International VACTRAIN/ 3rd Swedish-Ukrainian conference on cancer diseases

Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology (MTC)

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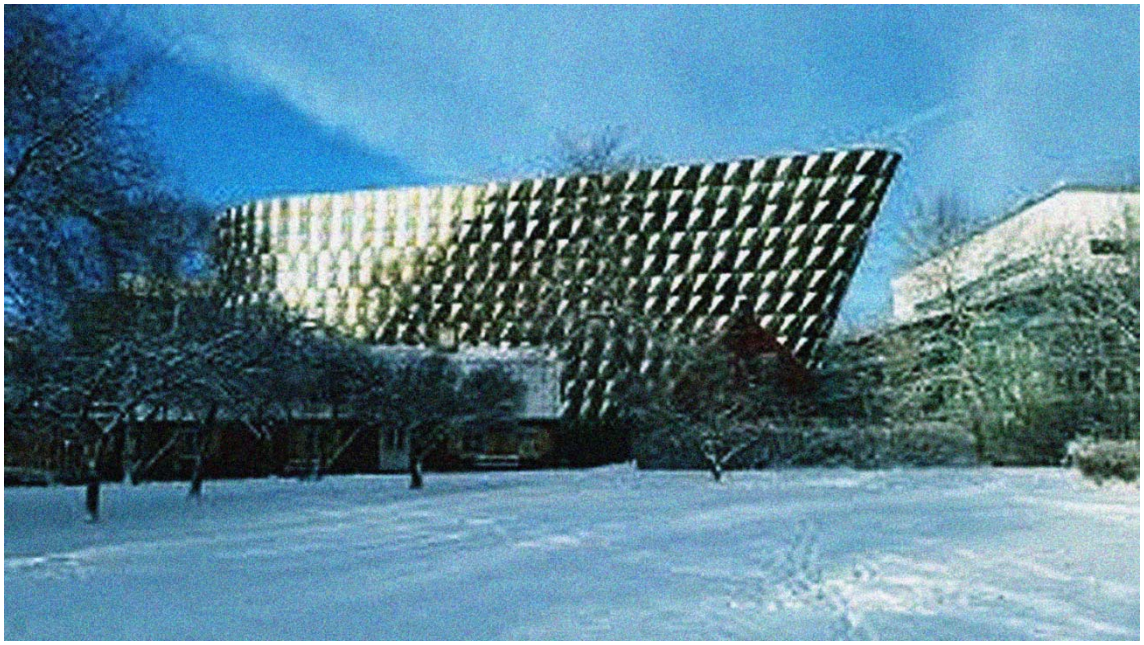

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VACTRAIN
TWINNING ON DNA-BASED CANCER VACCINES

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RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv

Monday, January 16th 2017 Radiumhemmet	
8.00 – 9.00	Registration, coffee, tea
9.00 - 9.20	Welcome by The Vice-Chancellor of KI Professor Karin Dahlman-Wright Welcome by H.e. Ambassador of Ukraine in Kingdom of Sweden, Igor Sagach Welcome by Academician Vasyl Chekhun, the Head of RE Kavetsky IEPOR of NASU Opening announcements
9.20 – 10.50	**Session 1: Molecular and cellular pathobiology** **Chair:** Ingemar Ernberg
30	TARGETING MISSENSE AND NONSENSE MUTANT P53 IN CANCER – FROM MOLECULAR BIOLOGY TO THE CLINIC? **Klas Wiman**
30	MECHANICAL FORCES CONTROL MORPHOGENESIS AND CANCER **Lars Holmgren** (p. 4)
12	REPROGRAMMING OF CANCER CELLS UPON INHIBITION OF IRE1-MEDIATED ENDOPLASMIC RETICULUM STRESS SIGNALING **Oksana Ratushna** (p. 5)
12	MYCN mediated metabolic plasticity and the pathogenesis in human neuroblastoma **Ganna Oliynyk** (p. 6)
10.50 – 11.15	Refreshments
11.15 – 13.10	**Session 2: Tumor metastases and role of cellular microenvironment** **Chair:** Elena Kashuba
30	ANTIANGIOGENIC CANCER THERAPY **Yihai Cao**
30	LAMININ ISOFORMS AS THERAPEUTIC TARGETS IN TUMOR INVASION AND METASTASIS **Manuel Patarroyo** (p. 7)
30	FROM A CELL BASED SMALL MOLECULE SCREEN TO THE CRYSTAL STRUCTURE OF A COMPOUND BOUND TO ITS TARGET **Sonia Lain**
12	THE ADAPTOR PROTEIN RUK/CIN85 PARADOXICALLY ENHANCES EMT OF TRIPLE NEGATIVE MOUSE BREAST ADENOCARCINOMA 4T1 CELLS **Iryna Horak** (p. 8)
12	BIOLOGICAL CHARACTERISTICS OF TUMOR CELLS AT THE DIFFERENT STAGES OF EMT UPON EXPOSURE TO ANTICANCER DRUGS AND CYTOKINES

