Abstracts
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Cancer Research Oral Presentations
Senior Scientist' Forum

Molecular theranostics in cancer metastasis

[1]Chung Leland W. K., [1]Yang Xiaojian, [2]Tong Rong, [2]Cheng Jianjun, [1]Wang Ruoxiang and [1]Zhau Haiyen E.

[1]Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center; Los Angeles, CA 90048, USA;

[2]Department of Materials Science and Engineering, University of Illinois, Urbana, IL61801, USA

Leland.Chung@cshs.org

Background: Cancer metastases are the major contributing factors to the lethality and morbidity of the patients. Thus far the majority of the new-targeted therapies are designed based on our understanding of inter- and intra-cellular signaling pathways between and within cancer cells and the unique biology of organ-specific expression of molecular targets. Since the mortality of cancer has not been improved significantly, new approaches are needed to reduce the occurrence and the progression of human cancer. In this context, we have developed a novel class of near infrared (NIR) organic heptamethine cyanine dyes (HMCD), which has unique properties of uptake and retention in cancer but not normal cells. We prepared chemical conjugates of these dyes and found them to be ideal theranostic agents for cancer imaging and therapy. These compounds are tested for their ability to eradicate prostate cancer growth and metastases in mouse models.

Objectives: We seek to achieve the following three objectives: 1) to develop a class of effective HMCD to image cancer cells; 2) to modify chemically HMCD to create a new class of theranostic agents for dual imaging and targeting of cancer cells in culture; and 3) to investigate the effectiveness of systemic HMCD-drug conjugates to eradicate preexisting prostate tumors grown in mice either at subcutaneous space or in mouse tibia.

Results: MHI-148 and IR-783 are non-toxic and effective imaging agents for human cancer cells in culture and human tumor xenografts in mice; a close structural analogy of the IR-783, the IR-780, was found to be less effective and more toxic. MHI-148 and IR-783 uptake into cancer cells through an organic anion transporter-mediated mechanism. We employed these dyes as carriers for the therapeutic drugs, which were covalently conjugated to the dyes to create novel theranostic agents. We synthesized two prototypes of dye-docetaxel conjugates, IR-MUT-1 and IR-MUT-2, and found both of these dye-drug conjugates highly effective against the growth of prostate cancer cells in culture and prostate tumor xenografts grown either subcutaneously or intratibially in mice.

Conclusion: A new class of HMCD-based imaging and targeting theranostic agents has been synthesized and tested in human cancer cells and human tumor xenografts. These conjugates were found to retain in tumor but not in normal tissues and exert cytotoxic effects against the growth of human tumor but not the regression of normal tissues. This class of novel theranostic agents may be applicable in human for the eradication of cancer and cancer metastases.

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Thiazolidinone motif in anticancer drug discovery. Experience of DH LNMU medicinal chemistry scientific group

Lesyk R.B., Zimenkovsky B.S., Kaminskyy D.V., Kryshchyshyn A.P., Havryluk D.Ya., Atamanyuk D.V., Subtel'na I.Y., Khyluk D.V.

Department of Pharmaceutical, Organic and Bioorganic Chemistry,
Danylo Halytsky Lviv National Medical University,
69, Pekarska St. Lviv, Ukraine, 79010

dr_r_lesyk@org.lviv.net; dankaminskyy@gmail.com

Background. Recently 4-thiazolidinone and related systems research area unexpectedly became interesting and promising in oncology field. The success in anticancer agents search among 4-thiazoldinones derivatives realized in identification the rows of molecular mechanisms and biological targets of mentioned compounds antitumor action. Discovery of new biological active compounds base on azolidinones and related systems refer to one of the most successful project of DH LNMU. It is based on 3 strategic vectors: organic synthesis, pharmacological research, rational design of "drug-like" molecules ((Q)SAR-analysis, molecular docking etc.) In spite of the series of perspective results, progress of the project brings to some research directions changes, notably it has focused on the search of new anticancer agents.

The **aim** was formation of some rational design directions of potential anticancer agents base on the 4-thiazolidinones and related heterocyclic systems anticancer activity data analysis.

Results. Synthetic research carried out in DH LNMU allowed us to propose a whole number of new chemical directions of biological active 4-thiazolidinones and related heterocyclic systems design and obtain directed library that numbers over 5000 of novel compounds. *In vitro* anticancer activity screening was carried out for more than 1000 compounds (DTP NCI protocol), among them 167 compounds showed high antitumor activity with low toxicity level. The desirable molecular fragments with crucial influence on activity level as well as main position for structure modification of core rings in each of synthesized 4-thiazolidinones sub-libraries were determined base on SAR analysis. For the purpose of optimization and rational design of highly active molecules with optimal "drug-like" characteristics, building the prediction models and discovering of possible molecular mechanisms of action QSAR, COMPARE analysis and molecular docking were carried out.

Conclusion. Based on systematic combination of pharmacological screening methods and *in silico* data the anticancer activity is determined as privileged for thiazolidinones and related heterocyclic systems that allowed identification of "hit-compounds" series. Some aspects of structure-activity relationships were determined in 4-thiazolidionones derivatives sub-libraries and structure rational design directions of anticancer agents were proposed. Among tested compounds 167 samples showed high antitumor activity level and their in-depth preclinical studies are in progress.

Cancer Research Oral Presentations
Young Scientists' Forum

Candidate SEREX-identified antigens for detection of breast cancer autoantibody profile

[1,2]Kostianets O. I., [1,2]Shyian M. A., [2]Demidov S. V., [1]Filonenko V. V., [1]Kiyamova R. G.

1 Institute of Molecular Biology and Genetics of the NAS of Ukraine,
150, Zabolotnogo St., Kyiv, Ukraine, 03680
2 Taras Shevchenko National University of Kyiv, Kyiv, Ukraine
64, Volodymyrska St. Kyiv, Ukraine, 01033

kostjanez@ukr.net; filonenko@imbg.org.ua

Autoantibodies to tumor-associated antigens can serve as reporters of molecular changes associated with cell transformation, which in turn opens up new possibilities for diagnosis, prognosis and monitoring of cancer. The production of autoantibodies reflects the immune response to a continuous remodeling of cells or tissues caused by a cell transformation process. The antibodies are the most appropriate cancer diagnostic tools, since these molecules are very stable, and its nano- and pico-molar concentration can be detected at the early stages of pathological process, including tumorogenesis, even before the onset of clinical symptoms.

