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S1.1

Anti-cancer activity of *Glycosmis parva* leaf extract on human colorectal cancer HT29 cell

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Aim: *Glycosmis parva* leaf extract was reported to have cytotoxicity and anti-inflammatory activities with the reduction of COX2 expression *in vitro*. This study aimed to investigate the effect of ethyl acetate extracts from leaves of *G parva* (GPE) on human colorectal cancer expressing COX2-HT29 cells and its underlying mechanisms of action. **Methods:** Cytotoxicity of 6.25–100 μg/mL GPE against HT29 was done by resazurin reduction assay. Induction of apoptosis was determined by AnnexinV/FITC and PI staining. Anti-proliferative study was performed using automated cell counter. Cell cycle pattern was analyzed by PI staining. The expression of Bcl-2 family proteins was examined by qPCR. COX2 expression was determined by RT-PCR. **Results:** GPE was cytotoxic to HT29 in a dose-dependent manner with IC_{50} values 69.50, 55.86, and 49.04 μg/mL at 24, 48, and 72 h, respectively. GPE at 50–100 μg/mL significantly induced HT29 apoptosis. At 25–100 μg/mL, it dose-dependently inhibited HT29 cell proliferation. GPE at 100 μg/mL caused G2M accumulation. It changed the expression of Bcl-2 family proteins. It also significantly decreased COX2 expression at 25–100 μg/mL. **Conclusion:** GPE exhibited cytotoxic activity, induction of apoptosis, inhibition of cell proliferation and arrest cell cycle in HT29 cells. The underlying mechanisms may involve decrease in COX2 expression and changes in the expression of Bcl-2 family proteins and which favor cancer cell death.

Keywords: *Glycosmis parva;* Colorectal cancer; COX2; Bcl-2; HT29

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S1.2

Role of tissue factor (TF)/protease-activated receptor-2 (PAR-2) signaling in pancreatic cancer progression

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Aim: Patients with advanced pancreatic cancer are associated with veous thromboembolism (VTE). tissue factor (TF) is a biomarker in initiation of thrombosis and cancer progression. The aim of this study was to investigate the pathological role of TF/ protease-activated receptor-2 (PAR-2) signaling in pancreatic cancer. **Methods:** First, effects of PAR-2 activation on gene expression investigated by microarray analysis. Then, PAR-2-mediated genes were confirmed by RT-PCR and Western blot analysis. VEGF-A levels were detected by a ELISA kit. For *in vitro* angiogenesis assay, HUVECs proliferation was measured by crystal violet staining; tube formation was examined by Matrigel tube formation assay. **Results:** According to the results of microarray assay, TF/PAR-2 signaling regulated an oncogenic pathway through increasing both HIF-1α and HIF-2α, resulting in enhanced transcription of TGF-α. Downregulation of HIF-α by siRNA or YC-1 resulted in depleted levels of TGF-α protein. Furthermore, the culture medium from PAR-2-treated pancreatic cancer cells enhanced HUVEC proliferation and tube formation, which was blocked by the MEK inhibitor PD98059. We also found that activated PAR-2 enhanced tumor angiogenesis through the release of VEGF from cancer cells, independent of the HIF pathways. **Conclusion:** The TF/PAR-2 signaling induced human pancreatic cancer progression through the induction of TGF-α expression by HIF-α, as well as through MEK/VEGF-mediated angiogenesis, and it plays a role in the interaction between cancer progression and cancer-related thrombosis.

Keywords: protease-activated receptor-2 (PAR-2); tissue factor (TF); HIF; VEGF; pancreatic cancer

S1.3

Cisplatin downregulates the aryl hydrocarbon receptor nuclear translocator to inhibit MDR1 expression

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Aim: Identification of molecular pathways essential for cancer cells is critical for understanding their underlying biology and designing effective cancer therapies. In this study, effect of expression of the aryl hydrocarbon receptor nuclear translocator (ARNT) on the development of acquired drug resistance in cancer cells was investigated. **Methods:** cisplatin-sensitive or acquired resistant cancer cells were treated with cisplatin for a period of time in ARNT knockdown or overexpression conditions. The cell survival and apoptotic markers such as expression of p53, caspase-3 activation and DNA fragmentation were then detected by CCK-8 assay, MTT assay, flow cytometric and Western blot analysis. The effect of ARNT on MDR1 expression was determined by luciferase reporter analysis, RT-PCR and Western blot analysis. In addition, the cooperation between Sp1 and ARNT in the regulation of MDR1 expression was examined by DAPA and luciferase reporter analysis. In a xenograft analysis of SCID mice, the effect of cisplatin on ARNT-deficient c4 tumors and ARNT-containing vT2 tumor formation was studied. **Results:** Cisplatin dramatically induced p53 expression and caspase-3 activation, resulting in degradation of the ARNT and cell apoptosis in drug-sensitive cells. ARNT interacted with Sp1 to enhance the expression of MDR1 through Sp1-binding sites in the MDR1 promoter, resulting in a reversal of the effect of cisplatin on cell death. In contrast, suppression of ARNT reversed the characteristics of cisplatin-resistant cells, making them cisplatin-sensitive, and significantly enhancing cell death with cisplatin treatment. In addition, cisplatin also efficiently inhibited ARNT-deficient c4 tumors but not ARNT-containing vT2 tumor formation. **Conclusion:** These results reveal previously unrecognized multifaceted functions of ARNT in establishing drug-resistant properties of cancer cells, highlighting its potential as a therapeutic target for an important subset of cancers.

Keywords: cisplatin; ARNT; MDR1; drug resistance

S1.4

The effect of high-efficiency immune modulators on inhibiting tumor growth and regulating immunity in S_{180} sarcoma mice

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Aim: To observe the inhibition effect of high-efficiency immune modulatorsits (HIS) on tumor growth in S_{180}-bearing mice and its regulation in immunologic function and improvement of tumour apoptosis. **Methods:** The second day of S_{180}-bearing mice model has established, mice were randomly divided into control group, model group, cyclophosphamide (CTX) group (50 mg/kg) and HIS group. After 10 days of different treatments, the mice's spleen index and inhibition rates of tumor were measured; the level of IL-6 was measured by Enzyme-linked immunosorbent assay (ELISA); The expression of IL-10 and Bcl-2 and Bax were detected by Western blot. **Results:** HIS can significantly inhibit the tumor growth with a 46.67% tumor inhibition rate. Compared with the model group, HIS group mice showed a decrease Bcl-2 (0.38±0.03, $P<0.01$) and an increase Bax (0.99±0.06, $P<0.01$) expression in S_{180} sarcoma. HIS can also improve immunologic function by increasing the spleen index in S180 sarcoma mice ($P<0.01$). The level of IL-6 in serum and the expression of IL-10 in S_{180} sarcoma tissue were down-regulated in HIS treated mice. **Conclusion:** HIS had an anti-tumor effect on S_{180}-bearing mice, and its mechanisms probably result of inducing the apoptosis of S_{180} sarcoma cells by the means of decreasing Bcl-2 and increasing Bax expression and improving the immunologic function via increasing the spleen index and downregulating the expression of IL-6 and IL-10.

Keywords: high-efficiency immune modulators; Bcl-2; Bax; IL-6; IL-10; tumor growth
Acknowledgements: This work was supported by grants from the Jiangxi Natural Science Foundation (2009GGY0014) and Bureau of Education (GJJ12557) of Jiangxi province.

S1.5

The role of microRNAs in tumor

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miRNA can function as either tumour suppressors or oncogenes in cancers by targeting genesa associated with tumor, such as anti-apoptosis genes, cell proliferation genes, senescence genes and cell cycle factors, *etc*. These sugegst

miRNAs can be used as predictive, diagnosed and prognosic biomarker. Moreover, based on the increasing number of studies demonstrate that the aberrant expression of miRNAs can predict the response to chemotherapeutic drugs, it is of great clinical value. miRNAs induced resistance to 5-fluorouracil in colorectal cancer by targeting some associated genes, including DNA, mismatch repair system, cell cyclefactors and apoptosis factors, *etc*.

Keywords: microRNA; oncogene; tumour suppressors; drug resistance
Acknowledgements: This work was supported by Special Major Research Projects of Jiangxi Province (No 20114013)

S1.6

4-Acetylantroquinonol B isolated from *Antrodia cinnamomea* inhibited prostate cancer cells growth and metastasis *in vitro* and *in vivo*.

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Aim: The 4-acetylantroquinonol B (4AAQB) isolated from the mycelium of *Antrodia camphorata* could inhibit proliferation of hepatocellular carcinoma cells HepG2 with very low IC_{50}. However, the detail molecular mechanism underlying this anti-tumor drug is poorly understood. We are interesting to investigate the *in vitro* and *in vivo* anti-proliferation and anti-metastasis effects of 4AAQB in prostate cancer. **Methods:** We first used MTT assay to determine the effect of 4AAQB on PC3 cell viability. Further, we used LDH release assay to verify the inhibition effect either causing cytotoxicity or approving apoptosis. Then, cell scratch motility assay was used to evaluate whether the 4AAQB would inhibit motility of PC3 cell line. To investigate the possible mechanisms involved in the inhibitory effect of 4AAQB, the phosphorylation of Akt, ERK and IκB was analysis by Western blot. 4AAQB IP administration was used to examine the anti-tumor and anti-metastasis effect *in vivo* model. **Results:** 4AAQB does-dependently inhibited the PC3 cell viability and there were no cytotoxicity at doses less than 3 μmol/L. In non-toxic concentration, 4AAQB does-dependently inhibited the migration of PC3 cells *in vitro*. Furthermore, 4AAQB dose and time-dependently affected the Akt, ERK and IκB phosphorylation in PC3 cells. 4AAQB also significantly inhibited tumor growth and metastasis in PC3 *in vivo* model. **Conclusion:** Taken together, these findings suggested that 4AAQB could reduce the growth and metastasis of PC3 cells *in vitro* and *in vivo*. 4AAQB may be a promising therapeutic agent for prostate cancer.

Keywords: 4AAQB; prostate cancer; proliferation; metastasis

S1.7

Anticancer effects of Gekko ethanol extract in human hepatocellular carcinoma HepG2 cells and in H22 bearing mice

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Aim: To explore the anticancer effects and underlying mechanism of Gekko ethanol extract (GEE) *in vitro* and *in vivo*. **Methods:** MTT assay was employed to evaluate the anti-proliferative effect of GEE *in vitro*. Hoechst 33258 fluorescence staining was performed to observe morphologic changes nucleus of HepG2 cells; Antitumor properties of GEE *in vivo* were tested in H22 tumor bearing mice. Tumor weight of each mouse was evaluated at the end. The expression levels of IL-6 and TNF-α were analyzed by ELISA assay. Western blotting analysis was carried out to detect the expression levels of caspase-3 and VEGF in tumors. **Results:** GEE has anti-proliferative effect in a time-dose dependent manner, with inducing typical apoptotic morphological changes ability in HepG2 cells. GEE resulted in significant decrease in tumor weight. The expression levels of IL-6 and VEGF were down-regulated, whereas TNF-α and caspase-3 were increased after treatment of GEE *in vivo*. **Conclusion:** Taken together, these findings suggest GEE has significant anti-liver cancer activities both *in vitro* and *in vivo*, which may be involved in antiangiogenesis and induction of apoptosis.

Keywords: Gekko ethanol extract; apoptosis; antiangiogenesis; hepatocellular cancer

S1.8

Potentiating effect of citral on anticancer activities of doxorubicin, vincristine, and etoposide in human B-lymphoma cells

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Aim: Citral is a mixture of terpene aldehydes in essential oils of several plants used for cooking such as lemongrass. This study intended to evaluate the potentiating effect of citral on anticancer activities of doxorubicin, vincristine, or etoposide against human B-lymphoma Ramos cells. **Methods:** Ramos cells were treated with each anticancer drug in the presence and the absence of citral. Cytotoxicity of these agents and the combination was determined by resazurin reduction assay. Proliferation and apoptosis of the treated cells were evaluated by an automated cell counter and AnnexinV/FITC and PI staining, respectively. The expression of Bcl-2 family proteins in the treated cells was performed by qPCR. **Results:** Citral had cytotoxicity on Ramos cells with IC_{50} value 85.6 μmol/L. At citral 10-40 μmol/L significantly increased the cytotoxic effects of doxorubicin, vincristine, and etoposide on Ramos cells, leading to the decrease in the IC_{50} value of each drug. It potentiated the apoptotic induction effects of these anticancer drugs. The combination of citral with doxorubicin significantly decreased the expression of anti-apoptotic Bcl-x_L when compared to doxorubicin alone. It did not change anti-proliferative effects of these drugs. **Conclusion:** Citral had potentiating effect on the Ramos cell cytotoxicity of doxorubicin, vincristine, and etoposide by increasing in apoptotic effects of these drugs. The results from this study strongly suggest that citral in several edible oils may have beneficial effect in patients with B cell lymphoma treated with chemotherapy.

Keywords: citral; anticancer; human B-lymphoma Ramos cells

S1.9

Differential protein expression of plumbagin treated CCA cell line

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Aim: Medicinal plant extracts are alternative sources of promising anti-cancer compound such as *Plumbago* extracts. Therefore, this study aims to investigate the effect of plumbagin on protein expression in CCA cell line namely CL6. **Methods:** The CL6 cells were treated with plumbagin compound for 48 h. The cells were harvested at 0, 1, 3, 6, 12, 24, and 48 h, protein extracted, and subjected for LC-MS/MS analysis. **Results:** Differential protein expression has been observed. To identification of up- and down-regulated protein will be analyzed by using DeCyder, MASCOT, and Mev software. **Conclusion:** This protein profile will be useful for anti-CCA drug development.

Keywords: plumbagin; cholangiocarcinoma; protein expression; LC-MS/MS

S1.10

12-*O*-tetradecanoylphorbol-13-acetate increases sensitibity of cisplatin to human lung adenocarcinoma cells

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Aim: To investigate whether 12-O-tetradecanoylphorbol-13-acetate (TPA) can increase the sensitivity of cisplatin on human lung adenocarcinoma cisplatin-sensitive cell line A549 and cisplatin-resistant cell line A549/CP. **Methods:** MTT assay was used to observe cell proliferation after treating by TPA alone or in combination with cisplatin. Intracellular cisplatin accumulation was measured by flameless automic absorption spectroscopy. Specific activities of Na^+, K^+-ATPase were detected according to the procedure of Na^+, K^+-ATPase activity detection kit. Assesment of Na^+, K^+-ATPase α and β subunits was done by Western blotting. **Results:** TPA at 8.1 nmol/L or lower didn't affect the cells' proliferation. TPA decreased IC_{50} of cisplatin by 77% in A549cells and 69% in A549/CP cells at 8.1 nmol/L after 96 h respectively. Intracellular cisplatin accumulation was increased after pretreated with TPA for 72 h in both cell lines. However, pretreatment with Na^+, K^+-ATPase activity inhibitor ouabain and TPA markedly reduced intracellular cisplatin accumulation. Na^+, K^+-ATPase activity was enhanced with increasing concentrations of TPA in all the cells. Western blotting results showed that Na^+, K^+-ATPase β1 subunit was induced in both cell lines. **Conclusion:** These results suggest that pretreatment of A549 and A549/CP cells with TPA increases the sensitivity of cisplatin by intracellular cisplatin accumulation possibly correlated with Na^+, K^+-ATPase.

