against HER2-neu, using a KPL-4 human breast cancer xenograft model (estrogen receptor-negative, HER2+) are presented.
Materials and Methods: The antitumor activity of optimal dose (OD) and $\frac{1}{2}$ OD C and D monotherapy was evaluated along with $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D or $\frac{1}{2}$ OD C + OD D. Since the results showed that $\frac{1}{2}$ OD C + OD D was toxic, OD C + OD D was not considered further. Both OD C + $\frac{1}{2}$ OD D and $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D, the optimal doublet from the initial study, were tested $\pm$ T in this HER2+ model.
Results: The initial investigation found that the tumor response (TR) and increased life span (ILS) were significantly better for $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D than for $\frac{1}{2}$ OD C, OD C, $\frac{1}{2}$ OD D, or OD D. Subsequent investigation found that TR and ILS were not significantly different for the $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D and OD C + $\frac{1}{2}$ OD D doublets; they were, however, better with the addition of T to each doublet. In comparing triplicates, TR was not statistically different, but survival was significantly better for the OD C + $\frac{1}{2}$ OD D + T group. At day 253, there were 1/10 complete responders (CRs) in the $\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T group (ILS = 267%) vs. 6/10 CRs in the OD C + $\frac{1}{2}$ OD D + T group (ILS > 837%, ongoing).

Treatment vs	Treatment	P (TGI)	P (ILS)
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D	$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T	0.021	0.0124
OD C + $\frac{1}{2}$ OD D	OD C + $\frac{1}{2}$ OD D + T	0.038	<0.0051
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D	OD C + $\frac{1}{2}$ OD D + T	0.002	<0.0016
OD C + $\frac{1}{2}$ OD D	$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T	0.273	0.0645
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D + T	OD C + $\frac{1}{2}$ OD D + T	0.241	<0.0237
$\frac{1}{2}$ OD C + $\frac{1}{2}$ OD D	OD C + $\frac{1}{2}$ OD D	0.064	0.2052

OD C = 400 mg/kg qd $\times$ 14; $\frac{1}{2}$ OD D = 10 mg/kg qweek $\times$ 3; T = 20 mg/kg 2x/week x 6. TGI = tumor growth inhibition.

Conclusions: The addition of T to non-toxic CD doublets increases TR and ILS. Results to date support the use of the most dense dose of C in triplicate combinations for sustaining CRs. Based on these results, the clinical testing of CD doublets with or without T in the neoadjuvant setting in HER2-negative and -positive breast cancer patients, respectively, is ongoing.

2013 POSTER

Bone marrow-derived TNF-a promotes tumour growth in a spontaneous model of mammary carcinogenesis

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Most solid tumors are composed by tumor cells sourrounded by infiltrating stromal cells, including immune cells and blood vessel cells, which play a key role in tumor development and progression. Cancer cells and infiltrating inflammatory cells communicate through a complex network of pro-inflammatory molecules, many of them still unknown. Recent evidences have highlighted a critical role for the transcription factor NF-κB and for the inflammatory mediator TNF-a in such multifaceted interaction that leads to cancer progression, in some tumor types.On this line, we are investigating the role of TNF-a in mammary carcinogenesis. Treatment with neutralizing Ab to TNF-a of mice injected s.c. with the mammary carcinoma cell line N2C, greatly reduces tumor growth; tumors grown in depleted mice show a less organized stroma and vasculature, with reduction of collagen type IV. To further study TNF-a role, we used the MMTV-HER-2/neuT transgenic mice, which, because of the expression of the mutated rat neu oncogene under the the MMTV promoter, spontaneously develop mammary carcinomas.Bone-marrow transplantation (BMT) experiments from TNF-a KO mice into NeuT significatively delay the onset and reduce mammary tumor growth, indicating a relevant role of TNF produced by cells of BM origin, likely macrophages. Performing BMT at different time points during tumor progression (8, 15, 20 weeks of age) indicates that TNF-a, differently form other models such as skin carcinogenesis where its role is mainly relevant for tumor initiation/promotion, is critical not only in the early steps of the carcinogenic process, but also at later time points when evident carcinomas in situ are already present. Whole mount analysis of mammary glands confirms the less sever tumor phenotype of mice transplanted with TNF-a KO BM in comparison with animals that have received wild type BM. Experiments with mice KO for TNF receptors are planned to identify the cellular target for TNF-a action and to further elucidate the mechanisms of its tumor-promoter activity in mammary carcinogenesis.

