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)

and 20 healthy control women by RT-PCR and immunocytochemisty. PBMC samples from all 30 BC patients at study entry showed the expression of heparanase, whereas no expression was observed for the 20 healthy women. Immunocytochemistry analysis demonstrated that heparanase was expressed in the lymphocytes of the PBMC of BC patients. Throughout follow up, heparanase expression by RT-PCR decreased significantly after surgery in patients treated with neoadjuvant chemotherapy ($P = 0.002$) and after tamoxifen treatment ($P = 0.040$), whereas it increased significantly with the advent of systemic metastasis ($P = 0.027$). In vitro, either serum from breast cancer patients or the medium originated from co-culture experiments of MCF-7 cells and lymphocytes of the PBMC from healthy women stimulated heparanase expression in normal lymphocytes. The results suggest that there is a tumor inducing effect on heparanase expression by lymphocytes present in the PBMC of BC patients which depends, in turn, on the interaction between tumor and normal lymphocytes.

2024 POSTER

Can angiogenic markers bFGF and VEGF predict prognosis in node-negative breast carcinoma?

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Background: Angiogenesis, or neovascularization, is a complex process leading to formation of new blood vessels from the pre-existing vascular network of the tissue. Actually, the switch from the avascular to a vascular phase of tumor is regulated by multiple biochemical and genetic mechanisms. It has been suggested that estrogen induces expression of various angiogenic factors, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). These factors are involved in tumorigenesis, angiogenesis and metastasis.
Patients and Methods: Basic FGF and VEGF levels were measured by ELISA in cytosol extracts of 135 node-negative breast carcinomas while ER levels were measured by classical biochemical method recommended by EORTC. In the present study the clinical follow-up of node-negative breast carcinoma patients has been made for period of 144 months. Nonparametric statistical evaluations were performed.
Results: A statistically significant positive association was found between: (a) bFGF and ER in pT1 ER-positive breast carcinomas ($p = 0.03$), (b) VEGF and ER in patients older than 59 years with postmenopausal status within ER-positive breast carcinomas ($p = 0.04$), (c) bFGF and VEGF protein levels younger than 45 years with premenopausal status. Breast cancer patients with low levels of bFGF (<median = 93.6 pg/mg) had significantly shorter disease-free survival (DFS) than patients with elevated bFGF (log rank test, $p = 0.03$). It is important to point out that the tumor size (pT1 vs. pT2, 3) was homogeneously distributed between the low- and the high-risk subgroups. The levels of VEGF did not correlate with prognosis of node-negative breast cancer.
Conclusions: Our results indicate that low bFGF levels in node-negative breast carcinoma are independent prognostic indicators of poor prognosis and disease recurrence. The adverse prognostic levels of bFGF levels in node-negative breast carcinoma may have relevant biological and clinical application.

2025 POSTER

The identification and validation of novel endogenous control genes for the analysis of gene expression data in breast cancer tissues by real-time quantitative PCR

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Background: Real-time quantitative PCR (RQ-PCR) has become the basis of many breast cancer biomarker studies and more recently, prognostic assays. RQ-PCR data normalisation is required to control for systematic variation. Endogenous control (EC) genes, used in this context, should ideally be expressed uniformly in all test samples. The aim of this study was to identify the most suitable endogenous control gene(s) from a panel of novel candidates identified by microarray analysis in addition to those previously cited in the literature such as GAPDH, ACTB, TFRC, PPIA, HPRT, RPLP0, B2M and GUSB. The effect of choice of EC on target gene expression was determined using transcripts including the oestrogen receptor alpha (ESR1).
Materials and Methods: Primary breast tumour tissues ($n = 20$) were obtained from consenting patients during primary curative resection in Galway University Hospital. Samples were divided into two age- and stage-matched groups according to the development of metastatic disease during 5 years of follow-up. Following RNA isolation and analysis, whole genome microarrays were performed using the Applied Biosystems 1700 platform. After quantile normalisation, probes showing fold change 1.0−1.2 ($P < 0.05$) were analysed to identify novel candidate EC genes. Gene expression was quantified in a second cohort of malignant ($n = 21$) and benign ($n = 8$) primary breast tissues by RQ-PCR using standard TaqMan® chemistry and the ABI Prism® 7000. Expression variability was analysed using geNorm and Normfinder. Bartlett's test was used to compare pooled variances within group for each EC and the variability of normalised target gene expression using different ECs.
Results: There was a significant difference in candidate EC variability within ($P < 0.01$) and between benign and malignant groups ($P < 0.01$). GeNorm and Normfinder identified the same two genes as most stable. GAPDH and many of the other endogenous control cited in the current literature were less stable than either of the two genes identified. ESR1 expression was estimated to be appreciably higher in malignant tissues than in benign tissues irrespective of which EC was used. Several genes previuosly used as ECs may be regarded as target genes in these tissues.
Conclusion: Two genes have been validated as good ECs for the normalisation of RQ-PCR gene expression data in these tissues. The identification of these genes facilitates increased accuracy of gene quantification by relative RQ-PCR in breast cancer studies.

Oral presentations (Wed, 26 Sep, 09.00−11.00)
Breast cancer – early

2026 ORAL

Results of the UK standardisation of breast radiotherapy (START) trials testing hypofractionation for early breast cancer – on behalf of the START trials centres

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Background: The START Trials (ST-A and ST-B) test the hypothesis that breast cancer is as sensitive to fraction (Fr) size as late reacting normal tissues, with an α/β value of about 3 Gy.
Methods: The phase III randomised START Trials tested hypofractionated post-operative RT in women with completely excised invasive breast cancer (T1−3, N0−1, M0). ST-A tested 50 Gy in 25Fr (5 ks) vs 41.6 Gy vs 39 Gy, both in 13Fr (5 wks). ST-B tested 50 Gy in 25Fr (5 wks) vs 40 Gy in 15Fr (3 wks). Stratification was by centre, surgery and boost. The primary endpoint was local-regional (LR) relapse. Late normal tissue effects (NTE) were assessed by breast photographs (in patients with conservative surgery), clinical examination and quality of life (QL) questionnaires. Survival analysis methods were used to estimate rates of relapse and NTEs, and hazard ratios (HR) (with 95% CI). Smoothed estimates of absolute differences were obtained from the rates in the 50 Gy arms and the HR.
Results: 2236 (ST-A) and 2215 (ST-B) patients were recruited from 35 UK centres during 1999–2002. Median follow-up is 5.1 years (ST-A) and 6.0 years (ST-B). There were 93 LR relapses in ST-A (4.1% at 5 years, 3.2−5.0%), with no significant differences between the regimens (table). The α/β estimate for tumour control was 5.0 Gy (−2.7−12.7). In ST-B, there were 65 LR relapses (2.8% at 5 years, 2.1−3.5%), with no difference between the schedules. Rates of change in photographic breast appearance, induration, telangiectasia and breast oedema were lower in 39 Gy (ST-A) and 40 Gy (ST-B) vs 50 Gy. The α/β estimate for change in breast appearance was 3.1 Gy (1.6−4.6). QL results were consistent with the clinical findings.
Conclusions: The fractionation sensitivity of breast cancer is comparable to that of late reacting normal tissues, confirming the results of a recent pilot trial. These results are consistent with the use of hypofractionated RT schedules for early breast cancer.