Keywords: 12-*O*-tetradecanoylphorbol-13-acetate (TPA); cisplatin; lung adeno-carcinoma cells; Na^+, K^+-ATPase

S1.11

Chrysin enhances sensitivity of BEL-7402/ADM cell to doxorubicin by suppressing PI3K/Akt/Nrf2 and ERK/Nrf2 pathway

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Aim: Nuclear factor-E2-related factor 2 (Nrf2) is a redox-sensitive transcription factor regulating expression of a number of cytoprotective genes. Recently, Nrf2 has emerged as an important contributor to chemoresistance in cancer therapy. Here, we investigated whether Nrf2 was associated with drug resistant in doxorubicin resistant BEL-7402 (BEL-7402/ADM) cells, and if chrysin could reverse drug resistance in BEL-7402/ADM cells. **Methods:** Reverse of drug resistance was assayed by MTT and Fluorometric analysis. Inhibition of Nrf2 signaling pathway by chrysin was detected through real-time quantitative PCR and western blot analysis. **Results:** We found that notable higher level of Nrf2 and its target proteins in BEL-7402/ADM cells compared to BEL-7402 cells. Similarly, intracellular Nrf2 protein level was significantly decreased in BEL-7402/ADM cells and ADM resistance was partially reversed by Nrf2 siRNA. chrysin is a potent Nrf2 inhibitor which sensitizes BEL-7402/ADM cells to ADM and increases intracellular concentration of ADM. Mechanistically, chrysin significantly reduced Nrf2 expression at both the mRNA and protein levels through down-regulating PI3K-Akt and ERK pathway. Consequently, expression of Nrf2-downstream genes HO-1, AKR1B10, and MRP5 were reduced and the Nrf2-dependent chemoresistance was suppressed. **Conclusion:** The results suggested that activation of Nrf2 is associated with drug resistance in BEL-7402/ADM cells and chrysin may be an effective adjuvant sensitizer to reduce anticancer drug resistance by down-regulating Nrf2 signaling pathway.

Keywords: chrysin; Nrf2; hepatocellular carcinoma; sensitivity; chemoresistance

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S1.12

Anticancer activity of celastrol in hepatocellular carcinoma

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Aim: Celastrol is an active ingredient of the traditional Chinese medicinal plant Tripterygium Wilfordii, which has anti-inflammatory and anticancer activities. The objective of the study was to evaluate the anticancer effects of celastrol in hepatocellular carcinoma. **Methods:** Sprague-Dawley rats were intragastrically administrated with 0.2% (*w/v*) DEN every 5 d for 16 wk (10 mg/kg) and maintained for an additional 4 weeks without administration of DEN, and were orally fed with celastrol since 10th week for 10 weeks. In addition, Bel-7402 cells, HCC cell line, were used to further explore the molecular mechanisms related to anticancer of celastrol, significantly model groupcelastrol showed a marked reduction in the above condition and subsequently. **Results:** Results showed that celastrol significantly reduced the numbers and volume of live tumor foci in DEN-induced HCC rats, decreased obviously the levels of AST, ALT, ALP and AFP in rat serum elevated by DEN. Meanwhile, celastrol induced apoptosis in Bel-7402 cells in dose- and time-dependent manners. Celastrol increased the expression of p53, inhibited the activity of MDM2, and elevated the ratio of Bax/Bcl-2 in Bel-7402 cells and in DEN induced rat liver. Furthermore, Celastrol induced the release of *cytochrome c*, increased the expression of FAS, FasL, cleavage of caspase-3, caspase-8 and caspase-9 in Bel-7402 cells. **Conclusion:** This study indicates that celastrol could prevent the progression of HCC development and its anticancer mechanisms is related to the induction of apoptosis, ie, celastrol may be a potential chemotherapy agent for HCC.

Keywords: celastrol; hepatocellular carcinoma; diethylnitrosamine; apoptosis; p53

S1.13

Role of osteopontin in human osteosarcoma

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Aim: Osteopontin (OPN), an extracellular matrix protein, has a functional RGD domain for binding to integrin and has been reported to be associated with malignant transformation. OPN plays a crucial role in tumor progression, invasion and metastasis. Here we investigated the effect of osteopontin on the regulation of glucose transporters in oateosarcoma. **Methods:** Protein expression was assayed by Western blot. Cell viability was assayed by MTT. Glucose uptake was assayed by flow cytomatry. Cell motility was assayed by trans well migration assay. **Results:** We found that OPN and glucose transporters expression were upregulated by hypoxia. The expression of Type I glucose transporters can be increased by osteopontin through αvβ3 integrin and CD44 receptor. PI3K-AKt, p38, and JNK pathways are involved in the signaling of osteopontin. OPN can also enhance migration of osteosarcoma cells migration. **Conclusion:** Osteopontin can regulate the expression of glucose transporter and control the supply nutrient, which will influence the viability of osteosarcoma cells. Oateopontin seems a good drug target for osteosarcoma therapy.

Keywords: integrin; osteopontin; osteosarcoma

S1.14

Involvement of the prostaglandin E receptor, EP_2, in paeoniflorin-induced human hepatoma cells apoptosis

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Aim: Prostaglandin E_2 (PGE_2) has been shown to play an important role in tumor development and progression. PGE_2 mediates its biological activity by binding any one of four prostanoid receptors (EP_1 through EP_4). The present study was designed to investigate the role of the EP_2 receptor during proliferation and apoptosis of human HepG2 and SMMC-7721 hepatoma cell lines and the effect of paeoniflorin, a monoterpene glucoside. **Methods:** The proliferation of HepG2 and SMMC-7721 cells was determined by methyl thiazolyl tetrazolium (MTT) after exposure to the selective EP_2 receptor agonists butaprost and paeoniflorin. Apoptosis of HepG2 and SMMC-7721 cells were also quantified by flow cytometry with annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining. The expression levels of Bcl-2 and Bax were quantified by western blotting and immunohistochemistry. The expression of the EP_2 receptor and cysteine-aspartic acid protease (caspase)-3 was determined by Western blotting. **Results:** Butaprost significantly increased proliferation in HepG2 and SMMC-7721 cells. Paeoniflorin significantly inhibited HepG2 and SMMC-7721 cells proliferation stimulated by butaprost at multiple time points (24, 48, and 72 h). Paeoniflorin induced apoptosis in HepG2 and SMMC-7721 cells that was quantified by annexin-V and PI staining. Our results indicate that the expression of the EP_2 receptor, Bcl-2 was significantly increased, Bax, cleaved caspase-3 was decreased in HepG2 and SMMC-7721 cells after stimulated by butaprost. Paeoniflorin significantly decreased the EP_2 receptor, Bcl-2 expression and increased Bax and caspase-3 activation in HepG2 and SMMC-7721 cells addition with butaprost. **Conclusion:** Our results suggest that the PGE_2 receptor subtype EP_2 may play a vital role in cell survival in both HepG2 and SMMC-7721 cells. Paeoniflorin, which may be a promising agent in the treatment of liver cancer, induced apoptosis in HCC cells by downregulating EP_2 expression and increased the Bax-to-Bcl-2 ratio, thus up-regulating the activation of caspase-3.

Keywords: paeoniflorin; butaprost; EP_2; hepatocellular carcinoma; apoptosis; caspase-3

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S1.15

Anti-tumor effect of three kinds of traditional chinese medicine formula on S_{180}-bearing mice

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Aim: To study the anti-tumor effect of three kinds of traditional chinese medicine(TCM) and discuss the mechanisms about sarcoma cell apoptosis and angiogenesis. **Methods:** The second day of S_{180}-bearing mice model established, mice were randomly divided into control group, model group, cyclophosphamide (CTX) group (50 mg/kg) and three TCM groups. After 10 days of different treatments, the tumor inhibition rate were measured, and the expression of Bcl-2, Bax, caspase-3 and VEGF protein in the tumor tissues were investigated by Western blot. **Results:** Bie Jia Jian Wan (BJJW) and Liu Wei (LW), especially BJJW, can significantly affect the tumor growth with 35.9% and 22.0%, respectively. While Fu Zhen TCM has no anti-tumor effect in S_{180}-bearing mice. Compared with the model group, BJJW and LW group mice showed decreases Bcl-2 and VEGF, and increases Bax and caspase-3 expression in S_{180} sarcoma. **Conclusion:** BJJW and LW have anti-tumor effects in S_{180}-bearing mice. Its mechanisms probably related with the induction of the apoptosis of S_{180} sarcoma cells through downregulation of Bcl-2 andupregulation of Bax and caspase-3expression, and disturb the tumor angiogenesis via downregulation of VEGF.

Keywords: traditional chinese drugs; tumor-bearing mice; anti-tumor; apoptosis; angiogenesis
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S1.16

Anti-angiogenic mechanisms of PPemd 25, a novel anthraquinone derivative

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Aim: Angiogenesis contributes to ischemic, inflammatory, infectious and immune disorders and also occurs during numerous malignancies. It is a balanced process that involves proliferation, migration, differentiation and tube formation of endothelial cells. As a critical factor in inducing angiogenesis, vascular endothelial growth factor (VEGF) has become an attractive target for anti-angiogenesis treatment. **Methods:** In an effort to develop inhibitors to block VEGF signaling and angiogenesis, we selected PPemd25, an anthraquinone derivative, and investigated its inhibitory mechanisms using human umbilical endothelial cells (HUVECs). **Results:** PPemd25 concentration-dependently inhibited VEGF-induced proliferation, migration and tube formation of HUVECs. PPemd25 also suppressed *ex vivo* VEGF-induced microvessel sprouting from aortic rings. In addition, PPemd25 inhibited VEGF-induced phosphorylation of focal adhesion kinase (FAK), Akt and ERK. Using an implanted matrigel plugs angiogenesis model, PPemd25 attenuated MDAMB-231breast cancer cells-induced angiogenesis. **Conclusion:** Together our findings suggest that PPemd25 inhibits VEGF-induced angiogenesis via downregulation of VEGFR2-mediated signaling. Results of this study suggest that PPemd25 may be a potential drug candidate in anti-cancer therapy, acting by suppressing tumor-induced angiogenesis.

Keywords: angiogenesis; endothelial cells; vascular endothelial growth factor (VEGF)

S1.17

WMJ-S-001, a novel HDAC inhibitor, induces HCT116 colorectal cancer cell death through AMPK/p38MAPK signaling cascade

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Aim: Histone deacetylases (HDACs) are often found over-expressed in human cancers, including colorectal cancer, and has been implicated in tumorigenesis. HDAC inhibition induces growth arrest and cell death in various transformed cell. However, the mechanisms by which this reduces cell viability in colorectal cancer cells remain to be elucidated. **Methods:** We explored the actions of a novel HDAC inhibitor, WMJ-S-001 in HCT116 colorectal cancer cells. **Results:** WMJ-S-001 dose-dependently decreased cell viability and proliferation in HCT116 cells. These results are associated with the modulation of survivin, Bax and p21. WMJ-S-001 activated AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (p38MAPK), whereas an inhibitor of AMPK or p38MAPK signaling abrogated WMJ-S-001's effects of increasing Bax and p21 levels. In addition, WMJ-S-001 induced p53 phosphorylation in a time-dependent manner. Furthermore, WMJ-S-001 also suppressed tumor growth in HCT116 colorectal tumor xenograft model. **Conclusion:** WMJ-S-001 may cause AMPK- and p38MAPK-mediated p53 activation, leading to cell death in HCT116 colorectal cancer.

Keywords: colorectal cancer; HDAC; AMPK; p38MAPK

S1.18

The study for apoptosis of human cervical cancer HeLa cell induced by celosin A from Celosiae Semen and its mechanisms

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Aim: To investigate the apoptosis of human cervical cancer HeLa cell induced by celosin A (CA) from the semen of *Celosia argentea L* and its mechanisms. **Methods:** The viability of HeLa cells was detected by the method CCK-8. The activity of LDH was measured by MTT assay. Immune colorimetry was used to test the proliferation of Hela cells. The change of morphology of apoptosis HeLa cells was observed by DAPI fluorescence staining. Western blot was used to detect the expressions of caspase-3/7 and NF-κB proteins of HeLa cells. **Results:** After treatment with CA, the leakage of LDH increased at dosage and time dependently. In the process of determination of BrdU and CCK-8, the inhibitory effect of CA to HeLa cell was stronger than that of the control, and showed clear inhibitory effect on the HeLa cell proliferation with the increasing of concentration and the acting time. By using the DAPI fluorescence dyes, HeLa cell cytoplasm concentrating, nucleolus dispersion and chromatin aglomeration were observed. The Western blot analysis showed that the expression of caspase-3/7 was significantly enhanced and the expression of NF-κB proteins was down regulation. **Conclusion:** These results suggested that celosin A could induce the apoptosis of HeLa cells and its mechanisms for activating the activity of caspase-3/7 and down-regulating the expression of NF-κB protein.

Keywords: *Celosia argentea L*; celosin A; HeLa cells; apoptosis; NF-κB
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S1.19

A betulinic acid derivative, SYK023, inhibits lung cancer formation

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Aim: Our previous study indicated that betulinic acid (BA) could inhibit lung cancer formation *via* inhibiting Sp1 level. Herein we try to screen BA derivative(s), which is more effective than BA itself. **Methods:** Various BA derivatives were used to treat the lung cancer cell lines at first to evaluate the therapeutic effect of these compounds, and then used the compound(s) effected in the cellular level to do the animal experiments including the xenograph and spontaneous induced lung cancer animal. Finally, the therapeutic molecular mechanism of the BA-derivative in lung cancer will be addressed. **Results:** After screen more than thirty BA derivatives in inhibiting the growth of lung cancer cells, several BA derivatives contain more cytotoxicity than BA itself in lung cancer cells. Moreover, cell viability by those BA derivatives was also decreased more than BA treatment by MTT assay. Subsequently, three more effective BA derivatives (SYK010, SYK019 and SYK023) were chose to study further. At first, that Sub-G_1 was found more in these BA derivatives treated cells than that by BA treatment. Second, these BA derivatives also affect the Sp1 level like BA treatment. Finally, the effects of these BA derivatives were then evaluated in animal experiments. We not only use the xerograph study, but also use the spontaneous induced lung cancer animal model, $Kras^{G12D}$-induced lung cancer mice, to address this issue. **Conclusion:** These results indicate that compound, SYK023, was more effective in preventing cancer formation than BA or SYK019 treatment, which might be benefit for the cancer therapy in the future.

Keywords: betulinic acid; SYKO23; Sp1; lung cancer

S1.20

Cotargeting autophagy and hsp70 for breast cancer treatment

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Autophagy is a conserved catabolic pathway in which autophagosomes fuse with lysosomes for protein degradation and amino acid recycling. The initiation of

autophagy is triggered by a lipid kinase (PI3KC3) signaling complex, consisting of Vps34, Vps15, and beclin1. Treatment of breast cancer cells with anti-tumor drugs inducing the metabolic and protein-misfolding stress results in heat shock proteins (hsp) expression and induction of autophagy to avoid cell death.

Hsp70 is a stress-activated multifunctional chaperone containing cytoprotective function to inhibit cell apoptosis and cause resistance in breast and other cancers. Suppression of Hsp70 sensitizes cancer cells to chemotherapy. In our previous studies, treatment of breast cancer MCF-7 cells with HDAC inhibitor LBH589 induces autophagosome formation companied by hyperacetylation of hsp70 which enhances the stability and activity of VPS34, via kap-1 dependent sumoylation, leading to the formation of VPS34-Beclin1 complex.

Recently, Chloroquine, a widely used antimalarial agent, has been revealed to have promising effects on cancer treatment in clinical trials. Here, we report that co-treatment with LBH589 and chloroquine or hsp70 inhibitor Pifithrin-α (PFTα) significantly enhanced lethality of breast cancer MB-231, MB-468, and MCF-7 cells. We found that both chloroquine and PFTα inhibited autophagy activation and hsp70 expression following LBH589-induced autophagy protecting cell from apoptosis. Importantly, combinatorial treatment with LBH-589 with hsp70 inhibitor chloroquine or PFTα resulted in the abrogation of LBH589-induced interaction of hsp70 with VPS34, and the sumoylation and activity of Vps34. Both chloroquine and PFTα targeting Hsp70 significantly delayed breast cancer growth *in vivo*.

Our findings demonstrate that a new strategy using agent, such as chloroquine and PFTα, cotargeting hsp70 and Vps34 has the potential benefits to improve treatment outcomes in cancer.

