Synergistic effect of Astragalus flavonoids on breast cancer chemotherapeutic agent cyclophosphamide based on the regulation of immune function

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The present study aimed to observe the synergistic effect of Astragalus flavonoids (TFA) on breast cancer chemotherapeutic agent cyclophosphamide (CTX) and its effect on immunologic function. 4T1 breast cancer bearing mice were established and then randomly into control group, model group, CTX group (80 mg/kg), CTX (80 mg/kg) combined with TFA (6 mg/kg) and TFA group (6 mg/kg). After 3 weeks of different treatments, tumor inhibition rates were measured; subpopulation of the splenic lymphocytes and marrow derived suppressor cells (MDSCs) in circulating blood were detected by flow cytometry. Compared with model group, both CTX and CTX-TFA can significantly inhibit tumor growth with inhibition rate of 55.61% (P<0.05) and 70.91% (P<0.01), respectively. However, compared to CTX group, CTX-TFA group showed a higher inhibition rate (P<0.05). CTX-TFA group showed an increased percentage of splenic CD3+, CD4+ T and CD8+ T lymphocytes (P<0.05, P<0.01) and decreased percentage of MDSCs (CD11b+Ly6Chi) (P<0.05, P<0.01), whereas the percentage of CD19+ cells remained unchanged. TFA could enhance the anti-tumor effect of CTX and the synergistic effect probably result from improving tumor immune-suppression via increasing the percentage of CD3+, CD4+ T and CD8+ T lymphocytes and down-regulating the percentage of MDSCs.

Keywords: Astragalus flavonoids; breast cancer; cyclophosphamide; immune function
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Anti-Warburg effect elicited by cAMP-PGC1α pathway drives differentiation of glioblastoma cells into astrocytes

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Glioblastoma (GBM) is among the most aggressive of human cancers. Although differentiation therapy has been proposed as a potential approach to treat GBM, the mechanisms of induced differentiation remain poorly defined. Here, we established an induced differentiation model of GBM using cAMP activators, which specifically directed GBM into astroglia. Next, transcriptomic and proteomic analyses uncovered oxidative phosphorylation and mitochondrial biogenesis were involved in induced differentiation of GBM. Further investigation showed dbcAMP reversed the Warburg effect evidenced by increase in oxygen consumption and reduction of lactate production. Stimulated mitochondrial biogenesis downstream of CREB/PGC1α pathway triggered metabolic shift and differentiation. Blocking mitochondrial biogenesis by mdivi or silencing PGC1α abrogated differentiation, reversely overexpression of PGC1α elicited differentiation. In GBM xenograft models and patient-derived GBM samples, cAMP activators also induced tumor growth inhibition and differentiation. This study shows mitochondrial biogenesis and metabolic switch to oxidative phosphorylation drive the differentiation process of tumor cells.

Keywords: differentiation therapy; metabolic reprogramming; Warburg effect; PGC-1 alpha; mitochondrial biogenesis
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Over-expression of SphK2 contributes to ATRA resistance in colon cancer through rapid degradation of cytoplasmic RXRα by K48/K63-linked polyubiquitination

S3.3

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The resistance mechanisms that limit the efficacy of retinoid therapy in cancer are poorly understood. Sphingosine kinase 2 (SphK2) is a highly conserved enzyme that is mainly located in the nucleus and endoplasmic reticulum. Unlike well-studied sphingosine kinase 1 (SphK1) located in the cytosol, little has yet understood the functions of SphK2. Here we show that Sphk2 over-expression contributes to the resistance of all-trans retinoic acid (ATRA) therapy in colon cancer through rapid degradation of cytoplasmic retinoid X receptor α (RXRα) by lysine 48 (K48)- and lysine 63 (K63)-based polyubiquitination. Human colon adenocarcinoma HCT-116 cells transfected with Sphk2 (HCT-116Sphk2) cells demonstrate resistance to ATRA therapy as determined by in vitro and in vivo assays. Sphk2 over-expression increases the ATRA-induced nuclear RXRα export to cytoplasm and then rapidly degrades RXRα through the poly-ubiquitination pathway. We further show that Sphk2 activates the ubiquitin-proteasome system through the signal mechanisms of (1) K48-linked proteosomal degradation and (2) K63-linked ubiquitin-dependent autophagic degradation. These results provide new insights into the biological functions of Sphk2 and the molecular mechanisms that underlie the Sphk2-mediated resistance to retinoid therapy.

