The First Tianjin Forum on Tumor Microenvironment Tianjin, China, July 2–4, 2010

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On July 2–4, 2010, Nankai University College of Pharmacy and The Laboratories of National Research Program (973 Project) on Cancer Microenvironment, headed by Professor Luyuan Li of Nankai, hosted the first Tianjin Forum on Tumor Microenvironment in Tianjin, China. The purpose of this meeting was to promote academic exchange on the cutting edge of contemporary cancer research area—the tumor microenvironment. It was China's first international academic event focused on this field. Leading researchers from North America, Europe and Asia gave 30 lectures on the latest findings in their own labs. About 300 participants from China and abroad attended the meeting. Dr. Zihao Rao, President of Nankai University, attended the opening session to extend his warm welcome to all the conference attendees.

Keynote Speeches

Several sessions focused on different aspects of the Tumor Microenvironment. The opening session included two presentations. Dr. Isaac Witz from Tel Aviv University and Editor in Chief of “Cancer Microenvironment” briefly reviewed in a historic perspective, how cancer researchers came to realize the importance of interactions between cancer cells and their microenvironments in the processes of cancer development and progression towards metastasis. He further pointed out that there are two types of microenvironments that cancer cells reside in: the microenvironment of the primary, local tumor and the microenvironment

of secondary, metastatic sites. The primary cancer cells and the metastatic cells differ from each other in several characteristics including gene expression patterns, functional phenotype and responsiveness to signals delivered by their different microenvironments. Tumor-Microenvironment interactions should thus be studied in the appropriate context. Another point raised in his presentation was that the interactions between tumor cells and their microenvironment are dynamic and bi-directional and that each interaction partner shapes the phenotype of the other partner. Dr. Witz illustrated these general remarks with supporting evidence using primary cancer and metastatic variants of Neuroblastoma and Melanoma.

Dr. Robert Kerbel from University of Toronto discussed his findings regarding circumstances by which certain cytotoxic drugs can rapidly alter the tumor microenvironment in such a way as to decrease their efficacy. Specifically, he summarized results showing that bolus injections of certain cytotoxic chemotherapy agents at maximum tolerated doses (MTDs) such as paclitaxel or cyclophosphamide, or microtubule inhibiting ‘vascular disrupting agents’ (VDAs) can cause a rapid mobilization of various cell populations from the bone marrow compartment which then migrate to and colonize the drug treated tumors. These retained cell populations include endothelial progenitor cells, but likely other cell types as well including monocytes, macrophages, and mesenchymal derived stem cells. Here these cell populations can promote tumor repopulation, at least in part, by stimulating tumor angiogenesis and vasculogenesis. This acute reactive host response appears to be driven by systemic induction of multiple chemokines and cytokines such as G-CSF and SDF-1. The mobilization/tumor colonization response by such BMDCs can be blocked, at least in part, by several pharmacologic maneuvers. These include administration of

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VEGF pathway targeting antibodies (which, incidentally, provides potential explanation about how administration of drugs such as bevacizumab may enhance the efficacy of chemotherapy, i.e., by suppressing such host BMDC responses and thus extending the duration of the tumor response), or by anti-SDF-1 antibodies. In addition, he summarized the fact that low dose ‘metronomic’ chemotherapy can also bring about a similar effect as the aforementioned agents. Indeed, metronomic chemotherapy not only avoids the rapid chemokine/cytokine/BMDC response, but can actually target certain BMDCs such as endothelial progenitor cells, and perhaps others as well. As such, this helps explain why lower doses of chemotherapy drug administered in a frequent, regular fashion with no breaks, despite being unable to induce rapid tumor regressions, might still possess anti-tumor activity that is equal or superior to conventional MTD chemotherapy regimens. Dr. Kerbel went on to summarize results employing new preclinical models of advanced metastatic disease showing remarkable anti-tumor effects of certain metronomic chemotherapy regimens used either alone or in combination with a targeted antiangiogenic VEGF pathway targeting drug. Some of these preclinical results have now led to the initiation and completion of phase II clinical trials evaluating similar therapeutic regimens in patients with advanced disease, with highly encouraging results, both in terms of efficacy and reduced toxicity, and as such, are leading to phase III clinical trial evaluation.

