as in the in-situ serous carcinoma of the FT. The down regulation results from hemizygous loss in many of the tumours and from activation of the PI3K/AKT and the Ras/MEK/ERK pathway, which targets FOXO3a for degradation, and in some cases due to up-regulation of miR-182. We managed to restore partial activity of FOXO3a using inhibitors of these pathways.
Conclusions: The immortalized benign FTSEC lines are an important asset for the identification of early-detection biomarkers and 'drugable' pathways in serous carcinoma. FOXO3a loss may be a key event in the progression into an invasive disease. It is possible to rescue FOXO3a activity with currently available experimental drugs.

1062 POSTER

Blood and Lymphatic Vessels: Early Crucial Players of Malignancy and Metastasis in Cervical Cancer

A. Cimpean[1], M. Raica[1], R. Ceausu[1], P. Gaje[1], V. Mazuru[2], L. Saptefrati[2]. [1]*Victor Babes University of Medicine and Pharmacy, Histology Angiogenesis Research Center, Timisoara, Romania;* [2]*Nicolae Testemitanu University of Medicine and Pharmacy Kisinev Moldavia, Histology, Kisinev, Moldova*

Background: Cervical neoplasia remains one of the most controversial issues for clinicians, pathologists and researchers. Screening programs reduced the incidence of invasive neoplastic lesions but did not change the rate of precursor lesions. Usually, malignant lesions of the uterine cervix are considered more important than precursor lesions.
Angiogenesis and lymphangiogenesis are accepted as important factors favouring tumour growth and metastases. But, questions about (i) startpoint of angiogenesis and lymphangiogenesis in cervical lesions, (ii) proliferative and/or activated status of cervical neovessels or (iii) the origin of lymph vessels and prognostic impact of lymphangiogenesis in precursor lesions of the uterine cervix still remain without a precise response.
Material and Methods: One hundred and twenty eight specimens of benign, premalignant and malignant cervical lesions were included in the present study. Co-localisation of Ki67 proliferation marker with CD105 in blood vessels endothelium and D2−40 in lymphatic endothelium was obtained by applying doublestain method followed by use of two different chromogens (3.3′diaminobenzidine for nuclear brown staining of Ki67 and aetil amino charbazole for cytoplasmic red staining of CD105 and D2−40).
Results: Specimens evaluation of normal, premalignant and malignant lesions of the uterine cervix revealed that activation and proliferation of blood vessels in cervical lesions are distinct processes. Activation of endothelial cells is an early event which predominate in benign and premalignant conditions of the uterine cervix while endothelial cell proliferation is observed in tumour vessels endothelial cell from cervical invasive carcinoma. Lymphangiogenesis is an early event in the pathogenesis of cervical lesions. The highest number of proliferative lymphatic vessels (D2/40+/Ki67+) was significant correlated with low grade intraepithelial lesions (LSIL, $p = 0.009$), high grade intraepithelial lesions (HSIL, $p = 0.044$), and microinvasive carcinoma ($p = 0.002$). The last correlation also persist in invasive carcinoma.
Conclusions: Our data showed that early lymphatic endothelial proliferation in preneoplastic stages of cervical lesions preceed the development of the angiogenic switch. Angiogenic process also begin in preinvasive lesions stages of cervical lesions and had different and distinct mechanisms.

1063 POSTER

Vasohibin-1 and Vasohibin-2 Are Expressed in Both Gastric Cancer Cells and Tumour-associated Macrophages and Play Roles in Anti-Angiogenesis Not Only as Intrinsic Inhibitors

Z.L. Shen[1], H. Seppänen[1], S. Vainionpää[1], Y.J. Ye[2], S. Wang[2], H. Mustonen[1], P. Puolakkainen[1]. [1]*University of Helsinki, Department of Surgery, Helsinki, Finland;* [2]*Peking University People's Hospital, Department of Gastroenterological Surgery, Beijing, China*

