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The 2nd International Conference for Cancer Metabolism and Therapy was successfully held in Wenzhou Awailou Resort from October 13 to 15, 2017 with the theme of "Cancer Metabolism and Therapy". The conference was organized by The First Affiliated Hospital of Wenzhou Medical with sponsorship from the Chemical Engineering and Environmental-Biological schools, Dalian University of Technology, the Hirshberg Foundation for Pancreatic Cancer (<http://pancreatic.org/>), UCLA Agi Hirshberg Center for Pancreas Diseases and participating industries, Bruker Daltonics, Agilent, Bio-Rad Laboratories, and Beijing Ancomol Biotechnologies Co Ltd. Cancer metabolism is intimately linked to drug resistance, which is currently one of the most important challenges in cancer therapy. Cell phenotype may be altered by proteome phenotype that results from altered genomic phenotype that may be regulated by metabolic phenotype initiated by early signals. Due to newly developed technology, we can readily use many high-tech diagnostic tools, new anticancer drugs, molecular targeting chemotherapy, less invasive and/or less expensive and effective therapy that were unavailable in the near past. Recent advancement of novel high-throughput technologies, such as

transcriptomics, proteomics and metabolomics, has significantly enhanced our understanding of metabolic properties related to malignancy, paving the way for selection of molecular targets for therapeutic interventions. Cancer therapy and research are in a new era of rapid and significant developments not only in the West but also in Asia as well. As the burden of cancer is increasing rampantly and posing challenges in early diagnosis and treatment, this international cancer metabolism and therapy conference is planned to be held every other year with the aim of identifying the genuine needs in the area and implementation for patient welfare. This conference covers a wide range of topics in cancer such as etiology, epidemiology, metabolic reprogramming, environment altered cellular metabolism, cancer prevention and vaccines, new drug development, multidisciplinary treatment i.e. surgical therapy, chemotherapy, and radiotherapy and cancer stem cell therapy. Scientists from all over the world shared their new ideas and thoughts with each other, especially young scientists who are able to communicate openly with the global leaders in the field, investigators, medical and surgical oncologists through presentations and vigorous discussion. The next meeting is planned to be held October 12–October 14, 2018 in Shanghai, China. The meeting venue will be in the Shanghai Baohua Marriott, Shanghai, China.

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These authors declare no conflict of interest.

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ABSTRACTS PRESENTED

Metabolic Regulation of Tumor Microenvironment Heterogeneity

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Background: Tumor Microenvironment is the cellular environment in which the tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix. The tumor microenvironment contributes to tumor heterogeneity. Exact mechanism of causing tumor heterogeneity is not clearly understood yet.

Results and Discussion: Data from our previous research and others suggest that tumor heterogeneity may be resulted from unsynchronized differentiation of cancer progenitor cells. We then hypothesized that cell phenotype may be altered by proteome phenotype that is resulted from altered genomic phenotype that may be regulated by metabolic phenotype initiated by early signals. In another words, heterogeneity of tumor microenvironment may be resulted from dysregulated metabolic phenotypes. To test our hypothesis, we developed analytical methods to measure quantitatively the signals and its initiated metabolic phenotypes in a cell. The methods developed in our group at both UCLA and Creighton University include stable isotopomer-based flux analysis and dynamic measurement of protein turnover in a cell, which extensively used in cancer research. In this oral communication, the details on how

these methods can be used will be delivered and an example based on the methods will be also illustrated.

Conclusion: Understanding the mechanism underlying tumor microenvironment heterogeneity regulated metabolically is key for development of anti-cancer drugs with minimum toxicity and maximal effectiveness.

Ubiquitylation of Autophagy Receptors: Multiple Modes of Regulation on Cellular Autophagic Flux

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Background: Ubiquitin (Ub) signaling regulates myriad of fundamental cellular activities. The alterations in cellular Ub homeostasis, known as Ub stress, feature and affect cellular responses in multiple conditions, yet the underlying mechanisms are incompletely understood.

Method: By biochemical and cell biology method we found that p62/SQSTM1 was polyubiquitylated in Ub+ stress conditions, and we identified its E2 enzyme Ube2d2 and Ube2d3 in yeast two hybrid screening, then we mapped the E2-dependant ubiquitylation sites by mass spectrum analysis. We further confirmed that polyubiquitylated p62 form large complex than unmodified p62 by electro-microscopy and dynamic light scattering analysis.

Results: Ub+ stress in cells induced by over-expression of Ub, prolonged proteasomal inhibition and heat shock could efficiently induce autophagy in dependence of autophagy receptor, p62/SQSTM1, p62 was increasingly ubiquitylated during those conditions. Subsequently, p62 was found to specifically interact with two Ub conjugating enzymes, Ube2d2 or Ube2d3, using a common E2-interacting region (EIR), and these E2s could support p62 ubiquitylation both in vitro and in the cell. Multiple Lys (K) residues in p62 were mapped as the sites for this E2-supported ubiquitylation, which include K420 in the UBA domain of p62. As previously reported, UBA domains in p62 formed stable dimers, which would prevent p62 from binding to polyUb chain in autophagy cargos; our in vitro polyUb-binding data showed that polyubiquitylated p62 in the presence of the E2s bind to polyUb chains much more efficiently than unmodified p62. Consistently, electro-microscopy and dynamic light scattering analyses indicated that polyubiquitylated p62 might predominantly form large complex with polyUb chains (~ two fold larger in diameter) than unmodified p62, suggesting that p62 might have indeed adopted an open conformation upon E2-supported polyubiquitylation.