The aim of our study was to identify and characterize novel tumor-associated antigens of medullary breast carcinoma (MBC) for the creation of test system for monitoring of autoantibody "signature" for diagnostics and prognosis of different types of breast cancer.

Medullary breast carcinoma despite the high degree of malignancy has a good prognosis for patients and characterized by heavily lymphocytic infiltration, that makes it an ideal target to search for tumor-associated antigens of breast cancer. In the course of this study using a modified SEREX methodology we have identified 42 genes, which products are involved in different cellular processes. Preliminary allogenic screening by phage expressed system allowed us to select 12 antigens for verification them as potential tumor-associated antigens in expanded allogenic screening in ELISA format. Up to date five MBC antigens were cloned, expressed, purified in bacteria and tested in ELISA with sera of breast cancer patients (n=18) and healthy donors (n=10). Increased levels of autoantibodies against three of them (RAD50, HMGN2, PDCL) were confirmed in sera of cancer patients compared with the control group. RAD50 (human Rad50 homolog of Saccharomyces cerevisiae), HMGN2 (high-mobility group nucleosomal binding domain 2) and PDCL (Phosducin-like protein) proteins have intracellular localization and are likely attacked by B-cells due to necrosis or apoptosis of cancer cells.

For creation of representative test-system for detecting of antibody "signature" with sufficient sensitivity and specificity more than three antigens should be included in this antigenic panel. So, further investigations of immunogenicity of the rest of MBC antigens should be continued in large scale ELISA screening.

Adaptor protein RUK/CIN85 influences hypoxia-inducible factor 1 alpha stability in tumor cells

[1]Kozlova N., [1]Samoylenko A., [2]Kietzmann T., [1]Drobot L.

[1]Palladin Institute of Biochemisry, National Academy of Sciences of Ukraine, 9, Leontovycha Str., Kyiv, Ukraine, 01601

[2]University of Oulu, Oulu, Finland

kozlovanina@gmail.com

Tumor hypoxia is a well-known micro environmental factor that causes cancer progression and resistance to cancer treatment. Such hypoxia is due to limited amount of vasculature, leaky or otherwise poorly functional tumor vessels with chaotic architecture. This involves multiple mechanisms, which are mediated through transcriptional gene activation by the hypoxia inducible factors (HIFs). Hypoxia-inducible factor-1 (HIF-1) is a DNA-binding protein that regulates transcription of a number of genes involved in maintaining biological homeostasis. HIF-1 is a heterodimer composed of an oxygen-destructible α-subunit and a β-subunit, levels of which are not dependent on the presence of oxygen. While the existing body of work provides a general picture of hypoxia-dependent gene expression, the roles played by individual molecular components are uncertain. We recently demonstrated that SH3 domain-containing adaptor/scaffold protein Ruk/CIN85 could induce the expression of plasminogen activator inhibitor-1 (PAI-1) gene via HIF-1. The aim of present study was to characterize the mechanisms by which Ruk/CIN85 influences HIF-1alpha-dependent gene expression. It was shown that transient as well as stable overexpression of Ruk/CIN85 induced HIF-1alpha protein levels and HIF-1 activity, while knocking down Ruk/CIN85 reversed these effects. To investigate whether Ruk/CIN85 may interfere with HIF-1α stabilization or transactivation, the cells were co-transfected with the luciferase reporter construct pG5-E1B-Luc that contains 5 copies of a Gal4 response element and vectors allowing expression of fusion proteins consisting of the Gal4-DNA binding domain (Gal4) and either HIF-1α N-terminal (TADN) or C-terminal (TADC) transactivation domains along with the Ruk/CIN85 expression vector. It was found that Ruk/CIN85 interfered with the prolyl hydroxylation-dependent HIF-1α protein destabilization but not with asparagine hydroxylation-dependent HIF-1α transactivation. It is well established, that HIFs are regulated in response to oxygen availability by a family of iron- and 2-oxoglutarate-dependent dioxygenases, the HIF prolyl hydroxylases (PHDs). PHDs inactivate HIFs in normoxia by activating degradation of the HIF-α subunit but release HIF activation in poorly oxygenated conditions. Since it was found that Ruk/CIN85 interfered with the prolyl hydroxylation-dependent HIF-1α protein destabilisation, we next investigated whether Ruk/CIN85 may interact directly with PHDs. By using co-immunoprecipitation assays it was shown that Ruk/CIN85 forms complex with PHD2. In conclusion our data show that Ruk/CIN85 is involved in modulation of the effects of hypoxia in tumour cells. Due to the fact that hypoxia is a well-known factor that causes resistance to cancer therapy, namely radiation therapy and also chemotherapy, and since Ruk/CIN85 influences HIF-1 alpha stability, it may be a potential agent in cancer targeting therapy.

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Analysis of potential ovarian cancer biomarker NaPi2b (*SLC34A2*) expression in ovarian cancer

[1,2]Shyian M. A., [1]Gryshkova V. S., [1,2]Kostianets O. I. [1]Filonenko V. V., [1]Kiyamova R. G.

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
150, Zabolotnogo St., Kyiv, Ukraine, 03680
[2] Taras Shevchenko National University of Kyiv, Kyiv, Ukraine
64, Volodymyrska St. Kyiv, Ukraine, 01033

m.a.shyyan@gmail.com; filonenko@imbg.org.ua

Identification and characterization of novel biomarkers as potential therapeutic and diagnostic targets in ovarian cancer is very important due to poor prognosis of this deadly disease. In recent publications, phosphate transporter NaPi2b was considered as potential biomarker for breast, thyroid and ovarian cancer. Sodium dependent phosphate transporter NaPi2b is coded by the *SLC34A2* gene and involved in maintaining of phosphate homeostasis in human body. Mutations in this gene and its aberrant expression are associated with several diseases including cancer. However, data about NaPi2b mRNA expression in different histomorphological types of cancer are controversial and restricted.

The main purpose of this study was to estimate the *SLC34A2* gene expression in ovarian cancer tumors of different histomorphological types and differentiation grades.