S1.21

The anti-proliferation effect of FSEO on human colorectal cell line HCT116 *in vivo* and *in vitro*

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Aim: To investigate the inhibitory effect and the anti-tumor mechanism of FSEO on human colorectal HCT116 cancer cell line *in vitro* and *in vivo*. **Methods:** Their morphological and cellular characteristics were observed by Wright Giemsa staining and HE staining. Growth inhibition was analyzed with MTT assay. Establish the xenograft tumor model of HCT116 cell line in naked mice. The expression level of mRNA or protein *in vitro* and *in vivo* (tumor tissue) was measured by RT-PCR or Western blotting. siRNA was used to silence the mRNA expression of *p53* gene in HCT116 cell line *in vitro*. After *p53* gene been silenced, cells were treated by high dose of FSEO for 24h. Then the mRNA expression was tested via RT-PCR method. **Results:** After treated with FSEO for 24 to 48 h, the proliferation of different cell lines were inhibited, and there was obvious dose-effect relationship of the inhibitory effect, IC_{50} of FSEO to HCT116 cells was 115.17±14.42 mg/mL. Apoptosis phenonminon in HCT116 cell line or in tumor tissue sections was observed after treated with different concentrations of FSEO. The *in vivo* anti-tumor trial illustrated all the tested doses of FSEO (1, 2, and 4 g/kg) had inhibitory effects on xenografted HCT116 tumor in naked mice. The inhibitory rates of each dose group are: 21.86% (P<0.05), 33.16% (P<0.05), 47.31% (P<0.05). There was a reduction of Bcl-2 and Cyclin d1 expression, up-regulation of p53 and Bax expression, down turn the Bcl-2/Bax protein ratio in FSEO groups under both *in vivo* and *in vitro* conditions(P<0.05). RNA interference trial results showed FSEO may via p53 dependent pathway to regulate the expression of Bcl-2 and Bax to induce the apoptosis. **Conclusion:** FSEO had antiproliferative activity against colorectal l cancer *in vitro* and *in vivo*. Its mechanism may be involved in apoptosis of cells induced by down-regulation of oncogenes and up-regulation of apoptotic genes.

Keywords: FSEO; HCT116 cell line; Apoptosis

S1.22

Effect of resveratrol on *RUNX3* gene in B16F10 murine melanoma cell

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Aim: To explore the effect and mechanism of RES on *RUNX3* gene in B16F10 cell line. **Methods:** The B16F10 cell line were treated with DMSO, different concentration of RES (20, 40, and 80 μmol/L) and positive compound [(5-Aza-CdR), 10 μmol/L] for 48 h. The difference of mRNA expression and protein expression and the promoter region methylation status of the *RUNX3* gene in B16F10 cell were measured by using RT-PCR, Western-Blotting and MSP, respectively. **Results:** Loss expression of RUNX3 mRNA and protein were found in the B16F10 cell. The methylation inhibitor 5-Aza-CdR up-regulates the expression of *RUNX3* mRNA and protein in the B16F10 cell. After the B16F10 cell treated by different concentrations of RES, expression of RUNX3 mRNA and protein were significantly up-regulated in a dose-dependent manner. *RUNX3* gene promoter was hypermethylated in untreated B16F10 cell. After treated by RES, the promoter of *RUNX3* gene was demethylated partly and such effect was concentration-dependent. **Conclusion:** RES could increase the expression of RUNX3 in melanoma B16F10 cells, suggesting that one of the potential mechanisms may be related with Demethylation of RES.

Keywords: Resveratrol; melanoma; RUNX3; Methylation

S1.23

Anticancer activity of cyclooxygenase-2 inhibitor on breast cancer *in vitro*

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Aim: Cyclooxygenase-2 (COX-2) plays a key role in breast cancer progression and metastasis by promotes carcinogenesis, tumor proliferation, angiogenesis, prevention of apoptosis, and immunosuppression. This study investigated the antitumor effects of celecoxib, the selective COX-2 inhibitor, and 5-fluorouracil on breast cancer cell *in vitro*. **Methods:** Human breast cancer HCC1937 cell (ATCC cat.no. CRL-2336) was tested with eight different concentrations of celecoxib or 5FU. The chemosensitizer effect of celecoxib and 5-fluorouracil was performed at fixed celecoxib 50 μmol/L with different concentrations of 5FU. The MTT assay was used to determine the growth inhibition of breast cancer cells *in vitro*. **Results:** The proliferation of HCC1937 cell was inhibited by celecoxib with dose dependent manner. The concentration that inhibited cell growth by 50% (IC_{50}) of 5FU and celecoxib were 5642 and 259 μmol/L, respectively. However, celecoxib does not reverse 5-fluorouracil sensitivity. **Conclusion:** Result suggests that celecoxib may be a potential candidate for breast cancer chemotherapy but could not use with 5FU.

Keywords: cyclooxygenase-2 inhibitor; breast cancer; celecoxib

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S1.24

Anticancer activity of the novel histone deacetylase inhibitor, MPT0G028, in human colorectal cancer cells *in vitro* and *in vivo*

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Aim: Overexpression of histone deacetylases (HDACs) were observed in many types of cancers, including colorectal cancer (CRC). Therefore, small molecules targeting HDACs may have benefits in developing new anticancer drugs for CRC patients. So, we investigated the anticancer activity of a novel synthetic HDAC inhibitor, MPT0G028, in CRC *in vivo* and *in vitro*. **Methods:** Anticancer activity was evaluated by SRB and MTT in HCT-116 and HT-29 CRC cell lines. Cell cycle distribution was accessed by flow cytometry and protein expression was examined by Western blotting. HDAC activities were measured by fluorogenic HDAC assay kit. HCT-116 xenograft model was used to examine the antitumor activity *in vivo*. **Results:** MPT0G028 showed a better anti-proliferation effect than vorinostat in human CRC and exhibited significant inhibitory effects against class I HDACs by using *in vitro* biochemical assay and repressed total HDAC enzymatic activity in HCT-116 cells. Moreover, MPT0G028 can induce apoptotic cell death in a time- and concentration-dependent manner. The data also showed that the repression of Wnt/β-catenin pathway might involve in MPT0G028-induced apoptosis. Finally, MPT0G028 has a potent inhibitory effect on the tumor growth in the HCT-116 xenograft model. **Conclusion:** MPT0G028 is a novel HDAC inhibitor with a better potency than the FDA-approved HDAC inhibitor, vorinostat, and induces dramatic apoptosis in CRC cells. We hope that this study will have contributions to the new drug development and clinical benefits for CRC patients.

Keywords: histone deacetylases (HDACs); MPT0G028; vorinostat; Wnt/β-catenin; colorectal cancer

S1.25

Study on the effect and its mechanism of Xihuang pill reversing tumor immune escape

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Aim: To investigate the effect and mechanism of Xihuang Pill reversing tumor immune escape of tumor-bearing mice tumor immune escape. **Methods:** 4T-1 tumor cell were inoculated into right armpit of BALB/c mouse and randomly divided into 6 groups: normal control group, model control group, positive control group (matrine) and Xihuang pill low dose group, midst dose group, high dose group. After the drug administration in 14 consecutive days, the inhibitory rate of tumor was observed. The tumor tissue was Stripping and weighing, the tumor-inhibitory rate was calculated. The level of IL-2, IL-6, IL-10, TGF-β, VEGF, IFN-γ in Serum were determined by enzyme-linked immunoadsordent assay(ELISA). Adhesion molecule B7-1 (CD80) and Treg, MDSC of peripheral blood were determined by flow cytometry (FCM). **Results:** The tumor-inhibitory rate of Xihuang pill at high dose group was 36.54 percent. Compared with the model group, the average level of IL-2, IFN-γ and the number of B7-1 of the Xihuang pill large dose group significantly up-regulated, however the number of IL-6, IL-10, TGF-β, VEGF, and Treg, MDSC significantly decreased (P<0.05). **Conclusion:** Xihuang pill can significantly inhibits tumor growth by interfere the tumor immune escape, the mechanism relate to the form of tumor immunosuppressive microenvironment by reducing the level of immunosuppressive factors and enhanceing immune clearance.

Keywords: Xihuang pill; 4T1 cell; BALB/c mice; tumor microenvironment; immune escape

S1.26

Anti-cancer effects of *Chaenomeles speciosa* flavonoids in murine sarcoma 180 cells

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Aim: In recent years, the use of natural plant products has intensively attracted attention among clinicians and the public for the prevention and treatment of various chronic diseases. *Chaenomeles speciosa* (Sweet) Nakai is one of the natural plants with high medicinal and dietary value, and comprised of amounts of various effective ingredients such as the total flavonoids, organic acid, and so forth, is originally from Anhui, Hubei and Sichuan of China. This investigation evaluates anti-tumor activity and its mechanism of total flavonoids from *Chaenomeles speciosa* (Sweet) Nakai (FLC). **Methods:** The sarcoma 180 (S180)-bearing mice were used to study potential anti-cancer activity of FLC. The tumor size, survival rate and immune responses were measured in S180 solid and ascites tumor. Molecular screening model of PD-1/PD-L1 was established and the FLC was used to investigate the inhibite activity. Lymphocyte proliferation was also assayed for the extract FLC. **Results:** Our data showed that the FLC could significantly inhibit the tumor growth and prolong the survival time in the S180 tumor bearing mice. The lymphocyte proliferation assay showed that the FLC could improve the specific lymphocyte proliferation. In the S180 ascites, the FLC enhanced tumor infiltrating lymphocytes (TIL) and inhibited programmed death-1-ligand 1 (PD-L1) expression. Using molecular binding assay FLC could inhibit the binding of PD-L1 to PD-1. **Conclusion:** These results suggest that FLC exhibited anti-cancer and immunomodulatory activities, which improved natural and adaptive immune functions, such as interfering PD-1/PD-L1 pathway.

Keywords: *Chaenomeles speciosa* (Sweet) Nakai; flavonoids; anti-cancer activity; immune regulation

S1.27

Effect of S003 on sulfur mustard-induced cutaneous injuries in the mouse ear vesicant model and human HaCaT cells

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Aim: Sulfur mustard (SM) can cause DNA damage and the activation of poly(ADP-ribose)polymerase (PARP). PARP inhibitor has been reported some protective effects in SM skin injury *in vivo* and in *vitro*. S003 is a PARP inhibitor synthesized by our institute. The aim of our study is to observe the effect of S003 in SM treated HaCaT cells and in SM induced mouse ear vesicant model (MEVM). **Methods:** HaCaT cells were exposed to SM 30 min and then cultivated in SM free medium with or without S003 for up to 6 h. Cell viability, NAD^+ and PAR contents, the percentage of apoptotic and necrotic cells, and caspase 3 activity were assayed. SM in propylene glycol (0.16 and 0.64 mg/ear) was applied to the right ear of KM mouse, S003 was ip at dose of 200 mg/kg. **Results:** The amount of intracellular PAR were decreased to 69% after exposed to 1000 mol/L SM about 30 min, and it was reduced to 11% by combined treatment with S003. NAD^+ levels were diminished to 11% and cell viability were reduced to 45% after 6 h exposure of SM. While, treatment with S003 increased the NAD^+ levels to 83% and cell viability to 78%. Meanwhile, the recuction of S003 on apoptosis and necrosis was also observed. While after 6 h exposure of 100 mol/L SM, S003 did not affect the cell viability, but significantly increased percentage of apoptotic and necrotic cells, and caspase 3 activation. In the MEVM, S003 significantly reduced relative ear weight about 30% in the 0.64 mg/ear SM injury, but showed no protective effect in the 0.16 mg/ear SM injury. **Conclusion:** S003 may have protective potentials in SM injury because of the protection of cell viability, the inhibition of PAR synthesis and intracellular NAD^+ depletion, and recuction on apoptosis and necrosis. S003 can reduce the ear edema of SM exposure, but showed different actions under different levels of SM injury.

Keywords: Sulfur mustard; PARP; S003; apoptosis; ear edema

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S1.28

3-bromopyruvate induces necroptosis and autophagy in breast cancer cell lines

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Aim: 3-bromopyruvate (3-BrPA) has antineoplastic activity on malignant cells. In this study, we investigated the toxicity of 3-BrPA on two breast cancer cell lines. **Methods:** The intracellular ATP level was measured by the ATP Assay Kit. Cell viability was measured using MTT assay. Cell death was assessed by flow cytometry with propidium iodide (PI) staining or PI/Annexin V staining. The ultrastructural details of two breast cancer cells treated with vehicle DMSO, 3-BrPA, or 3-BrPA plus caspase inhibitor (z-VAD-fmk) were analyzed by electron microscopy (EM). Small interfering RNA mediated RIP1 and RIP3 knockdown, and the expression of RIP1 and RIP3 were analyzed by Western blot. **Results:** 320 μmol/L 3-BrPA had significant toxicity on MDA-MB-231 cells, whereas no obvious effect was observed in MDA-MB-435 cells. In MDA-MB-231 cells, z-VAD-fmk switches the apoptotic response to necroptosis; meanwhile, knockdown of RIP1 or RIP3 block this form of cell death. Morphologically, autophagosome in insensitive MDA-MB-435 cells were observed. MDA-MB-231 cells treated with 3-BrPA plus z-VAD-fmk exhibit the features of necrosis. **Conclusion:** 3-BrPA can induce necroptosis in MDA-MB-231 cells and the signaling pathway requires the involvement of RIP1 and RIP3. MDA-MB-435 cells which are insensitive to relatively low-level concentrations of 3-BrPA may be related to activating the autophagy pathway.

Keywords: 3-bromopyruvate (3-BrPA); breast cancer cells; necroptosis; autophagy

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S1.29

The tumor suppression pharmacodynamics research of new drug MNF-211 in tumor-burdened (PC-3) nude mice

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Aim: To observe the tumor suppression effects of MNF-211 on tumor-burdened (PC-3) nude mice by gastric gavage. **Methods:** We first establish prostate cancer

model *in vivo* by subcutaneous injection of PC-3 cells into nude mice. Then, the nude mice were randomly divided into 5 groups, including the negative control group, positive control group and MNF-211 high, medium and low dose group, each group of 11, and then respectively give solvent, bicalutamide 35 mg/kg and MNF-211 70, 35, and 17.5 mg/kg, by gastric gavage for 28 consecutive days. **Results:** Each dose group had different degrees of weight loss, but no significant difference among the groups. After D17 gastric gavage, relative tumor volume (RTV) of bicalutamide group and MNF-211 high, medium, low dose groups reduced obviously (P<0.05). At d 28, compared with negative control group, bicalutamide group and MNF-211 high, medium dose groups were significant reduction (P<0.05). The bicalutamide group and MNF-211 high, medium, low dose groups, all the relative tumor proliferation rate less than 60%, was 58.92%, 37.51%, and 53.75%, respectively($P<0.05$). Compared with negative control group, the proliferating cell nuclear antigen in bicalutamide group and MNF-211 high, medium, low dose groups decreased obviously ($P<0.05$). **Conclusion:** New drug MNF–211 (35 and 70 mg/kg) and bicalutamide (35 mg/kg) has inhibitory effect on prostate cancer cells by subcutaneous inoculation into nude mice.

Keywords: prostate cancer; MNF-211; relative tumor proliferation rate; nude mouse; Proliferating cell nuclear antigen

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S1.30

Downregulation of PLK1 inhibits tumor cell metastasis through degradation of β-catenin in esophageal squamous cell carcinoma

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Aim: PLK1 is a serine/threonine kinase that regulates cell cycle and is overexpressed in many tumors. Our study aimed to investigate the biological functions and mechanisms of PLK1 in metastasis of esophageal squamous cell carcinoma (ESCC). **Methods:** The ESCC cell overexpressed PLK1 was screened by RT-PCR and Western blot. The effective PLK1siRNA fragment was screened by Western blot. Effects of PLK1siRNA on migration and invasion of ESCC cells *in vitro* were studied by wound-healing assay and Matrigel chemoinvasion assay. The effective interference fragment was constructed into lentiviral vector, which infected ESCC cells. Interference efficiency was detected by RT-PCR and Western blot. The recombinant lentiviral vector on invasion and metastasis of ESCC cells *in vivo* were studied by pulmonary metastasis model in nude mice and metastasis model in embryonic zebrafish. Related molecules in Wnt/β-catenin signaling transduction pathway which induce ESCC cells epithelial-mesenchymal transition(EMT)were detected by Western Blot and IP. **Results:** PLK1siRNA could obviously inhibit migration and invasion of ESCC cells *in vitro*. Lentivirus mediated RNA interference targeting PLK1 could significantly inhibit metastasis of ESCC cells *in vivo*. PLK1 inhibited expression of E-cadherhin by upregulating Snai, thereby induced ESCC cells EMT. In ESCC cells overexpressed PLK1, β-cateninin from the cell and the nucleus were upregulated. In the polymerized compound that degraded β-catenin, the expression of APC and Axin decreased in immune precipitate of GSK-3, and the expression of APC and GSK-3β also significantly decreased in immune precipitate of Axin. **Conclusion:** RNA interference targeting PLK1 could obviously inhibit invasion and metastasis of ESCC cells. PLK1 induced EMT to promote the metastasis of ESCC by regulating stability of β-catenin.