2014 POSTER

Serum proteome mass spectrometry analyses for identification of novel diagnostic biomarkers in breast cancer patients

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Background: Proteomics is the study of the proteome – a complete protein component of the cell. In contrast to the genome, the proteome is dynamic and its fluctuations depend on combination of numerous internal and external factors. Identifying and understanding changes in the proteome related to a disease development and therapy progression is a subject of clinical proteomics. Here we aimed to identify in the circulating blood a set of polypeptide biomarkers that could be used in diagnostics and monitoring of therapy of breast cancer patients.
Methods: Analysis of the low-molecular-weight region of the blood proteome (using either serum or plasma samples) by mass spectrometry (MS) methods is one of the basic approaches of clinical proteomics. Although no single peptide is expected to be a reliable bio-marker in such analyses, multi-peptide sets of markers selected in numerical tests have been already shown in a few studies to have prognostic and predictive value in cancer diagnostics. In our study we have analyzed low-molecular-weight serum polypeptides (<10 kD) using MALDI-TOF mass spectrometry.
Results: Blood samples were collected in the group of 100 breast cancer patients before the start of therapy, as well as in the group of 400 healthy controls. Specific patterns of low-molecular-weight polypeptides (1−10 kD) were identified due to mathematical analyses and cross-correlated between experimental groups. A multi-component set of polypeptides has been selected as a classifier that differentiate control and cancer samples.
Conclusions: Here we have presented report from the project aimed to identify a set of polypeptide biomarkers that could be used for diagnostics and monitoring of a therapy of breast cancer patients. Preliminary data showed that cancer-specific multi-component polypeptide pattern could be identified in serum of breast cancer patients. However, their importance for cancer diagnostics remained to be verified.

2015 POSTER

Functional analysis of the −2548G/ A leptin gene polymorphism in breast cancer cells

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Background: Leptin, a hormone produced mainly by the adipose tissue, regulates energy balance acting in the brain. In addition, leptin can stimulate mitogenic and angiogenic processes in peripheral organs. Recent data suggested that leptin can be involved in breast cancer progression, as it can induce proliferation, survival and anchorage-independent growth of breast cancer cells and is abundant in breast cancer tissues. The mechanisms of leptin overexpression in breast cancer are not clear.
The G to A substitution at −2548 in the leptin gene (Lep-2548G/A allele) in adipocytes correlated with a two-fold increase of leptin secretion and elevated circulating leptin levels. Furthermore, the occurrence of Lep-2548G/A in leukocytes correlated with increased susceptibility for different neoplasms, including breast cancer. However, molecular bases underlying this association have never been investigated. Here we asked whether occurrence of Lep-2548G/A in breast cancer cells could modulate transcriptional activation of the leptin gene.
Materials and Methods: We evaluated two different breast cancer cell lines, MDA-MB-231 and MCF-7. We used chromatin immunoprecipitation assays, DNA affinity immunoprecipitation, Western blot analysis and real time PCR.
Results: Lep-2548G/A was identified in MDA-MB-231, while it was absent in MCF-7 cells. DNA analysis revealed that Lep-2548G/A mapped near binding site for a transcriptional factor SP-1 and contained a motif for binding a transcriptional repressor nucleolin. Thus, we focused on the impact of Lep-2548G/A on the functional interactions of SP-1 and nucleolin with the leptin gene promoter Chromatin immunoprecipitation assays demonstrated that the existence of Lep-2548G/A improved efficient