	Natalia Bezdieniezhnykh (p. 9)
13.10 – 14.00	Buffet lunch
14.00 -15.45	**Session 3: Paradigm of personalized treatment in oncology** **Chair:** Svitlana Sydorenko
30	SUCCESSFUL NK CELL-BASED IMMUNOTHERAPY TO PATIENTS WITH HEMATOLOGICAL MALIGNANCIES **Hans-Gustaf Ljunggren** (p. 10)
30	RHO GTPASES IN CANCER CELL MIGRATION **Pontus Aspenström**
30	GLOBAL REGULATION OF EPIGENOME BY P53: A NOVEL FACET OF TUMOUR SUPPRESSION **Galina Selivanova**
12	ALTERNATIVE DIRECTION OF INHIBITION OF MALIGNANT PROPERTIES IN TUMOR CELLS IN VITRO AND IN VIVO BY GENE THERAPY WITH IFN-B GENE IN RECOMBINANT BACULOVIRUS VECTOR **Oleksandra Lykhova** (p. 11)
15.45 -16.10	Refreshments
16.10-17.40	**Session 4: Detection of potential targets for target therapy and cancer vaccines** **Chair:** Maria Issagouliantis
30	CD150/SLAMF1 AS A NEW POTENTIAL TARGET FOR ANTI-TUMOR THERAPY **Svitlana Sidorenko** (p. 12)
12	CD150/SLAMF1 ANTIGEN IN MOLECULAR PATHOBIOLOGY OF CHRONIC LYMPHOCYTIC LEUKEMIA **Inna Gordiienko** (p. 13)
30	DNA IMMUNIZATION TARGETING CARCINOEMBRYONIC ANTIGEN IN COLORECTAL CANCER PATIENTS **Britta Wahren** (p. 14)
12	CHANGES IN EXPRESSION OF MIRNA-122, -200B, AND 320A AS PROGNOSTIC BIOMARKERS FOR BREAST CANCER **Tetyana Borikun** (p. 15)
18.00	Dinner at restaurant Königs
Tuesday, January 17th 2016 Nobel forum	
8.00 – 8.30	Coffee, tea
8.30 – 10.30	**Session 5: Search for new markers for diagnostics of tumors with different genesis** **Chair:** Teresa Frisan

30	CANCER CELL HETEROGENEITY – AN ADDITIONAL LEVEL ANALYSED BY EBV-CELL MODELS **Ingemar Ernberg** (p. 16)
30	CHEMORESISTANCE OF LUNG ADENOCARCINOMA IS REGULATED BY TUDOR STAPHYLOCOCCAL NUCLEASE **Boris Zhivotovsky** (p. 17)
30	THE MRPS18-2 PROTEIN AS A PUTATIVE MARKER OF CANCEROGENESIS **Elena Kashuba** (p. 18)
12	HIGH EXPRESSION LEVELS OF MRPS18-2 AND PRESENCE OF THE RB PROTEIN ARE REQUIRED FOR THE MAINTENANCE OF THE STEM CELL PHENOTYPE **Muhammad Mushtaq** (p. 19)
12	IDENTIFICATION OF NOVEL MOLECULAR AND GENETIC MARKERS FOR EARLY DETECTION OF EPITHELIAL TUMORS AND PROGNOSIS OF THE COURSE OF DISEASE **Oksana Mankovska** (p. 20)
10.30 – 11.20	**Poster session** and Refreshments
11.20 – 13.10	**Session 6: Multidisciplinary cancer research** **Chair:** Britta Wahren
30	THE TYPHOID TOXIN MODULATES THE HOST INFLAMMATORY RESPONSE AND PROMOTES THE ESTABLISHMENT OF A PERSISTENT ASYMPTOMATIC INFECTION **Teresa Frisan** (p. 21)
30	CD4+T CELLS IN THE CONTROL OF EPSTEIN-BARR VIRUS INDUCED B CELL TRANSFORMATION **Noemi Nagy**
20	INTERFERON GAMMA IS A STAT1-DEPENDENT DIRECT INDUCER OF BCL6 EXPRESSION IN IMATINIB TREATED HUMAN CD34+ CHRONIC MYELOID LEUKEMIA STEM CELLS **Daniel Salamon**
20	SUSTAINABILITY AND ROBUSTNESS OF MINED BIOLOGICAL KNOWLEDGE: SOLUTIONS USING NETWORK-BASED PATHWAY ANALYSIS **Andrey Alexeyenko** (p. 22)
10	**Closing remarks**
13.10 – 14.30	**Poster session** and Buffet lunch

Mechanical forces control morphogenesis and cancer

Lars Holmgren

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Backround: Transmission of mechanical force via cell junctions is an important component that molds cells into shapes consistent with proper organ function. Of particular interest are the cadherin transmembrane proteins that play an essential role in connecting cell junctions to the intra-cellular cytoskeleton. Understanding how these biomechanical complexes orchestrate intrinsic and extrinsic forces is import for our understanding of the underlying mechanisms driving morphogenesis and invasion. We have previously identified the Amot protein family, which are scaffold proteins that integrate polarity, junctional, and cytoskeletal cues to modulate cellular shape. Methods: Analysis of protein expressin in human and mouse tumors. Using genetic inactivation approaches in endothelial cells, zebrafish and mice we have characterized the function of the protein family in normal development as well as in tumor progression.

Results: Expression analysis shows that p60 AmotL2 is regulated by hypoxia and is induced in colon, breast, prostate and glioma cancer patients. We provide a novel mechanism how tumor cells escape the mechanical constraint exerted by neighboring cells and become plastic and highly invasive.