Session 1: Tumor Fluids, Vasculature, and Lymphatics

Dr. Bruce Zetter of Harvard Medical School spoke about the fluid microenvironment in prostate cancer. Prostate cancer cells grow slowly in the prostate but quickly in the bone. Spermine—a polyamine that in the prostate is 100-times more than elsewhere—is responsible for the inhibition. The ductal epithelial cells surrounded by the fluid still differentiate early. With prostate cancer progression, the prostate cancer cells either move away from the spermine, or the cells dedifferentiate and stop making spermine, or become resistant to spermine. All three things happen. Ornithine decarboxylase is up regulated in the cell cycle in G1. It converts ornithine to putrescine, then to spermidine, then to spermine.

Dr. Luyuan Li from Nankai University, Tianjin, China, spoke about modulation of hematopoietic stem cell (HSC) differentiation into endothelial progenitor cells (EPC) by a TNF family member, VEGI (TNFSF15). VEGI is produced by endothelial cells, induces apoptosis in proliferating endothelial cells, and is down-regulated in tumor vasculature. Using a cell culture model to study differentiation of mouse bone marrow-derived HSC to EPC, a process

accompanied by decreasing stem cell markers and increasing endothelial cell markers, it is shown that VEGI inhibits EPC adhesion on fibronectin, laminin, and vitronectin, as well as EPC migration and capillary formation in vitro. VEGI induces apoptosis to E-selectin positive adhered EPC but protective effect on suspension cells. Death receptor-3 expression coincides with VEGI-induced cell death in EPC. Relationship between VEGI downregulation and inflammation is being investigated.

Dr. Zhou Wang of University of Pittsburgh, USA, spoke on regulation of angiogenesis by tumor suppressor U19/Eaf2 (ELL-associated factor 2), an androgen responsive gene in the prostate. U19—a potential transcription factor—is up-regulated by androgen and mainly localized in the epithelial cells of the rat ventral prostate. It is also found in the human bone marrow, lymph node, kidney and prostate. When over-expressed it induces apoptosis in prostate cancer cells. U19 is down-regulated in about 80% advanced prostate tumors. U19 knockout mice developed multiple types of tumors including lymphoma. U19 can inhibit angiogenesis via mechanisms involving hypoxia-inducible factor-1, thrombospondin-1 (TSP-1), and p53.

Dr. Shi-Yuan Cheng of University of Pittsburgh, USA, discussed mechanisms of PDGFR α -promoted glioma invasion and inhibition of growth and angiogenesis of brain gliomas. A hallmark of malignant glioblastomas is their intrinsic ability as single tumor cells to disperse throughout the brain, rendering the lethality to patients with gliomas and difficulty to treat. Work from his lab show that PDGFR α promotes glioblastoma invasion in the brain through Src-dependent phosphorylation of Dock180 at Y1811 within a regulatory domain, activating the CrkII-p130^{Cas}-Rac1 pathway and promoting glioma cell invasion. Aberrant activation of Rac1 signaling by constitutive p-Y of Dock180^{Y1811} in clinical glioblastomas could be a potential therapeutic target in treatment for these deadly brain cancers. Dr. Cheng also presented their recent results that a dual inhibitor for VEGFR2 and EGFR effectively inhibited brain glioblastomas that express a common mutated form of EGFR, EGFRvIII. This data could be of use for clinical applications in treatment of patients with glioblastomas that over express EGFRvIII.