We investigated *SLC34A2* gene expression level in serous (6), endometrioid (4), mucinous (2) benign tumors (6) and normal ovarian tissue (5) using Real-Time PCR analysis. Differences in gene expression were calculated as fold changes in gene expression in ovarian carcinomas compared to normal ovaries. It was found that *SLC34A2* gene was highly overexpressed in endometrioid tumors (20.2-fold increase) and serous tumors (116.9-fold increase), but not overexpressed in mucinous tumors compared to normal tissue. Benign tumors showed insignificant increase of *SLC34A2* mRNA expression (from 1 to 6-fold increase), except one case of mucinous cystadenoma (396.9-fold increase). Analysis of *SLC34A2* gene expression according to tumor differentiation grade (low, moderate or high) in the endometrioid and serous histological types of ovarian cancer showed that *SLC34A2* tend to reduce level of gene expression in less differentiated tumors.

The down regulation of *SLC34A2* gene expression may reflect cell dedifferentiation during ovarian cancer malignant transformation and could serve as potential marker for ovarian cancer diagnosis and prognosis.

Combination of arginine deprivation and canavanine treatment as anticancer approach: study in 3-D culture

[1]Vynnytska B., [1]Bobak Y., [2]Garbe Y., [1]Kurlishchuk Y., [2]Kunz-Schughart L., [1]Stasyk O.

[1] Department of Cell Signaling, Institute of Cell Biology, National Academy of Sciences of Ukraine, 14/16, Drahomanov St. Lviv, Ukraine, 79005

[2] OncoRay, Center for Radiation Research in Oncology, Faculty of Medicine Carl Gustav Carus, TU Dresden Fetscherstr. 74, PF 41,01307 Dresden

bozhena@litech.net; stasyk@cellbiol.lviv.ua

In vitro cancer cells are thought to be more sensitive than normal ones to the toxic effects of arginine deprivation. Unlike normal cells that enter quiescence upon arginine limitations and can be recovered by arginine supplementation, some cancer cells are unable to do so and exhibit apoptic signs under arginine-deprived conditions. Our data suggest that in monolayer culture cancer cells can also be divided into sensitive or resistant according to the differences in the cell behavior upon arginine limitation.

In the present study we applied 3-D culture system to check whether differences in cell sensitivity to arginine deprivation revealed in monolayer culture will be preserved during culturing in the form of multicellular tumor spheroids. We showed for the first time that in 3-D spheroid culture all tested cancer cells (despite their level of sensitivity to arginine withdrawal in 2-D monolayer culture) are much more resistant to arginine deprivation and retain growth potential even upon prolonged starvation.

Enhanced resistance of cancer cells to arginine withdrawal in 3-D spheroid culture, which most probably will be also well manifested under *in vivo* conditions, warrant the search for the approaches that could be used in combination with arginine starvation to accelerate its antineoplastic potency. That is why another task of present study was to estimate the antitumor properties and potential clinical efficacy of the combination of arginine deprivation and canavanine treatment (shown by us to be efficient in 2-D culture, Vynnytska et al., Anticancer Drugs, 2011) in 3-D culture. We demonstrated that although in the form of spheroids cells were more resistant to the application of arginine deprivation and canavanine treatment than respective monolayers, the proposed combinational approach was still efficient in reducing malignant cell growth, survival and recovery and retained its selectivity against malignant cells. These data approve further development of the combination of arginine deprivation and canavanine treatment as anticancer approach and warrant it's testing *in vivo* on animal models.

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Cancer Research Network Meeting
Oral Presentation

Synthesis and optical properties of new class of inorganic optical markers based on lanthanides emission for bio-medical applications

Podhorodecki A., Sojka B., Banski M., Misiewicz J.

Institute of Physics, Wroclaw University of Technology,
Wybrzeze Wyspianskiego 27, 50-370 Wrocław, Poland
artur.p.podhorodecki@pwr.wroc.pl

Introducing to medicine and biology concept of optical markers in tremendous way has changed the recent status of these two important disciplines. This was mainly due to strong development in imaging techniques which recently allow us to investigate both static as well dynamic properties of living cells, their components and their interactions with external factors or to investigate their response on drugs treatments.
However, recently used molecular markers including organic dyes, fluorescent proteins or chelates containing lanthanide ions have several significant limitations. Most of them emit light in visible spectral range, the emission is weak and characterize by efficient bleaching and blinking, obtaining varieties of colors is limited and needs to introduce different markers simultaneously, emission decay times are very short in most of the cases (ns) and many others. One of the alternatives for molecular markers is inorganic quantum dots (i.e. CdSe, CdS), which are recently in common use, in many academic works. However, even if they are much better from physico-chemical point of view, from the application point of view at this moment they are rather useless. This is mostly due to the fact that they are toxic from the definition and all these advantages are not worth to be used in the view of high risk during in vivo imaging and not know side effects caused by using these markers.
One of the solution combining advantages of both concepts [molecular markers and quantum dots] is to make nontoxic inorganic nanocrystals doped by lanthanide ions. These markers except close to zero toxicity are characterized by emission or excitation in infrared spectral range and by long emission decay times (μs or seconds). This strongly increases penetration depth of the light and improves emission signal quality. Both facts have strong consequences for application potential of these markers.
In this work, we will present results obtained for different inorganic markers doped by lanthanide ions [Au, Tb, ND, God]. All samples have been obtained by modified in our group co-thermolysis technique and characterized by many different structural and optical techniques. The aim of this work was to design and to synthesize these markers and to understand physical processes responsible for their emission and to optimize these processes to the physical limits. For this purpose, emission, absorption, emission decay times, micro-Raman, FTIR spectroscopies have been done for samples obtained in function of many technological parameters like ions concentration, core-shell architecture or different combinations of lanthanides in one nanocrystal.

Role of Wnt11 in regulating Alveolar Type II (ATII) differentiation: evidence from clinical samples and a three-dimensional lung tissue model

Bartis Domokos, Pongrácz Judit E.

Department of Medical Biotechnology, University of Pécs, Hungary
12 Szigeti ut, H-7524, Pécs, Hungary
domokos.bartis@aok.pte.hu

Adult stem cells are in the forefront of interest for lung tissue regeneration therapies in diseases resulting in excessive lung injury. Progenitors that are vitally important for airway repair of alveolar type I (ATI) cells which form the primary gas-exchange surface of the alveoli, are cells designated as alveolar type II (ATII) which can flexibly turn into ATI cells on physiological demand. Factors that can regulate ATII differentiation would have particularly high importance in regeneration therapy by increasing the number of ATII cells thus the regeneration potential of injured lung tissue. Since primary lung epithelial cells lose ATII characteristics in conventional 2-dimensional (2D) cultures, the construction of an in vitro human pulmonary 3-dimensional (3D) tissue-model was imperative to investigate and identify such factors.