Endoplasmic reticulum stress as a key factor of genome reprogramming in cancer cells

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The endoplasmic reticulum (ER) stress represents the unfolded protein response to cope with the accumulation of unfolded or misfolded proteins. It is required to maintain the functional integrity of the ER, which is a dynamic intracellular organelle with exquisite sensitivity to alterations in homeostasis. The unfolded protein response is a key player in the development of different malignant tumors. Depending on the duration and severity of the ER stress, it leads to cell adaptation or demise. This stress is a fundamental phenomenon which provides a secure protection of the cells from different environmental challenges and is transduced by three major ER resident stress sensors. Activation of these ER stress sensors leads to transcriptional reprogramming of the cells. The signaling pathways elicited by those stress sensors have connections with metabolic pathways and with other plasma membrane receptor signaling networks. As such, the ER has an essential position as a signal integrator in the cell and is instrumental in the different phases of tumor progression.

Inositol requiring enzyme 1 (IRE1) is the most conserved transducer of the unfolded protein response and produces either adaptive (preserve ER homeostasis) or death signals through both kinase and endoribonuclease, including unconventional splicing of XBP1 mRNA and regulated IRE1-dependent decay of mRNA (RIDD). Splice variant of XBP1 controls the expression of hundreds of the unfolded protein response-specific genes upon global translational repression to preserve ER homeostasis. We have shown that inhibition of IRE1 suppresses glioma cell proliferation and tumor growth by affecting the expression of genes encoding the tumor suppressors, TNF receptors and related proteins, key transcription factors and protein kinases, as well as numerous mitochondrial proteins. Moreover, inhibition of IRE1 endoribonuclease activity only has stronger effect on glial cell proliferation *in vitro* and also on glioma growth (in mouse orthotopic brain model) and gene expression profile. It concerns especially genes, encoding key regulatory factors, controlling cell proliferation, distinct from inhibition of both enzymatic activities of IRE1.

Furthermore, we have shown that hypoxia, which is an obligate interconnected component of malignant tumor growth, affects almost all studied genes. Thus, inhibition of IRE1 signaling network mostly modifies the expression of proliferation related genes, contributing to the rate of glioma cell proliferation. We have identified several perspective genes, whose expression was significantly changed in glioma cells with inhibited both, enzymatic activities of IRE1 and only its endoribonuclease. These genes, as well as IRE1 endoribonuclease and kinase can be perspective targets to the design the novel compounds for therapeutic strategies to manipulate levels of ER stress in diseases. A better understanding of the biological role of IRE1 signaling network is needed to build an integrated systematic view on IRE1 signaling. This will be applied to develop novel, innovative inhibitors and activators of this signaling enzyme.

MYCN mediated metabolic plasticity and the pathogenesis in human neuroblastoma

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Neuroblastoma (NB), which arises from the developing sympathetic nervous system, is one of the most aggressive solid tumors of early childhood. Amplification of the MYCN oncogene can be found in around 30% of NB patients and it is associated with rapid tumor progression and poor prognosis. Our recent findings show that a small chemical molecule, 10058-F4, previously identified as a c-MYC inhibitor also targets the MYCN/MAX complex resulting in apoptosis and neuronal differentiation in MYCN-amplified NB cells. Importantly, we demonstrated that inhibition of MYCN in NB cells results in metabolic changes including mitochondrial dysfunction leading to accumulation of lipid droplets. Similarly, treatment with the bromodamain inhibitor JQ1 leads to MYCN downregulation followed by lipid accumulation (Zirath et al, PNAS 100, 10258-10263, 2013).

To investigate downstream effects of MYCN targeting therapy we have performed quantitative proteomics of MYCN-amplified NB cells treated with 10058F4 or with JQ1. For comparison, downregulation of MYCN expression using short hairpin RNA followed by proteomic analysis was performed. We identified around 7000 proteins of which 6500 have been used for identification of novel pathways involved in NB pathogenesis and for investigation of potential MYC related biomarkers. Our data showed that primary metabolic processes including protein, lipid and nucleic acid metabolic processes were the most significantly affected upon MYCN downregulation. To investigate an impact of MYCN expression on glycolysis and mitochondrial metabolic capacity we have performed metabolic measurements using Seahorse XF analyser. We indicated that MYCN not only positively regulates the respiratory capacity in NB cells, but also significantly enhances glycolysis. Furthermore, we found that MYCN positively regulates the ability of NB cells to oxidize exogenous fatty acids.

Our findings show that MYCN expression enhances bioenergetics capabilities in NB cells, depending on the available nutrition. MYCN regulates metabolic plasticity in NB cells.

Taken together, we have highlighted important metabolic pathways in NB, which may be the basis for future therapies.

Laminin isoforms as therapeutic targets in tumor invasion and metastasis

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Tumor invasion and metastasis account for nearly 90% of all cancer-related deaths, and nearly 10 million people die from cancer every year. Laminins, a family of extracellular matrix proteins mainly found in basement membranes, are masters of tissue architecture, a property which is highly deregulated during tumor invasion and metastasis. Over 16 laminin isoforms are presently known and their expression is developmentally regulated and cell- and tissue specific. Laminins are recognized by integrins and other cell-surface receptors and promote cell adhesion, migration, survival, stemness and proliferation, cellular processes involved in the metastatic cascade. Although expression of laminin isoforms in tumors mostly reflects expression in their normal counterparts, distinct alterations of laminin expression and function occur during tumor invasion, particularly in epithelial-to-mesenchymal transition of the tumors cells and loss of the basement membrane barrier. During dissemination and metastasis cancer cells encounter vascular, neural, lymphoid tissue and other exogenous laminins. However, the malignant cells themselves are able to produce and secrete laminins and to use these endogenous molecules in an autocrine fashion. Recent studies have demonstrated participation of tumor cell-derived laminins in different steps of the metastatic cascade. RNA interference of particular tumor cell laminins inhibits tumor invasion and metastasis and mouse monoclonal antibodies to the laminins inhibit tumor cell migration and renewal. Expression of malignancy-associated laminins is much higher in malignant cells than in pre-malignant cells, and overexpression of these laminins in tumors significantly correlates with reduced patient survival. The contribution of tumor cell laminins to various steps of the metastatic cascade, such as tumor cell migration, survival, self-renewal and proliferation, will be presented, as well as the regulation of these laminins by oncogenic pathways and their potential as therapeutic targets.