recruitment of SP-1 to DNA, especially under insulin treatment, while SP-1 loading with or without insulin on DNA containing Lep-2548G/G was minimal. In contrast, nucleolin binding to Lep-2548G/A was downregulated in response to insulin, while it was not regulated on Lep-2548G/G. These results were confirmed by DNA affinity immunoprecipitation with specific Lep-2548G/A and control probes. Enhanced loading of SP-1 near Lep-2548G/A was paralleled by higher basal and insulin-induced expression of leptin mRNA in MDA-MB-231 cells.
Conclusions: The occurrence of Lep-2548G/A can enhance basal and insulin-induced leptin expression in breast cancer via SP-1- and nucleolin-dependent mechanisms.

2016 POSTER

Expression of the putative breast cancer gene BASE; relationship with microRNA-154* and estrogen receptor status

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Background: The role of micro-RNAs in the regulation of fundamental cellular processes such as proliferation, differentiation and apoptosis has advocated them as a novel molecular mechanism in the aetiology of carcinogenesis. It is estimated that 30% of human genes are regulated by micro-RNAs, many of which are cancer related. One such potential gene, BASE has been shown by in-vitro studies to be estrogen responsive and breast cancer specific. Little is known, however, about the associations or precise regulation of BASE expression in breast cancer tissues.
Aims: To quantify the expression of BASE and its putative targeting microRNA miR-154* in breast cancer tissues, and to establish potential correlations with clinicopathological variables.
Materials and Methods: Whole genome molecular profiling of gene expression and mi-RNA sequences was performed in 16 early stage, matched breast cancer specimens, to identify differentially expressed genes and micro-RNAs.
Expression of selected micro-RNAs including miR-154* were validated using RT Q PCR in a further 52 breast tumour samples. BASE was identified as a computationally predicted target of miR-154*, and its expression was also validated 52 breast tumour specimens and breast cancer cell lines. Associations between expression of BASE and miR-154* and clinico-pathological variables were examined
Results: BASE was expressed in 50% of tumour samples. A significantly higher proportion of tumours expressing BASE were estrogen receptor (ER) positive than ER negative ($p = 0.019$). BASE expression was also detected in the ER+ve cell lines but was not detected in an ER−ve cell line. MiR-154* was expressed in all breast tumour samples. The expression of miR-154* was significantly lower in ER+ve than ER−ve tumour samples ($p = 0.001$)
Conclusions: These findings suggest that the expression of both miR-154* and putative target gene BASE correlate with estrogen receptor status in breast tumours. This highlights the importance of these molecules breast cancer. Functional analysis to elucidate a possible interaction between these molecules is underway.

2017 POSTER

Prognostic value and response to chemotherapy of immunohistochemical phenotypes (IP) of 141 operable breast cancer patients (pts) included in phase III trials of adjuvant therapy

