Here we report that NHERF1 and PTEN interact with EGFR in a ternary complex, upon EGF stimulation, in metastatic breast cancer cell lines, regulating its degradation.

Materials and methods: Human mammary cell lines were transiently transfected with wild-type mouse NHERF1 cDNA inserted into the pcDNA vector and the experiments were conducted 48h later. After treatment monolayers were lysed in fractionation lysis buffer (HEPES 10mM pH 7.9, KCl 10mM, EDTA 0.1mM, EGTA 0.1mM). The nuclear fraction was obtained by centrifuging the homogenate at 600 X g for 10 min. The resulting supernatant was centrifuged at 3,500 X g for 10 min to obtain a pellet containing the endosomal fraction; the supernatant was centrifuged again at 100000 X g for 1.5 hr resulting in a plasma membrane pellet, the soluble cytoplasmic fraction in the supernatant. 50 µg of each cellular fractions were resuspended in ice-cold coimmunoprecipitation lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate,100 mM Na3VO4, and 1 mM NaF, protease inhibitors). 150 µg of total cellular protein was incubated at 4°C with 1 µg of primary antibody and protein A/G Plus-Agarose overnight. Immunoprecipitates were loaded in SDS sample buffer, run on 10% SDS-PAGE, analyzed by Western blotting.

Results: NHERF1 and EGFR signaling molecules interact after EGF stimulation. Interaction of endogenous NHERF1 with EGFR requires a NHERF1 functional PDZ1domain. The over expression of NHERF1 inhibits EGFR degradation, permits the interaction of the two proteins in not stimulated conditions. Moreover PTEN could be an important molecular integrator of the downstream events of EGFR.

Conclusions: Taken together, our studies suggest that NHERF1 senses signal of EGF and with PTEN regulates ligand induced degradation of EGFR.

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Validation of phosphoprotein array assay for determination of human epidermal growth factor receptors downstream signalling phosphoproteins in breast carcinoma

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Background: human epidermal growth factor receptors (HER) downstream signalling kinases have major consequences on tumor response to anti-HER monoclonal antibodies and tyrosine kinase inhibitors targeted therapy. The present study was designed to validate the use of phosphoprotein array assay to investigate HER downstream signalling functionality.

Materials and methods: the expression of phosphorylated EGFR and HER downstream signalling proteins (AKT, P70S6 kinase, ERK1/2, GSK3b) was measured by multiplex analysis using phosphoprotein array in 49 ductal infiltrating breast carcinoma frozen specimens taken at diagnosis and compared with western blot analyses. Based on routine immunohistochemistry, HER2 expression was overexpressed (3+) in 19 specimens. Ten tumors were triple-negative (HER2, estrogen and progesterone negative). Standard operating procedures were optimized regarding sample size, homogeneity, tumor content, freezing and protein extraction. The validation of the assay was based on the FDA guidelines for Bioanalytical Method Validation.

Results: linear regression and Bland-Altman analyses showed highly significant quantitative correlations between the phosphoprotein array assay and western blot analysis with regression coefficient values ranged from 0.790 to 0.852 ($P<0.001$). With limits of detection established at a signal-to-noise ratio of 3, great variations of phosphoprotein expression, up to several thousand-fold, were observed among the 49 tumor specimens. No significant variation in phospho-ERK1/2 as well as in phospho-AKT and related downstream phosphoproteins was observed between HER2 overexpressing and HER2 negative tumors. Lower expression of all HER downstream signalling phosphoproteins was observed in triple-negative tumors.

Conclusion: these results showed that before any treatment initiation, MAP kinase and PI3 kinase/AKT signalling pathway functionality can dramatically differ from patient to patient and could probably explain discrepancies in clinical response to signalling kinase targeted therapy even when based on individual immunohistochemistry target determination.

These results validate the use of multiplex phosphoprotein array assay in size-limited tumor specimens and warrant further prospective evaluation of HER downstream signalling phosphoproteins as predictive and/or surrogate marker for clinical response to anti-HER drugs.