Dr. Mengfeng Li of Sun Yat-sen University, China, showed that microRNA-mediated disruption of the I κ B-NF κ B negative feedback loop promotes tumor invasion and angiogenesis. A microRNA directly targets I κ B α 3-UTR, suppresses I κ B α expression and causes deregulated hyper activation of NF κ B, which consequently results in an up regulated expression of NF κ B-regulated genes, e.g., MMPs and VEGF. This drives aggressive invasion and angiogenesis of glioma cells. This mechanism is believed by the investigators to be clinically relevant as the I κ B α -targeting microRNA has been found to be over expressed in glioma

cells and highly correlates with malignant progression of the disease and poor patient survival. This molecule may therefore represent a new therapeutic target and prognostic marker.

Dr. Yongzhang Luo of Tsinghua University, China, spoke on the effect of pulmonary vascular destabilization in the premetastatic phase of lung metastasis. Data from his lab show that, in the premetastatic stage, angiopoietin 2 (Angpt2), matrix metalloproteinase (MMP) 3, and MMP10 are up-regulated in the lung by the primary B16/F10 tumor, which leads to the increased permeability of pulmonary vasculatures and extravasation of circulating tumor cells. Subsequent studies show that Angpt2, MMP3, and MMP10 have a synergistic effect on disrupting vascular integrity in both in vitro and in vivo models. He concludes that pulmonary vascular destabilization in the premetastatic phase promotes the extravasation of tumor cells and facilitates lung metastasis. These molecules may provide potential targets for clinical prevention of metastasis. Dr. Luo has also been working on endostatin, an antiangiogenic protein. He discovered that the N-terminal integrity and correct folding are very critical to the zinc binding and stability as well as biological functions of endostatin.

Dr. Tuomas Tammela of University of Helsinki, Finland, spoke about targeting angiogenesis and lymphangiogenesis in cancer. He pointed out that angiogenesis, the growth of new blood vessels from pre-existing vasculature, is insufficient in pathological conditions such as ischemic heart disease and critical lower limb ischemia. Lymphangiogenesis plays a major role in these disease conditions. VEGFR-3 is present on all endothelia during development, and in the adult it becomes restricted to the lymphatic endothelium. VEGFR-3 is activated by VEGF-C and VEGF-D. VEGF-C expression in human tumors has been correlated with poor prognosis, and increased incidence of lymph node metastases. Blocking VEGF-C/VEGFR-3 suppresses lymph node metastasis in preclinical tumor models. Intralymphatic tumor cells in metastatic transit inside lymphatic vessels serve as putative foci of tumor relapse in murine tumor models as well as in human melanoma, and their eradication using photodynamic therapy significantly reduced the incidence of tumor relapse in a murine melanoma model. Disruption of the Notch signaling pathway leads to widespread endothelial VEGFR-3 expression and excessive sprouting. These results highlight the utility of the VEGF-C/VEGFR-3 pathway as targets for anti-angiogenic and anti-metastatic therapy for cancer.

Dr. Zhi-Xiong Xiao of Sichuan University, China, showed that the p53-family member, p63, which is of crucial importance for the development and maintenance of all stratified epithelia, may play an important role in human cancer progression and metastasis. In order to identify

downstream targets of $\Delta Np63\alpha$, the predominant p63 isoform expressed in epithelia and low-grade cancers, associated with the maintenance of a non-invasive phenotype, his lab profiled gene expression of human breast cancer Hs578T cells stably expressing wild type $\Delta Np63\alpha$, or a mutant derivative defective in DNA binding or protein-protein interaction, showing that $\Delta Np63\alpha$ significantly up-regulates 160 genes and down-regulates 81 genes, including the up regulation of Mitogen Activated Protein Kinase Phosphatase 3 (MKP3), a negative regulator of MAP Kinase signaling whose expression is also decreased at advanced cancer stages. P63 knockdown in cancer cell lines leads to increased cell migration and invasion.

