prognostic biomarker and provide an attractive therapeutic target for TNBC.

**B38 Distinct functions for tumor-associated fibroblasts in melanoma growth.** LinLi Zhou, Kun Yang, Makoto M. Taketo, de Crombrugghe B, Yuhang Zhang. Division of Pharmaceutical Sciences, College of Pharmacy, University of Cincinnati, Cincinnati, OH, USA.

One origin of Tumor-associated fibroblasts (TAFs) is considered to be skin resident fibroblasts. Nevertheless, how local fibroblasts are stimulated to enter a continuous state of irreversible activation and establish an environment conductive for tumor development remains poorly understood. To address this, we inducibly deleted b-catenin in stromal fibroblasts in order to identify the molecular basis underlying the tumor-supportive functions of TAFs during the development of melanoma. Nuclear b-catenin was detected in TAFs inside and surrounding melanocytic neoplasm. Following constitutive expression of b-catenin in dermal fibroblasts, mouse rapidly developed skin abnormality and dermal fibrosis, including skin thickening, accumulation of collagen fibers and production of a large amount of growth factors, cytokine and extracellular matrix proteins, including EGF, FN1 and TNC, etc. By contrast, specific deletion of b-catenin in fibroblasts significantly decreased generation of those chemical factors as well as alleviated dermal fibrosis. Furthermore, Loss of b-catenin significantly blocked fibroblast growth and migration *in vitro*, and failed to support melanoma cells to grow. We first asked whether local dermal fibroblast support or inhibit melanoma growth at tumor initiation stage by grafting B16F10 melanoma cells to immunodeficient nude mice. Melanoma tumor grew bigger when β-catenin is ablated in fibroblasts than those with control wild type fibroblasts. This finding confirms that stromal fibroblasts in the vicinity of tumor cells initially repress malignant growth, and the loss of b-catenin would deprive their ability to counteract tumor development. However, when b-catenin was ablated in TAFs later after tumor was formed, melanoma development was significantly delayed, exhibiting a different effect on tumor development. Further studies showed that cell cycle progression and pRB signaling pathway are affected by secreted chemical factors from TAFs that significantly blocked melanoma cell growth. Thus, targeting Wnt/b-catenin signaling pathway in TAFs has the potential to activate pRB signaling and blocking cell cycle in melanoma cells, representing a novel therapeutic approach to conquer melanoma.

**B39 Cooperation of epithelial-mesenchymal transition and cellular stress response in promoting metastasis.** Yu-xiong Feng[1], Dexter X. Jin[1,2], Piyush B. Gupta[1-5]. [1]Whitehead Institute for Biomedical Research, Cambridge, MA, USA, [2] Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA, [3] Koch Institute for Integrative Cancer Research, Cambridge, MA, USA, [4] Harvard Stem Cell Institute, Cambridge, MA, USA, [5]Broad Institute, Cambridge, MA, USA.

Epithelial-to-mesenchymal transition (EMT) plays a central role in cancer progression. Through an EMT, cancer cells acquire a spectrum of malignant properties, including invasiveness, chemo-resistance and stem-like traits. With an aim to effectively target EMT-cancer cells, we previously performed a high throughput chemical and RNAi screen, and discovered that EMT-cancer cells are hypersensitive to perturbations of endoplasmic reticulum (ER) function, which represents a specific vulnerability of cancer cells that have undergone this cell-state transition. This increased sensitivity to ER stress of EMT-cancer cells is largely due to the increased synthesis and secretion of extracellular matrix (ECM) proteins. In line with this fundamental change, EMT-cancer cells constitutively activate the PERK-ATF4 branch of the unfolded protein response (UPR) pathway. Surprisingly, we found that the PERK pathway, in addition to mediating ER stress response, is required for acquisition of malignant features of the EMT-cancer cells. Mechanistically, the PERK-ATF4 signaling cooperates with the EMT transcription factors to induce a CREB family transcription factor, which is critical for maintaining the EMT-specific secretome. Inhibition of this factor markedly suppresses EMT-driven metastasis, while overexpressing this protein endows non-EMT cancer cells an increased metastatic capacity. In summary, we discovered a non-canonical role of the PERK-ATF4 stress response pathway in driving metastasis.

**B40 RhoE/ROCK signaling modulates chemoresistance in HCC through IL6/JAK2/STAT3 pathways.** W Ma, KM Sze, LK Chan, JM Lee, VC Cheung, TKW Lee, CCL Wong, IOL Ng. Department of Pathology, Li Ka Shing Faculty of Medicine and State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong.

Liver cancer (hepatocellular carcinoma, HCC) is a major malignancy worldwide and the second commonest fatal cancer in Southeast Asia and China including Hong Kong, due to the high prevalence of hepatitis B viral infection. HCC is highly chemoresistant, limiting treatment options to patients. There is an urgent need to delineate the underlying molecular mechanism of HCC chemoresistance so as to identify novel therapeutic targets for this aggressive cancer. Deregulation of Rho GTPase pathway is demonstrated to play important roles in HCC tumorigenesis. RhoE/Rnd3 belongs to the Rnd subfamily of the Rho GTPase which lacks the intrinsic GTPase activity. In our previous study, we