The adaptor protein Ruk/CIN85 paradoxically enhances EMT of triple negative mouse breast adenocarcinoma 4T1 cells

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To coordinate cellular responses, cell surface receptors employ receptor-associated adaptor proteins that are composed exclusively of domains and motives involved in intermolecular interactions. The assembling of adaptor proteins-mediated supramolecular complexes is regulated in dynamic and selective fashion, thereby influencing processing of information through signaling networks. Adaptor protein Ruk/CIN85 consists of three SH3 domains, four blocks of proline-rich motives and C-terminal coiled-coil region, and acts as a transducer platform that participates in control of various physiological processes, such as apoptosis, ligand-induced endocytosis of receptor tyrosine kinases, vesicular trafficking, cell adhesion, motility, and invasion. It has been shown that high levels of Ruk/CIN85 contribute to the conversion of weakly invasive human breast adenocarcinoma MCF-7 cells into a more malignant phenotype. However, a number of issues with respect to the role of Ruk/CIN85 in breast carcinogenesis remain still open.

To establish syngeneic mouse model suitable for experiments in vivo we stably overexpressed or down-regulated Ruk/CIN85 in triple negative mouse adenocarcinoma 4T1 cells (RukUp or RukDown cells respectively). As it turned out, RukUp cells acquired a rounded shape, whereas RukDown cells had a more distinct epithelial phenotype indicating that possible EMT/MET processes occurred in these cells. To elucidate this issue, RukUp and RukDown cells were studied for their adhesive and invasive properties in vitro and also for the expression of several marker EMT-associated genes.

Adhesion assay of 4T1 cells showed inverse dependence of adhesion to collagen type I and fibronectin on Ruk/CIN85 expression. We also demonstrate that overexpression of Ruk/CIN85 is associated with increased migration and invasion through Matrigel, collagen type I and fibronectin, as well as through endothelial cells layer. Importantly, Ruk/CIN85 down-regulation led to decrease in 4T1 cells motility and invasiveness in vitro.

Using western-blot analysis of main EMT markers (vimentin and E-cadherin) we found high levels of vimentin and low of E-cadherin in RukUp cells, and the opposite results in RukDown cells. We used RT2 Profiler PCR array (Qiagen) for 84 EMT-associated genes in order to find additional molecules involved in Ruk/CIN85-mediated EMT. Ruk/CIN85 was found to positively regulate expression of such transcription factors as Mitf, Zeb2, Twist, Sparc, as well as osteopontin, EGFR and TGFβ. Negative effect of Ruk/CIN85 was demonstrated for Bmp-7, Wnt, E-cadherin, FoxC, keratins genes Krt-7, Krt-14 and Krt-19, and MMP-9. These data suggest that adaptor protein Ruk/CIN85 is a critical regulatory component involved in EMT of breast cancer cells.

Biological characteristics of tumor cells at the different stages of EMT upon exposure to anticancer drugs and cytokines

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The aim: Formation of a highly malignant metastatic tumor cell phenotype and resistance to anticancer drugs is associated with the implementation of epithelial-mesenchymal cell transition (EMT). Thus, monitoring of EMT and the possible inhibition could be helpful to inhibit the tumor progression. It is quite often that anti-tumor treatment is not effective, so, new modalities and new targets to combat cancers should be developed.

Materials and methods: established cell lines, primary cultures of malignant cells obtained from biopsies or ascites of patients with epithelial cancers (breast, colorectal and ovarian), immunohistochemistry, statistical methods.

Results: We found that the majority of the primary tumor cells (of ascitic fluids) showed expression of mesenchymal markers, namely vimentin and N-cadherin. Upon culturing *in vitro,* on the adhesively active extracellular matrix, expression of mesenchymal markers, for example the Twist and Slug transcriptional factors was downregulated. At the same time, a number of cells expressing the epithelial markers, namely E-cadherin, pan-keratin, etc. was increased. Moreover, the cells altered their sensitivity to anticancer drugs, despite the various mechanism of drug action (taxanes, vinca alkaloids, antimetabolites). Modification of the extracellular matrix, i.e. growth on collagen or in spheroids, also significantly influenced EMT marker expression and sensitivity of cells to the drugs.

Next, we investigated the influence of components of the microenvironment, especially cytokines IFN, TNF, and ILs on the established cell lines (MCF-7, MDA-MB-231, Colo 205, HT-29) that show different EMT profiles. We wanted to find the most effective inhibitors of the EMT process, thus, reducing the malignancy of tumor cells. The anticancer drugs, such as platinum-containing, camptothecins, and some others were also tested.

Conclusion: We have observed a lability of phenotypic characteristics of the primary tumor cells, i.e. changes in morphology, expression of markers, associated with EMT, which correlated with their sensitivity to anticancer drugs. We identified a number of biologically active agents that might shift EMT to either side, thus inhibiting the malignant phenotype of tumor cells.

