1069 POSTER

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.

1070 POSTER

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.

1071 POSTER

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.

1072 POSTER

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.