 mg/kg/d, twice a week for 4 weeks, respectively) decreased tumor growth in a dose-dependent manner. STI571 (ip) at 10 mg/kg/d also inhibited tumor growth. Intriguingly, combinatory administration of low dose PS-341 (0.05 mg/kg/d, twice a week for 4 weeks) and STI571 (10 mg/kg/d) yielded a much more profound inhibition of tumor growth and even clearance of leukemic cells in mice compared to either monotherapy. Taken together, these results demonstrate synergic effects of PS-341 and STI571, and provide the rationale to evaluate PS-341/STI571 combination in treating CML aiming to further improve clinical outcome of patients.

***Keywords*:** leukemia; apoptosis; synergy; translational research

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Down-regulation of motility-related protein-1/CD9 gene in gastric cancer

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Gastric cancer is the second most common cause of cancer-related deaths in the world, being the first in China; it is a major cause of mortality. It is generally recognized that the development of gastric cancer arises gradually from premalignant lesions, chronic atrophic gastritis with intestinal metaplasia and dysplasia. With the development of molecular biology techniques, some cDNA fragments which are closely related to the development of human gastric cancer have recently been identified. However, very little is currently known about the genes that may cause a predisposition to gastric cancer. Fluorescent mRNA differential display (FDD) has been widely applied to identify cancer-related genes, a technique which is faster, safer and more cost-effective than the use of procedures that require radioactivity. Motility-related protein (MRP-1/CD9) is implicated in cell adhesion and motility and was shown to be clearly involved in tumor prognosis and angiogenesis. Elevated MRP-1/CD9 expression on tumor cells has been linked to a favorable prognosis of some kind of cancer. OBJECTIVE: To identify genes related to gastric cancer and to analyze their expression profiles in different gastric tissues. METHODS: Total RNA was extracted using TRIzol reagent, followed by treatment of the RNA with DNase Ⅰ. The differentially expressed cDNA bands were assayed by fluorescent differential display from gastric cancer specimens, matched with normal gastric mucosa and premalignant lesions. The cDNA fragments of interest were subcloned into pGEM-T vector and confirmed by *Eco*R Ⅰ digestion. Sequence analysis was performed with a CEQ8000 DNA sequencer. All nucleotide sequence databases maintained by the NCBI were searched for homologous sequences using BLAST. The motility-related protein (MRP-1/CD9) gene expression was studied by Northern blots and reverse transcription polymerase chain reaction (RT-PCR) in different kinds of gastric tissue. The procedure of hybridization was performed according to the DIG Northern Starter Kit's protocol. An antisense cRNA probe labeled with digoxigenin was generated from a digested cDNA insert by means of in vitro transctiption. RESULTS: Direct comparisons of mRNA fingerprints from 3 sets of gastric cancer, premalignant lesions and normal gastric tissues samples were made. In contrast, a 650-bp band, named G1, exhibited higher levels of expression in normal gastric mucosa and premalignant lesions compared to gastric cancer.

***Keywords*:** gastric cancer; mRNA differential display; motility-telated protein-1/CD9 gene; Northern blot

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Hypermethylation combining with loss of heterozygosity is the main mechanism of hMLH1 deregulation in non-small cell lung cancer

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To investigate the main mechanism of hMLH1 inactivation in NSCLC. METHODS: The samples were obtained from 40 patients diagnosed as primary NSCLC. The mean age of the patients was 61.7±5.6 years, 9 of them were female and 31 male. Twenty-eight of them had squamous cell carcinomas, 12 had adenocarcinomas. Sixteen had lymphnode metastasis. Genomic DNA was extracted from frozen samples of lung tumor issue and normal lung tissue using proteinase-K digestion and phenol/chloroform purification followed by ethanol precipitation. The molecular alteration examined included promoter methylation by Hpa II/Msp I-based PCR analysis, loss of heterozygosity (LOH) by D3S1621 locus PCR-electrophoresis-Silver staining, as well as the loss of protein expression by immunohistochemistry analysis. RESULTS: The frequencies of hypermethylation, LOH and loss of protein expression of hMLH1 were 67.5% (27/40), 65% (26/40), 72.5% (29/40), respectively. In 40 cases of NSCLC, there are 29 cases (72.5%) occurred loss of protein expression of hMLH1. The difference with the control group was significant (P<0.05). In this 29 cases, there are 20 cases (69%) of squamous cell carcinomas, 9 cases (31%) of adenocarcinomas. In 27 hMLH1 gene aberrant methylation cases, 26 cases (96.3%) had loss of protein expression of hMLH1, which is significantly higher than the group of un-methylation. (P<0.05). In 26 hMLH1 gene LOH (+) cases, 23 cases (88.5%) had loss of protein expression of hMLH1, which is significantly higher than the group of LOH (-). (P<0.05). In 29 cases occurred loss of protein expression of hMLH1, 21 cases (72.4%) had LOH and aberrant methylation, otherwise, in 11 cases occurred protein positive expression of hMLH1, 7 cases (63.6%) had neither LOH nor methylation. CONCLUSION: Aber-