Conclusion: By discovering E2-supported ubiquitylation of p62 as a novel mechanism for activating its autophagy receptor function under Ub+ stress conditions, our work has thus revealed a unique "sensor" function of p62 in modulating autophagy as part of the cellular responses to prolonged proteasomal inhibition, heat shock or Ub overexpression.

Identifying Clinically Actionable Alterations in the Era of P4plus Medicine: Challenges and Opportunities

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Background: The Chinese government recently launched two projects, i.e. "Healthy China 2030" and "Genetic testing for Chinese public health: science and social engineering". The programs center on chronic disease prevention and promoting personalized and positive lifestyle choices. Scientifically the identification of actionable alterations in gene, lifestyle or environmental factors will be the key for the diagnosis and prognosis of diseases and the improving of population healthcare.

Method: I will present our researches on the identification of actionable biomarkers for diagnosis of complex diseases and talk about our strategies for the biomarker discovery from big data to small data, based on the statistical analysis of the reported biomarkers, the mechanistic and functional analysis of biological networks.

Results: We first studied the network properties of gene society, and then applied the ideas to the identification of microRNA biomarkers for cancers, such as prostate cancer and leukemia. We cooperated with medical doctors to verify our findings by collect disease samples and experimental confirmation.

Conclusion: The future challenges and opportunities for the P4 medicine, i.e. predictive, preventive, personalized and participatory medicine include the data privacy, lifestyle, universal and experimental data characterization as well as the standardization of diverse unstructured data.

The Role of Hypermethylated in Cancer 1 (HIC1) in Tumorigenesis and Development

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Background: Hypermethylated in cancer 1 (HIC1) is a tumor suppressor gene and a transcriptional inhibitory factor, whose promoter is usually silenced in many solid tumor tissues and precancerous lesions (such as smokers' lung, colorectal polyps and other tissues). HIC1+/- mice studies also suggest that the gene may be associated with tumorigenesis, tumor development and metastasis.

Method: A variety of solid tumor tissues (including prostate cancer, breast cancer, lung cancer, etc.) were collected for analyzing HIC1 expression and methylation status. In vitro and in vivo (HIC1 cKO and transplant mice model) experiments were utilized to study the phenotypes as well as mechanisms upon HIC1 loss or reexpression.

Results: In recent years, our team have conducted a series of studies on HIC1 and found that hyper methylation of HIC1 promoters in a variety of solid tumor tissues (including prostate cancer, breast cancer, lung cancer, etc.) may lead to HIC1 silence, resulting in suppressive function loss or attenuation in these tumors. In vitro and in vivo experiments show that the recovery of HIC1 expression could significantly reduce the tumor proliferation, invasion, and metastasis. Mechanism studies disclose that HIC1 expression is widely modulated including ubiquitination, SUMOylation, as well as methylation.

Conclusion: These studies suggest that HIC1 may act as a potential tumor biomarker, which provides new ideas for the early diagnosis and treatment of tumors.

RP-MDM2-p53 Pathway Regulates Lipid Metabolism and Acts as the Checkpoint of Myc-induced Lymphomagenesis

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Background: The tumor suppressor p53 has recently been shown to regulate lipid metabolism through multiple mechanisms. However, the in vivo signaling pathways related to p53-mediated metabolic regulation remain largely uncharacterized.

Results: By using mice bearing a single amino acid substitution at cysteine residue 305 of mouse double minute 2 (Mdm2^{C305F}), which renders Mdm2 deficient in binding ribosomal proteins (RPs) RPL11 and RPL5, we show that the RP-Mdm2-p53 signaling pathway is critical for sensing nutrient deprivation and maintaining liver lipid homeostasis. Nutrient deprivation inhibits rRNA biosynthesis and triggers ribosomal stress, which increases RP-Mdm2 interaction, and induces p53-mediated transactivation of malonyl-CoA decarboxylase (MCD), leading to increased fatty acid oxidation. By contrast, Mdm2 mutant mice demonstrate attenuated MCD induction and increased lipid accumulation in the liver. Furthermore, to test whether RP-Mdm2-p53 pathway involved in the tumor suppressive function of p53, we crossed Mdm2^{C305F} mouse with Eμ-myc mouse, and found that interruption of RP-MDM2 interaction strikingly accelerates oncogenic Myc-induced lymphomagenesis.

Conclusion: Thus, the RP-Mdm2-p53 pathway appears to function as an endogenous stress sensor responsible for breaking down lipid storage for survival. However, in response to oncogene over-activation, this pathway acts as the checkpoint of excessive ribosome biogenesis.

miR-1207-5p Induced Metastasis by Regulating Trypsin-alpha-1 in Oral Cancer

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Background: Oral cancer metastasis is a life threatening health problem worldwide. Development of an effective strategy for treatment of oral cancer is a key for reducing the death rate of the disease. In recent years, microRNAs (miRNAs) as a group of small non-coded RNAs play a vital role in tumor metastasis. The goal of this study is to determine the regulatory role of miR-1207-5p and the mechanism underlying in oral cancer metastasis.

Methods: In this study, we systematically evaluated a large scale of miRNA profiles from current 24 oral pathological tissues, which including 8 benign samples, 7 local carcinoma samples and 9 distal metastasis carcinoma samples.

