Methods: Human CD14$^+$ monocytes (MOs) were isolated from peripheral blood mononuclear cells, and resuspended in RPMI medium supplemented with 10% foetal calf serum (Reference MOs). MOs were cultured (time course covered 12, 24 and 48 hrs) with tumor cell supernatants (TCSs) obtained either from growing or starving MD-MBA-231 cells. TCSs – conditioned MOs were then scrutinized for TEM8 expression levels and for the production of invasive/pro-angiogenic factors [i.e. urokinase system (uPAR/uPA), metalloproteinase 9 (MMP9), and chemokines (CXCL8/IL8, CXCL5)] by using well-established methods (i.e. Real-time RT-PCR, flow cytometry, western blotting, ELISA, and zymography).
Results: MOs' phenotype and functions were not substantially modified by the addition of serum in culture medium. A kinetic profile of TEM8 expression revealed that in response to TCS from growing MDA-MB231 cells, by 48 hrs, MOs expressed maximal levels of TEM8 mRNA (approx. 70 fold increase over reference MOs). Around this time point, conditioned-MOs showed also the highest production of membrane-bound uPR, and secretion of MMP9 and CXCL8/IL8, CXCL5 chemokine. Of note, these modifications were absent in MOs incubated with TCS obtained from starving MDA-MB231 cells.
Conclusions: We speculate that TEM8 is invlved in cellular mechanisms that foster both leukocytes-dependent inflammatory angiogenesis and tumor cell migration/invasion processes.

1065 POSTER

Metastasis-promoting S100A4 protein affects the EGFR signalling pathway

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Expression of S100A4, a member of the S100-family of calcium binding proteins, has been associated with tumor invasion and metastasis. Recently we described the suppression of tumor development and metastasis formation in S100A4 deficient mice. Immunohistochemical staining of tumors in these mice indicated an abnormal recruitment and distribution of immune cells. Since S100A4 is expressed and secreted from different cell types from the tumor environment, including macrophages and leukocytes, extracellular S100A4 could influence cell motility and affect the recruitment of immune cells as well as their function at the tumor site.
Our recent finding showing that extracellular S100A4 attracts mouse T-lymphocytes isolated from spleen in a transwell migratory assay, is supporting this hypothesis. However, the cell surface receptor recognizing S100A4 and the signal transducing pathways triggered by S100A4 are not known. To identify proteins binding extracellular S100A4, we screened a phage display peptide library using multimeric S100A4 as bait and identified a peptide motif that mimics the KCCY/F sequence present in the EGF domain of EGF receptor ligands. Binding studies confirmed selective binding between S100A4 and a number of EGF receptor ligands, with the strongest interaction to Amphiregulin. Furthermore, extracellular S100A4 enhanced EGFR/ErbB2 signaling and Amphiregulin-dependent proliferation of S100A4$^{(-/-)}$/fibroblasts. The S100A4 had no effect on ligand shedding, a process known to convert the transmembrane EGF-family pro-ligand to the active soluble form. Alternative mechanisms which could explain the observed S100A4 effects are currently under investigation. We speculate that extracellular S100A4 can affect tumor progression by interacting with the EGFR/ligand complex leading to an enhancement of EGFR signaling, increasing cell motility and proliferation.

1066 POSTER

A simple method to prepare tumour stem cells from the human breast cancer cell line MDA-MB 231

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Background: The commercially available cell line MDA-MB 231 is reported to contain considerable amounts of tumor initiating cells. As described in several papers these cells can be enriched by FACS or prepared via "mammo-spheres", three dimensional cellular aggregates. The procedures are all more or less time consuming and of limited efficiency. Here we present a very easy way to select a homogeneous cellular fraction of potential breast cancer stem cells.
Methods: The cells were cultured beyond confluency until viable cells escape from the monolayer into the culture medium. Then, the supernatant was transferred into a new culture flask and grown again beyond confluency.
Results: After 16 cycles, the suspension consisted of a homogenous cellular population presenting high CD44 and hardly detectable CD24 immunoreactivity, a generally accepted feature of breast cancer stem cells. The cells showed an intense staining of vimentin, a mesenchymal intermediate filament protein and no cytokeratin 18, the epithelial counterpart in breast epithelial cells. Time-lapse videography reveals that these cells are very motile but can be restrained by inhibition of the FAK system. Furthermore, a partial differentiation seems to be induced by FAK inhibition as indicated by the expression of cytokeratins.
Conclusion: Focusing on tumor stem cells in basic research and cancer therapy comes of age – here we present a convenient protocol to easily prepare the required cells for further experiments.