rant methylation and LOH were related to loss of protein expression of hMLH1. Biallelic inactivation of the hMLH1 gene by hypermethylation and LOH might cause loss of hMLH1 expression and play an important role in the development of NSCLC. Therefore, controlling and monitoring for hypermethylation of the hMLH1 gene may be partially useful for treatment and early diagnosis of NSCLC.

***Keywords*:** hypermethylation; LOH; hMLH1; NSCLC

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Establishment and identification of the high metastatic model of lung of mice histiocytosarcoma L–II

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The present study was declined to establish the high metastatic model of lung of mice histiocytosarcoma L–II by constant subculturing of the cells from the pulmonary metastasis *in vivo*. Methods We subcutaneously injected histiocytosarcoma L–II cells in the flank of mice, then isolate the cells in pulmonary metastasis and injected them in another mouse by the same methods. After several subculturing, we have established the high invasive metastatic strain of mice histiocytosarcoma L–II. We analyze the isolated cells by morphlogy analysis(light microscope, electron microscope), immunohistochemistry, karyotype analysis, flow cytometry, cell growth curve, mean life span with cancer, and the rate of lung metastasis. Results We have obtained the 7th generation now. There are no differences between the 1st and the 7th generation in morphology observation under light microscope. Chromatosome analysis show they are all hypotetraploid，and their modal number ambit are about 66-70. The cell cycles between the 1st and the 7th were: stage G1: 70.1±0.14/47.65±4.31; stage S: 18.55±3.75/28.45±1.06; stage G2: 11.35±3.89/23.90±3.25; respectively. Their DNA ploid were hypotetraploid. Mean life span with cancer was 31.5±6.38/25.75±7.1 day, respectively, (P<0.05). Immunohistochemistry show that 23 nm was weakly positive in the 1st generation and negative in the 7th generation. Also MMP-9 expression were more strong in the 7th generation than the 1st one. Lung metastasis was 30% (6/20)/65% (13/20), respectively; P<0.05. Conclusion We have established a high metastatic model of lung of mice histiocytosarcoma L–II, thus provided a ideal experimental model for the invasion and metastasis of tumor.

***Keywords*:** histiocytosarcoma; invasion; metastasis

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Co-localization of the heat shock protein94 and human immunoglobulin G in hepatocellular carcinoma: suggesting possible existence of an intracellular immune mechanism

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Heat shock protein (Hsp) and human immunoglobulin G (human IgG) are greatly important proteins in the protection of body from hurt of diseases. Recently, Some scientists argue that not only B cell and/or plasma cells but also cancer cells can secret IgG. In our previous work, we found hepatocellular carcinoma tissues or/and cell lines expressed both IgG and Hsp70 at the same time. In this article, we report our recent results that IgG co-localizes with Hsp94 in cancer cells. METHODS: Tissue arrays of 39 cases of hepatocellular carcinoma and 20 normal liver tissues were studied. Liver cancer cell lines included HCC, HepG2 and Smmc7721, and a normal liver cell line was QZG. Immunohistochemistry, both indirect immunoenzymatic method and double labeling Immunofluorescence staining with confocal microscopy scanning were used to detect the co-existance of IgG and Hsp94. RT-PCR was used to amplify the CDR3 region of human immunoglobulinG in 3 hepatocellular carcinoma cell lines. RESULTS: 15of 39 liver cancer tissues expressed Hsp94, and in the 15 positive tissues, 8 expressed human IgG. All of the 3 liver cancer cell lines, HCC, HepG2 and SMMC7721 expressed both Hsp94 and IgG. With double labeling Immunofluorescence staining and confocal microscopy scanning , we found that the Hsp94 and human IgG co-localized in the 8/39 liver cancer tissues and all the 3 liver cancer cell lines. And the RT-PCR proved that the IgG which co-localized with hsp94 was secreted by carcinoma cells themselves. CONCLUSION: The two protective proteins: Hsp94 and IgG co-localized in liver cancer tissues and cell lines . And on the basis of this result and our previous findings that the Hsp70 co-localized with IgG, we proposed a hypothesis that in the inner of cells exist a relatively independent immunity mechanism in which the Hsps and IgG play an important role.