The miRNAs expressed differentially in tumor cells were identified by using DChip technology, and peptides expressed remarkably in tumor core were identified by using LTQ/MS. The quantitative real-time PCR was applied to validate the level of miR-1207-5p and the target gene. Western blot analysis was used to verify the target protein expression of miR-1207-5p.

Results: The miR-1207-5p was significantly up-regulated in oral local carcinoma and distal metastasis carcinoma tissues, while trypsin-alpha-1 (TPSAB1) was dramatically up-regulated in oral local carcinoma tissues. By prediction analysis and reciprocally expression of both miR-1207-5p and its target TPSAB1 in oral tumor tissue, TPSAB1 was further confirmed as a target of miR-1207-5p. The regulatory role of miR-1207-5p in oral metastasis is still under investigation.

Conclusion: The data above indicate that miR-1207-5p may be potential biomarker or target of oral cancer metastasis and induce oral cancer metastasis by targeted Trypsin alpha-1.

The Expression of *MALAT1* in Endometriosis

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Objective: The article aimed to explore the expression and the meaning of metastasis-associated lung adenocarcinoma transcript1 (*MALAT1*) in endometriosis (EMs) and the serum.

Methods: The different gene was found between endometriosis tissues and normal tissues through GCB1, and the *MALAT1* was chosen among them. Extract the RNA from ovarian endometriosis and non-endometriosis as well as the serum, real-time PCR was applied to detect the relationship of *MALAT1* expression with menstruating cycle; the diagnostic efficacy of serum *MALAT1* was analyzed by the receiver operating characteristic curve (ROC).

Results: The *MALAT1* in EMs was down-regulated about 1.35 times; and the relative expression level of *MALAT1* in ectopic and eutopic endometrium with ovary endometriosis were significantly lower than those in non-endometriosis patients. The *MALAT1* in ectopic endometrium was lower than the eutopic endometrium with ovary endometriosis; The relative expression level of serum *MALAT1* in ovary endometriosis was significantly lower than that in non-endometriosis. The diagnostic sensitivity and specificity of endometriosis were satisfactory.

Conclusion: *MALAT1* played a critical role in the process of endometriosis, and the level of *MALAT1* in serum contributed to the diagnosis of endometriosis.

Tid1-S Regulates Metabolic Reprogramming in Clear Cell Renal Cell Carcinoma via SIRT3

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Background: Human Tid1 (hTid1) is a DnaJ chaperone protein and human homolog of *Drosophila* tumor suppressor Tid56. It has been reported that overexpression of hTid1-S, the short alternatively spliced isoform of hTid1, suppresses apoptosis induced by apoptotic stimuli. Altered expression of hTid1 is observed in various types of human cancers. However, the clinical significance of hTid1-S expression in clear cell renal cell carcinoma (ccRCC) remains unknown.

Methods: Western blot and qRT-PCR were used to detect protein and mRNA alteration of indicated molecules. Seahorse XF96 was performed to determine aerobic glycolysis and oxygen consumption rate. IHC was used to analyze Tid1-S protein level in ccRCC tumor tissues and adjacent non-cancerous tissues. Kaplan-Meier survival analysis was applied to analyze survival rate post-operation.

Results: In the present study, we found the mRNA and protein levels of hTid1-S were significantly lower in tumor tissues than adjacent non-cancerous tissues of ccRCC patients. Upregulation of hTid1-S in renal cancer cells significantly inhibits cell proliferation by increasing reactive oxygen species (ROS) production. In addition, we found that the interaction of Tid1-S with SIRT3 lead to the inhibition of mTOR/MAPK signaling and activation of AMPK- α signaling, they are both crucial processes in cell metabolism. Moreover, overexpressed Tid1-S represses aerobic glycolysis while mitochondrial respiration was enhanced, which indicates a switch of Warburg effect. Immunohistochemistry analysis showed that hTid1-S protein expression was decreased in tumor tissues. We further showed that hTid1-S expression level was much lower in later clinical stage (Stage III) than in early clinical stage (I and II) tumor tissues. Moreover, the expression level of hTid1-S was significantly correlated with patient's T stage ($P = 0.008$) and TNM stage ($P = 0.008$). We also

demonstrated that hTid1-S was an independent prognostic factor for overall survival of ccRCC.

Conclusion: Our findings suggest that hTid1-S could potentially be useful as a diagnostic and prognostic biomarker, as well as a novel therapeutic target candidate for ccRCC.

Deacetylation of Tumor Suppressor MST1 in Hippo Pathway Induces its Degradation Through HBXIP-elevated HDAC6 in Promotion of Breast Cancer Growth

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Background: Reduction or loss of tumor suppressor mammalian STE20-like kinase 1 (MST1) in Hippo pathway contributes to the tumorigenesis. However, the mechanism leading to reduction of MST1 in cancers remains poorly understood.

Methods: We used western blot, confocal, immunohistochemistry, and xenograft mice, et al. to investigate the mechanism of MST1 expression reduction in breast cancer.