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Background: Gene expression arrays and IP studies classified breast cancer in three distinct subtypes: basal, HER2/neu and luminal that are associated with different clinical outcomes.
Methods: In 141 pts with operable breast cancer, included in phase III trials of adjuvant therapy in our center, immunohistochemical staining was performed on 3μm sections of paraffin blocks, containing tissue-arrays of tumour tissue.A basal phenotype (BP) was defined by negative estrogen receptor (ER) and progesterone receptor (PR) and positive cytokeratin (CK) 5/6 or EGFR immunoreactivity. HER2/neu phenotype as positive c-erb B2 by HercepTest™ and luminal phenotype (LP) by positive ER, PR and CK 7/8 and negative HER-2. Survival curves were calculated by the Kaplan-Meier method. The differences between survivals were estimated using the log rank test. Multivariate Cox regression analysis was used to evaluate any independent prognostic effect of the variables on disease-free survival (DFS).
Results: Complete clinical follow-up information was available for 141 pts. The median follow-up period was 52 months (range 1–103 months). During this period, 13.8% pts died from breast cancer and 27.7% pts relapsed. At the time of the primary diagnosis 10.4% of the pts had lymph node negative disease and 89.6% had positive lymph nodes. 50.8% pts received standard chemotherapy with anthracycline and taxanes, 7.7% Trastuzumab, 62.3% radiotherapy and 61% pts received hormonotherapy. Positivity for LP was 65.2%, BP 9.9% and Her-2 phenotype 8.5%. 16.3% didn't fit for any of the three subtypes. Median DFS for BP: 24 moths, for LP and Her-2 phenotypes median DFS was not reached. 5 years DFS were; BP: 19%, LP: 63% and Her-2: 56%. Kaplan-Meier survival analyses demonstrated that the presence of a detectable BP was highly significantly associated with a worse DFS compared with the presence of a LP, log rank test ($p = 0.0001$). Multivariate Cox regression analyses estimated that the prognostic effect of BP in relation to DFS was independent of lymph node, stage and tumor size, HR: 0.12 95% CI (0.05−0.2). In the group of patients who received standard-based adjuvant chemotherapy, both DFS and OS were found to be significantly shorter in the BP ($p < 0.05$).
Conclusions: We found that expression of BP was associated with poor prognostic in the context of randomized phase III trials. Standard adjuvant chemotherapy seems to be less effective in these tumours and new therapeutic approaches are indicated.

2018 POSTER

Can differences in cellular antioxidant enzyme status predispose to breast cancer in women without a recognised increased risk?

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Introduction: Up to 10% of patients with breast cancer have a known genetic defect (eg. BRCA-1, BRCA-2) but the aetiological factors in the others remain unclear. We hypothesise that impaired expression of cellular antioxidant enzymes and subsequent reduction in the ability to counter DNA damage due to oxidative stressors could be, at least in part, important in the aetiology of breast cancer.
Method: We obtained whole blood and PBMNC from women with breast cancer ($n = 20$) and from an age matched control group without known risk ($n = 20$). Erythrocyte and plasma glutathione peroxidase-1 (GPX1) activity was determined in both groups using a spectro-photometric method. Aliquots of PBMNC were used to determine gene expression of redox enzymes in untreated, fresh cells using RT-PCR. Further aliquots of PBMNCs were incubated in autologous plasma for 24 hrs and stimulated with hydrogen peroxide (1 mM) for 15 minutes to assess inducibility of the selenium-dependent antioxidant enzymes (GPX1) and (GPX4).
Results: Neither GPX1 activity in plasma or erythrocytes nor mRNA expression in fresh, non-induced PBMNC differed significantly between groups although mRNA tended to be lower in the cancer group. However, GPX4 gene expression in fresh PBMNC was significantly (30%, $p < 0.004$) reduced in the cancer group. Percentage induction of mRNA by hydrogen peroxide was similar (30−40%) for GPX1 and GPX4 in both groups but absolute GPX4 induction was lower in the cancer group due to a lower un-stimulated, starting value.
Conclusion: Breast cancer patients do appear to have a lower redox enzyme expression than non-cancer patients which would be expected to impair their ability to counter free-radical damage to DNA resulting in greater risk of genetic mutations.

2019 POSTER

The role of primary stromal cell-derived chemokines in the breast tumour microenvironment

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Background: It is well established that within the breast tumour microenvironment, neoplastic epithelial cells coexist with stromal fibroblasts. Stromal cells secrete a variety of chemokines which may potentially mediate the reciprocal interactions between breast stromal and epithelial populations. However, the specific chemokines involved and their mode of action remain to be defined.
The aim of this study was to identify factors secreted by tumour stromal cells and elucidate their potential role within the tumour microenvironment.
Methods: Human breast tumour specimens harvested at surgery were separated into epithelial and stromal fractions for culture. Tissue harvested at reduction mammoplasty served as normal controls. Chemokines secreted by the stromal populations were detected using Chemiarray™, ELISA and RQ-PCR. Transwell® inserts were used to assess migration of breast cancer epithelial cell lines (MDA-MB-231 and MCF-7) in response to primary stromal cells.