A 3D micro-tissue model consisting of commercially available human adult primary cells has been set up to determine factors regulating ATII differentiation. Cellular differentiation factors in the micro-tissue model were determined using quantitative reverse-transcription polymerase chain reaction analysis, histology, and immuno-fluorescence microscopy.

Pulmonary epithelial cells in the environment of the micro-tissue model regain and maintain cellular differentiation of the ATII phenotype, as suggested by morphological changes as well as increased expression of differentiation markers Aquaporin-3, Thyroid transcription factor-1 (TTF-1) Surfactant proteins A and C, and E-cadherin. Furthermore, our studies provide the first evidence that Wnt11 is one of the main regulators of ATII type differentiation and added Wnt11 can increase expression of the above ATII markers. In contrast, silencing of Wnt11 resulted in decreased expression level of ATII markers and elevated levels of epithelial-mesenchymal transition (EMT) markers S100A4 and N-cadherin. Consistent with the findings in the tissue model, Wnt11 treatment of surgical lung tissue samples resulted in the up-regulation of ATII markers.

The 3-dimensional tissue model is highly applicable for studying intercellular interactions in the lung. In this easy maintained and flexible tissue system Wnt11 was identified as a factor that regulates ATII differentiation. This finding may designate Wnt11 as a potential therapeutic target, which enhances the repair potential of pulmonary epithelium.

Differential human cancer cells sensitivity to individual amino acid deprivation

[1]Kurlishchuk Y., [1]Vynnytska B., [1]Bobak Y., [2]Dittfeld C., [2]Kunz-Schughart L., [1]Stasyk O.

[1] Department of Cell Signaling, Institute of Cell Biology, National Academy of Sciences of Ukraine, 14/16, Drahomanov St. Lviv, Ukraine, 79005

[2] OncoRay, Center for Radiation Research in Oncology, Faculty of Medicine Carl Gustav Carus, TU Dresden

stasyk@cellbiol.lviv.ua

In this work we aimed to compare the effect of starvation for essential amino acids leucine, lysine and methionine with the lack of arginine (shown by us to be also essential for tumor cells *in vitro*; Bobak *et al*. Cell Biol Int, 2010) on cancer cells in 2-dimensional (2-D) and 3-dimensional (3-D) cultures. For this purpose, two human cell lines that differed in response to arginine deficiency in 2-D culture were chosen. Hepatocarcinoma cells HepG2 were considered sensitive to arginine deprivation due to the time-dependent apoptosis induction and loss of growth recovery after starvation. Contrary, lung adenocarcinoma cells A549 were referred as resistant because they retained ability for proliferation after durable arginine deficiency and showed no signs of apoptosis upon starvation.

It was shown that the absence of each of the chosen amino acids inhibited proliferation of tested cells in 2-D culture. Moreover, the lack of leucine or lysine did not influence cell viability during the whole experiment. By contrast, methionine starvation induced cell death already after 2 days of incubation. The deficiency of leucine or lysine had little effect on growth restoration ability of both lines tested and cells exhibited regrowth even after 3 days of starvation. The absence of methionine had the most prominent influence on malignant cell lines. Thus, HepG2 cells did not restore their proliferation already after 1 day of methionine starvation, whereas for A549 cells the time-dependent decrease of tumor cells response growth restoration potential was observed.

In order to evaluate tumor cells response to amino acids deprivation in 3-D culture, we investigated the effects of their limitation on the formation and growth of tumor spheroids. It was demonstrated that arginine deficiency completely prohibited aggregation of A549 cells, while HepG2 cells formed cell clusters under the same conditions, although of smaller size when comparing with control. The lack of leucine, lysine or methionine did not prevent aggregation of neither tested cells. In contrast with 2-D models, spheroids of both A549 and HepG2 cells were able to rescue growth even after prolonged (10 days) starvation. The molecular reasons for this phenomenon remain to be elucidated.

In summary, it was shown for the first time that spheroids of human epithelial cancer cells are significantly more resistant to single amino acids limitation in comparison with respective monolayer cultures. These data warrant application of the 3-D spheroid culture as a reliable testing platform for anticancer therapy based on amino acid restriction.

Drug design and structure-functional interrelationships underlying in molecular mechanisms of anticancer activity of novel thiazolidone derivatives

[1]Panchuk R. R., [1,2]Chumak V. V., [2]Fil' M. R., [3]Havrylyuk D. Ya., [3]Lesyk R. B., [1,2]Stoika R. S.

1 Institute of Cell Biology, NAS of Ukraine,
14/16, Drahomanov St. Lviv, Ukraine, 79005
2 Ivan Franko Lviv National University,
1, University Str, Lviv, Ukraine
3 Danylo Halytsky Lviv National Medical University,
69, Pekarska St. Lviv, Ukraine, 79010

rpanchuk@ukr.net; dr_r_lesyk@org.lviv.net; stoika@cellbiol.lviv.ua

Cancer chemotherapy represents a relevant challenge for chemists and oncologists. Thiazoles and thiadiazoles are useful heterocyclic compounds possessing numerous treatment actions including the anticancer one. Over 5000 novel thiazolidone derivatives were synthesized at Danylo Halytsky Lviv National Medical University, and three of them – Les-3120, Les-3166 and Les-3372 – were selected at National Cancer Institute (USA) as the most effective in inhibiting growth of carcinoma and leukemia cell lines. However, molecular mechanisms of their action were not studied.

Here we demonstrated that these compounds (5 uM, 24 h) caused splitting of anti-apoptotic PARP-1 and DFF45 proteins involved in DNA reparation. Their cleavage is mediated by the effector caspases-7 and -3 whose level was increased under action of mentioned drugs. However, while Les-3120 induced activation of these caspases in 12h, Les-3372 action was marked only in 24h. Les-3120 and Les-3166 also activated caspase-8 involved in receptor-mediated apoptosis and subsequent cleavage of Bid protein in 12h, while Les-3372 did not possessed such activities. However, Les-3372 induced activation of initiator caspase-9, which is key protein in mitochondria-related pathway of cell death, and release of pro-apoptotic protein AIF from mitochondria to cytosol. Thus, despite similar IC_{50} (5 uM) towards tumor cells, selected thiazolidone derivatives differ significantly in molecular mechanisms of their action: Les-3120 and Les-3166 induce receptor-mediated apoptosis, while Les-3372 switches on cell death via mitochondrial pathway.