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The alpha5beta1 integrin predicts glioblastoma chemotherapy outcome through modulation of p53 pathways

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Background - Of solid tumors, glioblastoma remains the most resistant to therapy and new therapeutic approaches are needed. The potential role of $\alpha5\beta1$ integrins in cancer has recently attracted much interest. Its overexpression in tumoral neovessels and in glioblastoma makes it a potential interesting therapeutic target. We investigated its role in glioblastoma carcinogenesis and chemoresistance.

Methods - The $\alpha5$ integrin subunit was overexpressed or knocked down in U87MG cells and proliferation and clonogenicity were examined. Ellipticine, a DNA intercalating agent and topoisomerase II inhibitor, which demonstrated specificity towards brain tumor cell lines, was investigated as a chemotherapeutic agent. Ellipticine effects on proliferation, cell cycling, apoptosis and senescence were investigated in the presence or absence of SJ749, a $\alpha5\beta1$ integrin specific antagonist.

Results - $\alpha5$-overexpressing U87MG cells exherted increased aggressivity as low expressing cells confirming a specific role of the $\alpha5\beta1$ integrin in glioblastoma tumorigenicity. Accordingly, blocking the integrin with SJ749 inhibited glioma cell adhesion to fibronectin and reduced cell proliferation / clonogenicity via a G0-G1 cell cycle arrest without induction of apoptosis. This effect was dependent on the $\alpha5$ subunit expression level. The main effect of ellipticine in U87MG cells was to induce premature senescence through the activation of wild type p53. By contrast, ellipticine mostly induced apoptosis in U373 cells expressing mutant p53. Association of chemotherapy (using ellipticine) and targeted therapy against $\alpha5\beta1$ integrin (using SJ749) led to an additive reduction of U87MG cell proliferation. Interestingly, combined therapy decreased senescence and increased apoptosis in U87MG but not in U373 cells. We also showed that knocking down the $\alpha5\beta1$ integrin in U87MG impaired the ellipticine-induced p53 activation (as observed with SJ749) but surprisingly drastically enhanced the senescent cell population.

Conclusions – The $\alpha5\beta1$ integrin is a marker of glioblastoma aggressiveness and modulates glioblastoma chemosensitivity through p53 pathways. p53 status and $\alpha5\beta1$ integrin expression level may predict outcome of combined therapies (chemo- and targeted therapies) for glioblastoma.

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Identification of therapeutic targets in uterine and soft tissue leiomyosarcomas

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Background: Leiomyosarcoma is a rare mesenchymal tumor, with poor prognosis and response to cytotoxic chemotherapy. It shares some similar phenotypic and histological features with gastrointestinal stromal tumors (GISTs) which the prognosis was dramatically changed with the selective tyrosine kinase inhibitor Imatinib mesylate. However, in contrast to GISTs, patients with advanced LMS do not benefit from Imatnib therapy. So, an effective treatment lack for this neoplasm. Thus, a better understanding of the LMS' biology needs to be elucidated in order to develop new therapeutic agents, notably tyrosine kinases inhibitors.

Objectives: We aim to clarify the molecular mechanisms of signal transduction of LMS by a large screening of phosphorylated protein kinases.

Methods: A screen of 630 phosphorylated protein kinases was performed on one Leiomyosarcoma tumor using the Kinex antibody micro array. Also, by Western Blot analysis, a more specific approach, we investigated the expression/activation of specific tyrosine kinases receptors like c-Kit, CSF1-R, and c-Met receptor tyrosine kinases in 13 primary LMSs.

Results: In our screen, we observed higher levels of phosphorylated Her2/Neu, FAK, Src, IRS1, MEK1 (MAP2K1), MEK3 (MAP2K3), ERK5, and MAPKAPK2a/b in the leiomyosarcoma tumor analyzed, than in the healthy muscle used as a control. Furthermore, western blot analysis revealed the expression of c-Kit, CSF1-R, c-Met in all cases but only Kit was phosphorylated, thus activated in these tumors.

Conclusion: To date, no causal event in the development of leiomyosarcoma was uncovered yet. A better understanding of the leiomyosarcoma biology will lead us to explain its non-response to Imatinib mesylate despite Kit activation, and especially to identify potential therapeutic targets as tyrosine kinases receptors.