Keywords: PLK; Esophageal squamous cell carcinoma; RNA interference; EMT; β-catenin

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S1.31

Paeoniflorin inhibited the apoptosis in human hepatocellular carcinoma cells via suppressing hedgehog/Gli signaling pathway

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Aim: To investigate the effect of paeoniflorin (Pae) on the apoptosis in human hepatocellular carcinoma (HCC) cells and its mechanism. **Methods:** Cells apoptosis was determined by flow cytometric analysis. The protein expression of B-cell CLL/lymphoma 2 (Bcl-2), p53, caspase-3 and shh, Gli-1 of hedgehog/Gli signaling pathway in $HepG_2$ and Bel-7402 was examined by Western blot analysis. **Results:** Pae (12.5, 25, and 50 μmol/L) significantly induced earlier period apoptosis of HCC $HepG_2$ and Bel-7402 cells (P<0.01); Pae (100 μmol/L) simultaneously led to later period apoptosis of $HepG_2$ and Bel-7402 cells; Pae (12.5, 25, and 50 μmol/L) significantly decreased the expression of shh and Gli-1 (P<0.01), while significantly decreased the expression of anti-apoptotic Bcl-2, increased the expression of caspase-3 and p53 (P<0.01). **Conclusion:** Pae can induce apoptosis of HCC cells, which maybe correlated with down-regulating hedgehog/Gli signaling pathway by Pae.

Keywords: paeoniflorin; human hepatocellular carcinoma; apoptosis; hedgehog/Gli signaling pathway

S1.32

The apoptosis of human breast cancer MDR cell line MCF-7/Adr induced by novel compound GA and realtedrelated mechanism

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Aim: To investigate the apoptosis of human tumor multidrug resistance (MDR) cell induced by a novel tetracyclic diterpenoid compound GA13315 and realted mechanism. **Methods:** This study was under taken using human breast cancer cell line MCF-7/Adr with typical multi-drug resistant (MDR) properties induced by doxorubicin (ADM) as model. Cells apoptosis induced by compoundGA alone or in combination with ADM were measured by flow cytometry (FCM) combined Annexin V/PI staining. Effects of compound GA alone or in combination with ADM ontranscription of MDR related genes: multidrug resistance-related protein (MRP), lung resistance protein (LRP), mutant p53, bax, bcl-2, and caspase-3 were detected by real-time fluorescencequantitative PCR (RQ-PCR). The proteins expression of MDR related genes MRP, LRP, mutant p53, bax, bcl-2, and caspase-3 were measured by Western blot. **Results:** Apoptosis of MCF-7/Adr was significantly induced by compound GA alone or in combination with ADM in a dose-dependent and time-dependent manner. After exposure of compound GA alone at concentration of 8, 16, 32, and 64 μmol/L to MCF-7/Adr for 24 h and 48 h, the apoptosis rate of cells were 8.92%±2.40%, 30.96%±1.37%, 41.89%±6.97%, 87.66%±3.74% and 18.35%±0.97%, 23.51%±4.24%, 63.92%±3.88%, 78.16%±2.25%, respectively. The treatment of the above same concentrations of GA combined separately with concentration of 18.4mol/L ADM exposure to MCF-7/Adr for 24 h and 48 h, the apoptosis rate of cells were 21.05%±5.74%, 26.45%±6.77%, 35.69%±6.34%, 53.98%±4.77%, and 23.60%±7.44%, 35.27%±4.58%, 54.28%±6.06%, 91.62%±4.12%, respectively. RQ-PCR results showed that after exposure of compound GA alone or in combination with ADM to MCF-7/Adr, the expression of mRNA of Bcl-2, mutant p53 were down-regulated; the expression of mRNA of bax and caspase-3 were up-regulated, but effects on the mRNA expression of MRP and LRP were unclear. Western blot results showed that GA alone and in combination with ADM could down-regulate the protein expression of Bcl-2, mutant p53 and LRP, and up-regulated the protein expression of bax at the same time, but treatment of GA alone and in conjunction with ADM had no obvious effect on the protein expression of MRP of MCF-7/Adr. **Conclusion:** Either treatment of MCF-7/Adr with GA alone or GA in combined with ADM, can significantly induced apoptosis of MCF-7/Adr cells. But GA does not always enhance the effect of ADM on cell apoptosis, the reason is not unclear. The mechanism of apoptosis of MCF-7/Adr induced by GA alone or GA in combined with ADM is involved in down-rengulation of the expression of bcl-2, mutant p53 and LRP, up-regulation of the expression of bax. But whether related to the effect on the expression of MRP and caspase-3 remain still uncertain.

Keywords: compound GA; tumor; multi-drug resistance; apoptosis

Acknowledgements: This work was supported by grants from the National Natural Science Foundation of China (81160405 and 21062029).

S1.33

***Pulsatilla chinensis* (Bunge) Regel saponins suppresses the proliferation of tumor cells and induces apoptosis and autophagy via inhibiting PI3k/Akt signalling pathway**

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Aims: To investigate the *in vitro* and *in vivo* anti-tumor effect of Pulsatilla saponins, the major components extracted from *Pulsatilla chinensis* (Bunge) Regel, and its underlying mechanisms. **Methods:** Human tumor cells were treated with Pulsatilla saponins, cell viability was assessed by MTT assay; cell cycle and apoptosiss were assessed by flow cytometry; cleaved-caspase3, LC3, PI3k, p-PI3k, Akt, and p-Akt were measured by Western Blot assay. Tumor xenograft model was used to further evaluate the anti-tumor effect. The T/C(%) value and tumor inhibition rate were calculated. **Results:** Pulsatilla saponins could inhibite the proliferation of tumor cells, it induced tumor cells S/G_1 phase arresting and apoptosis by increasing cleaved caspase3. It also induced the expression of LC3-II, which is a marker for cellular autophagy. Further study showed Pulsatilla saponins decreased the expression of p-PI3k and p-Akt. Pulsatilla saponins presented anti-tumor effect with 56.74% of tumor inhibition rate in A_{549} xenograft model, without reducing leukocyte number. **Conclusion:** Pulsatilla saponins induced tumor cells apoptosis and autophagy via inhibiting PI3k/Akt pathway, it inhibited tumor growth without reducing leukocyte number, may be a novel effective candiate as chemotherapeutic agent.

Keywords: Pulsatilla saponins; anti-tumor; apoptosis; autophagy; PI3k/Akt pathway
Acknowledgements: This research is supported by projects from National Science and Technology Major Projects for "Major New Drugs Innovation and Development" (No 2011ZX11102-001-19)

S1.34

Progress in mechanism of apoptosis induced by Chinese herbs and the relationship between autophagy in breast cancer cell

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Defects in programmed cell death (PCD) regulation contribute to tumor. Apoptosis as a PCD, is a barrier to cancer development. The autophagic program could degradate some cellular organelle to maintain homeostasis. Metabolites support survival in the stressed environments. Induction of autophagy also may operate independently of or in concert with apoptosis. Nutrient starvation and certain cytotoxic drugs can induce elevated levels of autophagy that are apparently protective for cancer cells. Autophagy seems to have conflicting effects on tumor cells. Recent studies suggested that many Chinese herbs are potential anti-tumor drugs. Herbs extract have multi anticancer effects, some could active apoptotic cell death and induce autophagy. There is increasing interest regarding interconnections between apoptositic and autophagic. Although there has been some progress in studying the apoptositic mechanism of herbs induction and the relationship of autophagy, little is known about the role autophagy plays in breast cancer cell. In this review, we stated the mechanism of apoptosis induced by Chinese herbs and the relationship between autophagy in breast cancer cell.

Keywords: herb; breast cancer; apoptosis; autophagy
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S1.35

Study of the antineoplastic role and its immune mechanism of Xihuang Pill Ethanol extract

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Aims: To investigate the antitumor activity and immunological mechanisms of Xihuang pill Ethanol extract on tumor-bearing rats. **Methods:** Walker256 tumor-bearing rats were randomly divided into 7 groups (n=10): normal control group, model control group, Xihuang pill Ethanol extract low dose group, midst dose group, high dose group, and lentinan group and Xihuang pill high dose group. After the drug administration in 14 consecutive days, the inhibitory rate of tumor was observed. FCM method was used to detect the number of $CD4^+$, $CD8^+$, B7-1. ELISA was used to dectect the level of IL-2, IL-6, IL-10, TGF-β, IFN-γ in serum. The comparison was made among groups. **Results:** The Xihuang pill Ethanol extract can effectively inhibit the growth of W256. The high dose group inhibitory rate of tumor was 36.8%. Compare with model group the serum level of IL-2, IFN-γ and the number of B7-1 $CD4^+$ $CD8^+$ of the Xihuang pill Ethanol extract group significantly up-regulated, however the number of IL-6, IL-10, TGF-β, significantly decreased (P<0.05). The Xihuang pill ethanol extract indicator was better than Xihuang pill in equivalents dose. **Conclusion:** Xihuang pill ethanol extract improve the Xihuang pill bioavailability, and Xihuang pill Ethanol extract has obvious antitumor activity. The mechanism is relate to the enhancement of immune function and reducing the level of immunosuppressive factors.

Keywords: Xihuang pill; ethanol extract; Walker 256 cell of rats mammary cancer; rats; immunological mechanism

S1.36

Bio-activity of novel Histone Lysine specific demethylase1inhibitorin human gastric cancer cell line MGC-803

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Aims: Histone epigenetic modifications including acetylation, methylation, phosphorylation and ubiquitination play important roles in structural changes of chromatin. Lysine specific demethylase1(LSD1) belongs to the flavin adenine dinucleotide (FAD)-dependent amine oxidase family which is a significant enzyme for post-translation modification and regulates the activation and silencing of gene transcription. Studies have shown that LSD1is highly expressed in a variety cancers. Moreover, it can suppress translation of tumor suppressor gene. In this study, modulation for activity of LSD1by its inhibitor 3e in MGC-803 was investigated. **Methods:** The novel LSD1 inhibitor 3e was screend and obtained by LSD1 recombinant based screen model. The bioactivity of 3e in cell level was investigated in human gastric cancer cell line MGC-803. After 48 h treatment of compound 3e, total lysates and total histone were extracted, denatured, and separated in SDS-PAGE. Then the results were analysed by Western-blot. In this study, LSD1, H3K4me1, H3K4me2, H3K4me3, and H3K9me2 were analysed by Western blot. LSD1 and snail were analysed by Crosslink Co-Immunoprecipitation. **Results:** The small molecule inhibitor specifically inhibites activity of LSD1 but does not influence expression LSD1 in MGC-803 cells. Meanwhile, it can interrupt the complex formation between LSD1 and snail, which results in the up regulation of E-cadherin. **Conclusion:** As expected, Crosslink Co-Immunoprecipitation of LSD1 revealed the association with Snail, but the interaction of Snail with LSD1 can be blocked by LSD1 inhibitor 3e. It might regulated the processes of epithelial-mesenchymal transition (EMT), and further research is still going on.

S1.37

ATM-mediated Chk1/2 and Cdc2 phosphorylation is involved in Jaridonin-induced cell cycle arrest in human esophageal cancer cells

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Aims: Jaridonin, a novel diterpenoid from *Rabdosia rubescens* has been shown previously to inhibit proliferation of esophageal squamous cancer cells (ESCC) through G_2/M phase cell cycle arrest. However, the involved mechanism is not fully understood. **Methods:** Cell cycle was analysed by flow cytometry. The related protein expressions were determined by Western blot. **Results:** In this study, we found that the cell cycle arrest by Jaridonin was associated with the increased expression of ATM and phosphorylation of Cdc2 at Tyr15. Jaridonin also resulted in enhanced phosphorylation of Cdc25C via activation of checkpoint kinases Chk1 and Chk2, as well as in increased phospho-H2A.X (Ser139). We also found that the increased activation of ATM is related to the DNA damage caused by Jaridonin. Furthermore, Jaridonin-mediated alterations in cell cycle arrest were significantly

attenuated in the presence of NAC, implicating the involvement of ROS in Jaridonin's effects. On the other hand, addition of ATM inhibitor caffeine reversed Jaridonin-related activation of ATM and Chk1/2 as well as phosphorylation of Cdc25C, Cdc2 and H2A.X and G_2/M phase arrest. **Conclusion:** These findings identified that Jaridonin-induced cell cycle arrest in human esophageal cancer cells is associated with ROS-mediated activation of ATM–Chk1/2–Cdc25C pathway.

Keywords: Jaridonin; diterpenoid; *Rabdosia rubescens*; ROS; ATM; cell cycle arrest; Chk1/2; Cdc2; esophageal cancer cells

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S1.38

Progress of studies on anti-tumor action of *Caulis Spatholobi*

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Caulis Spatholobi is a kind of herb with invigarating the circulation of blood and replenishing blood, which contains flavone, condensed tannin, sterides and so on. According to modern pharmacology, *Caulis Spatholobi* has anti-coagulants, improves hematopoiesis, antiviral and anti-tumor activities by improving cell death and inhibiting cell proliferation. This paper has reviewed anti-tumor components in Caulis Spatholobi and their pharmacological mechanism.

Keywords: *Caulis Spatholobi*; anti-tumor; herb

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S1.39

Gamma-linolenic acid trigger apoptosis in glioblastoma cells by regulating miR-22 and its corresponding gene Sirt-1 expression

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Aim: Gamma-linolenic acid (GLA) was reported had cytotoxic effect on gliobastoma cells. In this study, we investigated the direct cytotoxic action of GLA against gliobastoma cells and the underlying mechanisms. **Methods:** Glioblastoma cells (GBM2) were treated with 25, 75, and 150 μmol/L GLA for 24 h and the proliferation activity of cells were detected by MTT assay. The apoptosis effect and the change of cell cycle were determined by flow cytometry (FCM) technology. The Expression of microRNA-22 and mRNA levels of its corresponding tumor suppressor gene sirt-1 were analyzed by using QRT-PCR. **Results:** The apoptosis of glioblastoma cells was markedly enhanced when the concentration of GLA increased (P<0.05 between each group), and there was an inverse correlation between the concentration of GLA used and miR-22 expression tested by QRT-PCR. We also found an increased expression of Sirt-1 in the same treated cells.**Conclusion:** We conclude that GLA can trigger apoptosis in glioblastoma cells. The mechanisms might be in part due to regulating miR-22 and its corresponding tumor suppressor gene Sirt-1 expression. Our study highlights the potential application of GLA on the anti-cancer therapy of glioblastoma.

