486 POSTER

Phenotypic characterization of circulating tumor cells (CTCs) in triple negative breast cancer patients

G. Kallergi[1], S. Agelaki[2], A. Mathioudaki[1], D. Mavroudis[2], V. Georgoulias[2]. *[1]University of Crete, Medical School Laboratory of Tumor Cell Biology, Heraklion, Greece; [2]University General Hospital of Heraklion, Department of Medical Oncology, Heraklion, Greece*

Background: Molecular classification of breast cancer revealed that there are five different subtypes related to clinical outcome: luminal A, luminal B, normal-like, ERBB2-positive and basal-like. Triple negative patients (ER-negative, PR-negative, HER2-negative) belong to the basal-like subtype. Their tumors commonly express EGFR and are associated with poor prognosis. Circulating Tumor Cells (CTCs) have been proposed as a "real time liquid biopsy" in breast cancer patients. CTCs are associated with disease relapse and could serve as a target for molecular cancer therapies. The aim of the present study was, for the first time, the phenotypic characterization of CTCs in triple negative breast cancer patients.
Methods: We evaluated peripheral blood mononuclear cells (PBMC) cytospins from 40 triple negative patients and found CTCs in thirty one of them (15 early and 16 metastatic). The expression of Cytokeratins (CK), Estrogens Receptor (ER), Progesterone Receptor (PR), Epidermal Growth Factor Receptor (EGFR) and Human Epidermal Growth Factor Receptor (HER2) in CTCs was assessed using double immunofluorescent staining, confocal laser scanning microscopy and the Ariol system. PBMCs were stained with a monoclonal A45-B/B3 pancytokeratin antibody in combination with ER, PR, EGFR or HER2 antibodies, respectively.
Results: Our results demonstrated that ER, PR, EGFR and HER2 were expressed in 58%, 45%, 55% and 58% of the examined CK-positive patients. The respective proportions for early versus (vs) metastatic patients were 67% vs 50% for ER, 53% vs 38% for PR, 60% vs 44% for EGFR and 60% vs 56% for HER2. In addition ER, PR, EGFR and HER2 were expressed in 5.6%, 79.7%, 80.7% and 13.4%, respectively, of the total examined CTCs. Triple staining experiments with the Ariol system revealed no co-expression of CK/EGFR/ER in CTCs; however, there was only one patient with CTCs co-expressing CK/HER2/ER.
Conclusions: ER is expressed but in a minority of CTCs in triple negative breast cancer patients though the majority of CTCs revealed expression of PR and EGFR. However the clinical significance of these findings remain unknown and need further evaluation.

487 POSTER

The axon guidance molecule Slit2 regulates the motility of neuroendocrine cancer cells

K. Detjen[1], A. Göhrig[1], G. Hilfenhaus[1], M. Welzel[1], M. Schröder[1], B. Wiedenmann[1], C. Fischer[1]. *[1]Charité-Universitätsmedizin Berlin, Hepatology and Gastroenterology Campus Virchow Clinics, Berlin, Germany*

Background: Early blood-borne metastasis and a dense vascularization are characteristics for neuroendocrine tumors of the gastroenteropancreatic system (GEP-NET). Both events imply a central role for the interaction of blood vessels and cancer cells for the tumor biology of GEP-NET. Recently, a new function of the axon guidance molecule Slit2 and its receptors Robo1 and Robo4 has been established as a guidance factor for tumor and endothelial cells. Here we evaluated the role of the Slit2/Robo system in GEP-NET metastasis and angiogenesis.
Methods: Expression of Slit2, Robo1 and Robo4 on human GEP-NET tissue and cancer cell lines was determined by immunohistochemistry, RT-PCR and immunoblotting. We further analyzed the effect of vector-based overexpression of Slit2 on migration and agar colony formation of human GEP-NET cells using transwell and HTCA assays. HUVECs were incubated with tumor cell conditioned media to characterize the effect of Slit2 on endothelial cell migration and lamellipodia formation.
Results: Tissues from human GEP-NET as well as the corresponding neuroendocrine tumor cell lines BON and QGP showed variable expression of Slit2. However, in line with a consistent histological detection of Robo1 in epithelial cells of GEP-NET specimens, Robo1 was found abundantly expressed in BON and QGP cancer cell lines. In contrast, Robo4 was specifically expressed in endothelial cells of the tumor vasculature. Stable transfection of Slit2-deficient BON cells with a constitutive Slit2-pCMV vector substantially inhibited directed tumor cell migration and colony formation, while leaving cell proliferation unaffected. The effects of Slit2 were mediated in an auto-/paracrine manner, since Slit2 conditioned media also inhibited the migration of wild-type BON and QGP cells. Moreover, Slit2-mediated suppression of tumor cell motility involved restored E-cadherin expression and loss of vimentin expression in BON cells, indicating that Slit2 induced a mesenchymal-to-epithelial transition phenotype. Finally, tumor cell derived Slit2 repelled migrating primary endothelial cells by inhibiting VEGF-induced endothelial lamellipodia formation.
Conclusion: These data provide evidence for an intrinsic auto-/paracrine function of the Slit2/Robo system for the migration of GEP-NET cells as well as for their angio-regulatory interaction with endothelial cells *in vitro*. The differential expression of the Slit2 receptors Robo1 and Robo4 on tumor cells and the vascular compartment, respectively, thus imply a dual role of Slit2 in the process of both metastasis and angiogenesis of human GEP-NETs.

