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Role of Sodium Hydrogen Exchanger Isoform 1 (NHE-1) in Integrins-mediated Migration of Human Breast Cancer Cells

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Background: The Na^+-H^+ exchangers (NHEs) are a family of membrane glycoproteins which transport H^+ out of the cell in exchange for Na^+ with a stoichiometry of 1:1. In mammalian cells, the NHE family consists of nine isoforms, NHE-1 to NHE-9. NHE-1, the first one of the isoforms to be cloned, is ubiquitously distributed.
Several studies have shown both increased *activity* and *protein* of NHE-1 in transformed cells. Apart from its role as a principal regulator of intracellular pH (pHi) and cell volume, NHE-1 has been implicated in cell proliferation, transformation and migration.
Cell migration is a multi-step process that requires spatial asymmetry which is stimulated by Rho GTPases, phosphoinositides and actin polymerization. Integrin family of receptors is responsible for cell surface interactions with extracellular matrix (ECM).
NHE-1 may contribute to cell migration by: (a) affecting the cell volume, (b) regulating the intracellular pH and thereby the assembly and activity of cytoskeletal elements, (c) anchoring the cytoskeleton to the plasma membrane, and (d) by controlling cell adhesion. Disrupting NHE-1 function leads to impaired polarity of cells and their inability to migrate. Although NHE1 has been shown to affect cell migration through its various functions, a role for the exchanger in cell migration regulated by integrins has not been extensively studied. Fact that NHE1 binds several other proteins in the cytoplasmic regulatory domain, have led to the hypothesis that NHE1 can act as a plasma membrane scaffold that brings together many proteins so they can interact functionally. Thus, it is plausible to hypothesize the possible role NHE-1 might play by direct or indirect structural interaction with the assembly of cell adhesion molecules.
Materials and Methods:
1. Inhibition of NHE1 activity (using ethyl-isopropyl-amiloride) and its effect on pHi and cell viability (Measurement of intracellular pH, MTT assay)
2. Effect of NHE1 silencing (using siRNA transfection) and its effect on cell viability (Western blot analysis, MTT assay)
3. Effect of NHE1 silencing and pharmacological inhibition of its activity on cell adhesion and motility. (In vitro cell adhesion assay, In vitro cell migration assay)

Results: The data obtained shows that with either EIPA treatment or NHE-1 siRNA transfection, migratory capacity was impaired in MDA-MB-231 human breast cancer cells. Interestingly, pharmacological inhibition of NHE-1 did not significantly reduce the integrins-dependent cell adhesion in these cells. However, down-regulation of NHE-1 protein expression had significant effect on integrins-mediated cell adhesion to fibronectin.
Conclusion: This study shows that the effect of NHE-1 on integrins-dependent cell adhesion is independent of its activity. However, NHE-1 protein expression seems to be an important upstream event in the functional assembly of integrin receptors and may play an essential role in cancer cell adhesion to the extracellular matrix.

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mTOR Inhibition Arrests Selective Stages of Breast Cancer Progression in Vitro