Result: In this study, we explored the hypothesis that the oncoprotein hepatitis B X-interacting protein (HBXIP) is involved in the reduction of MST1 in breast cancer. Immunohistochemical analysis of tissue microarrays revealed that the expression of HBXIP was negatively associated with that of MST1 in 98 clinical breast tissue samples. Then, we found that HBXIP could post-translationally down-regulate MST1 in breast cancer cells. Mechanistically, we identified that MST1 could be acetylated in the cells. Strikingly, treatment with trichostatin A (TSA), an inhibitor of histone deacetylases (HDACs), markedly increased MST1 acetylation and protein level in the cells. Interestingly, oncoprotein HBXIP could significantly inhibit acetylation of MST1, resulting in the reduction of MST1 protein. Notably, we revealed that histone deacetylase 6 (HDAC6) could reduce protein level of MST1 through deacetylation of MST1 in the cells. Then, our data revealed that HBXIP up-regulated HDAC6 at the levels of mRNA and protein by activating transcription factor NF- κ B. Furthermore, we demonstrated that MST1 deacetylation promoted the interaction of MST1 with HSC70 in the cells, resulting in a lysosome-dependent degradation of MST1 via chaperone-mediated autophagy (CMA). Functionally, the reduction of tumor suppressor MST1 mediated by HBXIP promoted the growth of breast cancer cells in vitro and in vivo.

Conclusion: We conclude that the deacetylation of MST1 mediated by HBXIP-enhanced HDAC6 results in MST1 degradation in a CMA manner in promotion of breast cancer growth. Our finding provides new insights into the mechanism of MST1 reduction in Hippo pathway in breast cancer.

Correlation Between miR-21 and Pancreatic Cancer

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Objective: The article aimed to analysis the differentiate gene expression of pancreatic cancer to gain a core gene, namely, miR-21, clarifying the correlation between miR-21 and pancreatic cancer as well as regulating TGF β .

Methods: The miR-21 was screened by literature analysis and pancreatic cancer gene analysis. miR-21 was clinically verified by RT-PCR. RT-PCR and Western blot experiments were carried out to study the relationship between miR-21 and TGF β by overexpression and suppression of miR-21 in pancreatic cancer cells.

Results: miR-21 was highly expression in pancreatic cancer. The overexpression of miR-21 in PANC-1 cannot lead to the variation of TGF β ; the inhibition of miR-21 can cause the highly expression of TGF β .

Conclusion: miR-21 was overexpression in pancreatic cancer cells. The expression of miR-21 can regulate the expression of TGF β , which may be a mechanism of miR-21 in pancreatic cancer.

OLA1 Expression Varied in Pancreatic Cancer of Gemcitabine Resistance

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Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignant disease with a 5-year survival rate less than 5%. Gemcitabine alone or in combination with other therapeutics has been the main therapy for advanced pancreatic cancer, however the acquired drug resistance becomes a limiting factor in its clinical application. Since the resistance of pancreatic cancer negatively affects the therapeutic effects of gemcitabine, finding novel target that increase tumor sensitivity and overcome drug resistance to gemcitabine are needed. OLA1 (Obg like ATPase 1) is relative with resistance of gemcitabine in pancreatic tumor. The goal of this study is to determine the efficacy of OLA1 on overcoming drug resistance and the possible mechanism.

Methods: The sensitive of gemcitabine on viability of BxPC-3 and PANC-1 cell was measured with methyl thiazolyl tetrazolium (MTT) assay and microscopy examination. Flow cytometric assay (FCA) was used to detect apoptosis of these two cell lines with different dose and time. mRNA level of OLA1 was detected by Q-RT-PCR. The protein expression of OLA1 was determined by Western blot analysis. Tissue chip was carried to reveal the relationship between pancreatitis and acquired resistance of different tumors.

Results: Gemcitabine inhibited the proliferation of pancreatic carcinoma and induced apoptosis in a dose-dependent manner. BxPC-3 was more sensitive to gemcitabine than PANC-1 validated by morphology and MTT assay. The mRNA and protein level of OLA1 in gemcitabine resistance cells (PANC-1) is high-expressed. It's interesting that the pancreatitis tissue expressed more OLA1 compared with tumor by tissue chip.

Conclusion: Results of this project may lead to the establishment of a new anti-cancer strategy based on OLA1, an important post-translational regulator of many cellular process including proliferation, metastasis and invasion. Further studies are needed to clarify which signal pathway are relevant to OLA1 in regulating pancreatic cancer of gemcitabine resistance.

Involvement of the Fragile X Protein in Translation of STAT3 Promotes Hepatocellular Carcinoma Metastasis

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Background: The fragile X mental retardation protein (FMRP) is an RNA-binding protein that plays important roles in mRNA stability, mRNA export, location and translation. The most study of FMRP is focused on nerve diseases such as FXS caused by FMRP dysfunction, while how does it affect cancer progression is still unknown.

Method: We used RNA IP assay and FISH combined with IF to verify the relationship between FMRP and STAT3. We also stably knocked down the FMRP protein using siRNA system, then, RNA extraction, RT-qPCR array and western blotting were conducted to test the expression of STAT3. Using Co-immunoprecipitation, transwell, and immunohistochemical analysis to find the role of FMRP in HCCLM3 cells and human liver cancer tissue.

Results: FMRP protein colocalized with STAT3 (Signal Transducer and Activator of Transcription 3) mRNA in HCCLM3 cells using FISH and IF assay. Meanwhile, FMRP associated with STAT3 mRNA by RNA Immunoprecipitation assay. Interestingly, the protein level of STAT3 is increased while the expression of STAT3 mRNA is constant in the FMRP KD cells. Importantly, our results have shown that FMRP interacts with many translational complexes, such as TDP-43, eIF4E, and FMRP interact with protein kinase PI3K. Finally, we find that FMRP is overexpressed in liver cancer tissue compared to para-carcinoma tissues, and the invasion ability of HCCLM3 cell is decreased in FMRP KD cell.