Successful NK cell-based immunotherapy to patients with hematological malignancies

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Natural killer (NK) cells represent a unique subset of lymphocytes, distinct from classical T and B cells and more recently identified innate lymphoid cells (ILCs). Current insights into NK cell molecular specificity and function have suggested that it might be possible to treat human cancer with NK cells. To approach this goal, we have carefully addressed questions of importance for the development of NK-cell-based immunotherapies. Based on the questions addressed, we have designed and initiated clinical trials with NK cells in patients with treatment refractory hematological malignancies. We here report immunological and clinical results from one recently closed phase I/II study with HLA-haploidentical NK cell therapy given to patients with chemotherapy refractory high-risk myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). A lymphodepletive conditioning regimen consisting of intermediate doses of cyclophosphamide (Cy), fludarabin (Flu) and titrated doses of total lymphoid irradiation (TLI) was used. The NK cell-product was obtained from an over night IL-2-activated (16 h), CD3- and CD19-depleted leukapheresis product. Sixteen patients were included. The treatment was well tolerated and no severe non-infectious toxicity was observed. Nine patients responded to the treatment with six achieving a “major response”. Two patients had an “intermediate response” with stable disease (SD) but without hematological improvement and one had a “minor response”. In nine patients, donor NK cells were detectable by RT-PCR, 7-14 days after the infusion. 100% of the patients with a major response that were tested with RT-PCR at day 7 had detectable NK cells. Five patients became eligible for and proceeded to allogeneic stem cell transplantation (SCT). Three of the patients receiving SCT and one patient not proceeding to SCT are still free from disease 2-4 years after treatment. The results suggest that a combined lymphodepletive regimen followed by NK cell therapy may induce a temporary remission in patients with chemotherapy refractory high-risk myeloid malignancies and provide a possible bridge to allogeneic stem cell transplantation. Appropriately used, we predict that adoptively transferred NK cells will have a role, either directly or in combination with other treatment modalities such as, e.g., antibodies in future treatment of cancer.

Alternative direction of inhibition of malignant properties in tumor cells *in vitro* and *in vivo* by gene therapy with Inf-β gene in recombinant baculovirus vector

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Aim: To investigate the influence of recombinant baculovirus bearing the interferon-beta encoding gene (rBV/IFN) on phenotypic characteristics of tumor cells *in vitro*: morphology, growth, cytogenetic characteristics and expression of proteins associated with proliferative activity, cell cycle regulation, epithelial-mesenchymal transition (EMT), invasiveness, and the migration potential. Tumorigenicity and the metastatic potential of tumor cells after their transduction with rBV/IFN was studied in vivo, using the mouse models.

Methods: The mouse Lewis lung carcinoma cells (cell line LL) and melanoma cells (MM-4 cell line) were used.

Results: Transduction of melanoma cells and lung carcinoma cells with rBV/IFN leads to reduction of the cell number, reduction in the growth rate, migration ability, colony forming ability in soft agar, and also in inhibition of the ability of cells to grow in a serum-free medium *in vitro*. The LL/rBV/IFN and MM-4/rBV/IFN cells are arrested in G_0/G_1 and S phases. Transduction of these cells with the Inf-β gene was accompanied with the accumulation of apoptotic cells, an increased level of nuclear anomalies in MM-4 cells and the increased frequency of mitotic pathology in LL cells. Moreover, transduction of LL cells with rBV/IFN causes significant genotoxic effects: the levels of chromosome aberrations were increased, as were the frequency of appearance of nuclear protrusions, polyploidy and endoreduplication.

Also, the production of recombinant IFN-beta by MM-4 and LL cells was accompanied by changes in the expression of proteins associated with the cell cycle regulation, EMT, invasiveness and migration ability: expression of p19ARF and p21WAF1 was increased, and of N-cadherin was decreased in MM-4 cells. The number of E-cadherin-positive LL cells were increased then. In addition, transduction of lung carcinoma cells with rBV/IFN leads to significant inhibition of expression of Slug and Twist. These changes indicate a significant inhibition of EMT process and reversion of tumor cells to the “normal-like” epithelial phenotype.

In result, transduction of LL carcinoma and MM-4 melanoma cells with rBV/IFN *in vitro* inhibits their ability to form solid tumors and metastasize in the lungs of mice. Therapeutic intravenous administration of the rBV/IFN results in significant reduction of the number and volume of metastases in the lungs of mice.

Conclusions: Transduction of lung carcinoma and melanoma cells with rBV/IFN inhibits their sings of malignancy *in vitro* and *in vivo*, provides genotoxic effects in LL cells and leads to suppression of EMT in these cells.

CD150/SLAMF1 as a new potential target for anti-tumor therapy

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CD150/SLAMF1 is a prototype member of SLAM family within the immunoglobulin superfamily of surface receptors that are widely expressed on cells within hematopoietic system. Six of nine SLAMF receptors have a paired unique immunoreceptor tyrosine-based switch motif (ITSM) that serves as a docking site for SH2-containing proteins. In T and B lymphocytes, natural killer cells, macrophages and dendritic cells CD150 is a co-receptor molecule that mediates different signal transduction pathways depending on the availability of downstream signaling elements, especially, the adaptor protein SH2D1A/SAP. Due to highly glycosylated and sialylated extracellular Ig domains, CD150 is involved in homotypic interactions and could be considered as a pattern-recognizing receptor. It is a major entry receptor for several Morbilliviruses, including measles virus (MV), and also a bacterial sensor that control the killing of Gram-negative bacteria. Functionally it serves as a bridge between innate and adaptive immunity.