Keywords: gamma-linolenic acid; apoptosis; glioblastoma; miRNA-22; Sirt-1

S1.40

Anti-tumor effect of annexinA2 monoclonal antibody *in vitro*

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Aim: Annexins are a structurally related family of calcium and phospholipid-binding proteins that are involved in the regulation of a range of molecular and cellular processes. To explore the anti-tumor effect of annexinA2 (ANXA2) and its related mechanism, which provide the experimental basis for the tumor treatment *in vivo*. **Methods:** pET28a(+)-ANXA2 prokaryotic expressing vector was constructed and the expressed protein was purified, and then it was identified by SDS-PAGE and Western blot with His-tag antibody. Balb/c mice were immunized with the recombinant protein. Through cell fusion and selective culture, stable hybridoma cell strains were obtained. The antibody specificity and sensitivity were detected by indirect ELISA, Western Blot and immunoprecipitation. After ANXA2 antibody treated HepG2 cells, MTT detected the cell proliferation, FCM detected cell cycle, RT-PCR and real-time PCR detected cell cycle-related genes; Western blot detected cell cycle-related protein CDK6. Migration of HepG2 cells were demonstrated by Transwell method. **Results:** pET28a(+)-ANXA2 prokaryotic expressing vector was successfully constructed, and ANXA2 protein obtained and identified. Four hybridoma cell strains which secreted ANXA2 monoclonal antibody were obtained. A monoclonal antibody specifically recognizes the PC3 (human prostate cancer), ECV (human vascular endothelial cells), T24 (human bladder cancer), HepG2 (human hepatoma cells), PANC1 (human pancreatic cancer cells) and cancer tissue expressed ANXA2 protein was identified by Western blot. MTT showed that the ANXA2 monoclonal antibody could inhibit the growth of HepG2 cells. FCM displayed that the antibody inhibited the growth of HepG2 cells by extended G_1 phase, and shortened G_2+M phase time. RT-PCR results identified that the antibody inhibited cell cycle related genes hCCD1, hE2F1, CDK6 expression. Western blot results showed that the antibody decreased HepG2 cells cycle protein CDK6 expression. Transwell method results confirmed that the antibody treatment inhibited HepG2 cells migration. **Conclusion:** In this study, we obtained ANXA2 prokaryotic expression protein and high titers of specific monoclonal antibodies. The ANXA2 monoclonal antibody inhibited tumor cells growth and migration. These results hinted that the hinte expression has certain correlation with tumor occurrence and development and its antibodies may be used for tumor diagnosis and therapy.

Keywords: annexinA2; monoclonal antibody; cell cycle cell migration

S1.41

The novel tubulin-targeting agent MT-4 suppresses human ovarian cancer growth *in vitro* and *in vivo*

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Aims: In this study, the anti-cancer mechanisms of moscatilin(MT-4), have been examined in cisplatin-sensitive ovarian cancer cells (A2780) and multidrug resistance ovarian cancer cells (NCI-ADR-RES). **Methods:** MTT, flow cytometry, tubulin binding assay, and Western blot assay were used. **Results:** MT-4 inhibited cell viability in both ovarian cancer cells. Drug resistance ratios were also calculated as the ratio of the IC_{50} in the resistant cell line (NCI-ADR-RES cells) to the IC_{50} in the sensitive cell line (A2780 cells). The data demonstrated that, compared with A2780 cells, the NCI-ADR-RES cells displayed a 271.3-fold relative resistance to paclitaxel. In contrast, the A2780 cells and NCI-ADR-RES cells did not display any difference in their response to MT-4 (relative resistance ratio=3.7). We also demonstrated that MT-4 caused cell population at G_2/M phase arrest and then increased sub G_1 phase in both ovarian cancer cells. The data showed that MT-4 could disrupt microtubule dynamics and induce phosphorylation of G_2/M protein. Treatment of MT-4 induced apoptosis through catalytic cleavage of caspase-3, -7, -8, -9, and poly (ADP-ribose) polymerase (PARP). In addition, we also found that inhibition of p38 MAPK pathway and could reverse MT-4-induced apoptosis. We demonstrated that MT-4 (20 mg/kg, qd, iv) significantly inhibited tumor growth in xenograft mouse model. **Conclusion:** The MT-4 inhibited cell viability in *vitro* and *in vivo*. These finding indicated that MT-4 can be a potential anticancer agent for treatment of cancer.

Keywords: moscatilin (MT-4); resisrance; apoptosis; ovarian cancer cells; anti-cancer

S1.42

The inhibitory effect of celecoxib on Lewis lung carcinoma cell line by regulation of cyclooxygenase-2/cytosolic phospholipase A2

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Aim: Celecoxib is a potent nonsteroid anti-inflammatory drug (NSAID) that has demonstrated great promise in cancer chemoprevention and treatment. The goal of this study was to determine the inhibitory effect and mechanism of celecoxib on Lewis lung carcinoma. **Methods:** The effect of celecoxib on viability of Lewis lung carcinoma cells was assessed with methyl thiazolyl tetrazolium (MTT) assay. Apoptosis and the mitochondrial membrane potential were detected by flow cytometric assay. The protein expression of cytosolic phospholipase A2 (cPLA2), cyclooxygenase-2 (COX-2), and peroxisome proliferator-activated receptor gamma (PPARc) were determined by Western blot analysis. **Results:** Celecoxib inhibited the proliferation of Lewis lung carcinoma and induced apoptosis in a dose-dependent manner by breakdown of mitochondrial membrane potential. The protein expressions of cPLA2 and PPARc were upregulated, but COX-2 protein expression was downregulated in the Lewis lung carcinoma cells exposed to celecoxib. **Conclusion:** The major findings in this study are that celecoxib could

inhibit the viability of Lewis lung carcinoma cells by interference of the arachidonic acid pathway and upregulation of PPARc simultaneously, which are novel and important since the molecular mechanisms of celecoxib underlying the anti-neoplastic effects remain unclear.

Keywords: celecoxib; cyclooxygenase-2; arachidonic acid; prostaglandin E_2

S1.43

Anticancer activity of melanoxoin in human non-small cell lung cancer cells and in nude mice

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Aim: There are various anticancer drugs, which derived from natural products. In this study, we screened a serious of compounds isolated from *Pterocarpus santalinus* to identify their cell cytotoxicity and antitumor mechanisms. **Methods:** We performed the MTT assay to evaluate the cytotoxic effects of test compounds against several human cancer cell lines. We used Human OneArray® v5 to comprehensive analysis the DNA transcription of H1299 cells. Cell cycle distributions were analysied by flow cytometry. We performed the *in vitro* tubulin polymerization assay to examine whether the test compound affects microtubule organization. We used comet assay to measuring DNA strand breaks. We examined *in vivo* antitumor activity of melanoxoin in nude mice bearing human lung tumor xenografts. **Results:** Among the serious of compounds isolated from *Pterocarpus santalinus*, melanoxoin showed the highest cytotoxic effects with a IC_{50} of 1.98 μg/mL in the human non-small cell lung cancer H1299 cells. It showed that melanoxoin regulated the transcription of several cell cycle regulators in the H1299 cells. Melanoxoin inductions of tubulin depolymerization suggest that melanoxoin causes G_2/M arrest and then increased tumor cell apoptosis. The neutral comet assay revealed that the treatment with melanoxoin induced significant DNA damage in H1299 cells. Our mice xenograft models show the *in vivo* efficacy of melanoxoin. **Conclusion:** We are interested in the development the molanoxoin as a new source of anti-cancer drug.

Keywords: *Pterocarpus santalinus*; melanoxoin; apoptosis

S1.44

Compound *Astragalus* and *Salvia Miltiorrhiza* extract retards hepatocellular carcinoma progression by inhibiting fibrosis and PAI-1 transcription activity

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Aims: To investigate the effects of Compound *Astragalus* and *Salvia Miltiorrhiza* Extract (CASE) on the hepatocarcinogenesis in rats. **Methods:** Rats were randomly divided into control group, DEN (0.2%, ig ×14 weeks) group, and three dosages groups of CASE (60, 120, or 240 mg/kg, ig ×16 weeks). The rats were sacrificed at 12th week or 16th week after the start of DEN administration. Blood samples were collected for determination liver function. The extent of fibrosis of liver was determined by histopathologic examination. The expression of α-smooth muscle actin (α-SMA) and Glutathione S-transferase protein (GST-P) were detected by immunohistochemistry. PAI-1 transcription activity was analyzed by Real-time RT-PCR. **Results:** At 12th week, CASE significantly ameliorated the serum parameters of liver function, and decreased the extent of fibrosis and the overexpression of α-SMA. At 16th week, the elevated incidence and multiplicity of hepatoma, and GST-P expression were reduced by CASE treatment. CASE inhibited PAI-1 transcriptional activity in HepG2 cell induced by TGF-β_1 in a dose-dependent manner. **Conclusion:** These results suggested that CASE might retard DEN-induced hepatocarcinogenesis by inhibiting fibrosis and PAI-1 transcription activity.

Keywords: CASE; diethylnitrosamine; hepatic fibrosis; hepatocellular carcinoma; plasminogen activator inhibitor

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S1.45

Suppression of signal transducer and activator of transcription-3 signaling cascade by zerumbone inhibits the proliferation and induces apoptosis in renal cell carcinoma xenograft mouse model

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Aim: Signal transducer and activators of transcription 3 (STAT3) has been linked to the deregulated proliferation, survival, and angiogenesis in renal cell carcinoma (RCC). In this study we investigated whether zerumbone, a sesquiterpene, obtained from the plant *Zingiber zerumbet* Smith exerts its anticancer effect through the modulation of STAT3 activation pathway. **Methods:** The pharmacological effects of zerumbone on STAT3 activation, associated protein kinases and phosphatase, STAT3-regulated gene products and apoptosis was investigated using both RCC cell lines and xenograft mouse model. **Results:** We observed that zerumbone suppressed STAT3 activation in a dose- and time-dependent manner in RCC cells and was mediated through the inhibition of activation of upstream kinases c-Src, JAK1, and JAK2 and induced apoptosis in RCC cells. We found that zerumbone induced the expression of tyrosine phosphatase SHP-1 that correlated with the down-regulation of constitutive STAT3 activation. Deletion of SHP-1 gene by siRNA abolished the ability of zerumbone to inhibit STAT3 activation. The inhibition of STAT3 activation by zerumbone also caused the suppression of the gene products involved in proliferation, survival, and angiogenesis. Finally, when administered ip, zerumbone inhibited STAT3 activation in tumor tissues and the growth of human RCC xenograft tumors in athymic nu/nu mice. **Conclusion:** Overall, our results suggest that zerumbone is a novel blocker of STAT3 signaling cascade with potential for the treatment of RCC and other solid tumors.

Keywords: STAT3; JAK2; zerumbone; renal cell carcinoma; apoptosis.

S1.46

The anti-tumor activities of OP46, a novel ent-kaurene diterpenoid compound from Isodonrubescens, and its involving molecularmechanisms

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Aim: This current study is designed to determine the cytotoxic effects of OP46, a new ent-kaurene diterpenoid isolated from *Isodonrubescens* on human cancer cells and the underlying mechanisms. **Methods:** MTT assay and Trypan Blue exclusion method were used to evaluate the cell viability. Cell morphology, apoptosis, autophagy and cell cycle distribution were observed or analyzed by fluorescence microscopy or flow cytometry. **Results:** Compared with controls, OP46 caused strong anti-proliferative in human cancer cell lines MCF-7, PC-3, MGC-803, EC109 cells. OP46 also resulted in significant apoptotic, autophagy and G_2/M phase cell cycle arrest in human liver cancer cell SMMC-7721, but not in normal human liver cell L-02. These cytotoxic effects of OP46 on SMMC-7721 were associated with marked accumulation of reactive oxygen species (ROS), whereas a ROS scavenger, *N*-acetyl-*L*-cysteine (L-NAC), significantly attenuated these effects. **Conclusion:** OP46 could selectively inhibit the growth of cancer cells in vitro.The GSH depletion by OP46, associated with the generation of ROS, maybe a central mechanism of OP46-induced cytotoxicity in cancer cells.

Keywords: OP46; ent-kaurene diterpenoid; anticancer activity; GSH; ROS

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S1.47

Effects of *Astragalus polysaccharide* on enteric mucosal immunity in S180 tumor bearing mice

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Aim: *Astragalus polysaccharide* (APS) is a main immunocompetent constituent of *Radix Astragali.* In this study, the effects of APS on enteric mucosal immunity in S180 tumor bearing mice were investigated. **Methods:** Mice were subcutaneously

implanted with S180 tumor cells in the right axillary region. Then these mice were randomly divided into model group, diamminedichloroplatinum(DDP) group, APS low dose group, and APS high dose group. Another ten normal mice were used as normal control. The therapeutic effects of APS were determined by examining tumor size. The production of IgA in enteric mucosa was detected by Immunohistochemistry (IHC) assay. The number of Peyer's pathes(PPs) was detected by counting assay. **Results:** After 14 days of orally administration, APS could inhibit S180 tumor growth *in vivo*. Compared with model group, the number of PPs increased in APS group. In addition, production of IgA in the enteric mucosa was also improved in APS group, there was a significant difference compared with model group. **Conclusion:** The results indicated that APS might improve enteric mucosal immunity by regulating concentrations of IgA and PPs in S180 tumor bearing mice.

Keywords: *Astragalus polysaccharide*; S180 tumor; IgA; enteric mucosal immunity
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S1.48

Research advances in Bacillus Calmette-Guerin anticancer action

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Bacillus Calmette-Guerin (BCG) is the only available vaccine against tuberculosis, it is an attenuated live vaccine prepared from Mycobacterium boris. Now researches show that BCG has antineoplastic activity. This paper reviews applications and mechanisms of BCG anticancer action. BCG can be used to treat malignant tumors, including pediatric leukaemia, melanoma, cancer of colon, hepatoma, pulmonary carcinoma, renal cancer, and bladder cancer, by exclusive use of BCG or combined therapy to produce local antitumor action and general anticancer action. The mechanisms of BCG underlying antitumor therapy are invovled in the receptor responsible for the attachment of BCG to fibronectin, directly killing tumor cells, enhancement of antigen-presenting dendritic cells ability, cytotoxic T cells activated by $CD4^+$ and $CD8^+$, enhancing the antitumor activity of NK cells.

Keywords: BCG; cancer; anticancer; antitumortherapy

S1.49

Corilagin nanoparticle-induced apoptosis in human gastric cancer SGC-7901 cells via the mitochondrial pathway

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Aim: Corilagin is a novel member of polyphenol family which has been discovered from the medicinal plant Geranium in northeast China. Corilagin nanoparticle is a new physical dosage-form. This study was designed to investigate the effect of corilagin nanoparticle on the apoptosis of SGC-7901 cells. **Methods:** The cytotoxicity was assessed with SRB assay. The morphological changes were observed by transmission electron microscopy (TEM). The levels of mitochondrial transmembrane potential and ROS were analyzed by Laser confocal scanning microscopy (LCSM). The apoptotic rate was detected by flow cytometry (FCM). The expression of Bcl-2, Bax and Cytochrome c protein were detected by Western blotting, and caspase-3 activity was measured by a microplate reader. **Results:** The results showed that after treatment with corilagin nanoparticle, the expression of Bcl-2 was down-regulated and Bax was up-regulated compared to that in the control. Corilagin nanoparticle treatment reduced the mitochondrial transmembrane potential, increased the release of cytochrome *c* and induced caspase 3 activation. **Conclusion:** Corilagin nanoparticle can inhibit the proliferation of SGC-7901 cells and induce apoptosis via mitochondrial pathway.