have shown that RhoE is frequently downregulated in human HCCs and acts as a metastasis suppressor, whereas ROCK2 is upregulated in human HCCs. In this study, we aimed to investigate whether RhoE is also involved in the regulation of chemoresistance in HCC. Using short-hairpin RNA and a lentiviral approach, we knocked down RhoE in BEL-7402 and MHCC-97L HCC cells. RhoE knockdown cells displayed increased resistance to chemotherapeutic drugs, cisplatin and doxorubicin. Knockdown of RhoE also reduced cisplatin-induced apoptosis. On the other hand, addition of ROCK inhibitor, Y27632, sensitized the HCC cells to these two drugs. We further demonstrated that knockdown of RhoE enhanced chemoresistance in an *in vivo* subcutaneous injection model in nude mice; treatment with cisplatin significantly suppressed the tumor growth of the non-target control group while it had no effect in the RhoE knockdown group. In addition, treatment with ROCK inhibitor Y27632 sensitized HCC cells to cisplatin treatment *in vivo*; co-treatment of cisplatin and Y27632 suppressed subcutaneous tumor growth of BEL-7402 to a greater extent than cisplatin alone.

The downstream molecular targets of Rho/ROCK that regulates cell survival and chemoresistance remain largely unknown. Therefore we also investigated the molecular pathway which Rho/ROCK signaling might act on in mediating its pro-survival effect. IL6/JAK/STAT3 pathway has been shown to be important in regulating HCC chemoresistance, and previous studies have also suggested that Rho/ROCK pathway may interfere with the IL6/JAK/STAT3 pathway. We tested whether RhoE/ROCK modulated chemoresistance through IL6/JAK/STAT3 in HCC. Addition of Y27632 suppressed IL6 mRNA expression and IL6 secretion in BEL-7402 and SMMC-7721 HCC cells. We also observed up-regulation of JAK2 and STAT3 phosphorylation levels in the transient RhoE knockdown BEL-7402 cells using Western blot analysis. On the other hand, knockdown of ROCK2 and addition of Y27632 suppressed the phosphorylation of both JAK2 and STAT3. Dual luciferase reporter assay showed that the transcription activity of STAT3 was reduced upon Y27632 treatment in BEL-7402 and SMMC-7721. Immunohistochemical staining of the phospho-STAT3 in the subcutaneous tumors from nude mice treated with and without cisplatin and Y27632 confirmed that treatment of Y27632 also repressed STAT3 phosphorylation *in vivo*.

To conclude, downregulation of RhoE in HCC enhanced chemoresistance both *in vitro* and *in vivo* via upregulating the activities of ROCK. We identified the IL6/JAK2/STAT3 pathway to be a novel target of ROCK in promoting cell survival. Targeting the Rho/ROCK pathway may be a potential therapeutic in enhancing HCC treatment.

**B41 GPR56 inhibits melanoma progression by internalizing TG2 in extracellular matrix.** Liquan Yang[1,2], Nancy Corson[1], Lei Xu[1]. [1] Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY, USA, [2]Current Address: Winship Cancer Institute and Department of Neurosurgery, Emory University, Atlanta, GA, USA.

A critical component in tumor microenvironment is extracellular matrix (ECM). ECM is assembled by large polypeptides in the extracellular space. It forms a scaffold to support tissue structure and development and also serves as a repertoire of growth factors to promote cell growth. ECM proteins also directly bind to and signal through adhesion receptors, such as integrins, to regulate cell survival, proliferation, and migration. Excessive accumulation and crosslinking of ECM is a hallmark of cancer and has been shown to promote cancer progression by activating integrins and Rho GTPases. Its removal should have therapeutic benefits for cancer patients, but this potential has not been actively pursued. We recently discovered that a novel adhesion receptor, GPR56, inhibits melanoma growth and metastasis via removing its ligand, TG2, from ECM. GPR56 belongs to the family of adhesion G protein-coupled receptors (GPCRs), a group of important but poorly understood receptors. It was one of the down-regulated genes in the highly metastatic derivatives compared with the poorly metastatic melanoma parental line. Over-expression of GPR56 led to inhibition on melanoma growth and metastasis, and its knockdowns led to their enhancement. Results from later studies suggested that this inhibitory effect of GPR56 might apply to both BI-sensitive and BI-resistant melanomas, and probably occurs during the expansion of micrometastases into overt macrometastases. Our earlier work also discovered that TG2, a crosslinking enzyme in the extracellular matrix (ECM), binds to the N-terminus of GPR56, linking GPR56 with ECM remodeling during melanoma growth and metastasis. Indeed, studies using the immunodeficient *Tg2*$^{-/-}$ mice showed that TG2 itself promotes melanoma metastasis but GPR56 antagonizes it. Biochemical analyses were performed to understand this antagonism and showed that GPR56 removes TG2 from melanoma cell surface via receptor-mediated endocytosis, leading to impaired ECM deposition and adhesion. These results provided the first line of evidence that ECM removal may be feasible for cancer treatment, and GPR56 may serve as a target for this ECM-based therapy in treating metastatic melanoma.

**B42 MET suppresses epithelial VEGFR2 via intracrine VEGF-induced ER-associated degradation.** Tom T. Chen[1], Ellen Filvaroff[1*], Jing Peng[2], Scot Marsters[1], Adrian Jubb[3], Hartmut Koeppen[3], Mark Merchant[2], and Avi Ashkenazi[1**]. Genentech.

Hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) drive cancer through their respective receptors, MET and