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Annexin I regulation of breast cancer cell proliferation

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Background: Tissue microarray analyses of human breast carcinomas have linked decreased annexin I (ANXA-1) expression with breast cancer progression [1]. ANXA-1, a calcium- and phospholipid-binding protein, has been implicated in A549 lung cancer cell growth and apoptosis [2]. We sought to establish the effect of ANXA-1 protein knockdown on cell cycle regulation in the estrogen receptor (ER)-positive MCF-7 cells and ER-negative MDA-MB-231 breast tumour cell lines.
Materials & methods: MCF-7 and MDA-MB-231 cells were transfected with either ANXA-1 targeting stealth RNAi (siRNA) or its scrambled control sequence. After 24 hours, cells were stimuated with 5% FC. Cell lysates were obtained 24 and 48 hours post-transfection for analysis of ANXA-1 protein levels. Enumeration of viable cells was performed after a 48 hour incubation with FCS and cell cycle regulation was determined by flow cytometry of propidium iodide stained cells.
Results: At 24 and 48 hours post-transfection, ANXA-1 protein levels were significantly decreased by 28% and 55% respectively in MCF-7 cells. The reduction of ANXA-1 in MDA-MB-231 cells at 48 hours after transfection was 35% ($p < 0.05$). ANXA-1 knockdown reduced basal and FCS-induced increases in MCF-7 cell number, accompanied, at 48 by significant reduction in G2/M phase cells. Neither basal nor FCS-induced increases in cell number were influenced by ANXA-1 siRNA in the ER-negative MDA-MB-231 cell line.
Conclusions: These observations implicate ANXA-1 in the FCS-induced proliferation of MCF-7 cells.
1. Shen, D., et al., Decreased expression of annexin A1 is correlated with breast cancer development and progression as determined by a tissue microarray analysis. Hum Pathol, 2006. 37(12): p. 1583−91. 2. Croxtall, J.D. and R.J. Flower, Lipocortin 1 mediates dexamethasone-induced growth arrest of the A549 lung adenocarcinoma cell line. Proc Natl Acad Sci U S A, 1992. 89(8): p. 3571−5.

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NAV3 – a novel cancer biomarker

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Cancer is the leading cause of death worldwide and its incidence is growing. Therefore, also the need for new biomarkers in cancer diagnostics is increasing.
Neuron navigator 3 (NAV3) is a recently described gene, which contains functional domains related to cell signaling, gene expression, chromosome stability and segregation – all functions common for cancer related proteins. Although the exact function of NAV3 is currently not known, allelic loss of NAV3 gene has recently been shown to associate with cutaneous T-cell lymphoma (Karenko et al. 2005).
We have analyzed NAV3 gene copy number in a large clinical sample collection from different cancer types and also in different established cancer cell lines using NAV3 specific fluorescent in situ hybridization (FISH).
Our results show that NAV3 copy number changes in the form of allelic loss but also low level amplification were detected in various cancer types. NAV3 aberrations were common in colon carcinoma, breast cancer, bladder cancer, in different brain tumors and in B-cell lymphomas. Moreover, chromosome 12 polysomy showing strong correlation with NAV3 aberrations was frequently detected in these samples. Similar NAV3 copy number abnormalities were also detected in colon carcinoma and breast cancer cell lines. Furthermore, colon carcinoma cell lines showed various chromosomal translocations, which can cause the NAV3 aberrations.
In colon cancer studies, we used three different methods, loss of heterozygosity assay, array comparative genomic hybridization and fluorescence in situ hybridization (FISH), to look for NAV3 copy number changes. NAV3 deletions were detected with all three methods, but the FISH method revealing changes in as few as 2−3% of the cells studied, was the most sensitive and specific. In this study, both colon carcinomas and adenomas were analyzed. Notably, NAV3 specific FISH showed NAV3 aberrations already in adenoma stage, although the amount of NAV3 abnormal cells was higher in carcinomas. Furthermore, in breast cancer studies, the metastasis samples from sentinel node frequently showed high amount of NAV3 deleted cells.
These results indicate that NAV3 aberrations are common in several cancer types and these abnormalities can be detected already in pre-malignant stage and in metastasis samples. Thus, the NAV3 gene can be used as a potential new diagnostic marker to enhance the diagnosis of cancer at the early phase and to predict the progress of the disease.

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Selective inhibition of Stat3 expression induces apoptosis in human cutaneous T-lymphoma cell line Hut78