Taking into account these data, an idea appeared to combine these unique features of Les-3120 and Les-3372 in one molecule. To reach that goal, *in silico* design of novel anticancer drugs was performed and two novel isomeric compounds, Les-3661 (4-subsituted thiazolidone) and Les-3713 (2-substituted thiazolidone) were synthesized. Their anticancer activity was studied by annexin V/propidium iodide assay, DAPI staining and Western-blot analysis. We found that both Les-3661 and Les-3713 were 5-10 times more active towards leukemia and carcinoma cell lines comparing the parental compounds. Location of substitution of active groups in thiazolidone plays crucial role in mechanisms of action of novel "hybrid" molecules. Les-3661 is the most active among known thiazolidones, and its IC_{50} 0.5 uM is comparable with doxorubicin. It induced receptor-mediated apoptosis via activation of initiator caspases-8 and -10 in 6h, while its isomeric form Les-3713 possessed 2 times weaker cytotoxic effect and activated initiator and effector caspases only in 12h. Thus, combining active groups of two different anticancer drugs in one molecule dramatically increased their activity towards specific cancer cell targets.

Effect of autophagy modulation on human ovarian carcinoma response to arginine deprivation

[1]Shuvayeva G. Y., [1]Groholska O. B., [1]Igumentseva N. I., [2]Isidoro C., [1]Stasyk O. V.
[1]Department of Cell signaling, Institute of Cell biology, National Academy of Sciences of Ukraine, 14/16, Drahomanov St. Lviv, Ukraine, 79005
[2] Laboratory of Molecular Pathology and Nanobioimaging, Department of Medical Science, Amedeo Avogadro University, 6 Via Duomo Vercelli, Novara, Alessandria, Italy. 13100
shuvayeva@cellbiol.lviv.ua

Autophagy is a major mechanism of cellular protein and organelle recycling, induced in response to nutrient starvation or metabolic stress. The role of autophagy in cancer progression and in response to various therapies is a topic debate in current biomedical science.

Recently we showed that deprivation for such amino acids as arginine, lysine, leucine or methionine, strongly induces autophagic response in the human ovarian carcinoma SKOV3 cells, highly resistant to this metabolic stress. Arginine (ARG) deprivation achieved with recombinant ARG-degrading enzymes is currently considered as anticancer therapy. On the model of SKOV3 cells, we addressed the question, whether autophagy plays a pro-survival role upon ARG starvation and whether its modulation may enhance the therapy.

For this, we examined cell viability upon autophagy inhibition or over-induction in ARG-free medium and ability to restore proliferation upon ARG re-supplementation. Antimalarial and potential anticancer drug chloroquine (CQ) and 3-methyladenine (3-MA) were utilized as autophagy inhibitors, whereas resveratrol (RV) was applied as an autophagy-enhancing compound. In addition, we examined the effect of taxol, a drug currently used to treat human ovarian carcinomas, on cell survival under ARG deprivation and upon modulation of autophagy.

We demonstrated that autophagy over-induction by RV did not affect SKOV3 cells viability upon ARG deprivation. However, ARG re-supplementation inhibited of autophagy with 3-MA or CQ and decreased cell viability and proliferation. This effect was dramatically enhanced upon taxol treatment. Upon combined treatment with CQ and taxol in ARG -free medium, SKOV3 cells lost their ability to restore proliferation in ARG supplemented medium already after 2 days of incubation. We also observed that inhibition of autophagy upon cultivation in complete medium (either pharmaceutically or via siRNA silencing of autophagic protein Beclin 1) dramatically affected SKOV3 cell viability.

Although ovarian carcinomas were not considered as potentially sensitive to the therapy based on ARG deprivation, it was recently reported that tumors relapsed after treatment with platinum compounds concomitantly become sensitive to ARG deprivation due to the loss of the expression of ARG biosynthetic enzyme, argininosuccinate synthetase (ASS). Our results point at potential efficacy of the combinational approach based on ARG deprivation, autophagy inhibition and taxol treatment as a second line therapy for such hard to cure ovarian carcinomas.

Cancer Research Young Scietists' Poster Presentation

Glioma tumor markers - CHI3L1 and CHI3L2 in cell signaling and fate

Areshkov P, Avdeiev S, Kavsan V

Department of Biosynthesis of Nucleic Acids,
Institute of Molecular Biology and Genetics NAS of Ukraine,
150, Zabolotnogo St., Kyiv, Ukraine, 03680
chitotrio@gmail.com

Serial Analysis of Gene Expression revealed CHI3L1 and CHI3L2 among the genes with the most pronounced changes of expression in tumor cells (Kavsan et al., 2005). In contrast to CHI3L1, which has been characterized as a marker of inflammatory diseases, the features of it closest homologue - CHI3L2 were poorly described. Because they are closely related in size and sequence to each other, we summarize research on CHI3L1 as a background for CHI3L2 investigation.

CHI3L1 expression is increasing significantly under pathological conditions such as inflammation or tumors, notably glioblastoma. It was shown that CHI3L1 stimulates the growth of connective tissue cells similarly to IGF-1. This mitogenic activity is mediated by signaling through the MAPK and PI-3K cascades (Recklies et al., 2002).

To examine if CHI3L2 can stimulate the activation of MAPK pathway by phosphorylation of Erk1/2 similarly to EGF, we used human embryonic kidney (HEK293) and gliobastoma (U373) cell lines which don't produce endogenous growth factors. The results obtained, suggest that Erk1/2 phosphorylation was stimulated in these cells following addition of CHI3L2 or CHI3L1 in dose- and time-dependent manner. To determine whether CHI3L2 and CHI3L1 can enhance mitogenesis, cell proliferation and [3H]thymidine incorporation were evaluated. Unexpectedly, in opposite to CHI3L1, dose dependent decreasing of measured parameters was observed in HEK293 and U373 cells. In both cell types treatment with CHI3L2 gave more sustained than CHI3L1 MAPK activation with prolonged phospho-Erk-1/2 nuclear accumulation in HEK293. Moreover, our results indicate that CHI3L2 inhibit proliferative action of CHI3L1 and IGF-1 in tested cell lines.

