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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.
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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