***Keywords*:** heat-shock proteins; human immunoglobulin G; hepatocellular carcinoma; intracellular immune mechanism

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Histone deacetylase inhibitors induce apoptosis in both type I and type II endometrial cancer cells

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Endometrial carcinoma is the most common gynecologic malignancy. Current treatment options for women with serous carcinomas or advanced endometrioid adenocarcinomas generally include cytotoxic agents with associated short disease free intervals. In this report we characterize the activity of two histone deacetylase inhibitors in endometrial cancer cells *in vitro*. Compared to controls, there was a 95% reduction in the growth of Ark2 cells following administration of oxamflatin and this response was dose-dependent. These agents also caused profound morphologic changes and loss of mitochondrial membrane potentials consistent with the induction of apoptosis. Cleavage of PARP, caspase-9, and caspase-8 was detected by Western blot analyses, indicating the activation of apoptotic cascades in endometrial carcinoma cells. This effect is present in both serous and endometrioid cell types, suggesting a potential new therapy for women with this diagnosis.

***Keywords*:** histone deacetylase inhibitors; endometrial cancer; apoptosis

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ROS modulate Bax activation in apoptosis

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Mitochondria play essential role in regulating apoptosis. They receive pro-apoptotic protein signals from the cytosol and subsequently release apoptogenic factors from mitochondria to initiate apoptotic protease cascades for cell execution. Upon death stimuli, Bax, a pro-apoptoic protein of Bcl-2 family, could translocate to and then permeabilize mitochondrial out membranes leading to cytochrome c release. The signals that trigger Bax conformationl changes and subsequent translocation and homo-oligomerization are not clear. Here we showed that ROS modulate Bax translocation and homo-oligomerization by interacting with the cysteine residues. We transfected different Bax mutants (Bax, Bax C62S, Bax C126S, Bax C62/126S) into colon cancer lines and found that ROS can specifically induce wtBax and Bax C126S mutant, but not C62S & Bax C62/126S, to undergo conformational change, translocation to mitochondria and oligomerization. These events are closely associated with downstream execution of apoptosis. Under identical conditions, Bax C62S and Bax C62/126S mutants show no Bax activation and apoptosis. Our data suggest that ROS can directly or indirectly react with Cysteine 62 of Bax to modulate Bax conformational change, translocation and homo-oligomerization.

***Keywords*:** ROS; Bax; apoptosis

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Inhibition of human lung tumor cell proliferation and the mitogen activated protein kinase pathway by dexmethasone

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To investigate the effects of dexamethasone on human lung tumor cell A549 proliferation, cell cycles and cell mitogen-activated protein kinases (MAPKs) pathway. Dexamethasone was used at various concentrations in culture medium. Cell number was counted using a hemacytometer. Whole cell propidium iodide staining and flow cytometric analysis were performed to determine cellular DNA content. MAPK proteins and activation were tested by Western blot analysis with antibodies to extracellular signal-regulated kinase (ERK), phospho- ERK, c-Jun N-terminal kinase (JNK), phospho-JNK, p38 and phospho-p38. 1×10^{-7} mol/L and 1×10^{-6} mol/L dexamethasone suppressed the proliferation of A549 cell to 64% and 85% respectively than that of control. This suppression was dosage related. 5×10^{-7} mol/L Dexamethasone suppressed cell cycle with accumulation of cells in G1/G0 stage. It increased from 74 % to 86 % compared with that of control.

***Keywords*:** dexamethasone; cell cycle; mitogen-activated protein kinase

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APOCB2006-06-064

Regulation and function of vascular endothelial growth factor-C in prostate cancer