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Background: Kinase mTOR is one of the main links in signal transduction from variety of growth factors and hormones into the cell. mTOR participates in the regulation of protein synthesis, cell growth, proliferation etc. There are two functional complexes TORC1 and TORC2 whichregulate different cell events. Earlier it was demonstrated the overactivation of mTOR in numerous of malignant neoplasia. mTOR inhibitors are regarded as anti tumour drugs. But it is not clear which stage of tumour progression is critically depended from mTOR activation/deactivation. The elucidation of this issue will allow to detect the additional targets of anticancer therapy, which status is modulated by mTOR. It could provide the development of combined antitumour treatment.
Materials and Methods: Immuunofluorescent analysis was applied to detect subcellular localization of mTOR in MCF-7 breast cancer cells (2D and 3D cultures) and postoperative specimens of human breast tumour. The effect of 1 and 10 nM of rapamycin on cultured cells was tested by MTT-test, adhesion and spreading assay, migration test using “wound healing” model, zymography, actin detection with falloidin, confocal microscopy.
Results: Immunoflurescent analysis find out predominantly cytoplasmic localization of mTOR in postoperative specimens of breast cancer and MCF-7 cells. Also, additional positive reaction for mTOR was evident in nucleoli. According to our information this mTOR positive staining of nucleoli is revealed for the first time.
The process of tumour progression was hypothetically divided into several integral parts which were remodeled *in vitro* using breast cancer cell line MCF-7. Cell behavior under the condition of inhibited mTOR activity by rapamycin in concentration 1 and 10 nM was analyzed. It was detected the decrease of cell adhesion up to 40% at different time points. Besides, it was shown small but statistically significant reduction of cell spreading on the growth surface.
In the condition of mTOR inhibition there was up to 80% decrease of cell migration in “wound healing” model. Therefore the effect of rapamycin on cell cytosceleton reorganization was determined. It was shown the apparent change in actin cytoskeleton organization in paranuclear space using falloidin detection of F-actin. In addition some decrease of MMP-9 activity in the presence of rapamycin was confirmed by zymography method.
Conclusions: There is the first evidence of mTOR presence in nucleoli. The most prominent effect of mTOR activity inhibition was observed in the assay of migratory potential of cancer cells, as well as on the cytoskeleton remodeling. Further study of the role of mTORα and novel splicing isoform mTORβ in tumour progression will be developed.

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The Study of Cancer Metastasis Auxiliary Therapeutic Strategies, Using Metabolomics in Silico Simulation Methods

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Background: There are many distinctive differences among normal and cancerous cells, which can be assimilated in design of new strategies against cancer. When combined with proteome and genome studies, metabolite-profiling analyses reveals unanticipated insights into the cellular pathways. Old therapeutic patterns focus on simple single cause and effect relations despite of systematic over view of whole organized living system, and almost old treatment methods have their own unwanted effects on whole system organization.
Materials and Methods: Metabolic grids related to fatty acid and membrane component synthesis involved in the metastatic process of malignant cells and other raw materials extracted from databases (e.g. KEGG, PANTHER, etc.) and imported to the applications, CELLDESIGNER and GEPASI, as qualitative and quantitative simulators. Then further manipulations in initial values and parameters were performed to make an interactive simulation study leading to the functional steps.
Results: As the first result, we could obtain a real-time model of several metabolic grids which play a role in the living cell. This model can be used for further manipulations and tiny changes in its metabolite concentrations instead of expensive analytical methods. We made simulation variations in the quantity of two metabolites involved in the membrane integration and consequently monitored the resulting changes. Increasing the amount of such metabolites, could lead to decreasing the concentration of other metabolites which play a main role in membrane over fluidity.
Conclusion: This method is an economic, time and labor saving, multiple functional ways in pre-lab stage of biologic health and treatment studies. Also we suggest a new method to study and impose artificial manipulations computationally in the normal or malignant biologic systems such as disorders without any known causing stress for living systems.

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Silencing of DREV1 Promotes Cell Proliferation and Invasion in Lung Adenocarcinoma

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Background: We have previously identified that decreased expression of DREV1 was associated with shorter survival in patients with advanced non-small cell lung cancers (NSCLCs). The exact mechanism how reduction of DREV1 leads to shorter survival is still not known. The objectives of this study was to determine the functions of DREV1 in cell proliferation, apoptosis and cell invasion and to identify the related molecular pathways.