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Background: Cutaneous T-cell lymphomas (CTCL) are extranodal non-Hodgkin's lymphomas with pleomorphic skin lesions and distinct T-cell markers, of which Mycosis fungoides and Sézary syndrome are the most common. They are associated with severe morbidity and mortality with a poor prognosis and palliative therapy. CTCL cells have defective apoptosis; therefore, reversing resistance to apoptosis may provide a new therapeutic approach. Signal transducer and activator of transcription 3 (Stat3), an oncogene and a latent transcription factor, has been shown to play key role(s) in the development and progression of several human cancers by promoting cell proliferation and protecting against apoptosis. Our aim was to determine the involvement of Stat3 in CTCL and to test the hypothesis that Stat3 signaling could serve a novel therapeutic target.
Material and Methods: Selective inhibition of Stat3 expression in human CTCL cell line Hut78 was performed by small interfering RNA (siRNA) techniques. Effects of Stat3 knockdown on cell proliferation, apoptosis and gene regulation was assessed by standard biochemical methods and high content analysis techniques. Various parameters of apoptosis including nuclear morphology, DNA fragmentation assay by terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling were studied and quantified by FACS analysis.
Results: We demonstrate that expression and activation of Stat3 was significantly higher in the CTCL cell line Hut78 as compared to peripheral blood lymphocytes isolated from a healthy human volunteer. Specific knockdown of Stat3 expression in Hut78 cells induced morphological and biochemical changes including nuclear condensation, DNA fragmentation and Annexin V labeling indicating apoptotic cell death. Moreover, inhibition of Stat3 expression resulted in the reduction of the expression of Bcl2 family of anti-apoptotic gene Bcl-xL.
Conclusions: These results show that Stat3 is required for the survival of human cutaneous T-cell lymphoma. These studies suggest that targeting Stat3 using siRNA may serve a novel therapeutic strategy and can possibly open new horizons in the treatment of CTCL.

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CD147 expression correlates with monocarboxylate transporters 1 and 4 in cervical carcinoma

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Background: Due to the high glycolytic metabolism characteristic of solid tumors, there is an increased production of acids, however, cells maintain physiological pH through plasma membrane efflux of the accumulating acids. Acid efflux through MCTs constitutes one of the most important mechanisms involved in the maintenance of tumour intracellular pH, however, the molecular mechanisms underlying the regulation of these membrane proteins are not fully understood. We aimed to evaluate the co-expression of the MCT chaperone CD147 and the monocarboxylate transporters (MCT) isoforms 1, 2 and 4 in a large series of cervical lesions (neoplastic and non-neoplastic) and assess the clinico-pathological significance of CD147 expression. We also intended to observe if there were correlations between MCTs and both EGFR and COX-2 expressions.
Material and Methods: The series analyzed included 83 biopsy samples (28 chronic cervicitis, 26 low-grade squamous intraepithelial lesions and 29 high-grade squamous intraepithelial lesions) and surgical specimens of 126 invasive carcinomas from the uterine cervix (49 squamous cells carcinomas, 50 adenocarcinomas and 27 adenosquamous carcinomas). Analysis of CD147 expression was performed by immunohistochemistry

and results on MCT, EGFR and COX-2 expressions were previously described.
Results: Importantly, both MCT1 and MCT4 were found to be more frequently expressed in CD147 positive cases than in CD147 negative cases and we observed that the co-expression of CD147 with MCT1 was significantly associated with lymph-node and/or distant metastases in adenocarcinomas. Interestingly, we found positive correlations between COX-2 and both MCT2 and MCT4 expressions, as well as between EGFR and lack of MCT2 expression. Moreover, EGFR also correlated with CD147 expression.
Conclusions: In sum, our results contribute to the understanding of the metabolic alterations in cervical cancer and also provide evidences for the regulation of MCTs in human cervical samples, which could be of value in the development of new therapeutic strategies.

Phase I

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A phase I study of XL184, a MET, VEGFR2, and RET kinase inhibitor, administered orally to patients (pts) with advanced malignancies, including a subgroup of pts with medullary thyroid cancer (MTC)

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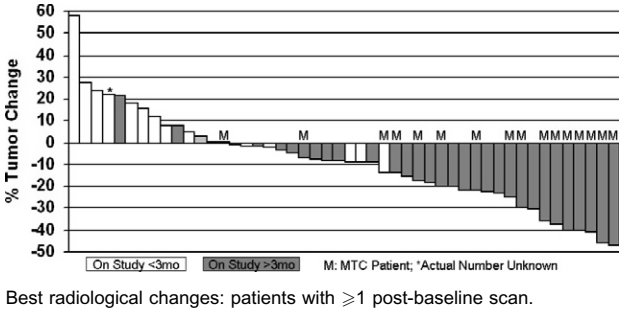
Background: XL184, a multi-kinase inhibitor, strongly inhibits proliferation in MTC cell lines harboring activated RET. Pharmacodynamic studies show substantial inhibition of RET & MET phosphorylation in the TT xenograft model of MTC.
Methods: XL184 was administered QD on Days 1–5 of 14 day cycles (5&9 schedule; cohorts (C) 1–9), or as continuous QD dosing (C10+). Initial mg/kg dosing using a suspension formulation changed to flat dosing using capsules. Response is assessed on day 28 & every 8 weeks (wks). Plasma markers reflecting anti-angiogenic therapy & RET status in blood & tumor samples are being analyzed.
Results: 70 pts (22 with MTC) have been treated across 13 dose levels: 0.08–11.52 mg/kg 5&9, (C1−9); 175 mg/d, 265 mg/d, 175 mg/d (capsules) & 250 mg/d (capsules). Eight DLTs include grade (Gr) 3 palmar/plantar erythema (PPE), & Gr 3 AST, Gr 3 ALT & Gr 3 lipase elevations at 11.52 mg/kg 5&9, Gr 2 & 3 mucositis at 265 mg/d resulting in dose reduction, & Gr3 AST elevation & G3 PPE at 250 mg/d using capsules. The capsule MTD is 175 mg QD. Frequent XL184-related AEs include diarrhea (25%), nausea (21%), fatigue (20%), mucosal inflammation (16%), anorexia & increased AST (13% each), hypertension & vomiting (11% each), increased ALT, hair color changes & PPE (10% each). Pharmacokinetic (PK) analysis suggests linear PK; the terminal half-life is ~100 hrs. XL184 resulted in statistically significant changes in pharmacodynamic biomarkers (PlGF, VEGF-A, sVEGFR2) in pts enrolled at the MTD & sMET, a potential biomarker of MET inhibition, was modulated.
Twenty-four pts have had SD $\geqslant$3 months including 9 pts with SD $\geqslant$ 6 months. One pt with neuroendocrine carcinoma had an unconfirmed partial response (PR). Eight of 16 MTC pts with measurable disease had a PR (50%, 5 confirmed) with all others experiencing prolonged SD; the overall disease control rate (PR + SD >3 months) is 100%. Three MTC pts have non-measurable disease & 3 are too early to evaluate. Three PRs in MTC pts were reported at the first radiographic evaluation. Most pts with MTC have had substantial reductions in plasma calcitonin & CEA. Best radiological changes are shown in the figure.