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Objective: (1) To understand the molecular mechanism of VEGF-C synthesis in prostate cancer when induced by androgen depletion. (2) To investigate the function of VEGF-C in stimulating metastasis of the prostate cancer cell. Results: Our results suggest a downregulation of the IGF-IR pathway in prostate cancer cells during androgen ablation, leading to the activation of the forkhead transcription factor, FOXO-1. FOXO-1 in turn increases the transcription of VEGF-C. We have also observed an increased production of reactive oxygen species (ROS) during androgen withdrawal in prostate cancer cells. Increased ROS generation activates Ras-like GTPase, RalA, whose downstream signaling events also upregulate VEGF-C mRNA and therefore provide an alternative mechanism for VEGF-C synthesis during androgen ablation. We have detected receptors for VEGF-C, in different prostate cancer cell lines, suggesting a possible autocrine function. We have shown an increase in the expression of the androgen receptor co-activator Bag-1L in LNCaP cells stimulated with VEGF-C. Bag-1L has been reported to enhance the trans-activation function of the androgen receptor with the help of Hsp70. Therefore, a VEGF-C-induced increase in Bag-1L indicates a possible mechanism for androgen receptor trans-activation in low androgen concentrations, leading to the generation of a more aggressive prostate tumor. We have also observed an increase in the cell migration of the androgen refractory LNCaP C4-2 clone in a transwell cell migration assay with wild type recombinant VEGF-C stimulation. These results therefore suggest that VEGF-C stimulation may increase the overall survival and the metastatic propensity of prostate tumor cells. Conclusion: VEGF-C upregulation may be a crucial stimulant for prostate cancer cell metastasis. Because systemic metastasis occurs during the androgen refractory stage of prostate cancer, it is possible that the elevated level of VEGF-C at this stage may be essential for an increase in the survival and overall metastatic propensity of prostate cancer cells.

***Keywords*:** vascular endothelial growth factor-C (VEGF-C); prostate cancer; androgen depletion

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The involvement of lipid rafts in epidermal growth factor-induced chemotaxis of breast cancer cells

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Metastasis is the major cause of morbidity and mortality in cancer. Recent studies reveal a role of chemotaxis in cancer cell metastasis. Epidermal growth factor receptors (EGFR) have potent chemotactic effects on human breast cancer cells. Lipid rafts, organized microdomain on plasma membranes, regulate the activation of many membrane receptors. In the current study, we investigated the role of lipid rafts in EGFR-mediated cancer cell chemotaxis. Our confocal microscopy results suggested that EGFR co-localized with GM1-positive rafts. Disrupting rafts with methyl-b-cyclodextrin (mbCD) inhibited EGF-induced chemotaxis of human breast cancer cells. Supplementation with cholesterol reversed the inhibitory effects. Pretreatment with mbCD also impaired directional migration of cells in an *in vitro* "wound healing" assay, EGF-induced cell adhesion, actin polymerization, Akt phosphorylation and protein kinase Cz (PKCz) translocation. Taken together, our study indicated that integrity of lipid rafts was critical in EGF-induced chemotaxis of human breast cancer cells.

***Keywords*:** lipid raft; EGFR; chemotaxis

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The reactivity of the human genome to chemotherapy

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The chromosomal aberrations in the peripheral blood proved to be the most important biomarker associated with cancer risk. We performed a study on peripheral blood lymphocytes in order to evaluate the cytogenetic and epigenetic changes induced by the ABVD (doxorubicin, bleomycin, vincristine, dacarbazine) chemotherapy regimen in patients with Hodgkin disease. To asses the cytogenetic damage rate we looked for chromosomal aberrations on metaphases chromosomes at cumulative doses of chemotherapy. For the molecular changes we evaluated the epigenetic effects e.g. hipo/hypermethylation of the ABVD regimen on DNA. Thus, 18 blood samples obtained from 2 patients with Hodgkin disease were analysed for cytogenetic and

epigenetic changes. Evidence of a direct link between chromosomal aberrations was found with the number of therapy cycles. The most frequent aberrations were the PCD (premature centromere divisions): 25 % for the first case and 15% for the second case, and the chromosomal fusions: 11% and respectively 17 %. A global imbalance of the methylation pattern was identified with a hypermethylation tendency. Further studies should clarify the epigenetic changes induced by these chemotherapy regimen.

***Keywords*:** ABVD regimen; cancer; chromosomal aberration

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Apoptosis induced by PGD2 in A549 cancer cells is caspase dependent

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Apoptosis is an important mechanism in both the development and homeostasis process which is distinct from necrosis; and mitochondria play a central role in the regulation of apoptosis. Cytochrome c, an apoptosis inducer could be released from mitochondria by numerous stimuli. Prostaglandins (PGs) are synthesized via cyclooxygenase (COX-1 and COX-2) pathway and have been involved in the processes of carcinogenesis and inflammation. Prostaglandin D2 (PGD2), one of the important COX metabolites in a variety of tissues or cells has been found to have significant effects in many physiological functions including platelet aggregation and vasorelaxation. To determine whether PGD2 is related to apoptosis, A549 cells (non-small cell lung carcinoma cell line) were used to treat with different concentrations of PGD2. Results showed that PGD2 induced apoptosis and caused chromatin condensation and DNA fragmentation in A549 cells. The PGD2-induced apoptosis also caused cytochrome c released in A549 cells and demonstrated that PGD2 was related to the process of apoptosis via mitochondria. Furthermore, activation of caspase-3 obtained by PGD2-induced apoptosis revealed that the apoptosis induced by PGD2 treatment is caspase dependent in A549 cells. All these results suggest that PGD2 is related to the regulation of apoptosis in A549 cancer cell line.