Material and Methods: Quantitative Real Time PCR (qPCR) and Tissue Microarray Immunohistochemistry (TMA-IHC) were performed to validate the expression of DREV1. Silencing of DREV1 was carried out using DHARMACON® SmartPooled Small Interfering RNA (Thermo Scientific) on the A549 lung adenocarcinoma cell line. Cell viability and apoptosis were measured using CellTiter-Glo® Luminescent Cell Viability Assay (Promega) and Caspase-Glo® 3/7 Assay (Promega) respectively. The effect of DREV1 silencing on cell invasion was studied using QCM® 24-well Collagen-Based Cell Invasion Assay − Colorimetric (Chemicon). The downstream genes and signal cascades were interrogated using Illumina HumanRef-8 v3.0 Expression BeadChips. Data analysis was performed using Genespring version 10.0.
Results: qRT-PCR confirmed that the expression of DREV1 was significantly higher in the long survival group (n = 8) compared to the short survival group (n = 8). TMA-IHC showed the DREV1 expression was reduced in advanced stages (Stage III and IV) compared to the early stages (Stage I and II) of NSCLC. Silencing of DREV1 significantly increased cell proliferation, reduced apoptosis and increased cell invasion. Microarray gene expression analysis revealed that silencing of DREV1 activated SRC, GNAQ and PIK3R, mediators of PAR1 and PKY2/ERK/MAPK pathway which are associated with increased cell proliferation, migration and cell invasion.
Conclusion: Reduced expression of DREV1 may contribute to poor survival in NSCLCs through increased cancer cell proliferation and cell invasion, and reduced apoptosis.

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17AAG Inhibits TGF-beta1-induced Cell Migration in Mv1Lu Cells

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Background: TGF-beta is well known to induce cell migration in various cell types. Recently, Heat shock protein 90 (HSP90) has also been reported to be associated with cancer cell invasion and metastasis. However, it is unknown if they share the common mechanism to increase cell motility. In the present study, we investigated the relationship between TGF-beta1 and HSP90 on cell migration using the specific HSP90 inhibitor, 17-allylamino-demethoxy-geldanamycin (17AAG) in Mv1Lu cells.
Materials and Methods: Mv1Lu cells were treated with 17AAG and/or TGF-beta1. We investigated the differences of TGF-beta1 signaling and cell migration by using western blot analysis and trans-well migration assay. Truncated form of HSP90 (ΔHSP90) and active Smad2/3 constructs were also used for verification.
Results: TGF-beta1 increased cell migration in Mv1Lu cells. However, we observed significant reduction of cell migration in Mv1Lu cells, pretreated with 17AAG or transfected with ΔHSP90 regardless of TGF-beta1 treatment. We also examined whether the inhibition of HSP90 by 17AAG or ΔHSP90 attenuate TGF-beta1 signaling through inactivation of Smads. Regardless of TGF-beta1 stimulation, Mv1Lu cells pretreated with 17AAG or transfected with ΔHSP90 showed the attenuation of phospho-Smad2 and phospho-Smad3. The attenuated Smads signaling was also confirmed by localization of Smad4. Additionally, transfection with constitutively active Smad2 (Smad2-EE) or Smad3 (Smad3-EE) significantly increased cell migration. Although Smads signaling was activated by Smad2-EE or Smad3-EE, cell migration was reduced upon HSP90 inhibition by 17AAG or ΔHSP90.
Conclusions: Thus, our data strongly suggest that HSP90 modulates TGF-beta1-induced cell migration through the regulation of Smads signaling.

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Effects of Zoledronic Acid and Denosumab on Human Vγ9Vδ2 T-cell-Mediated Cell Death of RANK-Expressing Breast Cancer Cells