Keywords: corilagin nanoparticle; human gastric cancer; apoptosis

S1.50

A novel apoptosis inducer WF-210 activates procaspase-3 through sequestering inhibitory zinc ions

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Aim: The direct induction of apoptosis has emerged as a powerful anticancer strategy, and small molecules that either inhibit or activate certain proteins in the apoptotic pathway have great potential as novel anticancer drugs. Increased expression of procaspase-3 had been found in numerous tumors. Described herein is the investigation of the effect and mechanism of a novel small compound WF-210. **Methods:** The procaspase-3 activate activity was detected using a modified molecular docking and enzymatic based *in vitro* method. The MTT method and Annexin-V/PI co-stain method were used to detect cytotoxic and apoptosis of U-937 cells. Western blot analysis was performed for detecting the expression of apoptosis-related proteins. To further confirm that the apoptosis induced by WF-210 was caspase-3-dependent, chemically synthesized siRNAs was used to silence caspase-3 in HL60 cells with high expression of procaspase-3. Transfection of HL60 cells with siRNAs reduced caspase-3 expression by 60%–70%. Eukaryotic expression plasmid encoding procaspase-3 was transiently transfected into MCF-7 cells which was procaspase-3 deficient. Further study of the procaspase-3 activate mechanism was assayed using recombinant human procaspase-3 protein in buffer with or without zinc. **Results:** In a modified molecular docking and enzymatic based assay, WF-210 markedly bound and activated procaspase-3 in a concentration-dependent manner with EC_{50} (0.85 μmol/L). WF-210 effectively induced apoptosis of U937 cells in a concentration-dependent manner. Co-treatment with different caspase inhibitor and WF-210 partly blocked cell apoptosis. The block rates of van-caspse and caspase-3 inhibitors were higher than caspase-8 and caspase-9 inhibitors. After treatment with WF-210, caspase-3, caspase-8, and caspase-9 were activated in HL60 and U937 cells, and procaspase-3 was activated at first. Silence procaspase-3 gene expression in HL60 could block apoptosis induced by WF-210. Transient transfection of caspase-3 into MCF-7 cells could restore apoptosis induced by WF-210. In further study, WF-210 markedly activated procaspase-3 in a concentration-dependent manner in buffer with zinc, showed no activity in buffer without zinc. **Conclusion:** Taken together, our results suggest that WF-210 activates procaspase-3 *in vitro* by sequestering inhibitory zinc ions, and inducing cell apoptosis in a caspase-3 dependent manner.

Keywords: Anticancer; Procaspase-3; Apoptosis; Zinc
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S1.51

Flavokawain B, a kava chalcone, induces apoptosis of non-small cell lung cancer by through Bax-initiated mitochondrial pathway

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Aim: Flavokawain B, a chalcone from kava root extracts, possesses strong anti-neoplastic activity against many cancer cells. Here the antitumor activity and molecular mechanisms in lung cancer cells were investigated. **Methods:** The anti-cancer potential of Flavokawain B in lung cancer H460 and A549 cells were tested *in vitro*. **Results:** FKB significantly inhibited cell proliferation and caused arrest of the cell cycle G_2-M in a dose-dependent manner. FKB also inducted apoptosis, which was associated with cytochrome *c* release, caspase-7 and caspase-9 activation and Bcl-x_L/Bax dys-regulation. FKB significantly down-regulated survivin and XIAP, and the inhibitory effect induced by FKB was greatly attenuated by through overexpression of survivin or $Bax^{-/-}$ MEFs. Furthermore, FKB activated the mitogen-activated protein kinases and the JNK inhibitor SP600125 significantly decreased the growth-inhibitory and apoptotic effects of FKB. **Conclusion:** These results suggest the anti-lung cancer potential of Flavokawain B for the prevention and treatment of lung cancer.

Keywords: apoptosis; Bax; Flavokawain B; JNK; lung cancer; survivin
Acknowledgements: This work was financially supported by the National Natural Science Foundation of China #81173095 (Ya-xiong TANG) and by the Major Research plan of the National Natural Science Foundation of China #91013006 (Jian SUN).

S1.52

Polyamine analogues tetrabutyl propanediamineon induced apoptosis of human leukemia HL-60 cells

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Aim: High concentration of polyamines are required for quick proliferation of tumor cells, and an exhaustion in intracellular polyamines will inhibit tumor cell growth and induce apoptosis. Polyamine analogues can promote polyamine degradation and thus to induce apoptosis in tumor cells. Therefor, it necessary to design and develop new polyamine analogues for clinical trail or mechanism research which target polyamine metabolism. In the present study, the impact of a new polyamine analogue, tetrabutyl propanediamine (TBP), on the proliferation and apoptosis of human leukemia HL-60 cells were observed. **Methods:** HL-60 cells were treated with 50 μmol/L TBP for 24, 48, 72 h. Proliferation rate of HL-60 cells was assayed by MTT. The flow cytometry was performed to identify cell cycle. The DNA fragmentation analysis was used to evaluate apoptosis. Western blot was used to identify the expression of apoptosis-related protein. **Results:** TBP dramatically inhibited proliferation of HL-60 cells and the inhibitory effect was in a time and dose dependent manner. The results from flow cytometry analysis indicated that TBP induced cell cycle arrest in G_0/G_1. After TBP treatment, typical DNA fragmentation for apoptotic cells was observed by agarose gel electrophoresis. Furthermore, the contents of proapoptotic protein Bax and cytochrome *c* in the cytosol were significantly increased. **Conclusion:** TBP can markedly inhibit the proliferation of human leukemia HL60 cells by arresting cell cycle and inducing apoptosis. These results first showed that TBP, as an asymmetric analogue of putrescine, has a potential value in clinical treatment of leukemia.

Keywords: polyamines; tetrabutyl propanediamineon; leukemia; apoptosis

S1.53

A standardized extract from *Paeonia lactiflora* and *Astragalus membranaceus* inhibits proliferation and induces apoptosis in human hepatoma cell lines

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Aims: Paeonia lactiflora and Astragalus membranaceus are two traditional Chinese medicines, commonly used in Chinese herb prescription to treat liver disease. Protective effects of the extract prepared from the roots of Paeonia lactiflora and Astragalus membranaceus (PAE) on liver fibrosis were demonstrated in previous investigations. The present study was designed to investigate whether PAE effect in the growth-inhibition and apoptosis-induction of human hepatoma cell lines HepG2 and SMCC-7721. **Methods:** After treatment with PAE, the proliferation of HepG2 cells and SMCC-7721 cells was determined by methyl thiazolyl tetrazolium (MTT) and the apoptosis of these two human hepatoma cell lines was measured by flow cytometry with propidium iodide (PI) technology. The expression of Bcl-2, Bax and cleaved caspase-3 was detected by Western blotting, while the expression of Bcl-2 and Bax was also detected by immunohistochemistry. **Results:** Treatment with PAE (10-200 mg/L) caused a inhibitory effect on cell proliferation in hepatoma cell lines HepG2 and SMCC-7721. Meanwhile, PAE induced HepG2 cells and SMCC-7721 cells to undergo apoptosis, which was demonstrated by PI staining. Furthermore, Western blotting and immunohistochemistry revealed that the expression of Bcl-2 was significantly decreased while Bax and cleaved caspase-3 was increased in HepG2 cells and SMCC-7721 cells after treatment by PAE (10-200 mg/L). **Conclusion:** Our results suggested that PAE inhibits hepatoma cell proliferation and induces cell apoptosis. PAE may induce apoptosis in HCC cells by regulating Bcl-2 families.

Keywords: PAE; hepatocellular carcinoma; apoptosis; HepG2; SMCC-7721

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S1.54

Cinobufacini inhibits proliferation of human cervical cancer Caski cells by inducing apoptosis

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Aim: To investigate the effects of cinobufacini on proliferation and apoptosis in human cervical cancer Caski cells. **Methods:** MTT assay was used to assay cell proliferation; the flow cytometry assay was used to determine cell cycle; DNA fragmentation assays was used to evaluate apoptosis; Western blot was used to analyze cytochrome c content in the cytosol; the level of reactive oxygen species (ROS) was determined by fluorescence histochemistry; the mitochondrial membrane potential was determined by Dioc6-staining method. **Results:** Cinobufacini significantly inhibited the proliferation of Caski cells in a time- and dose-dependent manner. The concentration of median inhibition (IC_{50}) at 48 h is 1.68 μg/mL. Cinobufacini treatment resulted in DNA fragmentation, elevated ROS levels, increased release of cytochrome *c* from mitochondria to the cytosol and a decrease in mitochondrial membrane potential. Meanwhile, cinobufacini inhibited cell division and lead to cell cycle arrest in G_2/M phase. **Conclusion:** Cinobufacini significantly inhibits the proliferation of Caski cells. The underlying mechanism is associated with the increase of ROS generation, which induced endogenous apoptosis and inhibited cell division. The results of this study suggest that cinobufacini has a potential value for the clinical treatment of cervical cancer.

Keywords: cervical cancer cell; apoptosis; cinobufacini

S1.55

Mitogen-activated protein kinases inhibitors exerts anti-fibrosis by mediating TGF-β/Smad signaling in HepG2 cells and HSC cells

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Aims: To define further the mechanisms of TGF-β_1/Smad signal transduction mediated by mitogen-activated protein kinases (MAPKs), including the extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 pathways, in HepG2 cells and HSC cells. **Methods:** In the absence or presence of three MAPK (ERK, JNK and p38)-specific inhibitors, HepG2 cells and HSC cells were stimulated with exogenous TGF-β_1 to activate Smad signalling. plasminogen activator inhibitor(PAI)-1 transcriptional activity was measured by real-time reverse transcriptase-polymerase chain reaction analysis. **Results:** TGF-β_1 induced PAI-1 mRNA expression in HepG2 cells and HSC cells; in addition, the three MAPK (ERK, JNK and p38)-specific inhibitors decreased PAI-1 mRNA expression. **Conclusion:** These results suggested that the ERK, JNK and p38 pathways mediate TGF-β_1/Smad signal transduction and might be considered as specific targets of drug therapy for hepatocellular carcinoma.

Keywords: HepG2 cells; HSC cells; mitogen-activated protein kinase; plasminogen activator inhibitor 1; Smad; TGF-β

S1.56

The effects of extracts from Astragalus membranaceue Bge(ABM) on HepG2 cell

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Aim: To observe the effect of extracts from Astragalus membranaceue Bge (AMB) on HepG2 cell and its molecular mechanism. **Methods:** HepG2 cells were cultured in 2 groups: control group and AMB group; MTT assay and RT-PCR were used to test the variation of the two groups. **Results:** We observed that the cell population and parapodium of HepG2 decreased transparently in AMB group compared with the control group, and some cell floated. The expression of p53 increased and Bcl-xLdecreased. **Conclusion:** The extracts of Astragalus membranaceue Bge (AMB) can restrain the growth of HepG2 cell, its mechanism may be the activation of p53 and the suppression of Bcl-x_L.

Keywords: *Astragalus membranaceue* Bge; HepG2; Bcl-x_L; p53

S1.57

Investigation of protective effect of Luobuma (*Apocynum venetum*) extract and its metabolites against tert-butyl hydroperoxide (t-BHP) induced human hepatoma HepG2 cell injury

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Aims: According to pervious publications, Luobuma *(Apocynum venetum)* extract showed protective effect for liver damage in *in vitro* and *in vivo* models, and the bioactive compounds were considered to be flavonoids that included in Luobuma

extract. However, after oral administration, flavonoids are extensively metabolized during absorption, which means only their metabolites instead of the mother flavonoids can reach liver and represent activities. In this work, we investigate the protective effect of Luobuma extract, as well as p-hydroxyphenylacetic acid (p-HPAA) and 3,4-dihydroxyphenylacetic acid (DOPAC) that are two metabolites of its main compounds (hyperoside and quercetin), against tert-Butyl hydroperoxide (t-BHP) induced human hepatoma HepG2 cell injury. **Methods:** HepG2 cells were per-incubated with Luobuma extract, p-HPAA or DOPAC for 2 or 24 h, and then treated with t-BHP for 3 h. Subsequently, cell viability and alanine aminotransferase (ALT) release were measured by MTT assay and fluorometric assay, respectively. **Results:** After 2 h pre-incubation, 40–80 μg/mL of Luobuma extract increased 25% cell proliferation and inhibited ALT release by almost 40%, but p-HPAA and DOPAC at tested concentrations showed no effect compared to the control. After 24 h pre-incubation, the protective effect of Luobuma extract was decreased, while DOPAC at concentrations of 0.5–5 μg/mL showed significant stronger beneficial effect compared to the cells treated t-BHP, no obvious change was observed in p-HPAA treated cells. **Conclusion:** Our results suggest that after biotransformation, Luobuma extract can protect hepatocytes from oxidant attack via its metabolites, which probably act through regulation of specific gene expression since the prolonged protective effect of the metibolites.

Keywords: *Apocynum venetum*; HepG2 cells; ethanol; paracetamol; t-BHP
Acknowledgements: This work was supported by the Ministry of Education and State Administration of Foreign Experts Affairs "overseas teacher" project (No MS2011XBNL057) and the Open Project Program of State Key Laboratory of Natural Medicines, China Pharmaceutical University (No SKLNMKF201221).

S1.58

Caffeic acid 3,4-dihydroxyphenethyl ester arrests colorectal cancer cells growth by upregulating phosphorylation of p38 MAPK

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Aims: Caffeic acid 3,4-dihydroxyphenethyl ester (CADPE) has been reported to possess a broad spectrum of antitumor activity; however, its antitumor mechanism is not well understood. In this study, we investigated the influence of CADPE on cell cycle and its underlying mechanisms using human colorectal cancer cell lines. **Methods:** First, the anticancer activity of CADPE in human colon cancer cells was determined by measuring the cell growth and the colony formation in the presence or absence of CADPE. Second, the effects of CADPE on cell cycle and the related cell cycle-regulating proteins were analyzed using flow cytometry and Western blot, respectively. Finally, a potential role of p38 MAPK in the anticancer activity of CADPE was explored by using chemical inhibitors of three components of MAPK signaling pathways and determining whether any of the inhibitors was able to abolish or attenuate the CADPE-induced alterations of above parameters. **Results:** CADPE inhibited cell growth in a variety of human colorectal cancer cell lines with IC_{50} values ranging from 4.06 to 17.60 μmol/L, and markedly reduced the colony formation of cancer cell lines. Consistently, CADPE significantly arrested cell cycle progression at G_0/G_1 phase in measured cell lines with a corresponding and robust decrease in the protein levels of Cdc25A, cyclin A, cyclin D1, cdk2, cdk4, and p-Rb (Ser780), suggesting a crucial role of inhibition of Cdc25A-Cdk2/4-Rb pathway in CADPE-induced cell cycle arrest. Furthermore, CADPE substantially activated p38 MAPK pathway as indicated by a considerable and sustained increase in the level of p-p38 MAPK (Thr180/Tyr182) and the inhibition of p38 MAPK with SB239063 substantially suppressed CADPE-induced arrests of colon cancer cell growth and cell cycle progression as well as CADPE-induced inhibition of Cdc25A-Cdk2/4-Rb pathway, whereas inhibition of ERK1/2 or JNK had no these effects. **Conclusion:** These results demonstrate that activation of p38 MAPK that leads to inhibition of Cdc25A-Cdk2/4-Rb pathway plays a key role in CADPE-induced cell cycle arrest as well as in the anticancer activity of CADPE.

Keywords: CADPE; human colon cancer cells; cell cycle; Cdc25A-Cdk2/4-Rb pathway; p38 MAPK

S1.59

Tangeretin enhances the therapeutic effect of 5-Fu against colon cancer *in vivo* and *in vitro* by inhibiting miR-21 at low concentration

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Aim: Tangeretin exhibits anti-proliferative, anti-invasive, anti-metastatic and antioxidant activities under several cancer models *in vivo*. In this study, we evaluate the effects of tangeretin alone or in combination with 5-FU on cell viability and apoptosis in colon cancer cell lines. **Methods:** Effects of tangeretin and 5-FU combination or alone on the cell proliferation were tested in colon cancer cells HCT-116 and SW-480 by MTT assay. Iso-bolographic analysis was employed to evaluate the combination effect of tangeretin and 5-FU. Apoptosis in cells were examined by DAPI-staining. The mRNA level of miR-21 (a signature of tumor aggressiveness) was determined by real-time PCR. Two different xenograft studies also used to evaluate the effect of co-administration of tangeretin and 5-FU. **Results:** Combination of 5-FU and tangeretin has a synergistic effect. Tangeretin at low concentration (25 μg/mL) could markedly enhance the therapeutic effect of 5-FU compared to 5-FU groups ($P<0.05$). Low concentrations of tangeretin and 5-FU promote apoptosis and decreased mRNA level of miR-21 ($P<0.01$). *In vivo* mouse xenograft studies showed that coadmisitration of tangeretin and 5-FU reduced tumor volumes without bodyweight losing. **Conclusion:** Our data indicate that combination of tangeretin with 5-FU could improve the efficacy of chemotherapy for colon tumors.

