Results: Tumour stromal cells were shown to secrete a range of chemokines including GRO, IL-6, IL-8 and MCP-1. The level of MCP-1 secreted by tumour populations was significantly higher (mean 951±158 pg/ml) than that secreted by normal stromal cells (mean 366±76 pg/ml). RQ-PCR analysis also revealed increased MCP-1 gene expression in tumour relative to normal stromal cells ($p < 0.05$). There were significant increases in migration of both MDA-MB-231 and MCF-7 cells in response to factors secreted by tumour, but not normal stromal cells [range 2−10 fold increase]. Significant inhibition (20−70% reduction) of migration in response to the stromal cells was observed in the presence of a monoclonal antibody to MCP-1.
Conclusion: Stromal cell derived MCP-1 stimulates epithelial cell migration and may play an important role in the breast tumour microenvironment. Increased understanding of the role played by stromal cells in breast cancer progression, and the specific mechanisms involved, may lead to the identification of novel therapeutic targets for treatment of the disease.

2020 POSTER

Downregulation of Wnt1 by siRNA induces apoptosis of breast cancer cells

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Background: Wnt family of secreted-type glycoproteins play key role in carcinogenesis and embryogenesis. Signals of glycoprotein Wnts are transduced through seven-transmembrane-type Wnt receptors encoded by Frizzled (Fzd) genes to the β -catenin- TCF pathway, the c-Jun-N-terminal kinase (JNK) pathway or the Ca2+-releasing pathway. In human breast cancer, evidence of β-catenin accumulation implies that the canonical Wnt signaling pathway is active in over 50% of carcinomas. The aim of present study was focused on the effect of Wnt1 gene silencing in triggering of apoptosis in breast cancer cells.
Materials and Methods: Light microscopy, viability/cytotoxicity tests, flow cytometry, Real Time-PCR and Western blotting were used for evaluation of the morphological features of cell death, percentage of apoptotic cells, Wnt1 mRNA and protein level. Breast cancer cells were transfected with fifteen siRNAs sequences specific to Wnt1 mRNA in concentration 50nM for 24−48h using Lipofectamine RNAi MAX. The sequences with the best efficiency in proliferation inhibition were used for further experiments.
Results: Breast cancer cells were transfected for 24−48 h with 20 nM of W15 siRNA. Among treated cells there were 64% apoptotic cells in comparison to cells treated with scrambled siRNA (4%) and control cells (7%) after 48 h. Flow cytometry analysis of Wnt1 expression showed that the percent of cells expressing Wnt1 is 3-times lower after transfection with W15 siRNA by comparison with cells treated with scrambled siRNA and control cells.
Conclusions: We show that silencing of Wnt1 in breast cancer cells can trigger apoptosis and this preclinical results indicate that siRNA specific to Wnt1 gene can be a useful strategy for breast cancer therapy.

2021 POSTER

Intensity-modulated proton- versus photon radiotherapy for locoregional, left sided breast cancer: a dose-comparison to heart and ipsilateral lung