More than 90% of lymphoid leukaemia and lymphomas in adults have B-cell origin. Within B-cell lineage cell surface receptor CD150/SLAMF1 is broadly expressed starting from pre-B cells with upregulation toward plasma cells. However, expression of CD150 is rather limited on the surface of malignant B cells with the block of differentiation at the different stages of maturation. The high level of CD150 surface expression is observed in hairy cell leukemia, classical Hodgkin lymphoma (HL), subtype of diffuse large B-cell lymphoma with activated B cell phenotype (ABC-DLBCL), primary cutaneous follicular centre B-cell lymphoma, and in 60% of chronic lymphocytic leukemia cases. Expression and functions of this antigen outside of the hematopoietic system were not fully explored. We found that CD150 was expressed in several tumors of ectodermal origin (e.g. squamous cell carcinoma of uterine cervix, rectum and oral cavity, basalioma), but not in their normal counterparts. Recently we found CD150 expression in malignant cells of CNS tumors. Although CD150 was not found in different regions of normal brain tissues, our immunohistochemical study revealed its expression in 77.6% of human CNS tumors, including glioblastoma, anaplastic astrocytoma, diffuse astrocytoma, ependymoma, and others. CD150 was detected in the cytoplasm, but not on the cell surface of glioma cell lines, and it was colocalized with the endoplasmic reticulum and Golgi complex markers. In addition to the full length mRNA of the conventional mCD150 splice isoform, in glioma cells we found a highly expressed novel CD150 transcript (nCD150), containing an 83 bp insert. The insert is derived from a previously unrecognized exon designated Cyt-new, which is located 510 bp downstream of the transmembrane region exon, and is a specific feature of primate SLAMF1. Since CD150 is not revealed in normal brain tissues, but is expressed in 77.6% of CNS tumors, CD150 could be considered as a novel diagnostic marker for CNS tumors and a potential target for the therapy of gliomas, especially MV-based oncolytic therapy.

CD150/SLAMF1 antigen in molecular pathobiology of chronic lymphocytic leukemia

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Recent finding indicate that CD150 cell surface expression can be used as a surrogate prognostic marker of CLL favourable outcome. It was shown that CLL patients with high CD150 expression level on malignant B cells has longer treatment free survival and overall survival compared to patients, which lost CD150 cell surface expression. However, the mechanisms that underlie dependent on CD150 expression biological properties of CLL B cells are not fully understood. Present study was focused on characterisation of CD150 topology and isoforms expression, as well as study of CD150 mediated signaling pathways in CLL B cells.

For the first time, we found that in cell surface CD150 negative (csCD150-) CLL cases CD150 antigen was expressed on the protein level and was localised in the cytoplasm. Within cytoplasm it was colocalised with markers of ER, Golgi apparatus and endosomes, but not lysosomes. Exclusive cytoplasmic CD150 expression in the part of CLL cases was not associated with ER stress or ceramid metabolisms. Ligation of CD180, but not CD40 or BCR, leaded to slight CD150 upregulation in csCD150- CLL B cells. CD150 cell surface expression was positively correlated with expression of cell death receptor CD95, BCR negative regulator - CD22 and receptor of toll-like family, potential prognostic marker of favourable outcome - CD180. The highest level of CD150 colocalisation on the cell surface of CLL B cells was observed for CD180. We found that conventional transmembrane mCD150 is a predominant CD150 isoform in CLL. However, in 20% of studied CLL cases nCD150 isoform was prevalent. Moreover, elevated mRNA expression of sCD150 isoforms was detected in all CLL cases, compared to normal B cells subsets. In csCD150+ CLL cases the basal level of tyrosine phosphorylation and phosphorylation of serine/threonine specific motifs, which are substrates for AMPK, Akt, PKA, PKC, CDK kinases, was higher compared to that in csCD150- CLL B cells. Outcome of CD150 mediated Akt and MAPK kinases phosphorylation in CLL B cells was dependent on basal phosphorylation level of these kinases and often demonstrated rapid p38MAPK phosphorylation and bimodal kinetics of Akt, ERK1/2 and JNK1/2 activation. Examining the transcription factors (TF) expression profile in CLL B cells revealed that high PU.1 protein expression level positively correlated with CD150 cell surface expression in CLL. Furthermore, CD150 signaling was involved in regulation of PU.1 mRNA level in csCD150+ CLL cases. We also found that signals via CD150, in contrary to BCR, CD40 and CD180, significantly downregulated mRNA expression levels of CCL3, CCL4 and IL-10 cytokines in CLL B cells. This may contribute to favourable clinical outcome of csCD150+ CLL cases.

Taken together, CLL cases are heterogeneous in cell surface CD150 expression as well as in CD150 isoforms expression.

DNA immunization targeting carcinoembryonic antigen in colorectal cancer patients

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Active specific immunotherapy targeting carcinoembryonic antigen (CEA) may induce antigen-specific humoral and cellular responses in cancer patients. Plasmid DNA, encoding tumor antigens, represents a novel approach of delivering conformational antigens. Here we report immune data of an explorative study using CEA66-DNA (non-glycosylated cytoplasmic CEA) and tetwtCEA-DNA (wild type glycosylated and secreted CEA) for immunization in combination with cyclophosphamide and GM-CSF in the adjuvant setting of radically operated colorectal cancer (CRC). 10 patients received intradermal (id) or intramuscular (im) CEA66-DNA delivered by needle-free Biojector at weeks 0, 2, 6 (part 1). 10 patients received tetwtCEADNA id by needle injection and electroporation at weeks 0 and 12 (part 2). In part 3, 6 patients were primed with CEA66-DNA and boosted with tetwtCEA-DNA. A significant increase of CD4+ effector memory, CD8+ effector and CD8+ effector memory T cells was seen in part 1. An immune response against CEA at at least one time point was noted in 15/20 (75%) patients in parts 1 and 2 together. The frequency of patients mounting a CEA-specific cellular immune responses was significantly higher in part 1 (100%) than in part 2 (50%) (p=0.03). In part 3, 5/6 (83%) patients showed a CEA-specific immune response after a prime-boost protocol. The higher CEA-specific T cell responses seen in part 1, may indicate reduced immunological tolerance induced by the non-glycosylated intracellularly produced CEA66-DNA immunogen. Humoral responses determined by ELISA were low. Further studies are warranted to optimize vaccination schedules to induce both cellular and humoral anti-CEA responses of clinical significance.

