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