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Background: To perform a treatment planning comparison between intensity modulated proton (IMPT) and intensity modulated photon radiotherapy (IMRT) for left-sided breast cancer patients and assuming 3 increasingly complex loco-regional irradiation volumes (PTV-1 to PTV-3). The study focused on the irradiated volumes of important normal tissues, namely heart and ipsilateral lung.
Materials and Methods: Comparative treatment planning was performed using planning CT scans of 10 consecutive left sided breast cancer patients following breast conservative surgery. For each scan 3 different PTV's were defined: whole breast (PTV-1), whole breast plus medial, lateral supraclavicular and level III axillary nodes (PTV-2), and PTV-2 plus internal mammary chain (IMC) (PTV-3). For each patient, 3 IMRT and 3 IMPT plans were calculated (total 60 plans) and each plan optimized for PTV coverage. Criteria for normal tissue comparison were radiation dose to heart (V22.5) and ipsilateral lung (V20 and V5).
Results: Both techniques met the required PTV coverage, with 95% of the PTV receiving more than 95% of the prescribed dose in all cases, although dose homogeneity was generally higher with IMPT. Statistically significant dose reductions were observed for left lung and heart using IMPT for all 3 PTV's. Effects of normal tissue sparing were most pronounced with increasing target complexity, i.e. increasing number of nodal areas, and thus maximally noted for PTV3, which included IMC's. For PTV3 mean V20 for the left lung was 30.35% (SD 2.97) and 15.98% SD 4.53) for IMRT and IMPT respectively, and mean V5 for the left lung was 95.17% (SD 3.79) and 28.72% (SD 6.28) for IMRT and IMPT respectively. Mean V22.5 for the heart was 17.62% (SD 7.23) and 2.33% (SD 1.69) for IMRT and IMPT respectively. Results correspond to a reduction of the ipsilateral lung doses (V20 and V5) by a mean factor of 2−3 with IMPT compared to IMRT and a reduction of the cardiac doses (V22.5) by a mean factor of 7 with IMPT compared to IMRT.
Conclusions: In this comparison-planning study IMPT significantly reduced irradiated volumes to ipsilateral lung and heart, specifically when several nodal chains require simultaneous inclusion in the target volume. Locoregional breast and nodal irradiation can pose a significant challenge and proton-radiotherapy might offer an attractive complementary alternative to photon irradiation.

2022 POSTER

Influence of cytokines on the expression of membrane-bound complement regulatory proteins and on complement-mediated lysis on breast cancer cell lines T47D und BT474

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Background: Clinical and experimental studies suggest that complement (C) may play a role in tumor cytotoxicity. Tumor cells avoid complement attack by several protective strategies, including over-expression of membrane-associated complement regulatory molecules (mCRPs).
Aim of this study was to investigate the possible relationship between complement-resistance of cancer cells and the expression of mCRPs and the potential impact of cytokines on these mechanisms.
Material and Methods: Here, we describe the expression of mCRPs CD46, CD55 and CD59 on two breast cancer cell lines BT474 and T47D. We examined the effect of IL-1β, IL-4, IL-6, IFN-γ, TGF-β und TNF-α on complement susceptibility of the cell lines using a novel non-radioactive cytotoxicity assay based on time-resolved fluorometry (Europium-TDA), and investigated the effect of these cytokines on the expression of these surface regulator proteins. Expression levels of mCRPs were analysed by flow cytometry. In addition, we examined the effect of Protein-kinase-regulators PMA and Calphostin C on complement-mediated lysis. Statistical analysis was done applying multifactorial, non-parametric analysis of variance.
Results: Basal levels of CD46, CD55 and CD59 were higher on T47D than on BT474. All cytokines augmented C-resistance of T47D, whereas enhanced expression of mCRPs was only observed after stimulation with TNF-α, TGF-β and IL-1β. On BT474 all cytokines but IFN-γ had an effect on C-mediated lysis, whereas expression of mCRP was enhanced by IL-1 β and TNF-α only.
Stimulation with PMA led to a decrease of C-mediated lysis on T47D. On BT474 it had no effect. Blocking of Proteinkinase C (PKC) led on both cell lines to increased complement lysis.
Conclusions: We conclude that membrane-bound complement inhibitors on breast cancer cell lines are differently regulated by the various cytokines applied. The difference in their effects on mCRP expression and on subsequent augmentation of resistance to C-mediated lysis suggests not only additional protective mechanisms but also a heterogeneity in resistance mechanisms, modulated in response to cytokines.
Our results also emphasize the role of PKC-based signal transduction pathways for cytokine regulated complement-resistance of cancer cells.

2023 POSTER

Heparanase expression in circulating lymphocytes of breast cancer patients as a marker of recurence and systemic metastasis

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Heparanase is an endo-beta-glucuronidase capable of degrading heparan sulfate chains of proteoglycans, generating a variety of bioactive molecules such as growth factors, chemotactic and angiogenic agents. The expression of heparanase was investigated in the peripheral blood mononuclear cell fraction (PBMC) of 30 patients with breast cancer (BC)