Activation of the MAPK is a normal response following engagement of growth factor receptors. However, the Erk1/2 pathway is frequently subject to inappropriate activation in a variety of tumor cell lines and tumor tissue as it was shown here for glioblastoma U373 cell line. Transient activation will therefore have very different consequences for gene expression compared with sustained activation in the cells of different origin because nuclear accumulation of active Erk1/2 will result in phosphorylation of different transcription factors. Determination of the cellular response depending on previous developmental events that determine which Erk-responsive transcription factors are present in the HEK293 and U373 cells is a purpose of further investigations.

Expression and biological role of adapter protein Ruk/Cin85 in human cervical adenocarcinoma

[1]Basaraba O., [1]Pasichnyk G., [1]Gerashchenko D., [2]Bobak Ya., [2]Shuvayeva G., [3]Volodko N., [1]Drobot L.

[1] Institute of Biochemistry, NAS of Ukraine, 9, Leontovycha Str., Kyiv, Ukraine, 01601

[2]Institute of Cell Biology, NAS of Ukraine, 14/16, Drahomanov St. Lviv, Ukraine, 79005

[3]Lviv National Medical University, Lviv.
olgabasaraba@gmail.com

Uterine cancer is a disease characterized by tumor-specific mutations and dysregulated signalling pathways. The characterization of molecular alterations in each single tumor is the basis for the development of personalized anticancer therapy. Adaptor/scaffold proteins are the key components of signalling networks involved in the control of cell physiology. In particular, by binding to numerous effector proteins adaptor/scaffold protein Ruk/CIN85 assembles multimeric complexes implicated in regulation of multiple cellular functions, including proliferation, adhesion, invasion, and survival.

Ruk/CIN85 expression in uterine tumours as well as conditionally normal tissues was analyzed using Northern- and Western-blot analyses. Serum-starved human cervical cancer HeLa cells were treated with 5α-dihydrotestosterone (DHT). Involvement of Ruk_l/CIN85 full-length form in rapid nongenomic effects of androgen was studied using Western-blot analysis, immunoprecipitation and confocal microscopy.

Increase of Ruk_l/CIN85 mRNA and protein expression levels was revealed in uterine tumours as compared to conventionally normal surrounding tissues. The characteristic feature of Ruk/CIN85 forms expression patterns in control tissues as well as in benign tumours was a high content of high-molecular-weight Ruk/CIN85 forms (140 and 130 kDa), while increase in the content of low-molecular-weight forms (40 and 30 kDa) was observed in samples of malignant tumours.

For the first time, androgen receptor was identified in human cervical cancer HeLa cells. It was established that the content of full-length Ruk_l/CIN85 form in Triton-X-100-soluble fraction of untreated human cervical adenocarcinoma HeLa cells in logarithmic growth phase was very low, whereas stimulation of cells with DHT resulted in its up-regulation. DHT-dependent complex formation between Ruk_l/CIN85 and androgen receptor as well as the role of Ruk_l/CIN85 in rapid non-genomic effects of androgen receptor was revealed using confocal microscopy, immunoprecipitation and Western-blot analysis.

The obtained results suggest that up-regulation of full-length form expression level as well as involvement of Ruk_l/CIN85 in rapid nongenomic effects of androgen might contribute to uterine carcinogenesis.

Studying mTORC2 architecture

Malanchuk O. M., Skorokhod O. M., Tykhonkova I. O., Palchevskyy S. S., Filonenko V.V.

Institute of Molecular Biology and Genetics NAS of Ukraine,
150, Zabolotnogo St., Kyiv, Ukraine, 03680

o.m.malanchuk@imbg.org.ua; filonenko@imbg.org.ua

Background. The signaling components upstream and downstream of the protein kinase mammalian target of rapamycin (mTOR) are frequently altered in a wide variety of human diseases. Upstream of mTOR key signaling molecules are the small GTPases Ras and Rheb, PI3K, PKB, known to be deregulated in many human cancers. Mutations in the mTOR pathway component genes *TSC1, TSC2, LKB1, PTEN, VHL, NF1* and *PKD1* trigger the development of the syndromes Tuberous sclerosis, Peutz-Jeghers, Cowden, Bannayan-Riley-Ruvalcaba, Lhermitte-Duclos disease, Proteus syndrome, von Hippel-Lindau disease, Neurofibromatosis type 1, and polycystic kidney disease, respectively. Recently, it has been recognized that mTOR is regulated by TNF-α and Wnt, both of which have been shown to play critical roles in the development of many human neoplasias. In addition to all these human diseases, the role of mTOR in Alzheimer´s disease, cardiac hypertrophy, obesity and type II diabetes is discussed. So, it is the hope of investigators and patients alike that a more detailed understanding of the mTOR signaling cascade will lead to new therapies for many different human diseases.
mTOR exists in two functionally and structurally distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 contains raptor, mLST8 and PRAS40 and controls cell growth via a rapamycin-sensitive signaling branch regulating translation, transcription, nutrient uptake, ribosome biogenesis, and autophagy. mTORC2 contains protor, rictor and sin1 proteins and controls the organization of the actin cytoskeleton through a rapamycin-insensitive signaling branch.
Objectives. The architecture of mTORC2 is discussed in the context of TORC2 assembly and regulation. We have focused on the characterization of mTORC2 architecture, notably, on the characterization of the interactions between mTOR and rictor. To analyze the specificity of these interactions prompted us to develop monoclonal antibodies, which would specifically recognize rictor (Mab against mTOR were produced previously).
Results. Recombinant His-GST-rictor/Nterm was used as an antigen and in hybridoma screening procedures. Generated antibodies work efficiently in various immunoassays, including ELISA, Western blotting and immunoprecipitation. Furthermore, the immunoblotting of the mTOR immune complexes with Mab against rictor clearly indicated that rictor is efficiently co-immunoprecipitated with mTOR in HEK 293 cells. Intriguingly, binding partners were not immunoprecipitated in *vice versa* experiment.
Conclusion. Data presented in this work indicate that mTOR interacts with rictor *in vivo*. In addition, N-terminal domain of rictor may implements an interaction with mTOR.

Comparison of the diagnostic efficacy of 18F-FDG PET/CT and 99mTc-MDP whole body bone scintigraphy in the detection of bone metastases in breast cancer patients

[1]Nagy Edit , [2]Hascsi Zsolt, [1]Varga Józse, [2]Szabados Lajos, [2]Garai Ildikó

1 Institute of Nuclear Medicine, University of Debrecen;
2 PET-CT Medical, Diagnostic Ltd.,
Nagyerdei krt. 98, Debrecen 4032, Hungary
edoedo0624@gmail.com

Background: In the therapeutic management of breast cancer patients' evaluation of the bone status is crucial, as it allows early detection of bone metastases, which have substantial influence on the patients' quality of life and on the treatment of choice administered.