Keywords: tangeretin; 5-FU; colon cancer; synergistic effect; miR-21

S1.60

Recent advances in anticancer research of homoharringtonine

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Homoharringtonine (HHT) was studied in our Institute for many years. In collaboration with other institutions it was proved as an effective antileukemic drug in China in 1978. Afterwards, In our Country HHT was manufactured in several pharmaceutical companies and used clinically as one of basic antitumor drugs approved by Chinese Ministry of Health in 1993. Meanwhile and later, more and more papers were published regarding its action mechanism of antitumor activity and clinical efficacy for treatment of different kinds of leukemias. In recent years in USA, several laboratories and clinical hospitals conducted systematic study on this drug and found some new uses for different malignancies. In particular, HHT is demonstrated as a good therapeutic drug for chronic myelocytic leukemia (CML) including patients who have developed resistance to targeting agents such as imantinib or dasatinib. It attracts much attention from medical circles. In October 2012 FDA approved this drug for treatment of CML patients. Semisynthetic preparation of HHT called omacetaxine mepesuccinate (ceflatonin, CGX-653, Myelostate) has been produced and recommended for clinical application in USA and other countries. When the drug is injected by subcutaneous (SC) route, the bioavailability is excellent. It has been reported that HHT was given as a single agent to 71 patients with late CML-CP (chronic phase of CML) patients at a daily dose of 2.5 mg/m^2 for 14 d during the remission induction phase, and for 7 d monthly during the maintenance phase. A complete hematologic response was obtained by 72%, and cytogenetic respone was achieved by 31%. The major toxicity was myelosuppression. The mechanism of action of omacetaxine is related to the induction of apoptosis in a Bax-independent fashion through mitochondrial disruption and release of cytochrome *c*, leading to caspase-9 and caspase-3 activation in leukemic cells. Gene expression analysis showed that the transforming growth factor-beta (TGF-β) and the tumor necrosis factor (TNF) signaling pathways appear to play an impotant role in induction of apoptosis by omacetaxine.

Keywords: homoharringtonine; chronic myelocytic leukemia; FDA approval

S1.61

Anti-cancer effects of triterpenoids isolated form *Alismatis Rhizoma* on HepG2 cells

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Aim: This study aims (1) to isolate different types of compounds from *Alismatis Rhizoma*, a well-known traditional Chinese medicine, (2) to compare the anti-cancer activities *in vitro*, and (3) to study the structure-function relationship. **Methods:** Silica gel column chromatography and preparative HPLC were used to isolate the compounds and spectroscopic techniques including UV, MS, ^{1}H-NMR, and

^{13}C-NMR were employed to elucidate the structures. MTT assay and observation of morphological changes were performed to evaluate the cell proliferation. Flow cytometry was used to detect cell cycle distribution in different phases. **Results:** Thirteen protostane triterpenoids, including alisol A, alisol A 23-acetate, alisol A 24-acetate, 16-di-hydroxy-alisol A 23-acetate, 16-di-hydroxy-alisol A 24-acetate, alisol B, alisol B 23-acetate, 11-deoxyalisol B, alisol C, alisol C 23-acetate, alisol F, alisol G, and alisol L, together with sitosterol, were obtained from the rhizome of *Alismatis Rhizoma*. The anti-cancer activities of alisol A, alisol A 24-acetate, alisol B, alisol B 23-acetate, alisol C, alisol C 23-acetate, alisol L, alisol F and sitosterol were screened, and alisol B showed the most effective anti-cancer activity as confirmed by the results of proliferation inhibition and cell numbers in sub-G1 section. Base on structure-activity relationship, C-16 hydrogen is critical to the anti-cancer activity of these alisol derivatives. **Conclusion:** The anti-cancer potential of alisol B is deserved further evaluation and C-16 hydrogen plays an important role for receiving their anti-cancer effects.

Keywords: *Alismatis Rhizoma*; triterpenoid; alisol B, cancer; structure-activity relationship.

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S1.62

Furanodiene induces G1 cell cycle arrest and activates the signaling molecules regulating apoptosis and autophagy, and slightly inhibits key steps of cell metastasis in 95-D lung cancer cells

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Aim: This study aims to systematically investigate the effects of furanodiene (FUR), a natural terpenoid isolated from *Rhizoma Curcumae*, on cell viability and significant processes of tumor progression in 95-D lung cancer cells. **Methods:** MTT assay and flow cytometry were used to detect the anti-proliferative effects and cell cycle distribution, respectively. Western blot assay was performed to detect the expression of proteins related to the different cellular signal pathways. Cell adhesion was tested by cell attachments to matrigel and fibronectin using a microscope. Wound healing and transwell assays were employed to investigate cell migration and invasion, respectively. **Results:** FUR concentration-dependently inhibited cell proliferation and blocked the cell cycle progressions in G_1 phase via down-regulating the protein levels of cyclin D1 and CDK6, and up-regulating those of p21 and p27. FUR also affected the signaling molecules that regulate apoptosis revealed by the down-regulation of the protein levels of PARP, caspase-7, survivin, and the expression ratio of Bcl-2/Bax. Further studies showed that FUR induced expression of light chain 3-II in the protein level, indicating autophagy is involved in this process. However, the adhesive ability of 95-D cells to matrigel and fibronectin was only slightly inhibited after FUR treatment for 1 h. FUR also exhibited slight suppression on cell migration and invasion in 95-D cells. **Conclusion:** FUR induced G1 cell cycle arrest and activated the signaling molecules regulating apoptosis and autophagy, while slightly inhibited cell metastasis in 95-D lung cancer cells.

Keywords: furanodiene; cell cycle; apoptosis; autophagy; metastasis

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S1.63

Study on the purification and identification for anti-tumor activity of DL1106 and its analogue

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Aim: To get enough quantity and quality of DL1106 and its analogue to evaluate their anti-tumor activity, the fermentation conditions of marine actinomycetes REL-3 were optimized. **Methods:** Estimating the effects of fermentation time and the pH value and salt concentration of fermentation broth on the yield, the fermentation conditions of REL-3 were optimized. After extracting the collection of the fermentation liquid with equal volume of acetic acid ethyl three times, the relative content of the DL1106 and its analogue in the concentrated extracts was detected by HPLC. The crude DL1106 and its analogue were purified by preparative HPLC. The MTT assay was used to evaluate the inhibitory ability of DL1106 and its analogue on the growth of tumor cells, such as A549, HepG2, Skov3 and SY5Y. **Results:** We got the most objective production until the sixth day in the following fermentation conditions: the salt concentration of fermentation is 2.8% andthe pH value is 7.2. We got DL1106 and its analogue with higher purity. DL1106 and its analogue have strong anti-tumor effect indicated by significant cytotoxicity in A549, HepG2, Skov3, and SY5Y cells. **Conclusion:** We got the optimal fementation conditions of REL-3 and obtained almost pure DL1106 and its analogue. The MTT assay showed that DL1106 and its analogue have a favourable anti-tumor activity *in vitro*.

Keywords: REL-3; DL1106; analogue; HPLC; anti-tumor

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S1.64

Gecko Peptides Mixture induce apoptosis in human esophageal squamous carcinoma EC109 cell line *in vitro*

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Aims: To investigate the effect of Gecko Peptides Mixture (GPM) on apoptosis of human esophageal squamous carcinoma EC109 cell line and the molecular mechanisms. **Methods:** EC109 cells were cultured *in vitro* and the inhibitory effect of GPM on their proliferation was measured by MTT assay. The apoptosis of EC109 cells was determined by Hoechst 33258 fluorescence staining. The effects of GPM on Bcl-2 and caspase-3 protein expression of EC109 cells were investigated by using immunohistochemical method. **Results:** GPM significantly inhibited proliferation of EC109 cells in a dose-dependent and time-dependent manner, the values of the IC_{50} for 24, 48, and 72 hwere 0.317 mg/mL, 0.227 mg/mL, and 0.183 mg/mL, respectively. EC109 cells treated with 0.1 and 0.2 mg/mL GPM for 24 h induced typical apoptotic morphological features, decreased Bcl-2 protein expression while increased caspase-3 expression in EC109 cells. **Conclusion:** GCPs exerts anti-tumor effects in vitro by inducing apoptosis, decreasing the bcl-2 expression and increasing caspase-3 expression.

Keywords: gecko peptides mixture; human esophageal squamous carcinoma; apoptosis; Bcl-2; caspase-3

S1. 65

Growth inhibition and apoptosis induction effects on human tumor cell lines by actinomycin D and its analog from Streptomyces parvus REL-3

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Aims: As an important natural product resource, the diversity and chemical complexity of marine microorganism are treasury for development of new antitumor drugs. In the present study, growth inhibitory and pro-apoptosis effects of actinomycin D (Act D) and its analog (DL1106-2) from *Streptomyces parvus* REL-3 were investigated *in vitro*. **Methods:** Growth inhibitory effect of actinomycin D and its analog in seven human tumor cell lines, including promyelocytic leukemia cell (HL-60), oral epithelial carcinoma cell (KB), cervical cancer cell (HeLa), hepatocellular carcinoma cell (HepG2, Bel-7402), colorectal carcinoma cell (HCT-8) and lung adenocarcinoma cell (A549), was measured by using the microculture tetrozolium (MTT) assay after 72 h of treatment. Apoptosis of cells were evaluated by Hoechst 33342 staining, DNA agarose gel electrophoresis and flow cytometry. **Results:** Following exposure of tumor cells to Act D or DL1106-2 for 72 h, Act D or DL1106-2 at the concentration of 0.3 μg/mL completely inhibited the growth of tumor cell lines mentioned above. The IC_{50} of DL1106-2 against cells were 4.3, 14.8, 3.3, 13.6, 13.4, 11.4, and 9.3 ng/mL, while the IC_{50} of Act D were 7.8, 29.0, 9.5, 35.8, 74.7, 36.1, and 27.2 ng/mL, respectively. These results showed that the

growth inhibitory effect of DL1106-2 in these tumor cells was stronger than that of Act D. Results from Hoechst 33342 staining, DNA agarose gel electrophoresis, and flow cytometry demonstrated that DL1106-2 concentration-dependently induced apoptosis in the tumor cells, and also had more remarkable apoptosis-inducing activity than Act D. **Conclusion:** DL1106-2 can inhibit the growth of many human tumor cell lines by inducing apoptosis, which is similar to Act D. These data suggest that DL1106-2 has the potential to be used as a new cytotoxic anticancer drug.

Keywords: actinomycin D; cancer; growth inhibition; apoptosis; Streptomyces parvus

S1.66

Drug release characteristics and antitumor activity of doxorubicin-hyd-PEG-folate conjugate

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Aim: Doxorubicin (DOX) is a highly potent chemotherapeutic agent that is widely used to treat various types of cancers, including breast and urothelial cancer. However, its severe side effects, such as cardiotoxicity, remain as a major problem. To overcome these limits, more effective and active targeting system is needed to enhance intracellular uptake of drug in cancerous cells at the tumor sites. The aim of this paper is to investigate the drug release characteristics and antitumor activity of doxorubicin-hyd-PEG-folate conjugate. **Methods:** A PEG based folate mediated active tumor targeting delivery systems for DOX was designed and synthesized. In this system, DOX was conjugated with PEG by hydrazone bond (DOX-hyd-PEG-FA) or by amide bond (DOX-ami-PEG-FA). The drug loading of the conjugates was detected by HPLC method, and the drugs release characteristic was performed in different pH media *in vitro*. The KB cells (folate receptor over-expression) and A549 cells (folate receptor low-expression) were selected to study the cytotoxicity of the conjugates, meanwhile the effect of exogenous folic acid on the cytotoxicity of the conjugates was also observed. The uptake of DOX and its conjugates by tumor cells were analyzed by flow cytometry and fluorescence microscope. The intracellular distribution of conjugates was studied by using laser scanning confocal microscope. **Results:** The rate and amount of DOX release from the DOX-hyd-PEG-FA were strongly dependent on the pH of the medium. DOX-hyd-PEG-FA showed a much faster DOX release at pH 5.0 than at pH 7.4. The rate and amount of DOX release from the DOX-ami-PEG-FA was independent on the pH of the medium. Compared with DOX-hyd-PEG, DOX-hyd-PEG-FA could increase the intracellular accumulation of DOX in short time and showed a DOX translocation from lysosomes to nucleus in KB cells. DOX-hyd-PEG-FA could deliver DOX to nucleus in KB cells in a time dependent manner. The cytotoxicity of DOX-hyd-PEG-FA on KB cells was much higher than that of DOX-ami-PEG-FA and DOX-hyd-PEG. Meanwhile, the cytotoxicity of DOX-hyd-PEG-FA on KB cells was much higher than that on A549 cells. The cytotoxicity and intracellular uptake of DOX-hyd-PEG-FA were inhibited by exogenous folic acid. These results indicate that the DOX-hyd-PEG-FA can provide excellent stability in blood circulation. FA presented in the DOX-hyd-PEG-FA plays an important role in enhancing the cytotoxic effect. Both passive and active tumor targeting abilities and acid sensitivity of DOX-hyd-PEG-FA were likely to act synergistically. **Conclusion:** DOX-hyd-PEG-FA can be selectively transported into the tumor cells that folate receptors are highly expressed via folate receptor-mediated endocytosis, and then the DOX is released and exert anti-tumor effect. Based on the results obtained in this study, the *in vivo* antitumor activity of DOX-hyd-PEG-FA is currently further evaluated on athymic nude mice bearing KB cells tumors.

Keywords: poly(ethylene glycol) (PEG); pH-sensitive; tumor-targeted; doxorubicin; folate receptor; cellular uptake; cytotoxicity

Acknowledgements: This work was supported by Shaanxi Science and Technology Innovation Project (2012KTCL03-18).

S1.67

Attenuation of tumor formation by *Ardisia crispa* root in murine skin carcinogenesis model

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Aim: *Ardisia crispa* has been traditionally used to treat inflammation-related diseases. In this study, chemopreventive effect of its triterpene- and quinone-rich fraction (TQRF) was investigated. **Methods:** Mice (groupI-IV; n=10) were initiated with single dose of 7,12-dimethylbenz(α)anthracene (DMBA, 100 μg/100 μL) followed by repeated promotion of croton oil (1%) twice weekly (20 weeks). Group I (anti-initiation) received TQRF (30 mg/kg) 7 d before and after DMBA; group II (anti-promotion) received TQRF 30 min before each croton oil application; group III (anti-initiation/promotion) was treated as combination of group I and II. Two other groups served as vehicle control (group V) and treated control (group VI). **Results:** Group IV (carcinogen control) showed highest tumor volume (8.79±5.44) and tumor burden (3.60±1.17). Group I revealed marked increment of tumor incidence (40%) with prompted latency period of tumor formation (week 7). Group II showed significant reduction of tumor volume (3.11), tumor burden (3.00) and tumor incidence (11.11%), along with prominent delayed of latency period of tumor formation (week 12). Group III revealed only 20% of tumor incidence, tumor burden (3.00±1.00) and tumor volume (2.40±1.12), which was significantly different from group IV. No tumor formation was observed in groups V and VI. **Conclusion:** This study evidences the inhibitory effects of TQRF during promotion period which further suggests the plausibility of each compound as chemopreventive agent.

Keywords: *Ardisia crispa;* skin cancer; chemoprevention; triterpene; quinone

S1.68

Molecular basis of *eIF3a* gene in therapeutic response of lung cancer patients to platinum-based chemotherapy

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Drug resistance is one of the major obstacles in successful chemotherapy of lung cancers. However, the mechanism of resistance is not yet clearly understood. Recently, eIF3a that plays an important role in translational control and regulation of gene expression was found to correlate negatively with prognosis in human cervical and esophageal cancers. We, thus, hypothesize that eIF3a may be an important factor in lung cancer response to chemotherapy by regulating the expression DNA repair proteins. Examination of 211 lung cancer tissue samples revealed that eIF3a expression negatively correlates with responses of the patients to cisplatin treatment. Ectopic over-expression or knockdown of eIF3a reduced or increased cellular resistance, respectively, to cisplatin, doxorubicine, and etoposide (VP-16) in cell lines as determined using MTT assay. Altering eIF3a expression also changed DNA repair activity as well as the expression of DNA repair proteins. We conclude that eIF3a possibly suppresses the synthesis of DNA repair proteins via translational control, which in turn contributes to the increased sensitivity to DNA-damaging anticancer drugs.