1067 POSTER

Characterization of primary tumour stromal cells and their potential role in the breast cancer microenvironment

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Background: The importance of the primary tumour microenvironment in breast cancer development and progression has emerged in recent years. Tumour-derived stromal cells have been shown to promote epithelial tumour cell growth, migration and invasion. Although the tumour promoting effect of stromal-epithelial interactions is recognized, the precise mechanisms involved are poorly understood. The aim of this study was to isolate primary tumour stromal cells from breast cancer specimens and investigate their potential mode of action in the breast tumour microenvironment, based on expression of genes associated with cancer progression.
Methods: Following written informed consent, specimens of human breast cancer were harvested from patients undergoing surgery. Cells were isolated from tumour- and tumour-associated normal regions of breast tissue. Breast tissue obtained from reduction mammoplasty served as normal controls. Following tissue dissociation and digestion, stromal cells were isolated by differential centrifugation and characterised. Following culture of stromal cells, RNA was extracted, reverse transcribed and relative quantitative PCR performed using primers targeting Fibroblast Activation Protein (FAP), Transforming Growth Factor β (TGFβ), Transforming Growth Factor β Receptor II (TGFβRII), Matrix Metalloproteinase 3 (MMP3), and Vascular Endothelial Growth Factor A (VEGF A).
Results: Expression of TGFβ, which is known to induce epithelial to mesenchymal transition (EMT), was upregulated in tumour compared to normal stromal cells, while there was no difference in expression of its principle receptor, TGFβRII. This was supported by changes in epithelial cell cytoskeleton, with reduced cell-cell adhesion and E-cadherin expression observed in epithelial cells cultured in the presence of tumour stromal cells. The proangiogenic factor VEGFA, and the invasion associated gene MMP3, were also upregulated in the tumour stromal cell population. In contrast, the level of FAP detected in tumour stromal cells was lower than that detected in normal stromal cells.
Conclusion: Tumour stromal cells have the potential to stimulate angiogenesis and epithelial to mesenchymal transition through secretion of paracrine factors such as VEGF A and TGFβ. Further understanding pathways involved in stromal cell induced tumour progression is essential to inhibit initiation of the metastatic cascade.

1068 POSTER

Increasing the efficiency of gene therapy by using protein transduction domains

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Background: Cancer is the main disease addressed by gene therapy. Despite the recent developments there are still some limitations. One important limitation of gene therapy strategies is that vectors are not efficient in vivo. It is necessary to deliver the gene products to every cell; otherwise, the remaining malignant cells will proliferate and disease will relapse. A promising approach to increase the efficiency of gene therapy is to increase the transmission of the gene product. The secretion of therapeutic agents from transduced tumor cells and the subsequent internalization by neighboring untransduced cells would increase the effect of gene therapy. This study aims to increase the transmission of therapeutic agents by using protein transduction domains (PTDs).
Methods: Cre recombinase – lox P system is selected as a reporter tool for examining the cargo delivery efficacies of several PTDs. Eukaryotic expression vectors were constructed to produce Cre fusion proteins with the protein transduction domain of HIV-1 TAT protein, and the herpes simplex virus (HSV) VP22. Reporter cells are transfected with Cre fusion protein vectors. To observe intercellular trafficking properties and subcellular distribution of the fusion proteins, transfected cells are examined by FACS and immunohistological staining.
Results: Cre mediated recombination in transfected cells indicated the functional protein production and successful transfer of the protein to the