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Background: Zoledronic acid (ZOL) inhibits osteoclast (OC)-mediated osteolysis by blocking farnesyl pyrophosphate synthase (FPPS), leading to accumulation of isopentenyl pyrophosphate (IPP), a phosphoantigen for anticancer gamma-delta T cells (Vγ9Vδ2). Indeed, interleukin 2 (IL-2) + ZOL stimulates expansion of Vγ9Vδ2 T cells from human peripheral blood mononuclear cells (hPBMCs), and ZOL induces IPP accumulation and secretion by breast cancer (BC) cells, resulting in activation and chemotaxis of Vγ9Vδ2 T cells to BC tumours. Denosumab (Dmab), a fully human monoclonal antibody against RANKL, inhibits osteolysis by blocking RANKL rather than FPPS. As RANKL may also play a role in activating BC cells, the effects of Dmab on BC are unknown.
Material and Methods: Effects of ZOL and Dmab on (1) the expansion of Vγ9Vδ2 T cells and (2) Vγ9Vδ2 T-cell cytotoxicity toward RANK-expressing BC cells were evaluated in vitro. hPBMCs were obtained from healthy donors. Expansion of Vγ9Vδ2 T cells in hPBMCs and RANK expression by BC cells were evaluated by flow cytometry, and IPP accumulation was measured by mass spectrometry. BC cell lines studied had high (T47D) or low (MDA-MB-231/B02; B02) FPPS activity.
Results: IL-2 + ZOL (1−10 μM) but not + Dmab (0.001−0.1 mg/mL) caused expansion of Vγ9Vδ2 T cells. Adding Dmab to IL-2 + ZOL did not block Vγ9Vδ2 T-cell expansion. This lack of Vγ9Vδ2 T-cell modulation was observed despite substantial in vitro activity of Dmab to inhibit RANKL- and macrophage colony-stimulating factor-induced OC differentiation from hPBMCs (⩾2.5× inhibition by 0.001−0.1 mg/mL Dmab treatment). ZOL (1−10 μM, 1h) caused high IPP accumulation in T47D but not B02 cells. RANKL-stimulated T47D BC cells were targeted for IPP-dependent cytotoxicity by Vγ9Vδ2 T cells after ZOL, but not Dmab, pretreatment. B02 cells were not targeted under any of these conditions. Moreover, Dmab pretreatment (0.01 or 0.1 mg/mL) neither induced nor blocked Vγ9Vδ2 T-cell cytotoxicity against RANKL-stimulated T47D cells induced by ZOL pretreatment.
Conclusions: These data suggest that BC cells producing high IPP levels after ZOL treatment are most likely to respond to Vγ9Vδ2 T-cell-mediated immunotherapy. Dmab had no immunomodulatory effects at concentrations that inhibit OC differentiation.

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The Clinical Significance of RCAS1 Expression in Primary Lung Neoplasms

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Background: RCAS1 (Receptor-binding Cancer Antigen expressed on SiSo cells) is a membrane protein that is expressed in different types of cancer. It halts the cell cycle and/or induces the apoptosis of the immune system cells within the tumour microenvironment. Hence, it is possible that this molecule is involved in the mechanism of the tumour cells' escape from the immune system surveillance (immunoescape).
Material and Methods: Patients with primary lung cancer, eligible for surgical treatment, were included in the study. The tissue samples (paraffin cubes) were processed using the Tissue Micro-arrays Method. Then, an immunohistochemical study followed, specific for the RCAS1 and the Ki-67 (a cell proliferation marker). The image analysis was feasible due to a special program. In addition, a database was created that included the clinical and pathological characteristics of the patients.
Results: In total, 108 patients were examined (81 men and 27 women), mean age 62 years old. Almost 44% of the cases were adenocarcinoma, 31% squamous cell, 9% large cell, and 16% other types of lung cancer. Associations between variables were analyzed by the application of Univariate Analysis Of Variance with SPSS v15.0 software (SPSS Inc., Chicago, IL, v.15.0). Two tailed p values ⩽0.05 were considered to be statistically significant. Statistical significance was identified correlating RCAS1 overall expression to grade III of the tumours (p-value 0.006) and in a positive correlation between RCAS1 and Ki-67 (p-value 0.005). Moreover, there is a trend of RCAS1 over-expression in advanced or metastatic stages. In contrast, protein expression was not strongly associated to tumour size, to histological type, to patient age or to gender.
Conclusions: The most important conclusions of this study are that there is an over-expression of RCAS1 protein mainly in grade III lung cancers and that there is a positive correlation between RCAS1 and Ki-67 expression which means that when the Ki-67 increases the expression of RCAS1 is higher.

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Overexpression of Calreticulin in Malignant and Benign Breast Tumours: Relationship to the Humoral Immunity

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Introduction: Calreticulin is a multicompartmental protein which regulates a wide array of cellular responses in physiological and pathological