Session 2: The Bone Microenvironment

Dr. Evan T Keller of University of Michigan Medical Center, USA, discussed the biology of prostate cancer bone metastases. Prostate metastasizes to bone, liver, lymph node, lung, soft tissue, dura, adrenal in that order. Bone resorption enhances carcinoma migration. Stromal-derived factor (SDF-1) and its receptor CXCR4 play a major role. Prostate cancer bone metastasis is heterogeneous. It favors the vertebrae, ribs, skull and proximal long bones, forming on trabecular bone surfaces at sites of previous bone resorption, coinciding with increased serum markers of resorption, and causing high morphometric resorption of bone. Tumors osteoclast bone interface, up to 10 osteoclast lesions per mm² with prostate cancer, 10-times of normal. Osteoprotegerin inhibits this. Phase III study of denosumab, an antibody to RANKL, in men with hormone-refractory prostate cancer is underway. Signaling pathways involving BMP and Wnt are discussed.

Dr. Cun-Yu Wang of University of California, Los Angeles, USA, spoke about molecular regulation of breast cancer bone metastasis by NF- κ B. Advanced breast cancers most frequently metastasize to distant organs such as lung and bone. NF- κ B plays a critical role in breast carcinogenesis. Elevated NF- κ B DNA-binding activities as well as IKK kinase activities were found in both mammary carcinoma cell lines and primary human breast cancer tissues. Work from his lab show that constitutive activation of NF- κ B promoted breast cancer bone metastasis by promoting osteoclast formation. Transducin- κ -like protein (TBL1) is found to be required for NF- κ B activation in breast cancer cells. Knock-down of TBL1 inhibits NF- κ B-dependent gene expression. TBL1 knock-down also attenuates cell invasive growth in vitro and tumor metastasis in vivo. Underlying mechanisms are being investigated.

Dr. David Roodman of University of Pittsburgh School of Medicine, USA, spoke on osteoblast suppression in myeloma. Multiple myeloma patients frequently have bone

lesions. Clinically, this results in appearance of lytic skeletal lesions that fail to heal even when patients have prolonged disease remissions. His lab developed a model of myeloma bone disease in which myeloma cells express both GFP and the thymidine kinase gene to allow his lab to kill myeloma cells either in *in vivo* or *in vitro* with ganciclovir and isolate cells from the marrow microenvironment to assess the mechanism involved in osteoblast suppression. Dr Roodman discussed the role of soluble factors produced by myeloma cells, including $\text{TNF}\alpha$ and IL-7, which suppress osteoblast differentiation, and do so by upregulating the transcriptional repressor Gfi-1. Experimental evidence from his lab suggest that Gfi-1 is an important transcriptional suppressor of Runx2 in osteoblast precursors in myeloma.

Dr. Xu Cao of Johns Hopkins University, USA, spoke about TGFb-1-induced migration of bone marrow stem cells to couple bone resorption and formation and implication for treatment of bone diseases including metastasis of tumors to the skeleton. Active TGFb1 is released to bone marrow microenvironment during osteoclastic bone resorption, induction migration of CD29+, Sca-1+, CD45- BMSC for bone formation. Migrations of injected mouse osteogenic BMSCs to bone surfaces and osteoblastic differentiation depends on TGFb1. Smad2/3 are required for migration of BMSCs and TGFbR1 inhibitor inhibits BMSCs migration. TGFb1 functions as a coupling factor for bone resorption and formation thus may be a potential target for inhibition of tumor metastasis to bone and other bone remodeling disorders.

Dr. Guozhi Xiao of University of Pittsburgh, USA, discussed ATF4-mediated regulation of osteoclast differentiation and its implication in skeletal metastases. Studies from his laboratory demonstrate that both *in vitro* and *in vivo* osteoclast differentiation was severely impaired by lack of ATF4 in a cell-autonomous manner and increased by osteoclast-targeted transgenic ATF4 expression. ATF4 favors osteoclast differentiation by a) directly activating the gene encoding the critical transcription factor NFATc1; b) modulating RANKL activation of multiple MAPK pathways in OCL progenitors; and c) mediating M-CSF induction of RANK expression on BMM, a critical step for early osteoclast differentiation. The lack of ATF4 caused a shift in OCL precursor to macrophage, thereby increasing macrophage. Furthermore, the level of ATF4 protein was largely modulated by M-CSF signaling and the PI3K/AKT pathways in bone marrow monocytes (BMMs). Finally, ATF4 was up-regulated by myeloma cells-derived factors such as $\text{TNF-}\alpha$ in primary mouse BMM cultures. These results demonstrate that ATF4 plays a direct osteoclast-intrinsic role in regulating osteoclast differentiation and suggest that it may be a therapeutic target for treating bone diseases associated with increased osteoclast activity.