***Keywords*:** apoptosis; mitochondria; A549 cells; PGD2; caspase

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Estrogen receptor gene silencing in breast cancer cells

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Introduction: Inhibition of estrogen receptor alpha (ERα) is one of the major strategies for prevention and treatment of breast cancer. However, failure to overcome development of endocrine resistance, arising despite continued expression of tumour ERα, limits this approach. Our aim was to produce a model system to investigate cellular consequences of loss-of-function of ERα, by establishing a breast cancer cell line with a permanent source of small interference RNA (siRNA) to specifically inhibit production of ERα protein. Methods: Three siRNA constructs(pI-III) targeting different sequences of human ERα, and a scrambled sequence, were cloned into the pRNA-U6.1/Neo GenScript vector. MCF7 breast cancer cells were transformed with 2 or 4μg of each plasmid (6 and 24h exposure), using lipofectin or Xtreme reagent conjugates. Transformants were rescued by growth in G418 selection medium. ERα mRNA levels were determined by Real Time RT-PCR of extracted RNA, and ERα protein by Western blotting; normalisation was achieved by simultaneous analysis of β-actin. Presence of plasmid DNA in transformants was verified with primers targeting various regions of the vector. RNA from w/t MCF7, plasmid II and scrambled plasmid transfected cells was hybridised to low density microarrays containing breast cancer related genes. Results: Stably transfected cells maintaining antibiotic resistance over several passages were established by continuous culture and eventually cloned. Linearised anti-ERα-siRNA construct pII most effectively down-regulated ERα (as evidenced by mRNA and protein analysis) in these cells as compared to G418 resistant transformants containing scrambled siRNA; complete knockdown was not observed. Whereas 4 μg produced more transformants, 24h exposure did not increase transformation efficiency. Array hybridisation indicated differential expression of a number of genes. Summary: Using ERα siRNA vector constructs, we have successfully established a long term culture of MCF7 breast cancer cells that exhibit decreased expression of ERα. This is hoped to provide a model system in which to study aspects of endocrine resistance and determine which other genes may be associated with this new phenotype. (Supported by KU Grant YS01/04).

***Keywords*:** breast cancer; RNA interference; estrogen receptor; endocrine resistance;

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Regulation of migration of breast cancer cell MCF-7 by uPA receptor

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A wide variety of tumor cells exhibit overexpression of serine proteinase urokinase plasminogen activator (uPA) and its Mr 55–60 kDa GPI-linked-receptor (uPAR). Expression of uPA and uPAR is essential for tumor cell invasion and metastasis. The binding of uPA to uPAR was demonstrated as an important trigger for migration, invasion and metastasis in many kinds of tumor through focal proteases hydrolysis of extracellular matrix (ECM). So the uPA/uPAR expression levels have been considered as a potential prognostic factor for many kinds of tumors especially in breast cancer. Previously we have shown that competent inhibition of binding of uPA to uPAR by amino-terminal fragment(ATF) of uPA can dramatically decrease the migration potential in vitro and metastatic capacity *in vivo* of highly metastatic human lung giant-cell carcinoma cell line (PG). In this study, we use small interfering RNA (siRNA) to down-regulate the expression of uPAR in breast cancer cell lines MCF-7. uPAR was down-regulated by transfection of CMV-driven siRNA expression vector pSilencer. Reverse transcription PCR (RT-PCR) and Western blot analyses indicated down-regulation at both the mRNA and protein levels. *In vitro* migration studies using Boyden's chambers indicated a decrease in the migration potential of cancer cells from treated cells when compared to the control. Furthermore, when uPAR was down-regulated, the apoptotic cascade was triggered as indicated by the up-regulation of caspases. This overexpression of pro-apoptotic caspases in relation to the RNAi-induced down-regulation of uPAR suggests the involvement of the uPAR in cell survival and proliferation in addition to their role in tumor progression.

