and phospho-Akt expression was also observed in lymph node metastasis specimens.
Conclusion: Overall, our studies suggest that invasion in BMP2-induced EMT is mediated through down-regulation of PI-3 Kinase/Akt pathway.

171 POSTER

Integrin LFA-1 expression regulates angiogenesis-stimulating potential of colorectal carcinoma cells at premetastatic niches in the liver

M. Valcárcel[1], A. Jaureguibeitia[1], C. Salado[1], A. Lopategi[2], B. Arteta[2], F. Muruzabal[1], L. Mendoza[1], F. Vidal-Vanaclocha[2]. *[1]Pharmakine S.L., Cell Biology Unit, Derio-Bizkaia, Spain; [2]Basque Country University School of Medicine, Cell Biology and Histology, Leioa Bizkaia, Spain*

The recruitment of vascular stromal and endothelial cells is an early pre-angiogenic event of premetastatic niches, but how the microenvironment created by avascular three-dimensional (3D) growth contributes to activation of the angiogenesis-stimulating potential in cancer cells is unclear. Herein, the proangiogenic profile of CT26 colon carcinoma cells was studied in seven-day cultured 3D-spheroids of <300 μm in diameter, produced by the hanging-drop method to mimic the microenvironment of premetastatic niches prior to hypoxia. Spheroid-derived CT26 cells increased VEGF secretion by 70%, which in turn increased in vitro endothelial cell migration by 2-fold. More importantly, spheroid-derived CT26 cells increased LFA-1-expressing cell fraction by 3-fold, and soluble ICAM-1, given to spheroid-cultured CT26 cells, further increased VEGF secretion by 90% via cyclooxygenase (COX)-2-dependent mechanism. Consistent with these findings, CT26 cancer cells also significantly increased LFA-1 expression at premetastatic niches within hepatic lobules. Angiogenesis also markedly increased in both subcutaneous tumors and hepatic metastases produced by spheroid-derived CT26 cells. Finally, two-dimensional electrophoresis plus mass spectrometry revealed that three-dimensional growth of CT26 cells led to the development of a VEGF-secreting cancer cell subset expressing a markedly proangiogenic protein profile, including 60S acidic ribosomal protein, ferritin heavy chain, phosphoglycerate kinase-1, estrogen-related receptor, vimentin and 14−3-3 epsilon alpha. Therefore, three-dimensional growth of cancer cells enriched the proangiogenic cancer cell phenotype needed for metastasis progression. The role of integrin LFA-1 and COX-2 in the microenvironmental activation of angiogenesis-stimulating potential of colorectal carcinoma cells potentially represents a new target combination for therapeutic strategies to block colorectal hepatic metastasis at premetastatic niches.

172 POSTER

Tumor-induced liver nerve growth factor (NGF): a new target for stromal cell inhibition during metastatic colorectal carcinoma growth

F. Basaldua[1], A. Lopategi[1], B. Arteta[1], A. Valdivieso[2], J. Ortiz de Urbina[2], F. Vidal-Vanaclocha[1]. *[1]Basque Country University School of Medicine, Cell Biology and Histology, Leioa Bizkaia, Spain; [2]Cruces University Hospital, Hepatobiliar Tumor Surgery Unit, Cruces-Baracaldo Bizkaia, Spain*

Besides its contribution to differentiation and survival of neurons, nerve growth factor (NGF) also plays a role in cancer progression. In the liver, expression of NGF is increased during tissue regeneration and hepatocellular carcinoma development, but its role during hepatic metastasis is not well understood. Herein, we investigated NGF and neurotrophin receptor expression by cancer and host cells in the hepatic metastasis microenvironment of murine and human systems. NGF immunostaining of metastatic colon cancer cells only occurred in 2 out of 24 patients with hepatic metastases, while around 80% of studied patients had hepatic metastases with NGF-expressing stromal cells. Not statistically significant correlation was demonstrated between NGF immunostaining of tumor-infiltrated stromal cells and cancer cell staining with anti-ki67 antibodies, suggesting that NGF was not involved in cancer cell proliferation. Hepatocytes and hepatic sinusoidal cells showed weak NGF immunostaining, while cholangiocytes had a high immunostaining in the hepatic tissue unaffected by cancer. Hepatic CT26 murine colorectal carcinoma metastases had an intense NGF immunostaining in those hepatocytes and myofibroblast-type stromal cells located at the invasion front of metastases. High NGF-expressing hepatocytes were preferentially located among cancer cells and had phenotypic features suggesting epithelial-to-mesenchymal transition. CT26 cancer cells did neither express in situ nor secrete in vitro NFG. p75-NTR had a low expression level in normal hepatic tissue, but it significantly increased in hepatocytes and HSCs located around and within hepatic metastases, while CT26 cancer cells were negative. Consistent with these in situ findings, NGF significantly increased by 3-fold in the hepatic blood obtained from livers affected by CT26 colorectal carcinoma metastases. NGF concentration was also 7 times higher in the supernatants from primary cultured HSCs than in those from hepatocytes, and it significantly increased in the supernatant of HSCs given C26 cancer cell-conditioned medium, and in those from cultured hepatocytes given tumor-activated HSC-conditioned medium. Recombinant murine NGF dose-dependent increased chemotactic migration, but not proliferation, of HSCs and some cancer cell lines in vitro. Moreover, HSC migration-stimulating activity induced by VEGF was NGF-dependent in vitro. Our results demonstrate for first time that hepatocytes and sinusoidal stellate cells express neutrotrophin receptor p75 and secrete NGF in response to specific stimulating factors released by cancer cell in the hepatic metastasis microenvironment of human and rodent colorectal carcinoma. Tumor-induced liver NGF contributed to intratumor stromal cell recruitment and potentially represents a promising target for tumor-activated stromal cells during metastatic colorectal carcinoma growth.