Dr. Chawnshang Chang of University of Rochester Medical Center, USA, discussed how differential androgen receptor signals explain why androgen deprivation therapy of prostate cancer fails. Androgen-deprivation therapy (ADT) for the suppression of androgens binding to the androgen receptor (AR) has been the norm of prostate cancer treatment, but it eventually fails, leading to recurrent tumor growth in a hormone-refractory manner, even though AR remains functional. Interestingly, some prostate cancer survivors who received androgen replacement therapy had improved quality of life without adverse effect on their cancer progression. These contrasting clinical data suggest that differential androgen/AR signals in individual cells of prostate tumors can exist in the same or different patients, and may be used to explain why ADT of prostate cancer fails. Data from his lab show that AR functions as a stimulator for prostate cancer proliferation and metastasis in stromal cells, as a survival factor of prostatic cancer epithelial luminal cells, and as a suppressor for prostate cancer basal intermediate cell growth and metastasis. These dual yet opposite functions of the stromal and epithelial AR may challenge the current ADT to battle prostate cancer and should be taken into consideration when developing new AR-targeting therapies in selective prostate cancer cells.

Dr. Li Fu from Tianjin Medical University spoke about the abnormal methylation and regulation mechanisms of epithelial mesenchymal transition in breast cancer of high invasion and metastasis. Dr. Fu's group had confirmed that invasive micropapillary carcinoma (IMPC) manifested an early stage action of EMT. By comparison of IMPC and invasive ductal carcinoma (IDC) through cDNA microarray and genome DNA methylation microarray assay, they found 26 genes with high methylation and 3 miRNA might play important roles in tumor invasion and metastasis. Specially, promoter region of FEZ1 gene is highly methylated. As a result, the expression of LZTS1 was absent or reduced more than 80% in the IDC and IMPC. The reduction significantly correlates with lymph node metastasis and poor prognosis.

Session 3: The Stroma and Immune Cells

Dr. Jeffery Pollard of Albert Einstein Medical College, USA, talked about macrophages Promoting tumor progression and metastasis. He pointed out that there is persuasive clinical and experimental evidence that macrophages promote cancer initiation and malignant progression. Macrophages enhance malignancy at the primary site by stimulating angiogenesis, inducing tumor cell migration, invasion and intravasation and by suppressing anti-tumor immunity. At metastatic sites macrophages promote tumor

cell extravasation, survival and subsequent growth. Each of these activities is stimulated by a different population of macrophages whose unique signaling pathways might represent new therapeutic targets

Dr. Limin Zheng of Sun Yat Sen University, China, also spoke about activated macrophages in human tumors. He showed that tumor microenvironments can alter the normal development of macrophages intended to trigger transient early activation of monocytes in the peritumoral region. Activated monocytes in peritumoral stroma suppressed tumor-specific T cell immunity by expressing B7-H1 molecules, which represent a novel link between pro-inflammatory response and immune tolerance in the tumor milieu. Proinflammatory Th17 cells are accumulated in HCC tissue, where they promote disease progression by fostering angiogenesis. Tumor-activated monocytes are significantly superior to the suppressive tumor macrophages in inducing expansion of Th17 cells from circulating memory T cells with phenotypic features similar to those isolated from HCC tumors. It is not inflammation alone but rather the inflammatory “context” that determines the ability of proinflammatory factors to facilitate or prevent tumor growth. By promoting the expression of B7-H1 and expansion of Th17 cells, tumor activated monocytes repurpose the inflammatory response away from anti-tumor immunity and towards tissue remodeling and proangiogenic pathways. Thus, selectively modulating the functional activities of monocytes/macrophages may provide a novel strategy for anticancer therapy.