488 POSTER

Cytoplasmic mislocalization of RUNX3 by activated Shh is correlated with the development of TGF-β resistance in gastric cancer

J. Kim[1], M. Kang[1], H. Kang[1], J. Kim[2], S. Oh[2], Y. Yoo[3]. *[1]Korea University, Graduate School of Medicine Korea University Colledge of Medicine, Seoul, Korea; [2]Korea University, Division of Oncology/Hematology Department of Internal Medicine Korea University College of Medicine, Seoul, Korea; [3]Korea University, Brain Korea 21 Program for Biomedical Science Korea University College of Medicine, Seoul, Korea*

RUNX3 that belongs to the RUNX family of transcription factors acts as a tumor suppressor in gastric cancer. The inactivated RUNX3 is associated with transforming growth factor-β (TGF-β)-resistant cell types, and TGF-β has been implicated in Sonic hedgehog (Shh)-induced cellular signaling in gastric cancer. We hypothesized that the relationship between Shh signaling and RUNX3 may be involved in the development of TGF-β resistance.
Cells transfected with control vector only or Shh were treated with control vehicle, TGF-β, or TGF-β plus cyclopamine (a specific inhibitor of Smo) and then growth inhibitory effect of TGF-β was assessed by MTT assay. The effects of N-Shh or cyclopamine on expression of RUNX3 mRNA were observed by RT-PCR analysis. Contribution of N-Shh or cyclopamine to RUNX3 stabilization was monitored by Western blotting for RUNX3 in cells treated with cyclohexamide or MG132 (a specific inhibitor of proteasomes). The localization of Runx3 was monitored by immunofluorescence staining and nuclear fractionation assay.
Treatment with TGF-β of control vector- and Shh-overexpressing cells produced different results; control vector-overexpressing cells exhibited a significant decrease in cell growth, whereas almost no decrease was observed in Shh-overexpressing cells. RT-PCR analysis showed that there was no significant difference in the expression levels of RUNX3 mRNA between cells treated with either Shh, cyclopamine, or control vehicle. Importantly, treatment with MG132 led to reduction of RUNX3 proteins in Shh-overexpressing cells, whereas blockade of the Shh pathway by cyclopamine resulted in accumulation of RUNX3 after MG132 addition. Confocal microscopy and nuclear fractional experiment showed that overexpression of Shh blocked nuclear translocation of RUNX3 by TGF-β. Moreover, RUNX3 sequestered in the cytoplasm by Shh overexpression is rapidly degraded through a proteasome-mediated pathway. On the contrary, treatment of Shh-overexpressing cells with cyclopamine induced TGF-β-mediated growth inhibition via the stabilization of RUNX3 and the induction of RUNX3 translocation to the nucleus.
These results indicate that suppression of TGF-β-RUNX3 signaling by activated Shh is correlated with the development of TGF-β resistance in gastric cancer.

489 POSTER

LC06, a novel angiopoietin-2 selective human antibody with potent anti-tumoral and anti-angiogenic efficacy in different xenograft models

M. Thomas[1], W. Scheuer[1], M. Baehner[1], K. Kaluza[1], C. Gassner[1], S. Seeber[1], H. Reiersen[2], A. Kavlie[2], J. Regula[1], C. Klein[1]. *[1]Roche Diagnostics GmbH, Discovery Oncology, Penzberg, Germany; [2]Affitech A/S, Antibody Engineering, Oslo, Norway*

Background: The Angiopoietins (Ang-1 and Ang-2) have been identified as agonistic and antagonistic ligands of the endothelial RTK Tie2, respectively. Ang-1/Tie2 signaling transduces survival signals, regulates mural cell recruitment, and controls the quiescent endothelial cell (EC) phenotype. In turn, Ang-2 destabilizes the vascular endothelium and primes EC responsiveness to cytokines and promotes tumor growth. Ang-2 is strongly expressed in the remodeling vasculature and almost not present in the quiescent vasculature making it a very attractive target for anti-tumor therapy. To investigate the functional role of blocking Angiopoietins, we generated Ang-2 selective antibodies that neutralize the binding of Ang-2 to its receptor Tie2 and Ang-1/Ang-2 crossreactive antibodies.
Material and Methods: Human antibodies against Ang-2 were generated by phage display. Affinity for human and murine Ang-2 and Ang-1 was determined by Biacore. The inhibition of the Ang-2-Tie2 and Ang-1-Tie2