VEGFD correlated with peritumoural LECP% (r = 0.61, p = 0.001) and with VEGFC (r = 0.78, p < 0.001). Linear regression analysis confirmed the expression of VEGFA as an independent predictor of ECP% in both PTs (β = 0.58, p = 0.03) and LN metastases (β = 0.90, p = 0.009) and of LECP% (β = 0.65, p = 0.09) in LN metastases. The expression of VEGFD (β = 0.88, p = 0.03), not of VEGFA independently predicted peritumoural LECP% in PTs.
Conclusions: Our results confirm existing data that in PTs angiogenesis and lymphangiogenesis are respectively driven by VEGFA and VEGFD. In LN metastases on the contrary, both processes seems to be driven by VEGFA. Lymphangiogenesis in PTs and in LN metastases might thus be driven by different factors.

314 POSTER

Investigation of the potential of human mesenchymal stem cells (hMSC) as vectors for therapeutic gene delivery to breast tumours

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Background: A variety of gene therapy strategies have been developed and evaluated for breast cancer treatment but clinical responses remain poor. Adenoviral vectors have been commonly used for gene therapy studies. One of the major barriers to effective therapeutic results using this system is the induction of an immune response. Targeting the vector to tumour sites is also a major challenge. The use of mesenchymal stem cells (MSCs) as systemic delivery vehicles for therapeutic genes has been proposed as a method to overcome both limitations as a result of their combined ability to home to the tumour site, and evade the host immune response. This study is aimed at investigating homing of human MSCs to breast cancer primary cultures and cell lines in vitro and in vivo, and to identify factors mediating this migration.
Materials and Methods: MSC migration in response to breast tumour cells was quantified using Transwell™ inserts. Chemokines produced by the tumour populations were identified using ChemiArray™ or ELISA. The role of specific chemokines in mediating cell migration was determined using blocking antibodies and recombinant standards of the ligands. An animal model of metastatic breast cancer was established using athymic nude mice, followed by an intravenous injection of fluorescently labelled MSCs. At varying timepoints following MSC administration, mice were sacrificed and tumour tissue harvested for detection of engrafted MSCs.
Results: There was a significant increase in migration of MSCs in response to all tumour cells examined, including whole primary tumour explants (2−10 fold increase). Tumour cells were shown to secrete a variety of chemokines including GRO, GROα, IL-6 & 8, MCP-1 and SDF-1α. Inclusion of antibodies to MCP-1 and SDF-1α in tumour conditioned medium caused a significant decrease (26−52%) in MSC migration. Successful engraftment of fluorescently labelled MSCs was detected in metastatic deposits of breast tumours in nude mice following systemic administration.
Conclusion: These promising preliminary results support a potential role for MSCs as vehicles for tumour-targeted delivery of therapeutic agents to breast cancer.

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Inhibition of AKT by novel tetracyclic triterpenoids induces cell cycle arrest and triggers apoptosis in human prostate cancer cells

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Background: Akt are serine/threonine kinases, which control pathways involved in cell metabolism, proliferation and apoptosis. Akt play an important role in progression and chemoresistance of prostate cancer. Indeed, loss of the 'phosphatase and tensin homolog deleted on chromosome ten' (PTEN) expression, a phosphatase inhibiting Akt, is associated with aggressive behaviour of prostate cancer.
Materials and Methods: We analyzed the expression and function of Akt isozymes in androgen-dependent LNCaP and androgen-independent PC-3 and DU 145 prostate cancer cells.
Results: Akt1 and Akt2, the major isoforms expressed, are constitutively active in all three cell lines. Three structurally different Akt inhibitors exerted cytotoxic effect on LNCaP and PC-3 cell lines indicating that the Akt pathway is indispensable for cell viability. Various Boswellia species contain a mixture of mono- and triterpenoids that possess biological activities including antitumor properties. In search for well-tolerated and stable Akt inhibitors, we have isolated several tetracyclic triterpenoids from the oleogum resin of Boswellia carterii and purified them to chemical homogeneity. 3-Keto–tirucallic acid, alpha-acetyl-tirucallic acid and beta-acetyl-tirucallic acid potently inhibited the activities of human recombinant Akt1 and Akt2 in in vitro kinase assays. Similarly, the triterpenoids inhibited Akt activity immunoprecipitated from PC-3 cells, but did not affect the activity of immunoprecipitated IKK. The triterpenoids inhibited the phosphorylation of cellular Akt and glycogen synthase kinase-3beta, whereas extracellular signal-regulated kinase 1/2 phosphorylation was increased. Further, the compounds inhibited nuclear accumulation of p65/relA, androgen receptor, and the expression of the cell cycle regulators cyclin D1 and c-myc, followed by hypophosphorylation of retinoblastoma protein. These events culminated in cell cycle arrest and induction of apoptosis. Similarly, selective downregulation of Akt1, but not Akt2 expression, by siRNA induced marked inhibition of cell proliferation and apoptosis. In addition, the triterpenoids induced inhibition of proliferation and apoptosis in tumors grafted onto chick chorioallantoic membranes.
Conclusions: These results suggest that the inhibition of Akt activity is sufficient to trigger apoptosis in prostate cancer cells. Tetracyclic triterpenoids inhibiting Akt might provide a novel approach for the treatment of human prostate cancer.

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Oncogenic H-Ras V12 promotes anchorage-independent cytokinesis

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During carcinogenesis the cell achieves specific characteristics due to critical genetic changes. These changes cause transformation creating a cell with defect cell cycle control and the ability to divide without attachment to extracellular matrix (ECM). Since loss of cell anchorage to ECM induces untransformed cells to arrest in the cell cycle G1-phase this phase has been suggested to possess the major control of cell anchorage to ECM. A second point at which cell anchorage influences cell cycle progression is during cytokinesis. When non-transformed tissue cells are cultured in suspension they become binuclear.
We hypothesized that cancer cells capable of anchorage-independent growth must overcome controls in all anchorage-controlled cell cycle phases. Therefore we investigated the progression of primary human fibroblasts through each cell cycle phase when cultured without anchorage. Cells were synchronized at the start of different cell cycle phases and the cells were cultured either in suspension or attached to ECM followed by analysis of their cell cycle progression.
We show that cell anchorage to extracellular matrix do not control progression through the S and G2 phases in primary human fibroblasts, which also progress through most of mitosis with normal morphology. The cells in suspension initiated cytokinesis by forming midbodies with Aurora B, Rho A and alpha-Tubulin localized as in attached cells. The suspended cells also formed cleavage furrows and initiated but were unable to complete contraction, and instead collapsed and became binuclear. However, Ras-transformed fibroblasts and two cancer cell lines progressed through the entire cell cycle without anchorage to ECM. We therefore suggest that the ability to progress through cytokinesis without anchorage is achieved during carcinogenesis and might be a prerequisite for anchorage-independent growth.

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APC, CDH1 and CTNN1B promoter CpG islands methylation patterns during ductal breast carcinoma progression

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Background: In spite of earlier detection and better management, mammary tumors are still the primary cause of cancer deaths among women. The advent of mammography screening has led to an increased detection of pre-invasive mammary lesions, and to a better elucidation of the pathological events that precede the development of invasive breast carcinoma. Among the pathogenetic events leading to breast tumorigenesis, CpG island hypermethylation is emerging as one of the main mechanisms for inactivation of cancer related genes. In this study