Conclusion: In this study, we identified STAT3 mRNA as a new FMRP binding target which involved STAT3 mRNA translation. FMRP knock down decrease the HCCLM3 metastasis, and the FMRP protein level is elevated in Hepatocellular Carcinoma tissue, suggesting that FMRP may affect the Hepatocellular Carcinoma Metastasis though regulating expression of STAT3.

Exosomes: A Breakthrough in Pancreatic Cancer Multidrug Resistance

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Background: Pancreatic cancer is one of the most lethal malignancies on a global scale and its morbidity increases year by year. Prognosis for patients with locally advanced or metastatic disease is poor (5-year survival ratio <5%). This occurs not only in that it lacks a reliable marker for early detection, but also in that it can easily develop resistance to the anticancer drug.

Discussion: Gemcitabine has been considered standard of care for treatment of advanced pancreatic cancer since 1997. Compared to 5-fluorouracil, Gemcitabine is found obviously to prolong patients' survivals and improve clinical benefit response, however, Clinical treatment of pancreatic cancer soon develops resistance against gemcitabine. There are many studies on multidrug resistance of pancreatic cancer currently and lots of biological molecules have been found to be associated with drug resistance of pancreatic cancer, but it is still not clear how drug resistance occurs and spreads. As a kind of cell secretions, exosomes are composed of protein, RNA and lipid molecules. It is widely distributed and exists in various body fluids. It carries and transmits important information molecules, which is an important part of cell information transmission. Because of this property, it can be a good marker for early cancer detection and an entrance to the research of chemoresistance. MicroRNAs or alterations in intercellular communication play a dominant role in chemoresistance development, and recently several reports have found that in pancreatic cancer cells with drug resistance, the composition of exosomes have undergone significant changes. This is of great importance for the research of the production and spread of drug resistance in pancreatic cancer. In addition, exosomes are considered good drug carriers, which means it has great potential in terms of drug delivery.

Conclusion: In this review, we have compiled the current research on the chemoresistance of pancreatic cancer and introduced the origin of the exosomes, its composition and its role in the cell growth. We discuss the relationship between exosomes and pancreatic cancer drug resistance, and we believe that further study on the composition of exosomes and its mode of action in the intercellular will help the diagnosis and treatment of pancreatic cancer.

Monoclonal Antibody Against Protein Disulphide Isomerase Anterior Gradient-2 as a Novel Agent in Suppressing Tumor Growth and Metastasis

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Background: Anterior gradient 2 (AGR2) is a protein disulfide isomerase-like protein widely expressed in many normal tissues as well as cancers. AGR2 is overexpressed in multiple cancers, particularly those arising from lung, breast and prostate tissues, and higher levels of AGR2 are associated with earlier patient death. Metastasis is one of the areas which need immediate attention. There have been lots of proteins reported to have helped in the progress of metastasis. Anterior Gradient -2 (AGR2) is one of them. AGR2 protein has been demonstrated to interact with C4.4A and DAG-1 proteins which are associated with metastasis formation since these transmembrane proteins are involved in cell and matrix interactions between cancer and normal cells.

Method: Knowing the importance of AGR2 in the cancer development, we designed, synthesized and purified a novel monoclonal antibody 18A4 against AGR2. We investigated the anti-metastatic and anti-tumorigenic activity of MAb 18A4 in experimental pulmonary lung melanoma metastasis model and non-small cell lung carcinoma (NSCLC) xenograft models.

Result: Our results showed that 18A4 successfully suppressed B16F10 melanoma pulmonary lung metastasis in C57/BL6 mice and suppressed tumor growth in NSCLC models.

Conclusion: We also studied MAb18A4 mode of action against AGR2. It is reported that AGR2, to be functionally active needs to be in the form of a dimer. Our findings suggest that MAb18A4 have an important role to play in reducing the dimerization of AGR2. We are also keen on finding out the pathways MAb18A4 chose to act upon AGR2.

Berberine Reverses Hypoxia-induced Chemoresistance in Breast Cancer Through Regulating AMPK

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Background: The hypoxic tumor microenvironment has been considered to be a major obstacle in chemo-resistance for breast cancer treatment. AMP-activated protein kinase (AMPK), a key metabolic enzyme regulating cellular energy, is activated in hypoxic condition to enhance mitochondrial oxidative phosphorylation. Berberine, a traditional Chinese medicine that modulates the energy sensor AMPK, has been proved to be efficient and safe in cancer therapy. However, a molecular-level understanding of how

different dose of berberine influences the modulation of drug resistance and apoptosis in hypoxic breast cancer has not been shown yet.

Methods: The sulforhodamine B (SRB) assay is routinely used for cytotoxicity determination. Cell death and apoptosis were detected by using an Annexin V-FITC Apoptotic Detection Kit by flow cytometry. Western blot was used to determine the protein expression. All data were expressed as mean $\pm$ SD. Statistical significances among groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post. A value with $p < 0.05$ was considered as statistically significant.

Results: The expression of the phosphorylated AMPK (p-AMPK), HIF-1 α and P-gp was significantly increased in the hypoxia condition, indicating that hypoxia induces the activation of AMPK. Then we discovered that different doses of berberine reduced AMPK activation, reversed drug resistance and elicited anticancer effects in hypoxic MCF-7 cells. Moreover, our study demonstrated that low dose of berberine (5 μ M) could enhance DOX sensitivity through AMPK-HIF-1 α -P-gp pathway, while high dose of berberine (40 μ M) could activate AMPK-HIF-1 α -p53 pathway and significantly activate mitochondrial apoptosis pathway with a series of proteins releasing, including BAX, Cytochrome c, Cleaved-Caspase 9, Cleaved-Caspase 3 and cleaved PARP.

