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.