Translational Research and Drug Development

Inhibition of malignant features of 293_*CHI3L1* Cells

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Aim: To find the inhibitors of malignantly transformed 293 cells.

Methods: *CHI3L1* knockdown approach by siRNA transfection of 293 cells, which stably produced CHI3L1 oncoprotein.

Results: Among the genes with the pronounced increased expression in glioblastoma, the most aggressive form of brain tumors was *CHI3L1*, encoding the secreted chitinase 3-like 1 protein. CHI3L1 can decrease the doubling time of 293 cells, allows the anchorage independent growth in soft agar and in addition, stable *CHI3L1* expression made 293 cells tumorigenic: these cells stimulated the initiation of tumors after their transplantation into the rat brains. 293_*CHI3L1* cells had activated extracellular signal-regulated kinases (ERK1/2)-mediated MAPK and AKT-mediated phosphoinositide 3-kinase (PI3K) pathways; phosphorylated ERK1 and ERK2 were localized in both cell cytoplasm and nuclei while AKT localized only in cytoplasm. 293_*CHI3L1* cells differed from 293 cells transfected by an "empty" vector in their size and ability to adhere to the culture plate. Thus, the overexpression of *CHI3L1* is likely to have an important role in tumorigenesis and orthotopic implantation of transformed human cells with overexpressed human oncogene *CHI3L1* to the rat brain presents a new model of human brain tumor which can be used as a target for anticancer drug development.

To dissect the relationship between CHI3L1 and tumorigenesis, we employed a *CHI3L1* gene knockdown approach by siRNA transfection in 293 cells, which stably produced CHI3L1. Seventy two hours post-transfection, a noticeable protein blockade (80-90%) was obtained in 293 cells producing CHI3L1 with two different siRNAs to *CHI3L1* at concentrations ranged from 10 to 100 pM as compared with the control cells, which were transfected by control siRNA. It was confirmed by Western blotting in both 293_*CHI3L1* cells and U87MG cells. To identify further the influence of CHI3L1 on the intracellular signaling pathways, we then measured levels of pERK1 and pERK2. An active level of pERK1/2 was significantly reduced in 293 cells, which overexpressed *CHI3L1*, after transfection with siRNA_*CHI3L1* relatively to control cells. Despite reduction of *CHI3L1* expression in 293_*CHI3L1* cells we did not observe any morphological changes in these cells. CHI3L1 suppression 5,5-fold reduced the colony-forming ability of 293_*CHI3L1* cells after specific inhibition of CHI3L1 production as compared to control cells. For delivering of the specific siRNAs to the tumor, they were conjugated with nanoparticles which could penetrate through blood-brain barrier.

Conclusions: The obtained results demonstrate that activity of CHI3L1 mediated by pathways involved ERK1/2 and AKT has a growth-promoting role during tumorigenesis and indicate that efforts to inhibit its activity should be considered during cancer therapy.

The main milestones on the way to thiazolidinone based anticancer agents search

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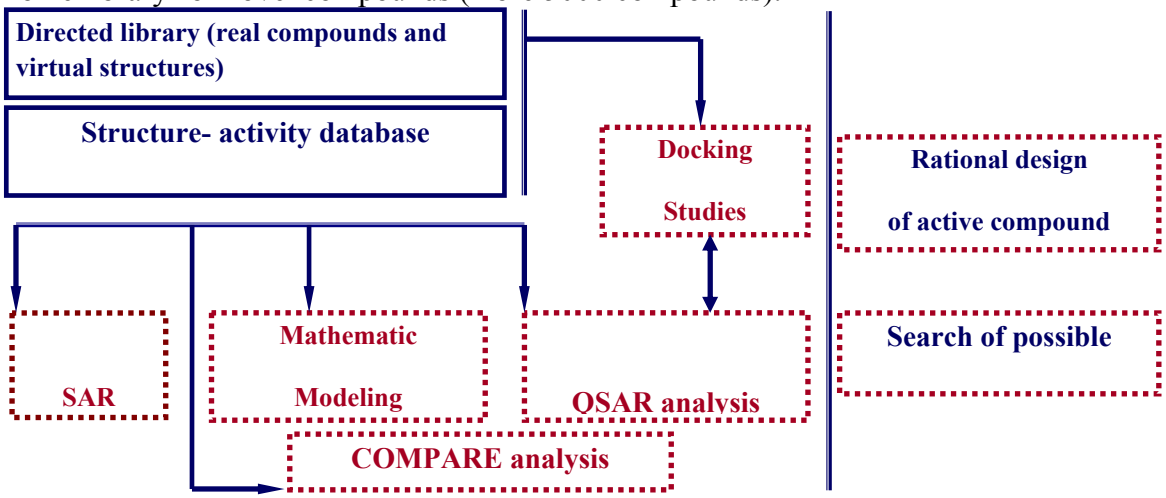
Aim. New biological active small molecules design based on the 4-thiazolidinone matrix as well as the related heterocyclic building-blocks remains the main research area of our scientific group for decades. Encouraged by our previous results and current achievements in the study of thiazolidinones as representatives of privileged scaffolds in new drugs creation we have focused on the search of innovative anticancer agent with unique mechanism of action and satisfactory toxicological profile. Presented projects are based on 3 strategic vectors: organic synthesis, pharmacological research and rational design of "drug-like" molecules. **Results**: Chemical diversity of 4-thiazolidinones and related heterocyclic systems allows to obtaine the directed "in-home library" of novel compounds (more 5000 compounds).