Conclusion: Indeed, our study shed light on a new strategy that targets AMPK in drug-resistant breast cancer treatment based on berberine to achieve the most efficient therapy, which sheds light on a new strategy in hypoxia-induced drug resistance breast cancer treatment.

Brusatol Strengthens the Efficacy of Gemcitabine in Pancreatic Cancer: Involvement of NRF2 and NF- κ B Signaling Pathways

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Background: Gemcitabine is the standard chemotherapy treatment for advanced pancreatic cancer, due to deep rooted chemoresistance, the benefit is quite limited. However, the underlying mechanism remains unveiled. Recently, Nrf2 and NF- κ B have emerged as significant contributors to gemcitabine chemoresistance of pancreatic cancer. Brusatol is deemed as a unique inhibitor of the Nrf2 pathway, through previous researches we found out brusatol inhibits growth and induces apoptosis in pancreatic cancer cells and meantime restrain NF- κ B pathways. Due to the above studies, we speculate brusatol could reverse the activation of Nrf2 and NF- κ B and have question in its further potential in accelerating gemcitabine efficacy in the treatment of pancreatic cancer.

Method: Cell viability and apoptosis were detected by Cell Counting Kit 8 assay and Annexin V-FITC/PI double staining assay, respectively. The mRNA expression levels were investigated by real-time PCR analysis. The expression of proteins was investigated by western blotting, immunofluorescence staining and Immunohistochemical staining.

Results: In this study, we firstly proved brusatol could effectively inhibit Nrf2 signaling pathway in pancreatic cancer cells. Next, we demonstrated that Brusatol is able to abrogate the activation of Nrf2 and NF- κ B caused by gemcitabine in pancreatic cancer cells. And the following we discovered brusatol potentiates gemcitabine-induced growth inhibition and apoptosis in human pancreatic cancer cells. Additionally, in PANC-1 xenografts, treatment with combination of brusatol and gemcitabine could reduce vivo tumor growth drastically compared with control or treatment of either brusatol or gemcitabine alone. The immunohistochemical staining also showed that both Nrf2 and NF- κ B reduced in brusatol-treated xenograft tumor tissues. To sum up, our results suggest that through the suppression of Nrf2 and NF- κ B pathways, brusatol is capable of potentiating the antitumor effects of gemcitabine.

Conclusion: In conclusion, our current findings demonstrate that brusatol can inhibit the Nrf2 pathway in pancreatic cancer cells. Moreover, brusatol enhances the antitumor efficacy of gemcitabine in both pancreatic cancer cells and PANC-1 xenografts, which are partly due to inactivation of Nrf2 and NF- κ B pathways.

miR-205 as an Effective Biomarker for Early Diagnosis in Cancer

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Background: Cancer poses a major threat to public health and early diagnostic accuracy is becoming increasingly significant. MicroRNA (miRNA) expression profiles are being intensively investigated in the regulation of

basic cellular processes such as cell proliferation, differentiation, and apoptosis of every tumor type examined. We summarized the early diagnostic value of mature microRNA-205 (miR-205) in cancer.

Discussion: miRNAs are a group of small non-protein-coding RNAs that can modulate gene expression and are easily accessible, quantifiable, detected in the blood, so miRNAs have promise to be potential, sensitive, specific, accurate biomarkers. Detecting cancer at an early stage anticipates better disease outcome and prolonged patient survival. Two major experimental techniques are applied to the study: the quantitative real-time PCR is applied to validate the level of miR-205 and the target gene, and Western blot analysis is used to verify the target protein expression of miR-205.

Results: The miR-205 is significantly up-regulated in cancer tissues and cancer cells, while protein products of tumor-suppressor genes as E-cadherin are reduced in some types of cancer, and oncogenic proteins are increased. These results suggest that miR-205 is a tumor suppressor in cancer. However, due to the need for an optimized detection strategy, miR-205 has not yet been clinically utilized as disease-specific marker.

Conclusion: Overall, miRNA expression levels can be used as prognostic markers cancer. miR-205 miRNA as potential cancer early diagnosis biomarker is underscored by their involvement in the regulation of basic cellular processes such as cell proliferation.

Quercetin Inhibits Epithelial-Mesenchymal Transition, Decreases Invasiveness and Metastasis by Inhibiting STAT3 Signaling in Pancreatic Cancer Cells

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Background: Quercetin, a flavone, is multifaceted, having anti-oxidative, anti-inflammatory, and anticancer properties. The objective of this study was to evaluate the effects of quercetin on the epithelial-mesenchymal transition (EMT) and invasion of pancreatic cancer cells and the underlying mechanisms.

Method: We investigated the effect of quercetin in pancreatic cancer cells using CCK8 assay, Transwell invasion and migration assays, Wound healing assays, Real-time RT-PCR, Western blot analysis and Immunofluorescence microscopy.

Results: Quercetin exerted pronounced inhibitory effects in PANC-1 and PATU-8988 cells. Moreover, quercetin inhibited EMT and decreased the secretion of matrix metalloproteinase (MMP). STAT3 phosphorylation decreased following treatment with quercetin. The EMT and MMP secretion increased with activation of the STAT3 signaling pathway by using interleukin-6 (IL-6), and quercetin reversed IL-6-induced EMT, invasion, and migration.