Dr. Wolf-Herve Fridman, Cordeliers Research Centre, INSERM, University Paris Descartes and University Pierre et Marie Curie, France, spoke about immune control of human tumors. He pointed out that the microenvironment of human tumors differs, from patient to patient in their lymphocytic infiltration. In addition, lymphocytes are not randomly scattered within the tumor but appear organized in the center of the tumor, its invasive margin and in tertiary lymphoid follicles adjacent to the tumor nests. His lab has particularly analyzed large cohorts of colorectal and lung cancers and have shown that a high infiltration of memory T cells, with a Th1 and cytotoxic orientation, both in the center and the invasive margin of the primary tumors is a major prognostic factor since it controls tumor recurrence, probably through an immune control of potential metastatic cells. He and colleagues recently analyzed some of the factors essential to shape an efficient immune control, which is likely to originate in the tertiary lymphoid follicles, and identified chemokines and cytokines produced in the microenvironment which structures a T cell specific response. These data allow drawing an integrated picture of how an immune reaction develops in the tumor microenvironment to control cancer spread and recurrence.

Dr. Michael Lotze, University of Pittsburgh School of Medicine, USA, discussed damage associated molecular pattern molecules (DAMPs) that drive altered tumor metabolism and inflammation. His lab’s recent discovery of cytosolic HMGB1 as an inducer of autophagy and others of cytosolic p53 as an inhibitor of autophagy has placed these two molecules at the crux of metabolism. HMGB1 is a cytosolic activator of autophagy and associated with post-translational modifications of p53. HMGB1 serves as a damage-associated molecular pattern molecule [DAMP] interacting with the RAGE, TLR2, TLR4 or TLR9 central to sepsis, arthritis, myocardial and hepatic ischemia reperfusion, and cancer. He pointed out a novel role for HMGB1 as a cytosolic factor promoting autophagy and mitophagy, enhancing aerobic glycolysis, rapid ATP generation, and limiting apoptosis, and concluded that HMGB1 serves as a critical link, regulating metabolism, the response to stress, and cellular survival.

Dr. Zhihai Qin, Institute of Biophysics, Chinese Academy of Sciences, China, discussed the role of fibroblasts in tumor environment. He pointed out that tumor stromal cells, especially fibroblasts, play important roles during the initiation and progression of tumors. Stromal fibroblasts can not only synthesize extra-cellular matrix, which is crucial for the encapsulation of the tumor, but also secrete a variety of cytokines regulating tumor angiogenesis, recruitment and activation of different immune cells. His data show that fibroblasts-involved “foreign body reaction” against tumor cells is responsible for the inhibition of carcinogenesis. Correspondingly, in DMBA/TPA skin-painting model where carcinogen cannot be encapsulated as ‘foreign body’, IFN γ promotes tumorigenesis by up-regulating Th-17-associated inflammation. Using a model by co injection of tumor cells and gene-modified mouse embryonic fibroblasts, he demonstrates that fibroblasts inhibit tumor growth; this relies on IFN γ -mediated down-regulation of VEGF expression by these cells.