Screening investigation of more than 1500 compounds provided to powerful structure-activity database formation which became the basis for a number of *in silico* studies (QSAR, docking, COMPARE etc). Formation and validation of a number of hypotheses regarding structure optimization in combination with pharmacological data allowed obtaining 170 compounds exhibiting high levels of antitumor activity; 14 of these compounds are considered as lead-compounds with probable apoptosis-dependent mechanism of action not typical for known anticancer agents. Further research allowed to form a number of requirements to rational design of thiazolidinone-based compounds including probable pharmacophore model creation, identification of desirable molecular fragments etc. The next phase of research that requires the closest cooperation with experts in experimental pharmacology and molecular biology is in progress and will clearly define the mechanisms of anticancer effects of highly active compounds. **Conclusions**: Real directed library of 4-thiazolidinone and related heterocyclic derivatives was obtained. Powerful structure-anticancer activity data is considered as the basis for highly active anticancer agents rational design in the row of above mentioned heterocycles using the whole arsenal of medicinal chemistry approaches. Investigation of the molecular mechanisms of lead-compounds anticancer effects realization as well as the drug-like properties optimization is the present stage of our project.

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Antiviral activity of sophorolipid a novel biosurfactant

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Aim: Testing antiviral activity of Sophorolipid with acidic and lactonic conformation (SLLA) against plus single stranded RNA, double stranded DNA, and segmented single negative stranded RNA viruses.
Methods: SLLA was synthesized using *Candida bombicola*. Its antiviral activities were tested in 3 different modes - virucidal or direct virus treatment, rapid culture assay for Influenza virus and treatment of host cell lines with SL prior to viral challenge. Challenge viruses used for the studies were CV (B1-CVB6, CA7, CA9), murid gammaherpesvirus, strain MHV-68, Influenza virus strain A/Mississippi/1/85 (H3N2). Cell lines used for testing the activity were GMK and Hep-2 for CV, VERO, BHK and 3T3 for MHV-68, MDCK for IAV.
Results: Direct treatment of virus indicated 1 $\log_{10}$- 4.5 $\log_{10}$ reduction in the virus titers. Pretreatment of cell cultures GMK and Hep-2 prior to CV infection showed a reduction in virus titer 1$\log_{10}$ - 2 $\log_{10}$. Similar results were obtained on the VERO, BHK and 3T3 cells with gamma-herpesvirus MHV -68. Visible but mild reduction of IAV replication on MDCK cells was obtained at the concentration 100 μg/ml of SLLA.
Conclusions: We conclude that the SLLA showed virucidal activity. This can be attributed to the amphiphilic structure of SL that may act directly on the virus capsid proteins or disturb the viral envelope. Relatively less activity was observed on different cell lines infected with CV, MHV-68 and IAV at a concentration 100 μg/ml SLLA.
Keywords: coxsackieviruses, murid herpesviruses, influenza virus, antiviral, sophorolipids

A role of calcium/calmodulin-dependent protein kinase ii in diabetic neuropathy

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Aim: Abnormalities in peripheral nerve and dorsal root ganglion (DRG) have been noted in early stages of experimentally-induced diabetes. The enzyme calcium/calmodulin-dependent protein kinase II (CaMKII) has been linked with neuropathic pain. The aim of this study was to compare expression of total CaMKII (tCaMKII) and its phosphorilated alpha isoform (pCaMKII-alpha) in rat models of diabets mellitus type 1 (DM1) and II (DM2), as well as their pain-related behavior.