Conclusion: Our results demonstrate that quercetin triggers inhibition of EMT, invasion, and metastasis by blocking the STAT3 signaling pathway.

TOM70 Involved in Localization of STAT3 to Mitochondria and Promoted HCC Metastasis

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Background: Cancer cell motility plays an important role in the spreading of tumors throughout the human body, a process known as metastasis. Cellular motility requires the function of mitochondria in the cell. Recent evidence shows that transport of protein factors to mitochondria of cancer cells promotes their motility. STAT3 (Signal transducers and activators of transcription-3) is a key transcription factor and plays an important role in cancer invasion and metastasis. Recent results have shown that STAT3 had been identified in mitochondria. How STAT3, which translated in cytoplasm, localizes to mitochondria and promotes tumor progression is still unexplored.

Method: HCCLM3 were stimulated by IL-6 (10ng/ml) for different minutes, then we extracted and isolated mitochondria and cytosolic fractions from HCCLM3. Western blot analyzed expression of STAT3 and pSTAT3 (Tyr705) in different fractions of HCCLM3. We applied LC-MS/MS to measure possible proteins that interact with STAT3. The interaction between TOM70 and STAT3 was verified by using co-immunoprecipitation and immunofluorescence.

Results: We extracted the mitochondria and found the STAT3 factor in mitochondria. Localization of STAT3 to mitochondria increased after inducing STAT3 phosphorylation on tyrosine 705 by IL-6 (10 ng/ml). Knocked down STAT3 in HCCLM3, resulting in decreased amount of its expression in mitochondria and prevents HCC cells proliferation, ATP production, and cell migration and invasion. Interestingly, STAT3 interact with TOM70, an important component of the TOM complex, suggesting that TOM complex is required for STAT3 import to mitochondria.

Conclusion: Focusing on liver cancer, which is the third most common cancer in the world and is highly prevalent in China, our studies have shown that pSTAT3(Tyr705) as an important factor in mitochondria and involved in liver cancer metastasis and invasion. In this process TOM complex plays an essential role in import of STAT3 to mitochondria.

CircHIPK3 Promote the Epithelial-mesenchymal Transition and Invasion in Pancreatic Cancer

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Background: Accumulating evidences shows that Circular RNAs (circRNAs) as a new non-coding RNA can modify gene expression in the pathogenesis of various carcinomas. However, the function and mechanism of circHIPK3 in pancreatic cancer has not been elucidated.

Method: Herein, we compared the expression of circHIPK3 in duct epithelial cells of the pancreas and several wild-type pancreatic cancer cells. Then the model of circHIPK3 knockdown in Patu-8988 and Panc-1 were generated using siRNA of circHIPK3 to figure out its function in pancreatic cancer. Meanwhile, we make intratumoral injection of cholesterol-conjugated circHIPK3 siRNAs in a xenograft animal model to explore the role of circHIPK3 in pancreatic cancer progression.

Results: The results of PCR showed that circHIPK3 is upregulated in pancreatic cancer cell lines and silencing of circHIPK3 inhibits pancreatic cancer cells proliferation and promotes apoptosis. In addition, invasion and EMT related markers between si-circHIPK3 and NC groups were compared using RT-PCR and Western blotting assays, which indicates that circHIPK3 promoted invasion and EMT in pancreatic carcinoma cell lines. Interestingly, in agreement with the data of RT-PCR and Western blotting, the fluorescence intensity results and transwell assay indicated that downregulation of circHIPK3 observably decreased invasive and migration capacity of pancreatic cancer cells by contrast with NC groups. Moreover, according to the growth rate of xenograft tumors and IHC (ki67 and MMPs) between si-circHIPK3 and NC groups, we found that intratumoral silencing of circHIPK3 suppresses pancreatic cancer growth and metastasis *in vivo*.

Conclusion: Silencing of circHIPK3 could suppress cell proliferation, migration and invasion, reverse EMT as well as inhibit tumor growth and metastasis *in vitro* and *in vivo*, suggesting that circHIPK3 would be a promising target of diagnosis and therapy in pancreatic cancer.

Effects of miR-17-5p in Human Hepatocellular Carcinoma Cells by Modulating STAT3 Expression

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Background: Messenger RNA localization is linked to translational control to avoid translation of the mRNA while being transported. And local translation is involved in a variety of processes, including cancer cell protrusions development which are critical for cell migration and metastasis. MicroRNAs are ideal regulators of mRNA translation since their activity can be easily reversed by modulating the RNA silencing complex (RISC), that uses them as guide for RNA recognition. This kind of control can provide a local and rapid production of proteins required for migration and pseudopod formation. Several miRNAs have been reported to target STAT3. And miR-17-5p is an important member of miR-17-92 cluster that was described as oncogenic microRNA oncomiR.

Method: We will study the localization of miRNAs and their targets in pseudopods. We will use as model the STAT3 mRNA and the miRNAs miR-17 which is known for regulating STAT3 translation. We will use the FISH technology from Affymetrix to visualize miRNAs and its target (view RNA miRNA ISC cell Assay). The impact on STAT3 regulation of the miRNAs will be studied using antagonomirs. We will measure STAT3 levels and cell migration in a Transwell migration assay after antagonomir treatment. We anticipate that deregulated STAT3 expression as a consequence of lack of miRNA regulation will affect cell migration.