Session 4: The Stroma—Other Factors and Signaling Pathways

Dr. Yifan Dai from Nankai University discussed the tumor inhibitory effect of Omega-3 fatty acids. He pointed out that Omega-3 fatty acids (ALA, EPA and DHA) are essential fatty acids to all mammals and have to be taken from diet. In last 100 years, the industrialization in food and farm animal production resulted in severe imbalance of intake of omega-6 and omega-3 in human diet. The current omega-6/omega-3 ratio in human diet is 20:1 instead of the healthy 1:1 ratio. The unhealthy ratio of omega-6/-3 causes a high inflammatory status in the human body and thus results in many contemporary human diseases such as obesity,

diabetes, coronary heart diseases as well as cancer. By using the fat-1 transgenic mouse model, who can convert omega-6 to omega-3 fatty acids in their body, Dr. Dai showed that omega-3 fatty acids have a significant inhibitory effect on various cancers including hepatocellular carcinoma, colon cancer, prostate cancer and breast cancer. The potential mechanisms of the tumor inhibitory effect of the omega-3 fatty acids were discussed.

Dr. Duanqing Pei, from Guangzhou Institute of Biomedicine and Health, Chinese Academy of Science discussed the new culture technique for induced pluripotent stem cells (iPSCs). Somatic cells can be reprogrammed into iPSCs by over-expressing defined combinations of transcription factors that are highly expressed in embryonic stem cells (e.g. Sox2, Klf4, Oct4 and c-Myc). He demonstrated that vitamin c (Vc) enhances iPSCs generation from both mouse and human somatic cells. The mechanisms of Vc functions were also discussed. He pointed out that Vc acts at least in part by alleviating cell senescence, a recently identified roadblock for reprogramming. Furthermore, Vc accelerates gene expression changes and promotes the transition of pre-iPSCs colonies to a fully reprogrammed state. Dr. Pei's results highlight a straightforward method for improving the efficiency of iPSCs generation and provide additional insights into the mechanistic basis of the reprogramming process.

Dr. Qimin Zhan from Chinese Academy of Medical Sciences discussed the mechanisms of BRCA1-regulated centrosome stability. He demonstrated that BRCA1 physically interacts and colocalizes with Nlp (Ninein like protein). Nlp centrosomal localization depends on normal cellular BRCA1 functions. Suppression of Nlp results in aberrant spindle formation, failure of chromosomal segregation and cytokinesis, and aneuploidy. Nlp exhibits strong oncogenic property and induces NIH3T3 fibroblasts transformation. Importantly, Nlp transgenic mice mimic the phenotypes of disrupted BRCA1, including centrosome amplification and spontaneous tumorigenesis. Thus, Nlp may cooperatively act together with BRCA1 in mitotic machinery and abnormalities of Nlp leads to genomic instability and tumorigenesis.

Dr. Zhinan Yin from Nankai University discussed the role of microRNA *let-7x* in breast cancer invasion and metastasis. He found that diminished expression of *let-7x* was significantly associated with lymph node metastasis and poor survival. Abrogation of *let-7x* expression in otherwise non-invasive mammary carcinoma cells elicited rapid metastasis from orthotopic location. He identified GAB2 as a novel *let-7* target mediates tumor invasion through increasing MAPK/ERK activity. Treatment with estrogen and EGF specifically reduced *let-7x* expression and thereby increased GAB2 expression. Conversely, inhibition of MAPK/ERK activity abrogated estrogen or EGF stimulated *let-7x* down-regulation. Dr. Yin's study

revealed a *let-7* family member as the prognostic biomarker in breast cancer. In addition, he also presented a novel paradigm utilized by etiological endocrine factors to promote breast cancer invasion and metastasis.

Dr. Jian Zhang from University of Michigan discussed the role of uPA (urokinase plasminogen activator) and its receptor (uPAR) signaling in the development and progression of prostate cancer. He constructed tumor-bearing models with uPA and uPAR knockout mice. Tumor volume was significantly diminished in both uPA^{-/-} and uPAR^{-/-} mice compared to WT controls. Greater inhibition of tumor volume was also observed in uPA^{-/-} mice compared to uPAR^{-/-} mice suggesting the important contribution of stromal uPA in the tumor growth. Tumors in uPA^{-/-} and uPAR^{-/-} mice displayed significantly lower proliferative indices, higher apoptotic indices, and fewer neovascularity compared to the tumors in WT mice. In addition, tumors in uPA^{-/-} and uPAR^{-/-} mice displayed significantly less monocyte infiltration compared to the tumors from WT mice. In consistence with these findings, the in vitro macrophage chemotaxis to RM-1 cell conditioned media was significantly reduced in the peritoneal macrophages collected from uPA^{-/-} and uPAR^{-/-} mice compared to the macrophages from WT mice, indicating that extratumoral macrophages may play a key role in the tumor progression in this model. These findings suggest that the uPA/uPAR axis acts in both autocrine and paracrine manners in the tumor microenvironment, and activation of uPA/uPAR axis is essential for macrophage infiltration into prostate tumor.