Best radiological changes: patients with $\geqslant$1 post-baseline scan.

Conclusions: XL184 appears generally well tolerated & the daily dosing MTD using capsules has been defined. Antitumor activity has been observed in pts with various cancers and 50% of response-evaluable MTC pts achieved a PR while all 19 evaluated MTC pts derived clinical benefit. A Phase 3 study of XL184 in MTC is planned.

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Transcriptional and metabolic response associated with acute doxorubicin cardiotoxicity in perfused rat heart

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Background: Doxorubicin (DXR) belongs to the most efficient anticancer therapeutics. However, its application is limited by the risk of severe cardiotoxicity, molecular mechanisms of which are not yet completely understood. While fast selective down-regulation of the several cardiac specific genes have been implicated in development of DXR cardiotoxicity, general impact of the drug on heart gene profile is less characterized.
Material and Methods: Here we use a genome-wide DNA microarray approach to analyse the acute transcriptional response of the perfused Wistar rat heart to the low DXR dose. In addition, to better understand gene-function relationships, we focus on a group of genes involved in cardiac energy metabolism and analysed in more detail the corresponding phenotype: mitochondrial respiration in permeabilized cardiac fibers and levels of high energy phosphates.
Results: We show that perfusion of the rat hearts with 2 microM DXR during 2 hours induced moderate but significant hemodynamic dysfunction as well as distinct transcriptional reprogramming associated widespread downregulation of gene expression in DXR treated hearts. Selective upregulation of individual genes/gene sets was also observed; upregulation was however less sound both in term of fold changes and statistical power. For several genes our unbiased analysis converged with previous candidate oriented studies but we identified new potentially interesting DXR-responding genes/gene sets as well. Though in our model only minor changes were observed in general energy status (ATP, PCr level) and in the respiratory activities measured in permeabilized cardiac fibers, the upregulation of glycolytic and Krebs cycle genes seems to be a compensatory mechanism triggered by the onset dysfunction.
Conclusions: Doxorubicin rapidly induces widespread repression of gene expression in heart. Induction of some genes/gene sets escaping this repressing tendency can be, at least in a part, due to action of compensatory mechanism. Functional consequences of the transcriptomic changes can be of meaning both for cardiotoxic but and anticancer action of DXR.

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Detecting EGFR mutations in NSCLC by mutant specific antibodies

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Background: Patients of non-small cell lung cancer (NSCLC) carrying the somatic mutation of epidermal growth factor receptor (EGFR) have been shown to be hyperresponsive to the EGFR tyrosine kinase inhibitor Gefitinib and Erlotinib. The most common NSCLC associated EGFR mutations are the 15-bp nucleotide in-frame deletion in exon 19 (E746_A750del) and the point mutation replacing leucine with arginine at codon 858 in exon 21 (L858R), accounting for 85–90% EGFR mutations. The ability to detect mutated gene products in cancer cells can identify patients most likely benefit from such therapies, and make clinical trials more efficient and informative.
Methods: We generated rabbit monoclonal antibodies (RmAb) against EGFR with E746-A750 deletions and L858R point mutation. We tested the antibodies by western blot, Immunofluorescence (IF) and immunohistochemistry (IHC).
We used the antibodies staining 40 molecularly pre-typed NSCLC tumor samples by IHC. Then, we used IHC by a panel of four antibodies (two mutant antibodies, wtEGFR and pan-keratin antibodies) to screen 340 cases of NSCLC patient tumor samples without information of phenotypes.
Results: The western blot, IF and IHC were confirmed that the antibodies can specifically detect the mutant EGFR proteins. 40 molecular pre-typed