Keywords: sphingosine kinase 2 (SphK2); retinoid therapy resistance; cytoplasmic RXRα; polyubiquitination; rapid degradation; lysine 48 (K48); lysine 63 (K63)
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S3.5 The application of "Warburg effect" in chemosensitization—a case report
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Otto Warburg discovered in 1924 that cancer cells are dependent on exclusively glycolysis for production of energy even in the presence of oxygen ("Warburg effect"). The "Warburg effect" is the scientific basis for Positron emission tomography (PET-CT) which has revolutionized cancer detection. During chemotherapy, cancer cells can increase the levels of multi-drug resistance proteins and cause treatments failure. Since multi-drug resistance proteins require energy for its operation, we explored lowering blood glucose by insulin for potential clinical efficacy for chemotherapy of an advanced pulmonary adenocarcinoma patient with multiple metastases. A 64-year-old male was admitted to our department due to irritating cough and multiple bone pain. PET/CT with F-18 fluordeoxy glucose (FDG) showed multiple hypermetabolic foci in the right hilum, right upper lung, double shoulder blade, thoracic vertebrae, lumbar, sacrum, bilateral iliac crest and the pelvis. The patient received insulin-induced hypoglycemia combined with reduced doses of chemotherapy 56 times. For each treatment, 0.2 units per kilogram of body weight of insulin were injected intravenously. After the blood glucose level reached about 2.5–3.0 mmol/L, navelbine 10 mg, cisplatin 10 mg and S-FU 250 mg were injected iv during a period of about 10 min. The patient's blood glucose level was returned to normal with iv injection of 20 mL 50% glucose solution. After the eight months of chemotherapy treatments, the patient received two additional PET/CT follow-ups. The results showed that the levels of 18F-FDG uptake in all the lesions has been reduced. In addition, the patient experienced improved appetite, weight gain and reduction of pain and cough. The values of tumor markers also declined gradually. Our results suggest that treatment of controlled, mild hypoglycemia can be safely combined with reduced chemotherapy drugs to provide clinical benefit for a late stage NSCLC patient.
Keywords: Warburg effect; insulin; hypoglycemia; chemotherapy
Acknowledgements: This study was supported by the Science and Technology Planning Project of Changzhou, Jiangsu Province (No CE20160502).

S3.6 Investigating the platelet-derived growth factor B regulation network in gastric cancer diagnosis and treatment
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Gastric cancer is one of the most common cancers in Asian countries and is the fourth most commonly occurring cancer worldwide. In the past decade, numerous groups have attempted to profile the expression changes in gastric cancer tissues using proteomic approaches, in search of diagnostic and prognostic biomarkers. However, investigating signalling network with proteomics analysis is rarely reported. In this study, we aimed to investigate the PDGFB regulation network in gastric cancer with a label free proteomics approach. To validate the network, the PDGFB was silenced in gastric cancer cells with shRNA based approach. The predicted proteins were validated with Western blot in the PDGFB knockdown cells. To evaluate the effect of PDGFB on gastric cancer cells, cell proliferation was measured in PDGFB knockdown cells. In our study, a total of 2280 unique proteins were identified. Among these, 87 were potentially differentially expressed between gastric cancer and normal gastric tissues. Gastric cancer tissues had an obvious up-regulation of PRDX5, CALR, and CTSD, and a marked down-regulation of S100A6. Furthermore, by applying novel pathway analysis, we found upstream reference proteome containing 919 proteins. Biostatistics analysis indicated that 61 proteins presented a significant expression difference between two groups. The subsequent integrative bioinformatics analysis based on both qualitative and quantitative proteomics results predicted the over-expression of the platelet-derived growth factor B (PDGFB) in tumor tissue and supports the hypothesis of the PDGFB signaling network as a key upstream regulator in PCa progression. The over-expression of PRDX2, PDA3, HNRNPL and PDGFB in tumor tissues were validated in a small cohort with immune-histochemistry staining (n=56) and were further confirmed as three novel PDGFB-regulating proteins with immunoblot in shRNA-based PDGFB knockdown PCa cells. Furthermore, we demonstrated that Crenolanib, which is a novel PDGFB receptor inhibitor, inhibited PCa cell proliferation in a dose-dependent manner. We revealed the importance of PDGFB regulatory network in PCa progression, which will assist to understand the role and mechanisms of PDGFB in promoting the cancer growth and provide valuable knowledge reference in the future research on anti-PDGFB therapy.
Keywords: prostate cancer; animal model; quantitative proteomics; PDGFB
Acknowledgements: This work is supported by the National Natural Science Foundation of China (No 81450049) and the Medical Science and Technology Development Plan of Shandong Province, China (No 2014WS0486).