Results: We utilized Boyden chamber cell fractionation method to separate cell protrusions from cell bodies in the metastatic Hepatocellular Carcinoma cell line HCCLM3. Then We identified and quantified total miRNA isolated from CB (cell body) or PS (protrusion). The expression level of miR-17-5p in CB and PS is markedly different. We identified that miR-17-5p was expressed lower in high metastatic capability HCC cell lines HCCLM3 and MHCC97H than low metastatic HCC cell lines HepG2 and Huh7 by real-time (RT)-PCR. In this study, we found that the potential target of miR-17-5p could

be STAT3 whose subcellular localization and local translation plays an important role in protrusion development. And western blotting assays revealed that miR-17-5p downregulate the expression level of STAT3. And we will further confirm whether miR-17-5p will act on protrusion localization and formation by modulating STAT3 expression.

Conclusions: Our studies have shown that miRNAs may involve in mRNA protrusions localization and its translation by interaction with 3'UTR of target mRNA, and this mechanism by which play an important role in cancer metastasis and invasion.

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Pancreatic Exocrine-Endocrine Interrelationship: Its Clinical Relevance

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Background: The pancreas regulates metabolic homeostasis via two inter-related functions: exocrine pancreas secretion of digestive enzymes into the gastrointestinal tract for food digestion and nutrient absorption and the endocrine pancreas secrete metabolic hormones, insulin and glucagon to the bloodstream in control of blood glucose level and nutrients utilization and metabolism. Circulatory and function link occurred between the endocrine and exocrine pancreas and play a significantly physiological role for nutrient metabolism. This metabolic pathway is of clinical relevance in the pathophysiology and treatment of diabetes mellitus, pancreatitis, and pancreatic cancer and their relationship.

Results and Discussion: The US National Institutes of Health has developed the consortium of chronic pancreatitis-diabetes and pancreatic cancer program to support research into this clinically complex interrelationship (www.cdpcc.mdannderson.org). The goal of this consortium is to understand the mechanism of risk of pancreatic cancer from diabetes mellitus and pancreatitis in order to develop strategies for early diagnosis, interdiction, and prevention of pancreatic cancer, which is now the third among cancer mortality. This pancreatic exocrine-endocrine relationship is now also involved in the control of food intake and the mechanism of action and possible modulation in the pathophysiology and treatment of obesity. It is now well established that the pancreatic exocrine-endocrine relationship plays a key role in nutrient metabolism that regulates metabolic homeostasis both in health and disease at the organ system and cellular molecular level.

Cancer Metabolism From a Systems Biology Perspective

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Recent advances have focused on molecular regulation of cancer metabolism by oncogenes and tumor suppressor genes. Alteration of these genes are associated with changes in central glucose metabolism particularly aerobic glycolysis, the Warburg effect, which is the main theme of cancer metabolism. As its name implies, the main function of aerobic glycolysis is the production of lactate and energy (reducing equivalents and ATP) at the expense of glucose. The role of providing substrates for macromolecular synthesis is relegated to the mitochondria. How these processes are coordinated is poorly understood. In the past decade, the application of tracer-based metabolomics has provided evidence of system-wide changes in cancer metabolism in response to metabolic inhibitors, giving cancer metabolism a systems biology perspective.

Since biochemical reactions in the Warburg effect place competing demands on available precursors, high energy phosphates and reducing equivalents, the cancer metabolic system must fulfill the condition of balance of flux (in physiological terms, homeostasis). Such a principle can be demonstrated by experiments with individual metabolic inhibitors demonstrating system-wide effects on cancer metabolism. It is hypothesized that anticancer treatments that target molecular signaling pathways or inhibit individual metabolic enzymes alter the invasive or proliferative behavior of the cancer cells by their systems biology effects on the balance of flux (homeostasis) of the cancer cells.

The Effect of Deuterium Depletion on Cancer Cell Metabolism: Therapeutic Perspectives

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Background: Deuterium, the heavy non-radiating isotope of hydrogen, blocks mitochondrial energy production by damaging ATP synthase and thus induces molecular crowding and the Warburg effect. Western diets and lifestyle, among other factors, promote deuterium accumulation in human cells with far reaching implications in cancer incidence and clinical management.

Method: Oxygen sensitive breast (MCF7) as well as glycolytic, Warburg type pancreatic (MIA-PaCa) epithelial cells were cultured in the presence of [1,2-¹³C₂]-D-glucose as the single metabolic tracer and increasing doses of deuterium oxide. We determined ¹³CO₂ release and ketoglutarate formation in the TCA cycle via malic acid recycling.

Results: Exchange of protons by deuterons in cultures of epithelial breast cells using 50 ppm (0.005% D), 100 ppm (0.01% D) and 150 ppm (control;

0.015% D) increased complete [1,2-¹³C₂]-D-glucose oxidation to ¹³CO₂ and malate recycling to [3,4,5-¹³C₃]-L-glutamate. Proof of mitochondrial malate coupling in oxygen sensitive MCF-7 cells was obtained by the high correlation coefficient, 0.97, between ¹³CO₂ release and malate recycling by malate dehydrogenase using [3,4,5-¹³C₃]-L-glutamate formation as the surrogate marker. Malate recycling was not coupled in MIA-PaCa cells but a significant branching of the TCA cycle at citrate occurred by heavy ¹³C palmitate labeling via new synthesis.

Conclusion: Deuterium depletion by natural low deuterium ketogenic substrate oxidation is essential to produce deuterium depleted matrix water for mitochondrial health as deuterium free rotations of the ATP synthase nanomotor protein increases substrate intake and TCA cycle turnover to prevent molecular crowding to treat cancer.

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