Changes in expression of miRNA-122, -200b, and 320a as prognostic biomarkers for breast cancer

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High heterogeneity and diversity of breast tumors make the molecular characterization based on new biological markers very important. Recent studies have shown that not only genes and proteins are important players in cancer development, but also a new class of potential epigenetic tumor markers - miRNAs. An important advantage of miRNA is their stability in both, plasma and tumor tissues.

Aim: To identify the features of oncosuppressive miR-122, -200b, and -320a in breast cancer patients and to monitor the possible correlation between their expression and cancer progression.

Materials and methods: The study was conducted, using 110 tumor samples and serum of the breast cancer (BC) patients and 14 serum samples of healthy volunteers. The levels of miRNAs were assessed, using q-PCR. The expression levels of ER, PR, Her2/neu, Ki-67, E-cadh, and N-cadh were monitored by immunohistochemical analysis.

Results: Each of miRNAs has a great network of targets involved is cellular processes, that makes them very promising diagnostic markers. Among variety of cancer-related miRNAs we choose several that can be used as molecular markers in BC prognosis and are responsible for migration, proliferation, EMT, and drug sensibility. We have found that the majority of BC tissues are characterized by a significant decrease in expression of miR-122 and -200b (in 93.2% and 83.1% of cases, respectively). Down-regulation of miR-122, -200b, and -320a in BC tissue was associated with a higher staging, enhanced proliferation, and also with the absence of hormone receptors ($p<0.05$). Serum levels of miR-122 and -200b were associated with the pathological stage ($p=0.005$ and $p=0.01$, respectively). Decrease of mir-320a levels in cancer tissue compared to normal samples was associated with the TNBC as well, and also with the active proliferation, hence, with cancer aggressiveness. Low levels of miR-320a in serum were observed in patients with lymph node metastases ($p=0.03$).

Conclusions: Established profile of circulating and tumor microRNAs is associated with aggressive clinical course of BC. It makes the determination of expression of the miR-122, -200b, and 320a in the serum the base for development of noninvasive screening tools for BC prognosis.

Cancer cell heterogeneity – an additional level analyzed by EBV-cell models

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Genetic and to some extent epigenetic heterogeneity within a cancer cell population are well established phenomena. But there is a third level of singular cell heterogeneity which results from stochastic noise in transcription and in the intracellular network.

This observed intercellular heterogeneity within a clonal cell population can be mapped as dynamical states clustered around an attractor point in gene expression space, owing to a balance between homeostatic forces and stochastic fluctuations. These dynamics have led to the cancer cell attractor conceptual model, with implications for both carcinogenesis and new therapeutic concepts. Immortalized and malignant EBV-carrying B-cell lines were used to explore this model and characterize the detailed structure of cell attractors.

Any subpopulation selected from a population of cells repopulated the whole original basin of attraction within days to weeks. Cells at the basin edges were unstable and prone to apoptosis. Cells continuously changed states within their own attractor, thus driving the repopulation, as shown by fluorescent dye tracing. Transcriptome analyses suggest that these forces result from high-dimensional dynamics of the gene regulatory network.

We propose that this phenomenon can be generalized to all cancer cell populations and represent intrinsic behaviors of tumor cells, offering an additional characteristic governing phenotype.

Chemoresistance of lung adenocarcinoma is regulated by Tudor staphylococcal nuclease

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Lung cancer is the main cause of all cancer-related deaths in the world, with lung adenocarcinoma (ADC) being the most common subtype of this fatal disease. Lung ADC is often diagnosed at advanced stages involving disseminated metastatic tumors. This is particularly important for the successful development of new cancer therapy approaches. The high resistance of lung ADC to conventional radio- and chemotherapies represents a major challenge to treatment effectiveness.

Earlier we found that Tudor staphylococcal nuclease (SND1 or TSN) is overexpressed in ADC lines and tissues, and is important for maintaining of ADC chemoresistance. Downregulation of TSN by RNAi in ADC cells led to strong potentiation of cell death in response to cisplatin.

In order to identify potential molecular targets involved in ADC sensitization to cisplatin, the global gene expression analysis was performed. Widespread transcriptional changes were observed upon TSN knockdown: 391 unique genes demonstrated a greater than twofold average change in expression, with 234 transcripts under- and 157 transcripts overexpressed compared to scrambled transfected control samples. Using the Ingenuity Pathways Analysis (IPA) program and gene ontology category enrichment analyses, we selected several major networks containing genes that were closely associated with autophagy and apoptotic cell death as well as survival, DNA damage response and Ca2+ signaling. The expression of shortlisted genes was further analyzed by q-RT-PCR, confirming microarray data. ON the top of the list was S100A11. Silencing of TSN was accompanied by a significant decrease in S100A11 expression at both mRNA and protein level. Downregulation of S100A11 by RNAi resulted in enhanced sensitivity of NSCLC cells to cisplatin, oxaliplatin and 5-fluouracil.