Dr. Zhi Yao from Tianjin Medical University discussed the effects of raf kinase inhibitor protein (RKIP) on the metastasis of breast cancer. He found that RKIP expression was significantly higher in normal breast duct epithelia, hyperplastic breast tissue and ductal carcinoma in situ than that of invasive ductal breast carcinomas. The intensity of RKIP positive cells was significantly reduced in paired lymph node metastases than that of primary invasive ductal breast carcinomas. High RKIP expression significantly suppressed the invasiveness, migration, adhesion, and IL-8 secretion in vitro. Accordingly, low RKIP expression significantly increased the invasiveness, migration, adhesion and IL-8 secretion. Furthermore, high RKIP expression significantly suppressed angiogenesis and decreased the number of lung metastases. In contrast, low RKIP correlated with increased angiogenesis and the number of lung metastases. These data suggest that RKIP can be a prognostic biomarker for occult metastases.

Dr. Yu Jiang from the University of Pittsburgh discussed the molecular mechanisms underlying the oncogenic activities of Rheb. He identified a FK506-binding protein, FKBP38, which can interact directly with mTOR and Bcl2/Bcl-XL. FKBP38 inhibits both mTOR mediated cell growth and Bcl-2/XL mediated survival. He also demonstrated

Rheb as a negative regulator for the FKBP38 functions. Association of Rheb with FKBP38 abrogates its inhibition on both mTOR and Bcl-2/XL pathways, thus promote the growth and survival of the cells.

Finally, Dr. Jilong Chen from Chinese Academy of Sciences presented his studies on the mechanisms of oncogene Abl in the modulating of cytokine signaling. He found that co-expression of BCR-ABL with SOCS-1 resulted in tyrosine phosphorylation of SOCS-1. Phosphorylation of SOCS-1 was associated with its binding to BCR-ABL. Moreover, BCR-ABL-dependent phosphorylation of SOCS-1 decreased SOCS-1 interaction with the Elongin BC complex and inhibited SOCS-1-mediated JAK degradation. These data suggest that the BCR-ABL may regulate the inhibitory function of SOCS-1 through mediating SOCS-1 phosphorylation. His group also found that the AKT1(E17K) mutant greatly increased the v-Abl transformation efficiency as compared to wild-type AKT1. Furthermore, AKT1(E17K) promotes survival of the Pim-deficient cells, indicating a functional link between AKT and Pim in v-Abl transformation. In addition, AKT1(E17K)

delays loss of JAK/STAT-dependent Pim-1 and Pim-2 protein expression on v-Abl inactivation, which suggests that there exists reciprocal signaling between AKT and Pim in v-Abl transformed cells.

The audience consisted of medical researchers and graduate students from universities and research institutions from other parts of China, including Beijing, Shanghai, Guangzhou, Chongqing, and Chengdu, to mention a few large cities, as well as from various academic institutions and hospitals in Tianjin. Many felt that the lectures were highly informative, and that the meeting served to demonstrate what the frontiers in the field, in China and abroad, are thinking about. It would seem that the meeting served its intended purpose: to help establish a TME research field in China. Some commented that this was one of the best international conferences they have attended in China lately. Dr. Isaac Witz, current president of the International Society for Tumor Microenvironment, congratulated the colleagues from the academic institutions of China for the high quality of their presentations.