Background: Recently, Vasohibin-1 and vasohibin-2 are found in endothelial cells and considered as two intrinsic anti-angiogenesis factors. However, So far, we don't know whether they are expressed in cancer cells themselves and tumour-associated macrophages (TAMs) which have been confirmed to contribute to tumour progression.
Materials and Methods: Realtime RT-PCR were used to quantitatively investigate the vasohibin-1 and vasohibin-2 expression in four gastric cancer cell lines including non-metastatic cell line AGS and metatatic cell lines HGC-27, Hs-746T and NCI-N87 with or without co-cultured with TAMs, as well as their expressions in TAMs under normal or hypoxia condition. Furthermore, the correlation between vasohibin-1, vasohibin-2 and VEGF-A expressions were analyzed under different culture condition.
Results: Both vasohibin-1 and vasohibin-2 were expressed in four gastric cancer cell lines and TAMs. Under normal condition, vasohibin-1 and vasohibin-2 expression were up regulated significantly by macrophages in four gastric cancer cell lines. Under hypoxia condition, both vasohibin-1 and vaosohibin-2 expression were decreased significantly in distant metastasis cancer cell line Hs-746T ($P < 0.001$), moreover, the increase induced by macrophages was also down regulated significantly in Hs-746T cell line($P < 0.001$). The regulations for vasohibin-1 and vasohibin-2 expression by macrophages and hypoxia had correlation with VEGF-A expression. In addition, hypoxia induced vasohibin-1 and vasohibin-2 significant up-regulations in TAMs co-cultured with metastatic cancer cell lines ($P < 0.05$).
Conclusions: Both vasohibin-1 and vasohibin-2 was expressed in gastric cancer cells and TAMs, and their expression were regulated by TAMs and hypoxia. Vasohibin-1 and vasohibin-2 might not only be an intrinsic angiogenesis inhibitors in endothelial cells, but also play important roles in anti-angiogenesis as an extrinsic inhibitors mediated by TAMs. Vasohibin-1 and vasohibin-2 might be as a novel anti-angiogenesis target in the treatment of gastric cancer.

1064 POSTER

Adaptive Exploitation of Stromal Cell Metabolism by Tumour Cells

B. Patel[1], Y.I. Rattigan[2], E. Ackerstaff[3], G. Sukenick[3], J.W. Glod[4], D. Banerjee[4]. [1]*Cancer Institutue of New Jersey, Pharmacology, New Brunswick,* [2]*Johns Hopkins University, Medicine, Baltimore,* [3]*Memorial Sloan-Kettering Cancer Center, Medical Physics, New York,* [4]*Cancer Institute of New Jersey, Pharmacology, New Brunswick, USA*

Background: Tumour cells secrete factors to recruit and activate stromal cells in the tumour microenvironment (TME) leading to reciprocal paracrine support of tumour growth by stroma-derived growth factors. This is an important means by which tumours adapt their microenvironment to facilitate their growth. Indeed, breast cancer development and metastatic progression is highly dependent on stromal support, particularly from carcinoma associated fibroblasts (CAFs). As a result of aerobic glycolysis, tumour cells produce and secrete high levels of lactate, thought to be a toxic byproduct that needs to be extruded into the tumour milieu.
Using *in vitro*-generated CAFs, we investigated the role of lactate in CAF-mediated support of tumour growth. In addition to extruding lactate as a byproduct of glycolysis, we suggest that tumour cells secrete it to recruit and subsequently exploit stromal cells to recycle lactate into utilizable metabolites, such as pyruvate, to fulfill metabolic demands of tumour cells.
Materials and Methods: We used a lactate analyzer (Roche Diagnostics) to quantify lactate in media; transwell migration assays were used to measure lactate-induced *in vitro* migration; RT-PCR was used to determine expression of genes involved in lactate transport; ^{13}C NMR spectroscopy was used to track the metabolic fate of lactate; luciferase assays were used to monitor growth of tumour cells.
Results: We find that MDA-MB-231 breast cancer cells (MDAs) secrete significantly higher levels of lactate under hypoxia, and that lactate recruits mesenchymal stem cells (MSCs), the precursors of CAFs. Lactate is transported by monocarboxylate transporters (MCTs); cells take up lactate via MCT1 and efflux it via MCT4. Expectedly, MDAs display low expression of MCT1 while exhibiting high expression of MCT4. However, CAFs show high expression of MCT1 while displaying low expression of MCT4, indicating that lactate extruded by the tumour cells is taken up by stromal cells, in a source-sink manner. NMR analyses indicate that ^{13}C-lactate is metabolized via the Krebs cycle in stromal cells. Finally, pyruvate-mediated tumour cell growth assays indicate that CAFs may serve to evacuate lactate from the TME, thereby reducing lactate-mediated inhibition of stroma-derived pyruvate influx into tumour cells.
Conclusions: Thus, stromal cells in the TME (1) have the capacity to take up tumour-secreted lactate and use it as an energy source, and (2) may provide subsequent/surplus metabolites, such as pyruvate, to tumour cells as a secondary source of energetic and biosynthetic precursors. To our knowledge this is the first *in vitro* model system demonstrating tumour/stroma metabolic coupling by which tumour cells exploit stromal cells. A better understanding of the molecular mechanisms governing metabolic cooperation within the tumour milieu will potentially identify new targets for therapeutic intervention.