S1.69

2-Methoxyjuglone induces apoptosis in HepG2 human hepatocellular carcinoma cells and exhibits *in vivo* antitumor activity in a H22 mouse hepatocellular carcinoma model

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In order to discover anticancer agents from natural sources, an ethanol-soluble extract of the root bark of *Juglans cathayensis* was investigated and showed cytotoxic effects against various human cancer cell lines. A subsequent phytochemical study on the EtOAc-soluble fraction determined 2-methoxyjuglone (1) as one of the main active constituents. Compound 1 was shown to be cytotoxic against HepG2 cells. Morphological features of apoptosis were observed in 1-treated HepG2 cells, including cell shrinkage, membrane blebbing, nuclear condensation, and apoptotic body formation. Cell cycle analysis with propidium iodide staining showed that **1** induced cell cycle arrest at the S phase in HepG2 cells. Flow cytometric analysis with annexin V and propidium iodide staining demonstrated that **1** induced HepG2 cell apoptotic events in a dose-dependent manner (0-8 μg/mL). Western blot analysis of apoptosis-related proteins revealed that **1** induces HepG2 cell apoptosis through mitochondrial cytochrome c dependent activation of the caspase-9 and caspase-3 cascade pathway (intrinsic pathway). An *in vivo* experiment using tumor-bearing mice showed that treatment with **1** at 0.5 and 1.0 mg/kg per day decreased the tumor mass by 56% and 67%, respectively.

Keywords: *Juglans cathayensis*; 2-methoxyjuglone; apoptosis; intrinsic pathway; *in vivo* antitumor effect

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S1.70

Src mediates extracellular signal-regulated kinase 1/2 pathway and autophagic cell death induced by cardiac glycosides in human non-small cell lung cancer cells

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Aim: Our previous work demonstrated that extracellular signal-regulated kinase 1/2 (ERK1/2) pathway regulates autophagic cell death induced by representative CGs digoxin or ouabain in human non-small-cell lung cancer (NSCLC) cells. In this study, the role of Src in ERK1/2 signaling pathway as well as autophagy induced by the CGs was examined in A549 and H460 cells. **Methods:** Src inhibitor PP2 or siRNA were used to knock down phosphorylation or mRNA level of Src, respectively, along with the CGs treatment. Cellular viability was performed with MTT assay. Level of intracellular reactive oxygen species (ROS) was detected with H_2DCFDA staining, followed by flow cytometry. **Results:** Src activation was significantly observed simultaneously with mitogen-activated protein kinase kinase 1/2 (MEK1/2) and ERK1/2 activation upon the CGs treatment. Src inhibitor PP2 or siRNA knockdown could block either drug-induced MEK1/2 and ERK1/2 activation, and autophagic cell death in the cells. In addition, the level of ROS was markedly increased by CGs in a time-dependent manner, and this increase was significantly attenuated with PP2 pretreatment. **Conclusion:** CGs induces autophagy in human NSCLC cells through activation of Src-mediates ERK1/2 pathway. Besides, Src also plays an important role in CGs-induced ROS generation.

Keywords: cardiac glycosides; autophagic cell death; Src; lung cancer; reactive oxygen species

S1.71

Aspirin enhanced ABT-737-induced apoptosis via regulation of PI3K pathway in human cancer cells

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Aim: Observational studies show that regular use of aspirin after diagnosis was associated with longer survival among patients with mutated-PIK3CA colorectal cancer, but not among patients with PIK3CA wild-type cancer. ABT-737, a potent small-molecule inhibitor of antiapoptotic members of the Bcl-2 family, is a promising therapeutic agent for multiple malignancies. We examined the anti-cancer activity of 2 chemotherapeutic agents (aspirin and ABT-737) in several PIK3CA wild-type cancer cells. **Methods:** We analyzed the anti-tumor efficacy of aspirin and ABT-737 in human cancer cells *in vitro* by SRB cytotoxicity assay. Apoptosis and cell cycle were determined using flow cytometry analysis. Western blotting analysis was carried out to examine the expression of related molecules. AO staining was used to visualize Acidic autophagic vacuole. **Results:** In this study, we showed that combination treatment with clinically achievable concentrations of aspirin and ABT-737 could induce a synergistic growth arrest and apoptosis in several human PIK3CA wild-type cancer cells. In addition, we found that the combination of aspirin and ABT-737 resulted in a blockade of the PI3K signaling pathway. Furthermore, our data showed increased autophagy correlated with the resistance to aspirin or ABT-737 as single-agents, the enhanced autophagy induced by aspirin plus ABT-737 switched from a cytoprotective signal to a death-promoting signal. **Conclusion:** Our study indicated that the combination of aspirin and ABT-737 exerted synergistic anti-proliferative effects against PIK3CA wild-type cancer cells *in vitro*. We hope that this synergy may contribute to failure of aspirin cancer therapy and ultimately lead to efficacious regimens for cancer therapy.

Keywords: aspirin; ABT-737; solid tumors; apoptosis; autophagy

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S1.72

Heparan sulfate proteoglycans and cancer

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Heparan sulfate proteoglycans (HSPGs) are widely distributed in extracellular matrix (ECM) of mammalian tissues and involved in a number of processes related to malignancy. They are composed of a core protein to which attached chains of the glycosaminoglycan,heparan sulfate(HS). HSPGs interact with many proteins including growth factors, chemokines and structuralproteins of ECM to influence cell growth, differentiation, migration, and the cellular response to the environment. Recently, HSPGs are reported to have close relationship to cancer, especially tumor cell migration. This review describes current understanding of HSPGs in cancer and highlights new possibilities for therapeutic control.

Keywords: heparan sulfate proteoglycan; tumor; metastasis; growth factors

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S1.73

Temozolomide analogs overcome tumor resistance and induce DNA damage

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Aim: Based on the molecular and resistance mechanism of alkylating agent temozolomide (TMZ), TMZ analogs's mechanism of action was investigated by in vitro anti-tumor activity assay, cell cycle and apoptosis analysis and DNA damage assay in glioam cells. **Methods:** the anti-tumor activity of temozolomide analogs was investigated by MTT assay with SNB19, U373 cells and the paired O6-methylguanine-methyltransferase (MGMT) transfected cells lines SNB19M and U373M; also the corresponding acquired resistance glioma cell lines SNB19VR and U373VR and the intrinsic TMZ resistance cell line HCT116 (mismatch repair gene *hMLH1* loss) were used as the screening models; the cell cycle analysis of TMZ analog 14112 were performed by flow cytometry in the SNB19V and SNB19M glioma cell lines; DNA double strand breaks (DSBs) induced by 14112 was detected by Western blot and immunofluence with SNB19V and SNB19M cells. In addition, DNA single strand breaks and DNA repair kinetics were analysized by comet assay. Furthermore, the apoptosis caused by 14112 was investigated by PARP antibody in HCT116 cells. **Results:** TMZ showed highly resistant to MGMT overexpressed cells, however, TMZ analogs were very active in TMZ resistance cell lines, showing equi-activity in the low and high MGMT expressed cells. Analog 14112 showed the most activity in the tested cell lines. Furthermore, TMZ analogs showed anti-tumor activity in the TMZ acquired resistant cell lines SNB19VR and U373VR. Analog 14112 caused cells arrest at G_2/M and apoptosis. Morevoer, DNA strand breaks was observed with treatment time increased, the excision repair of 14112 was the similar to that of TMZ. The double strand breaks induced by 14112 were earlier than that of TMZ indicated by γH2AX expression. **Conclusion:** The TMZ analogs can overcome TMZ resistance, the DNA damage induced by analog is not repaired by MGMT.

Keywords: glioma; resistance; temozolomide; analogs; DNA damage

S1.74

Jaridonin, a novel anticancer drug, induced human gastric cancer cell line MGC-803 autophagy and its possible mechanisms

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Aim: Jaridonin, a novel ent-kaurane diterpenoid compound from *Isodon Rubescens* in our laboratory. We designed the current study to determine the effects of Jaridonin on human gastric cancer line MGC-803 autophagy and its possible mechanisms. **Methods:** After treatment by Jaridonin for 24 h, the effect of Jaridonin on the MGC-803 cell viability was examined using MTT assay. Used the fluorescence

microscope to observe the autophagosome and then used the flow cytometry to determine the effects of Jaridonin on the ratio of the autophagy. Finally we used Western blot to detect the expressions of the proteins related with cell autophagy. **Results:** Jaridonin caused strong antiproliferative on MGC-803 cells in a dose- and time-dependent manner. Jaridonin also induced the produce of autophagosome in the MGC-803 cells. After the treatment of Jaridonin with MGC-803 cells 24 h, the expression of LC3I, LC3II were up-regulating, and the rate of LC3I/LC3II was increasing too, but the expression of PI3K, Akt and the phosphorylation of Akt were down-regulating. **Conclusion:** Jaridonin can significantly inhibit the growth of MGC-803 cells, and induce autophagy. The antitumor activity of Jaridonin might be attributed partly to the upregulation of LC3I, LC3II and thedownregulation of PI3K, Akt, and the phosphorylation of Akt.

Keywords: ent-kaurene diterpenoid compound; anticancer; autophagy; molecular mechanism

S1.75

Rules of treatment and prescription-herbs on lung cancer in chinese medicine literatures

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Aim: To explore the herbal administration rules, the syndrome and pathogenesis in TCM literatures. **Methods:** Collecting the prescriptions and herbs during and before the period of the Republic of China, analyzing data by frequency analysis. **Results:** Phlegm-dispelling and cough-suppressing medicinal, tonifying and replenishing medicinal, toxic herbs, qi-regulating medicinal, interior-warming medicinal, dampness-draining diuretic medicinal, dampness-resolving medicinal, exterior-releasing medicinal and blood-activating and stasis-resolving medicinal are the main types of herbs, qi and yin–tonifying medicinal are more commonly used. The herbs mainly belong to lung, spleen and stomach channels and characterized by the qi and flavor of pungency, sweetness, bitterness and warmth-heat. **Conclusion:** Reinforcing the healthy qi and restoring deficiency, dispelling phlegm and eliminating dampness, regulating qi and activating blood circulation and treating with poisonous agents are the main methods of treatment in TCM literatures. The qi and flavor of herbs is mainly pungency, sweetness, bitterness and warmth-heat. The channels of herbs is mainly lung, spleen, stomach. For further analysis, we preliminarily infer that lung cancer is the disease of root deficiency with insufficiency of qi and yin and branch excess with phlegm, dampness, blood stasis and toxin.

Keywords: TCM literatures; lung cancer; prescription-herbs; frequency analysis
Acknowledgements: The study was supported by the Capital Medical Development Foundation (SF-2007-I-08) and the Capital Public Health Project (Z111107067311044)

S1.76

Antiproliferation activity of Triterpenoid Saponins AG4 from *Ardisia Gigantifolia Stapf* on MCF-7 cells

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Aim: AG4 is a anti-tumor compound derived from *Ardisia gigantifolia Stapf*. The aim of this study was to investigate the influence of AG4 on different kinds of tumor cells and the possible mechanisms. **Methods:** We exposed 9 kinds of tumor cells to different concentrations of AG4 in order to investigate the effect of AG4 on cell viability by using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The appearance of MCF-7 cells treated by AG4 was measured by fluorescence microscopy. The apoptosis and cell cycle were detected by the flow cytometry. Activities of caspase-3 and caspase-9 were measured using activity quantitative detection kit. **Results:** AG4 showed proliferation inhibition in A549, BeL-7402, BGC-823, C6, EJ, HeLa, HepG2, MCF-7, and LS180 cells, and significantly in MCF-7 cells. Compared with control, the appearance has been changed and apoptosis apparently in MCF-7. Significant production of MDA was observed, the results also showed that AG4 down-regulated SOD activity and GSH level, and the activities of caspase-3 and caspase-9 had significant raise. Simultaneously, AG4 significantly blocked the cell cycle in S phase. **Conclusion:** These results indicated that the inhibition on proliferation of MCF-7 cells was possibly through reactive oxygen species evoked, cycle blocking, and mitochondrial path way activation by AG4.

Keywords: *Ardisia gigantifolia Stapf*; cancer cells; apoptosis; cell cycle; caspase; redox system

S1.77

The anti-tumor effect of Sedum Lineare Thunb alcohol extract on S_{180}-bearing mice and its mechanism

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Aim: To observe the effects of Sedum Lineare Thunb (SLT) alcohol extract on tumor growth and to study its antioxidant ability and regulation of immune function. **Methods:** The second day of S_{180}-bearing mice model established, mice were randomly divided into control group, model group, cyclophosphamide (CTX) group (50 mg/kg) , SLT low and high dose groups (4 and 8 g/kg), 10 per group, and continuously administrated for 14 d. The index of the inhibition rate of tumor weight was used to observe the anti-tumor activity of SLT. The levels of serum interleukin-6 (IL-6) and interleukin-10 (IL-10) were detected by Enzyme-linked immunosorbent assay (ELISA). Biochemical method was used to measure the activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA). Flow cytometer was used to survey the ratio of neutrophil and lymphocyte percentage in the white blood cell, the percentage of $CD3^+$ positive cells in the lymphocytes and the percentage of $CD4^+$ or $CD8^+$ cells in the T-Lymphocytes, and then calculate the ratio of $CD4^+/CD8^+$. **Results:** The inhibition rate of the tumor growth in the SLT low and high dose groups (4 and 8 g/kg) are 84.26% and 62.24%, obviously higher than CTX group 29.28%. After treated with SLT, the thymus index was up-regulated, while the spleen index was down-regulated; the levels of serum IL-6 ($P<0.05$) and IL-10 ($P<0.001$) were increased significantly; the activity of SOD in serum was enhanced, but the content of MDA was reduced; the percentage of neutrophils was inhibited yet the percentage of lymphocytes and $CD3^+$ lymphocyte were improved significantly; $CD4^+$ lymphocyte T was obviously higher than that of the model group, however $CD8^+$ T lymphocyte was lower; and $CD4^+/CD8^+$ was increased markedly. **Conclusion:** SLT alcohol extract had an anti-tumor effect on S_{180}-bearing mice, and its mechanism probably result of improving the antioxidant ability and immune function.

Keywords: Sedum Lineare Thunb; S_{180}-bearing mice; cytokine; antioxidation; immune function
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S1.78

Synthesis and *in vitro* anti-cancer activity of four metal - fluorouracil complexes

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Aim: In order to synthesize new efficient and low toxic metal-fluorouracil (5-FU) complexes anticancer drugs. **Methods:** Copper, iron, zinc salts and fluorouracil were used to synthesize copper, iron and zinc-fluorouracil complexes. Preliminary chemical structures of the 4 complexes were confirmed by elemental analysis and mass spectrometry. Their inhibitory activity on human cancer cells was measured by MTT colorimetric assay. **Results:** $[Cu(5\text{-}FU)_2Cl_2]$, $[Cu(5\text{-}FU)_2(NO_3)_2]$, $[Fe(5\text{-}FU)_3SO_4]$, and $[Zn(5\text{-}FU)_2Cl_2]$ were successfully synthesized. The IC_{50} values of $[Zn(5\text{-}FU)_2Cl_2]$ and $[Fe(5\text{-}FU)_3]SO_4$ on HCT-116 cells were 9.2 and 18.1 μg/mL, lower than those of 5-FU and their metal salts counterparts. **Conclusion:** $[Zn(5\text{-}FU)_2Cl_2]$ and $[Fe(5\text{-}FU)_3]SO_4$ exhibited a synergic inhibitory effect with fluorouracil on cancer cell proliferation, their anti-cancer activity is worthy of further study.

Keywords: copper; iron; zinc-fluorouracil complexes; synthesis; anti-cancer activity