Methods: Sprague-Dawley rats were injected with streptozotocin to get DM1, while DM2 was induced with combination of high-fat diet and low-doze streptozotocin. Pain-related behavior was analyzed with thermal and mechanical stimuli. Rats were sacrificed 2 weeks or 2 months after the induction of diabetes. Tissues were perfused with fixative and DRGs were sectioned. For detection of tCaMKII and pCaMKII-alpha, immunofluorescence was used and measured with Metamorph software.

Results: Two weeks after diabetes induction there wre no consistent changes in pain-related behavior in both types of rats. Significant changes were observed in hyperalgesic and withdrawal rsponses in DM rats compared to control after 2 months.

In DM1 rats we observed significant difference between control and diabetic rats in tCaMKII expression, while this difference was not observed in DM2 model. In both DM models pCaMKII-alpha expression was increased. Increase in pCaMKII expression ws significant in small, medium and large DRG somata.

Conclusions: Differences in pain-related behavior were observed in rat diabetes models after longer duration of diabetes. Experimentaly-induced diabetes increases expression of tCaMKII (in DM1) and pCaMKII-alpha (DM1 and 2) in sensory neurons. This may indicate involvement of this enzyme in tramsission of nociceptive input early in diabetes. CaMKII may be a suitable pharmacological target for diabetic neuropathy. The experiments are continued in DM1 and DM2 rats that will live for 2 months, 6 months and 12 months. The aim of the project is to study long-term effects of diabetes on neuropathic pain-related behavior and CaMKII.

Keywords: diabetic neuropathy, diabetes type 1, diabetes type 2, pain, CaMKII

Tumorigenic potential of adaptor/scaffold protein Ruk/CIN85 under hypoxia

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Aim: The expression of numerous genes, products of which are involved in tumorigenesis, is regulated by oxygen. While a large body of work concerning hypoxia-inducible factors, in particular HIF-1, has been carried out, a number of issues with respect to the role of certain adaptor proteins remains still open. The adaptor/scaffold protein Ruk/CIN85 plays important roles in various physiological processes such as apoptosis, ligand-induced endocytosis of receptor tyrosine kinases, cell adhesion and motility. The aim of the present study was to investigate the involvement of Ruk/CIN85 in the hypoxia-dependent regulation of gene expression.

Methods: Western-blot analyses were used to study the expression levels of corresponding proteins in MCF-7 breast adenocarcinoma cells. Ruk/CIN85-PHD2 interaction was shown by GST pull down and co-immunoprecipitation assays. HIF-1 activity was demonstrated by luciferase reporter gene assay.

Results: Transient or stable overexpression of Ruk/CIN85 induced HIF-1α protein levels and HIF-1 activity while knocking down Ruk/CIN85 reversed these effects. Knocking down HIF-1α abolished not only the hypoxia-dependent but also the Ruk/CIN85-dependent induction of plasminogen activator inhibitor-1 (PAI-1) that is known to be one of the major hypoxia-regulated genes. It is known that oxygen sensitivity of HIF-1 is primarily regulated on the level of α-subunit hydroxylation by prolyl hydroxylases (PHD). It was shown by using various Gal4-HIF1α fusion constructs that Ruk/CIN85 interfered with the destabilization of proline hydroxylation-dependent HIF-1 protein. Our data also indicate that Ruk/CIN85 reduced half-life of PHD2 which then leads to HIF-1α stabilization. By using GST pull down and co-immunoprecipitation assays we further showed Ruk/CIN85-PHD2 interaction.

Conclusions: Together, our findings indicate that Ruk/CIN85 is involved in the regulation of gene expression under hypoxia.

Keywords: carcinogenesis, adapter proteins, Ruk/CIN85, hypoxia.