***Keywords*:** migration; breast cancer; uPAR; siRNA

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PML-RARα fusion protein and impaired protein kinase A signaling account for the increased reactive oxygen species generation and susceptibility to arsenic cytotoxicity in acute promyeloytic leukemia-derived NB4 cells

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Acute promyelocytic leukemia (APL) cell and APL-derived cell line NB4 are characterized by the oncoprotein PML-RARα and the high responsiveness to the arsenic cytotoxicity. We found that the higher sensitivity of NB4 cells to arsenic-induced apoptosis is closely related with higher production of reactive oxygen species (ROS) in cells. Neutrophils cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling pathway may exert inhibitory effect on nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation and the consequent ROS generation. However, whether the same mechanism exists in leukemia cells remains unknown. Based on these clues, the present study was designed to investigate the association of PML-RARα with cAMP/PKA signaling pathway, NADPH oxidae and ROS in leukemia cells. A pair of myelocytic leukemia-derived cell lines, NB4 and U937, were used. In addition, PR9 cells, also named U937/PR that define a cell clone of U937 stably transfected with the inducible PML-RARα expression vectors, were used. PR9 cells, which would stay as ordinary U937 cells without induction, became PML-RARα-expressing cells after being induced for a few days, thus acquired the major pathological features of NB4 cellst. Results showed that the difference in susceptibility between NB4 and U937 cells to arsenic cytotoxicity was related to the discrepancy in the inherent cellular ROS level. NADPH oxidase-derived superoxides constituted the major contribution to the discrepancy in ROS level between NB4 and U937 cells. After PR9 cells were induced to express PML-RARα, they displayed the increased sensitivity to arsenic toxicity, the augmented cellular ROS level and more substantial superoxide generation by NADPH oxidase upon PMA stimulation. On the other hand, the basal intensity of cAMP/PKA pathway differed between NB4 and U937 cells, and pre-induced- and post-induced PR9 cells, demonstrating that PML-RARα-expressing cells had an impaired cAMP/PKA/CREB signaling pathway. Using a variety of cAMP elevating- and PKA stimulating agents, we found the activation of this pathway could reduce the inherent and NADPH oxidase-derived ROS generation in NB4 cells. In summary, the present study proves that PML-RARα that renders NB4 cells various pathological features, also paradoxically endows the basis of these cells with apoptosis susceptibility to ROS generating drug arsenic trioxide. It is reported for the first time that NADPH oxidase can be activated by PMA to generate ROS in NB4 cells, and its higher ROS level is related to the impaired cAMP/PKA signaling pathway. These findings may be helpful to arsenic-related redox

manipulation and cancer treatments.

***Keywords*:** PML-RARα; reactive oxygen species; NADPH oxidase; cAMP; PKA; leukemia

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Apoptotic effect of P2X7 receptor on human leukemia cells

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The P2X7 mediated apoptotic effect in different types of cells ($P2X7^-$, $P2X7^+$ with calcium response, $P2X7^+$ without calcium response) was studied. The results showed that P2X7 agonists, ATP or BzATP, dose-dependently reduced the cell viability in all $P2X7^+$ cells tested, including J6-1, LCL, and Namalva cells, which were negative for P2X7 mediated calcium response, though the effects were weaker than what was observed in KG1a cells, which had normal P2X7 functions. The cytotoxic effect could be blocked by P2X7 antagonists, oATP and KN62. In addition, externalization of phosphatidylserine could be detected in a time-dependent manner and apoptotic morphological changes could be observed after the activation of P2X7 receptor in KG1a and J6-1 cells. Furthermore, P2X7 mediated pore formation could be detected in KG1a and J6-1 cells under low ionic conditions, but not under low divalent conditions. These effects could not be observed in $P2X7^-$ Ramos cells. We have previously reported that the expression and calcium response of the P2X7 receptor in a panel of hematopoietic cell lines. $P2X7^+$ cell lines, such as KG1a and J6-1, and $P2X7^-$ cell lines, such as Ramos, were identified at both mRNA and protein levels. Furthermore, lack of calcium response under normal conditions was found in J6-1, LCL and Namalva cell lines, which were P2X7 positive. In the present study, we investigated whether the activation of P2X7 resulted in the inhibition of cell proliferation or induction of cell apoptosis in these cells. Decreasing of cell viability and induction of apoptosis could be detected both in KG1a and J6-1 cells after they were treated with P2X7 agonists, which suggested that induction of apoptosis through P2X7 receptor in leukemia cells was independent of calcium response.