Aim: The aim of the present study was to retrospectively compare the diagnostic accuracy of the PET/CT and bone scintigraphy (BS), which is the current gold-standard method for the detection of bone metastases in breast cancer patients. The relationship between FDG uptake, CT morphology and abnormal findings on BS was also analyzed.

Methods: The inclusion criteria for patients were: BS and FDG PET/CT examinations performed within 60 days, available imaging datasets and verified bone status, which was confirmed either by corresponding findings of the baseline BS and PET/CT examinations, or by follow up imaging examinations (BS, PET/CT, x-ray, CT, MR) corroborating the original findings. Patients, who were considered metastasis free, were followed clinically for at least two months. Additionally, tumor marker levels were also measured.

Based on PET/CT a total of 235 lesions were analyzed, out of which 164 were concordant with BS.

Results: During the past 26 months 1546 breast cancer patients were examined with PET/CT at our center, and 98 of them met the inclusion criteria. Bone metastases were verified in 35 patients. The sensitivity of PET/CT and BS was 97.14% and 91.43%, respectively, whereas the specificity of methods was 96.83% (PET/CT) and 85.71% (BS). Positive predictive values were 94.44% (PET/CT) and 78.05% (BS), whereas negative predictive values were 98.39% (PET/CT) and 94.74% (BS). The overall accuracy of PET/CT and BS was 96.94% and 87.76%, respectively.

On lesion-based analyses significant difference were observed between FDG uptake and CT morphology ($p<0.0001$), and diameter of metastases. We also find an unexpected difference between activity on BS and CT morphology; lytic lesions have higher activity on BS than sclerotic ones ($p<0.0001$).

Conclusions: Our results suggest that the diagnostic value of PET/CT to detect bone metastases in breast cancer patients is higher than that of BS. PET/CT is superior to BS as it also provides full staging, although at a substantially higher cost.

C_{60} fullerene causes mitochondrial photo damage in transformed T lymphocytes

[1]Palyvoda K., [1]Samoylenko A., [1]Drobot L., [2]Matyshevska O.
1Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine,
9, Leontovycha Str., Kyiv, Ukraine, 01601
2Taras Shevchenko Kyiv National University,
64, Volodymyrska St. Kyiv, Ukraine, 01033
zuav@ukr.net

Background: Nowadays we can observe increasing interest in the possible application of carbon nanospheres, known as fullerenes, in biomedicine. C_{60} fullerenes are nanodimensional molecules consisting of 60 atoms of carbon. C_{60} fullerene molecules act as prooxidant due to the formation of ROS after UV/VIS photo excitation. Since generation of ROS is known to be involved in the development of apoptotic cell death, using of fullerenes for cancer photodynamic therapy seems to be promising. It is known that ROS generally led to a rapid apoptotic outcome with mitochondrial damage and release of cytochrome *c* into the cytosol. The appearance of cytosolic cytochrome *c* triggers an apoptotic response *via* the sequential activation of caspase-9 and caspase-3. Nevertheless, the mechanism of C_{60} fullerene-induced cell death in oncotransformed cells has been poorly studied.

Aim: The aim of the present work was to study the markers of mitochondria-dependent apoptosis in transformed T cells irradiated with UV/VIS light in the presence of C_{60} fullerenes.

Materials and methods: Cell viability was assessed by the MTT reduction assay. Level of cytochrome c in cytosolic fraction and caspase-3 activation was analyzed by Western-blot analysis. Colloidal aqueous solutions of C60 fullerenes were prepared in Technical University of Ilmenau (Germany).

Results: The cytotoxic effect of C_{60} fullerene was detected in human transformed T cells (Jurkat cell line) after irradiation with UV/VIS light (λ=320-600 nm). After short-time irradiation of Jurkat cells pre-incubated for 1 h with $5 \cdot 10^{-5}$ M C_{60} fullerene cell viability was decreased by 50±4% after 6 h incubation. The cytotoxic effect of photoexcited C_{60} fullerene in transformed cells was proved to be dose- and time-dependent. There was no significant change in the level of cytosolic cytochrome *c* after UV/VIS illumination, but a substantial increase was observed by combined action of C_{60} fullerene and UV/VIS illumination after 1 h incubation. UV/VIS irradiation alone is followed by caspase-3 activation in Jurkat cells at 6 and 24 h. However, in cells, irradiated with UV/VIS light in the presence of C_{60} fullerene, caspase-3 processing was dramatically intensified – the content of 17 and 19 kDa intermediate cleavage products was increased already at 6 h. After 24 h, caspase-3 activation was increased by 20 fold as compared to control.

Conclusion: Photo excited C_{60} fullerene causes apoptotic cell death accompanied by cytochrome *c* release into the cytosol and subsequent caspase-3 activation in transformed T cells.

The effect of arginine deprivation on cytoskeleton remodeling and invasiveness of human neuronal tumor cells

[1,2]Pavlyk Julia, [1]Rzhepetskyy Yuriy, [2]Wasik Anna, [2]Redowicz Maria Jolanta, [1]Stasyk Oleh

[1]Institute of Cell Biology, NAS of Ukraine,
14/16, Drahomanov St. Lviv, Ukraine, 79005
[2]Nencki Institute of Experimental Biology,
3 Pasteur Str., 02-093, Warszawa,Poland
stasyk@cellbiol.lviv.ua

It was shown previously that certain cancers may be auxotrophic for a particular amino acid, and amino acid deprivation seems to be one of the methods to treat such tumors. Arginine is a semi-essential amino acid for normal cells in adult humans but it is essential for rapidly proliferating tumor cells. Thus arginine deprivation, alone or in combination with other chemotherapeutics, may offer a new powerful tool in the control of tumor growth.

Our preliminary data indicate that arginine-deprivation in monolayer culture (obtained via addition of recombinant arginase), unlike lysine-deprivation, caused significant decrease in human glioma U251 cells migration and invasiveness. Using confocal and scanning electron microscopy we have also observed evident changes in cell morphology: the zone of the dense lamellipodial network at the leading edge was reduced to half in width in cells incubated without arginine relative to cells grown on complete medium. The cells grown in arginine-deprived medium were more elongated that the cells cultured in lysine-deprived and control media and had reduced potential to form colonies.