1065 POSTER

An in Vitro Comparative Study of Fulvestrant and Tamoxifen in Breast Cancer Cells

E. Giannopoulou[1], D. Lymperatou[1], A. Koutras[1], H.P. Kalofonos[1]. [1]*University of Patras, Clinical Oncology Laboratory Medical School, Patras, Greece*

Aim: In the current study, two selective antagonists of the estrogen receptor (ER), fulvestrant which suppresses and degrades the ER (SERD, selective estrogen receptor down regulator) and tamoxifen which modifies the function of the ER (SERM, selective estrogen receptor modulator) were

compared. We focused on the effect of the two agents in the migration capacity of the human breast cancer cells. There are two mechanisms of cell migration, the single cell migration that occurs during metastasis and collective single migration that occurs during invasion.
Materials and Methods: Two hormone-dependent human breast cancer cell lines MCF-7 and T47D were used. Cells were treated with fulvestrant, tamoxifen, and the two metabolites of tamoxifen, endoxifen and 4-OH-tamoxifen. The proliferation of the cells was determined using the MTT assay. The migration capacity was evaluated using the boyden chamber and the scratch wound assays.
Results: The tested agents inhibited the proliferation of both cell lines after stimulation of ER by estradiol with a dose dependent manner. Then single cell migration was studied using boyden chamber assay and it was found that fulvestrant was superior than tamoxifen and its metabolites. The scratch wound assay reflects collective cell migration and it was found that the inhibitory effect of tamoxifen and its metabolites was greater than fulvestrant.
Conclusions: The results of the current study confirm the antitumour effect of fulvestrant and tamoxifen in hormone dependent breast cancer cells. However, it is the first time that the two agents are compared according to their effect on breast cancer cell migration. The results demonstrate that there are more than one ways for breast cancer cell migration and the two agents affect different mechanisms. Further research on the pathways that control the different mechanisms of cell migration are necessary and are ongoing.

1066 POSTER

Interleukin-6 Increases Metastasis Formation Through Mobilization of Immature Myeloid Cells to the Pre-metastatic Niche

M. Andre[1], M. Zhang[2], H. Peinado[3], M. Papaspyridonos[3], R. Kaplan[4], J. Bromberg[2], D. Lyden[3]. *[1]Portuguese Institute of Cancer, Department of Medical Oncology, Lisbon, Portugal; [2]Memorial Sloan-Kettering Cancer Center, Department of Medicine, New York NY, [3]Weill Cornell Medical College, Department of Paediatrics, New York NY, [4]National Cancer Institute, Center for Cancer Research, Bethesda MD, USA*

Interleukin-6 (IL-6) is a key inflammatory cytokine that has been shown to link immune cell function with cancer progression. IL-6 produced by tumour cells is associated with increased tumour cell proliferation and regulation of the immune system with reduction of mature dendritic cells through activation of STAT3. However, the systemic effects of host-derived IL-6 have not been delineated. More specifically, the role of this cytokine in the modulation of maturation and recruitment of immature myeloid bone marrow-derived cells (BMDCs) and formation of the pre-metastatic niche has not yet been addressed. To demonstrate the role of IL-6 in this process, wild-type (WT) and IL-6 knock-out (KO) C57Bl/6 mice were injected either subcutaneously with B16 melanoma cells or orthotopically with EO771 breast adenocarcinoma cells. For both tumour models, there remained no differences in primary tumour growth, however, a decrease in lung metastasis was observed in IL-6 mice as compared to WT mice at 3 and 5 weeks respectively post tumour injection. By immunohistochemistry, there was an increase of pSTAT3+ cells and CD45+ cells in the pre-metastatic lung in WT as compared to IL-6 mice. In further analysis of this cell population by flow cytometry, we observed an elevation of CD11b+Gr1+ cells in the lungs and in the circulation of WT mice compared with IL6 KO mice. To confirm that STAT3 activation promotes the mobilization of BMDCs, we used a conditional STAT3 overexpression transgenic mouse model. After 5 days of doxycycline treatment, we observed an increase of recruited STAT3+ cells and CD45+ cells to the lungs and of CD11b+Gr1+ progenitor cells in the circulation and to the lungs. When these mice were crossed with IL-6 KO mice, there was a complete reversion of this phenotype. Our results suggest that host IL-6 promotes metastatic growth in the lungs through mobilization and recruitment of immature myeloid cells to the pre-metastatic lungs, and that this mobilization and recruitment is dependent upon STAT3 activation.