Quantitative proteomics in pre-clinical and clinical research

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Advanced proteomic technologies are capable of identifying thousands of protein species in a single proteomic analysis. This makes it highly perspective for answering important questions regarding the human health and disease at molecular level. However, to obtain significant and unambiguous results, all phases of the proteomic project have to be planned with consideration, with special emphasis on selecting the best-suited proteomic approach. In our contribution we would like to provide a basic overview of high-throughput quantitative proteomic approaches that are well applicable to the pre-clinical and clinical research and summarize their pros and cons. We further want to share our experience with implementing proteomics into biomedical research and show results recently achieved in our lab, with emphasis on characterization of differences in protein quality and quantity in pre-clinical and clinical samples.

Keywords: preclinical and clinical proteomics

Influence of testosterone propionate on urea, total protein and protein fractions levels in serum, kidney and heart tissues of rats with food deprivation

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On the white lab-rats breed male aged 5-6 months, weighing 260-270h, which held in extreme conditions (food deprivation for 9 days), The influence of testosterone propionate on the content of urea, total protein and protein fractions in serum, tissues kidney and heart of rats. It is established that the level of urea in the blood serum of rats, which used intramuscular testosterone propionate significantly greater than in the control group to 1.830 mmol / l and compared with the intact group at 2.685 mmol / liter. The content of total protein in serum intact animals less than controls at 1.253 mg / ml and compared with experimental group at 2.373 mg / ml (P> 0,05). Also the most important content decreases β-globulin fractions of 14.27% and γ-globulins at 11.19%, and the contents of AL and α-globulin in serum increased by 14.7 and 15.07%. The level of low-protein (prealbumines) in serum of rats is reduced by 2.74 - 2.83%.
When food deprivation in rats under the influence of testosterone propionate, the level of total protein in kidney tissues more than in the control group to 1.79 mg / ml and less than in intact animals at 0.80 mg / ml (P> 0.05) . There is a lower content of β-globuline fraction of proteins in kidney tissue and a significant increase in α-globulins and γ-globulins.The content of total protein in rat heart tissue homogenate was greater than in the control and intact groups at 2.18 and 0.39 mg / ml (P> 0.05).
We have found significantly lower levels of β-globulin in heart tissue homogenate in the experimental group of rats compared with controls at 4,0% (P <0,05). Impact (F) between two gradations (II / III group) significantly from 7.778., Impact strength (η2) is 52.6%. The content of protein fractions AL; α-globulin was also lower than in the control group, respectively by 5.4 and 4.3% (P> 0.05). The level of protein fractions pAL and γ-globulin in experimental group larger than controls at 5.55 and 8.15% and greater than the intact group at 11.08, 9.42% - respectively (P> 0, 05).
Keywords: Testosterone propionate, food deprivation, protein, protein fraction

Difference in virulence phenotype of Coxsackieviruses B4 and B5 isolates

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Aim: To study the differences in the virulence phenotypes of coxsackievirus (CV) isolates from stool samples of aseptic meningitis cases and sewage isolates from the area of domicile of the patients.
Method: 2 patient's stool isolates and 2 isolates from treated sewage identified as CVB4 and CVB5 were accessed in this study. CD1 outbred mice were infected orally. Histopathological changes and presence of viral RNA in heart, pancreas and brain at days 5, 10 and 49 post infection (p.i) were studied. Sequencing of 5' non coding region (NCR) was done. The sequences were aligned using the ClustalW method, BioEdit or commercial Lasergene. Sequences were compared with standard sequences in the world database by Nucleotide Blast.
Results: Infected mice showed presence of viral RNA in pancreas, brain and heart at days 5 and 10 p.i. Persistence of viral RNA was observed in the brain of the mice till day 49 p.i. Hyperemia and oedema was observed in the brain of infected mice. Pancreas of mice infected with CVB4 isolates from the sewage showed inflammation. Differences in the VP1 sequenced region were absent.
Conclusions: In conclusion we suggest that the presence of virus and persistence of viral RNA in the organs of CD1 mice was related to the origin of the virus strain. Furthermore our results suggest that the virus shedded in the sewage, a CVB4 serotype, had undergone a genetic change not located in the 5'NCR, but creating a pancreotropic phenotype inducing pancreatitis in mice.
Keywords: coxsackievirus, viral RNA, inflammation, aseptic meningitis