S3.7 Quantitative proteomics of transgenic prostate cancer mice reveals that PDGFB-regulatory network plays a key role in prostate cancer progression
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Prostate cancer (PCa) is one of the most common cancers in men and is still one of the most intriguing challenge in oncology due to the lack of knowledge of disease progression mechanisms on the molecular and cellular levels. Transgenic adenocarcinoma of the mouse prostate (TRAMP) mice is a widely used transgenic animal model of human prostate cancer (PCa). To investigate the regulatory network associated with PCa progression, we performed a label free quantitative proteomics analysis combined with a careful bioinformatics analysis on the entire prostate protein extraction from TRAMP mice and compared with WT littersmates. From totally 2379 identified proteins, we here presented a modest mice prostate reference proteome containing 919 proteins. Biostatistics analysis indicated that 61 proteins presented a significant expression difference between two groups. The subsequent integrative bioinformatics analysis based on both qualitative and quantitative proteomics results predicted the over-expression of the platelet-derived growth factor B (PDGFB) in tumor tissue and supports the hypothesis of the PDGFB signaling network as a key upstream regulator in PCa progression. The over-expression of PRDX2, PDA3, HNRNPL and PDGFB in tumor tissues were validated in a small cohort with immune-histochemistry staining (n=56) and were further confirmed as three novel PDGFB-regulating proteins with immunoblot in shRNA-based PDGFB knockdown PCa cells. Furthermore, we demonstrated that Crenolanib, which is a novel PDGFB receptor inhibitor, inhibited PCa cell proliferation in a dose-dependent manner. We revealed the importance of PDGFB regulatory network in PCa progression, which will assist to understand the role and mechanisms of PDGFB in promoting the cancer growth and provide valuable knowledge reference in the future research on anti-PDGFB therapy.
Keywords: prostate cancer; animal model; quantitative proteomics; PDGFB
Acknowledgements: This work is supported by Taishan Scholars Construction Engineering; National Natural Science Foundation of China (Ng 81407711 and 8117303), Shandong Provincial Natural Science Foundation (Ng.ZR2014HL033), Shandong provincial science and technology Plan (Ng J14LE01 and J15LK03).

S3.8 FOXC1 is associated with estrogen receptor and affects sensitivity of tamoxifen treatment in breast cancer
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FOX1 is a member of Forkhead box transcription factors that participates in embryonic development and tumorigenesis. Our previous study demonstrated that FOX1 was highly expressed in triple-negative breast cancer. However, it is not clear what relation between FOX1 and ERα is and if FOX1 regulates expression of ERα. To explore relation between FOX1 and ERα and discover regulation of ERα expression by FOX1 in breast cancer, we analysed data assembled in the Oncomine and TCGA, and found that there was significantly higher FOX1 expression in estrogen receptor negative (ER-) breast cancer than that in estrogen receptor positive (ER+) breast cancer. Overexpression of FOX1 reduced expression of ERα and reduced cellular responses to estradiol (E2) and tamoxifen in the MCF-7 FOX1 and T47D FOX1 cells while knockdown of FOX1 induced expression of ERα and improved responses to estradiol (E2) and tamoxifen in BT549 FOX1 shRNA and HCC1806 FOX1 shRNA cells. In addition, overexpression of FOX1 reduced expression of progesterone-receptor (PR), insulin receptor substrate 1 (IRS1) and XBP1 (X-Box Binding Protein 1) and significantly reduced luciferase activity.
caused by E2 using ERE luciferase reporter assay. These results suggested that FOXC1 regulated expression of ERα and affected sensitivity of tamoxifen treatment in breast cancer, and that FOXC1 may be used as a potential drug target in ERα negative breast cancer.

**Keywords:** breast cancer; estrogen receptor; FOXC1; TCGA; triple negative

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### S3.9

**NRG1-1 stimulates DJ-1 secretion from human breast cancers by disassociation from HER3**

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It is demonstrated that breast cancer cells secrete DJ-1. However, the physiological and pathological significance of DJ-1 secretion is not clearly understood. In fact, a high level of DJ-1 protein has been detected in peripheral blood of patients with breast cancers, which could be a biomarker candidate for breast cancer. We previously found that NRG-1 promoted the decoupling of DJ-1 with HER3 and activated the heterodimerization of HER2/HER3. In this study, we found that the detectable DJ-1 protein expression is decreased, but the HER3 expression is increased, in tumor tissue with the progression of breast cancer disease. There is a significant rise of DJ-1 in the supernatant of MCF-7 cells and in serum in vitro after stimulation of NRG-1 in the normal control. Furthermore, we found that the level of DJ-1 in supernatant or serum of HER3 knockdown group was not detected both with and without NRG-1. While DJ-1 level in supernatant or serum of HER3 over-expression group increased significantly than that in normal group with NRG-1 stimulation. These results suggest that NRG-1 improves the exocrine of DJ-1 protein, which was significantly affected by HER3 level in vivo and in vitro. Moreover, our findings indicate that serum DJ-1 level is a potential serum biomarker for predicting HER3-positive breast cancer patients.