***Keywords*:** P2X7 receptor; cytotoxicity; apoptosis; calcium response; leukemia cells

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Expression of nucleotide receptor P2Y1 and P2Y11 in leukemic cells

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P2-receptors mediate the actions of extracellular nucleotides such as ATP or UTP. The receptors play a physiological or pathophysiological role in a variety of tissues or cells. This includes vasoconstriction due to sympathetic nerve stimulation, blood platelet aggregation, pain transmission and chloride ion secretion in airway epithelia. Two families of P2-receptors are known: P2X-receptors, which represent a totally new class of ligand-gated ion channels with two transmembrane domains, and P2Y-receptors, which belong to the superfamily of G-protein coupled receptors with seven transmembrane domains. However, P2Y receptors' expression in leukemic cells were not fully elucidated. Semi-quantitative RT-PCR was used to investigate P2Y1 and P2Y11 expression in 4 human hematopoietic cell lines (Jurkat, J6-1, K562 and HL-60), representing different lineages. The PCR amplified fragments were verified by DNA sequencing. The results showed that P2Y11 mRNA was detected in 3 cell lines except for K562 cells. The expression levels varied among these cells, HL60 cells showed high expressions of P2Y11, J6-1 and Jurkat cells showed moderate expression. None P2Y1 receptor expression was found in these leukemia cells. These results showed that different expression styles of P2Y receptor were found in leukemia cells, and this maybe involved in the formation and development of leukemia.

***Keywords*:** RT- PCR; P2Y receptors; leukemia cells

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Elevation of serum L-lactate dehydrogenase B correlated with the clinical stage of lung cancer

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To identify potential biomarkers related with lung cancer metastasis, conditional media (CM) proteins collected from a primary non-small cell lung cancer (NSCLC) cell line NCI-H226 and its brain metastatic subline H226Br were analyzed by one-dimensional electrophoresis (1-D PAGE) and matrix-assisted laser desorption/time of flight mass spectrometry (MALDI-TOF-MS). Twelve biomarkers were identified, of which L-lactate dehydrogenase B (LDHB) chain was significantly up-regulated in the CM of H226Br cell and was further validated in 105 lung cancer, 93 non-lung cancer, 41 benign lung disease, as well as 65 healthy individuals sera using enzyme-linked immunosorbent assay (ELISA). It was found that the levels of LDHB were specifically elevated in NSCLC sera compared with other groups and were progressively increased with the clinical stage. At the cutoff point 0.260 (OD value) on the Receiver Operating Characteristic (ROC) curve, LDHB could comparatively discriminate lung cancer from benign lung disease and healthy control groups with sensitivity 81%, specificity 70% and total accuracy 76%. These findings demonstrated that secretome could open up a possibility to find, identify, and characterize novel biomarkers related with invasion and metastasis.

***Keywords*:** LDHB; NCI-H226 cell line; H226Br cell line; metastasis; proteome

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Biochemical reconstitution of a mitochondria-initiated apoptotic pathway

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A variety of apoptotic stimuli including the most commonly used chemotherapeutic agents, induce apoptosis in human cells through a mitochondria-initiated caspase activation pathway. In response to these stimuli, cytochrome c is released from mitochondrial intermembrane space into cytosol, where it binds to Apaf-1 and leads to the formation of a caspase-9 activating complex named apoptosome. Activated caspase-9 in turn cleaves and activates the executioner caspases such as caspase-3 and caspase-7. Our laboratory has recently identified several additional proteins factors that play regulatory roles in this pathway. These factors include an oncoprotein and a tumor suppressor protein that either negatively or positively regulates this pathway. In this meeting, I will report the total reconstitution of this caspase activation pathway with seven recombinant proteins. This allowed us to study the detailed biochemical mechanism of caspase activation and its implications in tumorgenesis and cancer therapy.

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Construction of oxidative mannan-conjugated adenovirus-mcyclin B1 and induction of anti-tumor effect *in vivo*

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To construct the replication-deficient recombinant adenoviruses inserted mouse cyclin B1 (mcyclin B1) cDNA drived by CMV promoter using homologous recombination in bacteria provided by AdEasy system. Then to synthesize oxidative mannan-conjugated adenovirus-mcyclin B1 (OX-M-AdmCLB1) and further elucidate its anti-tumor activity. The shuttle plasmid pShuttle-CMV-mcyclin B1 (pSh-C-mCLB1) in which mcyclin B1 cDNA was inserted into the downstream of CMV promoter was established by ligation. Then the linearized pSh-C-mCLB1 was co-transformed with backbone vector pAdEasy-1 to obtain the recombinant adenoviral plasmids pAdmCLB1 by homologous recombination. After packed in HEK-293 cells, the recombinant adenovirus AdmCLB1 was obtained. To further confirm AdmCLB1, its genomic DNA was isolated and used as template to gain mcyclin B1 cDNA by PCR amplification. AdmCLB1 was expanded and purified. To synthesize OX-M-AdmCLB1, OX-M was mixed with AdmCLB1. Dendritic cells (DCs) were infected with OX-M-AdmCLB1 in vitro and the expression of mcyclin B1 in DCs was evaluated through RT-PCR amplification. Being treated with OX-M-AdmCLB1 one week later, BALB/C mice were challenged with CT26 colon carcinoma (CT26) cells. Then the tumor growth and survival of mice were observed. The virus titer of AdmCLB1 was 2.1×10^{11} pfu/ml. The expression of mcyclin B1 in DCs infected with OX-M-AdmCLB1 was higher than AdmCLB1 group. The potential for attenuating tumor growth and sustaining survival benefits in mice treated with OX-M-AdmCLB1 has been displayed. OX-M-AdmCLB1 we constructed could induce the anti-tumor activity *in vivo* successfully, which might be connected tightly with the activation of immune system through the target recognition between DCs and OX-M-AdmCLB1.