We suggest that this effect of arginine deprivation is connected with changes in the polymerization of β-actin. To confirm this hypothesis, we performed a biochemical fractionation of lysates from U251 glioma cells by differential centrifugation. We found that the major difference between extracts of cells incubated in arginine-sufficient and deprived media was observed at the highest sedimentation speed, which separates F- and G-actin. For arginine-deprived cells (but not lysine-deprived) we found dramatic reduction in the level of polymeric fraction of β-actin. This effect was reversed upon re-supplementation of the medium with arginine.

The current study takes the first steps toward uncovering the mechanism behind arginine-dependent regulation of neuronal cell motility and invasiveness and metastatic potential, issues not addressed in the previous studies. The obtained results point at profound alterations in actin cytoskeleton organization, probably also associated with arginylation of actin, a process known to regulate actin cytoskeleton remodeling, and are very important for design of efficient anticancer therapies based on arginine deprivation.

Enhancement of analytical characteristics of enzyme multibiosensor for simultaneous carbohydrates determination

[1]Pyeshkova V. N. , [1]Soldatkin O. O., [2]Mikhal A. A., [2]Melnik2 V. G., [1]Dzyadevych S. V.

[1]Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, 150, Zabolotnogo St., Kyiv, Ukraine, 03680
[2]Institute of Electrodynamics of National Academy of Sciences of Ukraine, 56, Peremogu Av., Kyiv, Ukraine, 03680

victoriya.p@gmail.com

Quantitative analysis of carbohydrates has significant importance for diagnosis and treatment of different diseases connected with metabolism disorders. Measurement of glucose, major metabolite, is critical for people who suffer from diabetes. Determination of lactose is also of interest in medicine. For example, excessive amount of lactose in blood (>600 mM) can indicate gastrointestinal malignancy. This type of cancer is widely spread in the world. Abnormal amount of lactose, sucrose and maltose in human fluids indicates different metabolism disorder such as carbohydrate intolerance etc.

The great demand for the determination of carbohydrates in human fluids (blood, urine etc.) has led to the development of a variety of analytical methods. The majority of these methods (spectrophotometry, chromatography etc.) is tedious and time consuming, may require expensive equipment, in addition to considerable technical skills. In this context, biosensors have the potential to overcome disadvantages of conventional methods: they are simple, accurate, high specific, sensitive, fast, convenient, rather cheap and small devices. Among different biosensor systems multibiosensors are especially attractive because they enable simultaneous measurement of several analytes in a single sample.

The main aim of our work was improvement of analytical characteristics of new enzyme conductometric multibiosensor for simultaneous quantitative analysis of glucose, maltose, lactose and sucrose in liquid samples. To create bio-selective membranes for analysis of maltose, lactose and sucrose three enzymes (glucose oxidase, mutarotase and suitable glycosidase) were immobilized on electrodes using glutaraldehyde. Single-enzyme glucose oxidase membrane was applied for glucose determination. The optimal concentrations of enzymes and their ratio in bio-selective membranes were found. 10 mM phosphate buffer solution, pH 6.0 as working solution proved to be optimal for functioning of multibiosensor. The developed sensor system showed a linear response to carbohydrates within the concentration range from 0.001 to 1.5-10 mM depending on enzyme composition and thickness of bio-selective membrane. The time of carbohydrates determination was 1-2 min. The adapted multibiosensor is characterized by high operational stability; show good selectivity and high signal reproducibility. Proposed multibiosensor can be applied in medical diagnostics.

Expression of phospholipase Cε in patients with myeloproliferative disorders

Tyutyunnykova A., Dubrovska A., Telegeev G.

Institute of Molecular Biology and Genetics of NAS of Ukraine,
150, Zabolotnogo St., Kyiv, Ukraine, 03680
anna.tyutyunnykova@gmail.com

Background. Bcr-Abl, the product of a chromosomal translocation t(9;22), has been demonstrated to be a key protein responsible for the pathogenesis of Ph-positive leukemia. Depending on the breakpoint region of the bcr gene, three Bcr/Abl proteins have been observed: p190 Bcr-Abl, p210 Bcr/Abl, and p230 Bcr-Abl. The only structural difference between various Bcr-Abl chimeras is the presence of Dbl homology (DH) and pleckstrin homology (PH) domains in p210 Bcr-Abl and p230 Bcr/Abl. p210 Bcr-Abl and p230 Bcr-Abl are responsible for chronic myelogenous leukemia while p190 Bcr-Abl is associated with more aggressive acute lymphoid leukemia suggesting the important role of the DH and PH domains in leukemogenesis. Our resent data demonstrated that Bcr-Abl PH domain binds a number of proteins including phospholipase Cε (PLCε). However, the signaling consequences of such association remain unclear. Previous studies have shown that PLCε is both target and regulator of the Ras superfamily of GTPases, key players in p210-BCR-ABL-dependent leukemogenesis. Also it has been shown that PLCε transcripts are not expressed in normal peripheral blood leucocytes. However, the role of a PLCε in leukemia progression is not clear yet.

Aims. The aim of this study is to analyze the relationship between PLCε expression and both the presence and the type of Bcr/Abl rearrangement in patients with myeloproliferative disorders (MPDs).

Results. We have analyzed PLCε expression in 12 patients with different MPDs. The cDNA was obtained from the peripheral blood leukocytes by one-step RT-PCR using total RNA as template. PLCε expression and the presence and the type of Bcr/Abl transcript were identified using gene specific primers. PLCε expression was detected using oligonucleotide primers specific to Ras/Rap-associating (RA) domains that are a unique distinguishing feature of PLCε among other phospholipase C isozymes. The specificity of PCR product was confirmed by direct sequencing. Bcr/Abl rearrangement was detected by nested RT-PCR analysis. Our data showed that all patients have detectable level of PLCε expression and 9 of them demonstrate the presence of p210 Bcr/Abl translocation.

Conclusion. Our results suggest that expression of PLCε in peripheral blood leucocytes of patients with MPDs correlates with the presence of p210 Bcr/Abl. Therefore, the PLCε expression in leukemia patients could be a marker of oncogenic transformation during CML development. Further analysis of a molecular mechanism underlying a crosstalk with Bcr/Abl and PLCε signaling may lead to the development of novel therapeutic angles for Ph-positive leukemia.