**Keywords:** serum DJ-1; HER3; NRG-1; breast cancers; MCF-7 cells

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### S3.10

**Plk1 promotes esophageal squamous cell carcinoma cell metastasis by increasing YAP**

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Polo-like kinase I (Plk1), a key regulator of cell cycle progression, is over-expressed in most cancers and can promote proliferation and metastasis. The present study aimed to investigate the function of Plk1 in esophageal squamous cell carcinoma (ESCC) cells metastasis and its potential mechanism. Cell migratory and invasive capabilities were examined by wound scratch assay and Matrigel assay in Eca-109 cells transfected with siPlk1. The pulmonary metastasis model in nude mice were established using TE-8 cells transfected with Plk1 expression plasmid or a combination of Plk1 expression plasmid and siYAP. The effect and mechanisms of neferine reversal of multi-drug resistance in BEL-7402/CDDP and BEL-7402/FOL cells

**Keywords:** Plk1; esophageal squamous cell carcinoma; metastasis; YAP

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### S3.11

**Research progress in exosome-mediated interaction between tumor cells and microenvironment**

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Exosomes represent a class of cell-derived bilayered membrane-bound nanovesicles defined by size, surface protein and lipid composition, and the ability to carry bio-information. Their contained mRNAs, miRNAs and proteins are important mediators of intercellular communication. Thus, exosomes participate in many normal and pathological processes. They appear to be promising new tools for the clinical diagnostics and potentially for novel therapeutic strategies. More and more studies focus on the roles of exosomes in cancer development, metastasis, drug resistance, diagnosis and cancer treatments. This article bases on my studies on exosome, as well as covers the current evidence concerning exosome-based cancer research.

**Keywords:** exosomes; cancer; metastasis; therapy resistance; diagnosis; treatment

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### S3.12

**The effect and mechanisms of neferine reversal of multi-drug resistance in BEL-7402/CDDP and BEL-7402/FOL cells**

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The present study aimed to observe the reversing effects of neferine on multidrug resistance of BEL-7402/CDDP and BEL-7402/FOL cells, as well as a preliminary investigation on their mechanism of action. The current clinical regimen FOLFOX4 was used to induce the BEL-7402/FOL cell line by an improved method based on pulsed exposure to chemotherapy drugs in time-step-wise increments and the same method was used in cisplatin-induced CDDP-resistant cell line BEL-7402/CDDP. Drug-resistant cell lines displayed cross-resistance to cisplatin, 5-fluorouracil, oxaliplatin and doxorubicin. Results from the cell counting kit-8 method showed that neferine significantly inhibits the proliferation of parental and drug-resistant cells in a time- and dose-dependent manner. Flow cytometric analysis displayed that neferine induced the cells apoptosis and the further experiment showed that neferine inhibited the cells migration. Subsequently Western blot showed that neferine could downregulate the expression of drug resistance-associated protein (P-gp, MRPI-3) in drug-resistant cells. These results demonstrated that neferine could effectively reverse MDR in drug-resistant cells.

**Keywords:** neferine; reversal; hepatocellular carcinoma; multidrug resistance

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### S3.13

**TRPM7 as a potential novel drug target for the treatment of glioblastoma**

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Glioblastoma (GBM) is a brain tumor consisting of malignant glial cells, and represents the most aggressive and common cancer originating in the CNS. Prognosis is dismal, and median survival is ~1 year. This poor outcome can in part...
be attributed to the lack of effective treatment; the currently used chemotherapeutic agent temozolomide is non-specific, highly cytotoxic, and ineffective. There is an urgent need for new therapeutic agents, which first requires the identification of effective drug targets. TRPM7 aberrant expression has been strongly linked with promoting GBM cellular functions. We previously reported that TRPM7 inhibition suppressed GBM cellular functions.

The aim of this study is to evaluate TRPM7 as a drug target for GBM treatment by potentiating TRPM7 with the recently discovered agonist (naltriben), and examining outcomes of GBM cellular functions (proliferation, migration, and invasion).

With patch-clamp electrophysiology, we observed in the human GBM cell line U87 that naltriben further potentiated the endogenous TRPM7 current. Next, with Fura-2 calcium imaging, we found that there was robust calcium influx in response to naltriben perfusion. GBM cellular functions were then examined with the MTT assay, scratch wound assay, and Matrigel Transwell assay (to assess proliferation, migration, and invasion, respectively). We found that naltriben significantly enhanced U87 migration and invasion, but not proliferation. To examine the underlying mechanism, we employed Western immunoblotting to detect the protein levels of p-Akt/t-Akt, and p-ERK1|2/t-ERK1|2. Naltriben-application significantly upregulated ERK signaling, but not Akt signaling.

The current study verifies the involvement of TRPM7 in GBM cellular functions, and provides evidence that TRPM7 activity can potentially be further augmented even in GBM. Treatment options for GBM patients should be considered cautiously in order not to upregulate TRPM7 activity.

Keywords: glioblastoma; GBM; TRPM7; naltriben

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