173 POSTER

Arf6-AMAP1 pathway in invasion of lung cancer and malignant mesothelioma cell lines

T. Menju[1], S. Hashimoto[2], A. Hashimoto[2], E. Ogawa[3], H. Wada[4], H. Date[1], H. Sabe[2]. *[1]Kyoto University Hospital, General Thoracic Surgery, Kyoto, Japan; [2]Osaka Bioscience Institute, Molecular Biology, Osaka, Japan; [3]Jujo Rehabilitation Hospital, Respiratory Medicine, Kyoto, Japan; [4]Sakazaki Clinic, Respiratory Medicine, Kyoto, Japan*

Backgrounds: Distant metastses are the major problem in cancer therapeutics. For most carcinomas, metastases begin with invasion of cancer cells into the basement membrane or the stromal environment. We have shown that a small GTPase Arf6 and its effector AMAP1 play pivotal roles in invasion and metastasis of significant populations of breast cancers. It has been well documented that lung cancers show even more invasive and metastatic tendency clinically than breast cancers.
Tumor cells, including those of epithelial origin, exhibit two distinct phenotypes for their invasion, namely mesenchymal type and amoeboid-like type. The former requires activities of matrix proteases and calpain, while the latter ROCK, a Rho-dependent kinase. Here we examine whether Arf6 and AMAP1 are involved in invasive activities of lung cancer cells and mesothelioma cells, together with analysis on what types of invasiveness each of these cells exhibits.
Materials and Methods: Non-small cell lung cancer cell lines (H1299, Lu99, H460, A549, PC9, PC14, H1650, H441, H522, H1975 and H520 (and malignant mesothelioma cell lines (211H, H2052 and H28) were used.
To examine types of the invasiveness, we used ALLN (a calpain inhibitor) and Y27632 (a ROCK inhibitor). We also used a cocktail of protease inhibitors, which contains GM6001 (a multi metalloprotease inhibitor), E-64 (a cystein inhibitor), pepstatin A, leupeptin, and aprotinin. We performed a matrigel chemoinvasion assay to measure invasive activities, using Biocoat Matrigel chambers (Becton Dickinson). Protein knock-down was done by the siRNA technique using RNAiMAX (Invitrogen). Cell viability was measured using Cell Countin Kit-8 (Dojindo Molecular Technologies).
Results: Six of 14 cell lines we examined (H1299, Lu99, PC9, PC14, 211H and H2052) showed appreciable matrigel invasive activities in vitro, while other three cell lines (H460, A549 and H1650) also exhibit less but detectable levels of invasive activities. Among the former 6 cell lines, we found that siRNA-mediated knockdown of Arf6 and AMAP1 both significantly inhibits invasion of H1299, Lu99, PC14 and 211H. On the other hand, AMAP1 knockdown, but not Arf6 knockdown, inhibited invasion of PC9 and H2052. We moreover found that H1299, Lu99, PC9 and H2052 exhibit the typical mesenchymal-type of invasion, while PC14 and 211H are not.
Conclusions: Consistent with previous studies, our results also suggest that invasive phenotypes are highly diversified among different lung cancer cells and mesothelioma cells. Still then, one can consider Arf6 and/or AMAP1 as molecular targets for the adjuvant therapy of some lung cancers and mesotheliomas.

174 POSTER

Epigenetic changes of tumor suppressor genes and therapeutic implications in glioblastoma

L. Sooman[1], J. Gullbo[2], J. Lennartsson[3], S. Bergström[4], E. Blomquist[4], M. Bergqvist[4], S. Ekman[4]. *[1]Uppsala University, Department of Oncology Radiology and Clinical Immunology, Uppsala, Sweden; [2]Uppsala University Hospital, Department of Medical Sciences, Uppsala, Sweden; [3]Uppsala University, Ludwig Institute for Cancer Research, Uppsala, Sweden; [4]Uppsala University Hospital, Department of Oncology, Uppsala, Sweden*

Background: Glioblastoma is the most common and aggressive type of primary brain tumor and there have been little improvements of its poor survival rate during the last decades. Aberrant DNA methylation, including