1067 POSTER

Study of the Effects of Dietary Flavonoids, Luteolin and Quercetin on the Reversal of Epithelial-mesenchymal Transition in A431 Epidermal Cancer Cells

M. Lee[1], Y. Lin[2], P. Tsai[2], C. Kandaswami[3], F. Ke[4], J. Hwang[5], P. Lee[1]. *[1]Academia Sinica, Institute of Biological Chemistry, Taipei, [2]National Taiwan University, Institute of Biochemical Sciences, Taipei, Taiwan; [3]Castle Hills Health, R&D, Lewisville, USA; [4]National Taiwan University, Institute of Molecular and Cellular Biology, Taipei, [5]National Yang-Ming University, Institute of Physiology, Taipei, Taiwan*

In our earlier studies, we documented the antiproliferative and anti-metastasis effects of luteolin and quercetin. Appreciation of the diverse anticancer activities exerted by these two flavonoids prompted us to evaluate their impact on events such as tumour progression and invasion. Highly invasive A431-III cells derived from parental A431-P cells, were isolated via three successive passages through Boyden chamber with matrigel-coated membrane support (*Anticancer Research.* 28: 2109–2120, 2008). The greater invasion potential exhibited by A431-III cells was owing to increased ability for spreading, migration and enhanced MMPs activity. Comparison of tumour progression events evoked by A431-P cells with those manifested by A431-III cells could emerge as a useful strategy for evaluating of EMT. These cells might afford a reliable model for the evaluation of tumour metastasis events (*Cancer Science*, 102: 815–827, 2011). Employing this approach, we evaluated the effects of luteolin and quercetin, with the A431-P/A431-III EMT model. These flavonoids reversed cadherin switching, downregulated EMT markers and nullified the invasion ability of A431-III cells. Overexpression of MMP-9 resulted in inducing EMT in A431-P cells and this could be reversed by treating with luteolin or quercetin. Co-treatment of A431-P and A431-III cells with EGF plus luteolin or quercetin caused these cells to become more epithelial-like in morphology, led to a fall in the levels of EGF-induced markers of EMT and the restoration of cell-cell junctions. E-cadherin was decreased by EGF, but increased by luteolin and quercetin. Our emerging data from the present investigations suggest that luteolin and quercetin are potentially beneficial agents to intercept and prevent EMT occurrence in epidermal carcinoma cells and manifest activities relevant to the attenuation of tumour progression in A431-III cells. In conclusion, luteolin and quercetin may have inherent potential to function as chemopreventive and anti-neoplastic agents in abating tumour progression through the reversal of EMT in cancer.

1068 POSTER

Preconditioned Monocytic Endothelial Progenitor Cells Reduce Formation of Melanoma Metastases Through SPARC-driven Cell-cell Interactions

F. Defresne[1], C. Bouzin[1], M. Grandjean[1], C. Kupatt[2], A.K. Hatzopoulos[3], O. Feron[1]. *[1]UCL Institute of Experimental and Clinical Research (IREC), Angiogenesis and Cancer Research Lab, Brussels, Belgium; [2]Ludwig-Maximilians University, Klinikum Großhadern, Munich, Germany; [3]Vanderbilt University, Cell and Developmental Biology, Nashville, USA*

Background: Tumour progression is associated with the release of signaling substances from the primary tumour into the bloodstream. Tumour-derived cytokines are known to promote the mobilization and the recruitment of cells from the bone marrow, including endothelial progenitor cells (EPC). Here, we examined whether such paracrine influence could also influence the capacity of EPC to interfere with circulating metastatic cells.
Materials and Methods: We examined the effects of preconditioned EPC by the tumour cell secretome on metastases formation using mouse EPC and B16 melanoma cells as well as human EPC and a highly metastatic human breast cancer cell line. Luciferase expression by tumour cells was used to track metastases while 2D-DIGE proteomic analysis and gene silencing strategies were used to identify relevant changes in the EPC phenotype.
Results: We injected EPC pre-stimulated by tumour conditioned medium (CM-EPC) and B16 melanoma cells to mice. A net decrease in metastases spreading (vs non-stimulated EPC) led us to carry out a 2D-DIGE proteomic study to identify possible mediators of EPC-driven protection. Among 33 proteins exhibiting significant changes in expression, osteonectin/SPARC (Secreted Protein, Acidic and Rich in Cysteine) presented the highest induction after EPC exposure to CM. We then showed that contrary to control EPC, SPARC-silenced EPC were not able to reduce the extent of metastases when injected with B16 melanoma cells. Using adhesion tests and the hanging drop assay, we further documented that cell-cell interactions between CM-EPC and melanoma cells were promoted in a SPARC-dependent manner. This interaction led to the engulfment of melanoma cells by CM-EPC, a process prevented by SPARC silencing and mimicked by recombinant SPARC. Finally, we showed that contrary to melanoma cells, the pro-metastatic human breast cancer cell line MDA-MB231-D3H2 reduced SPARC expression in human EPC and stimulated metastases spreading.
Conclusions: Our findings unravel the influence of tumour cells on the EPC phenotype through a SPARC-driven accentuation of macrophagic capacity associated with limitations to metastatic spread, thereby adding a new layer of complexity in the role of so-called endothelial progenitor cells in tumour progression.