***Keywords*:** cyclin B1; oxidative mannan; recombinant adenovirus; tumor; dendritic cells

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The growth Inhibition and the apoptosis of HeLa cells with TCS

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Objective: The purpose of the current study is to investigate the growth inhibition and the apoptosis of human cervical cancer HeLa cells inducing with TCS, and to explore the underlying molecular mechanism. Methods: The antiproliferative effect of TCS on HeLa cells was measured with CCK-8. The FCM analysis was carried out to examine the effect of TCS on the cycle distribution of HeLa cells. Electron microscopy and FCM analysis was employed to observe the apoptosis of HeLa cells. The protein expression of Caspase-3 was determined by Western blot. The activity of Caspase-3, 8, 9 was determined by a Caspases colorimetric assay kit. Results: (1) The proliferation of HeLa cells was significantly inhibited with TCS. (2)TCS led a S-phase arrest in HeLa cells. (3) HeLa cell apoptosis was induced with TCS, which was characterized by chromatin condensation and apoptotic bodies. And the apoptotic rate of HeLa cells increased with time. (4) The expression of Caspase-3 protein was decreased significantly ($P<0.01$) in a dose- and time-dependent manner and the activity of Caspase-3 was increased with a time-dependent, which clearly indicate that Caspase-3 was activated in the apoptosis of HeLa cells. (5) The activity of Caspase-8, 9 also had a time-dependent increase in the experiment. In terms of temporal sequence of the Caspase activation, Caspase-8 was the first Caspase triggered at 24 h following 100 μg/ml TCS treatment, which is earlier than the activation of Caspase-3. These results indicated that the activation of Caspase-8 led to Caspase-3 activated. Conclusion The results of our study showed that TCS inhibit the growth of HeLa cell through cell cycle arrestted in S phase and the apoptosis of HeLa cells. And further studies demonstrated that the activation of Caspase-3 is a common pathway mediating the apoptosis of HeLa cells induced with TCS, in which Caspase-8, 9 critically involve.

***Keywords*:** Trichosanthin; HeLa cells; apoptosis; cell cycle; Caspases

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Small interfering RNAs targeting cyclin D1 or cyclin E inhibit growth of breast cancer cells *in vitro*

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Cyclin D1 and cyclin E play essential regulatory roles in the G1 phase of the cell cycle. Increasing evidence indicates that the deregulation of cyclin D1 or cyclin E is involved in mammary tumorigenesis, suggesting the suppression of cyclin D1 or cyclin E could be an attractive strategy for inhibiting proliferation of breast cancer cells. The purpose of this study was to determine whether targeted inhibition of cyclin D1 or cyclin E, using small interfering RNA (siRNA), alters proliferation in the Breast cancer cell line MCF-7. The siRNA targeting cyclin D1 or cyclin E was chemically synthesized and transfected into MCF-7 cells by Oligofectamine, siRNA targeting cyclin D1 or cyclin E gene could suppress the mRNA expression and protein level by quantitive PCR and Western blot analysis in MCF-7 cells. Lacking cyclin D1 or cyclin E expression exhibited significantly inhibited cell proliferation by CCK-8 assay. MCF-7 cells in G1 phase increased after transfecting with siRNA-cyclin D1 or siRNA-cyclin E, but cells in S phase decreased. Furthermore, MCF-7 cells showed less ability of colony forming after siRNA-cyclin D1 or siRNA-cyclin E treatment. These results indicate that siRNA targeting cyclin D1 or cyclin E has been shown to be a useful tool for silencing expression of cyclin D1 or cyclin E and inhibiting cell proliferation in breast cancer cell line.

***Keywords*:** Cyclin D1; Cyclin E; siRNA; RNAi; breast cancer

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