S100A11 interactions were analyzed using Interactive pathway analysis of complex'omics data and S100A11-related pathways involved in apoptosis and cell resistance to cytotoxic treatment were selected for further analysis. We found that in cell cytoplasm S100A11 interacts with Annexin A1 and Annexin A2 and inhibit phospholipases A2 (PLA2), a superfamily of enzymes involved in arachidonic acid (AA) release. A PLA2 inhibitor or silencing with siRNA strongly abrogated chemosensitization upon silencing of S100A11 suggesting that PLA2 inhibition by S100A11 governs the chemoresistance of ADCs. Thus, we present the novel TSN-S100A11-PLA2 axis regulating superoxide-dependent apoptosis, triggered by platinum-based chemotherapeutic agents in ADCs that may be targeted by innovative cancer therapies.

The MRPS18-2 protein as a putative marker of cancerogenesis

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Endometrial cancer (EC) is one of the most frequent causes of cancer death among women in developed countries. Histopathological diagnosis and imaging techniques for EC are limited, thus new prognostic markers are needed to offer patients the best treatment and follow-up.

We showed that the level of mitochondrial ribosomal protein MRPS18-2 (S18-2) increased in EC compared with the normal endometrium and hyperplasia, based on a study of 42 patient biopsies. Importantly, high expression of free E2F1 in EC correlates well with high S18-2 expression. The EC cell line HEC-1-A, which overexpresses S18-2 constitutively, showed an increased proliferation capacity in vitro and in vivo (in SCID mice). Moreover, pan-keratin, beta-catenin and E-cadherin signals are diminished in these cells, compared to the parental HEC-1-A line, in contrast to vimentin signal that is increased. This may be associated with epithelial-mesenchymal cell transition (EMT).

We conclude that high expression of S18-2 and free E2F1, and low pan-keratin, beta-catenin, and E-cadherin signals might be a good set of prognostic markers for EC.

High expression levels of MRPS18-2 and presence of the RB protein are required for the maintenance of the stem cell phenotype

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Background: We have found that S18-2 is involved in regulation of the RB-dependent pathway. It binds to both hypo- and hyperphosphorylated RB. The binding between RB and S18-2 is promoted when cytoplasmic S18-2 is targeted to the nucleus, and this disrupts the association of E2F1 with RB, as indicated by the increased level of free E2F1 in the nucleus. This presumably lifts the RB-dependent block to S-phase entry in the cell cycle. We have also found that overexpression of the human S18-2 immortalized primary rat embryonic fibroblasts (REFs) that showed properties of embryonic stem cells. Elevated expression of S18-2 in stem cells (our findings and analysis of published microarray data) raises the question of whether this protein co-operates with the RB protein in differentiation and cancerogenesis. The aim: We wanted to seek a connection between the expression of RB and S18-2 in -/-Rb1 MEFs and stemness. We hypothesized that simultaneous expression of both proteins at the high levels might support stemness. Methods: Transfections, inoculation into SCID mice, directed differentiation, q-PCR, immunostaining, immunohistochemistry, western blotting. Results: We showed that S18-2 protein, together with RB, plays a crucial role in cell de-differentiation. We have found that overexpression of S18-2 and RB is needed for maintenance of cell stemness. Such cells can differentiate into various cell lineages under certain conditions. Conclusion: The presence of RB and simultaneous expression of S18-2 at high levels are required for the cell stemness.

Identification of novel molecular and genetic markers for early detection of epithelial tumors and prognosis of the course of disease

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The main topic of our research is the identification of molecular and genetic markers for the early detection of epithelial tumors and prognosis of the course of disease. Early detection of carcinogenic process can lead to more successful treatment, thus, significantly increasing the chances for recovery.

The investigation of non-invasive diagnostic tools, which can be used in clinical practice, is now the main focus of oncology. This problem can be solved by using of molecular nucleic acid markers that are present in biological fluids (serum, urine, semen etc).

We have developed several useful and effective approaches for investigation of molecular and genetic markers. We are studying both, genetic and epigenetic changes in cancers of different types, using a broad scale screening of their presence in clinical samples.

The techniques and methods we use allow us to obtain reliable results and propose them for implementation in clinical practice.

The typhoid toxin modulates the host inflammatory response and promotes the establishment of a persistent asymptomatic infection

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Epidemiological evidence links several bacterial infections with increased risk of tumor development. Several Gram-negative bacteria are able to produce genotoxins, which induce DNA damage in the target cells and possess carcinogenic properties in vitro. The mechanisms by which bacterial infection can promote carcinogenesis are poorly understood. Likely the production of DNA damaging toxins may enhance the tumor promoting effects of chronic inflammation.

To address this issue, we have developed a unique model of acute and chronic infection with S. enterica, serovar Typhimurium (S. Typhimurium) expressing either the toxigenic typhoid toxin or the non-functional genotoxin. We have chosen this bacterium since it is the only genotoxin producing bacterium that induce chronic asymptomatic infections, which are associated with an increased risk of cancer development in humans.

We demonstrated that the expression of a functional typhoid toxin reduced the mortality rate of the host and prevented intestinal inflammation in the early phase of the infection, while it promoted the establishment of persistent asymptomatic infection in the liver associated with a chronic mild inflammatory response.

Our data suggest that one of the effects of these bacterial effectors that cause DNA damage is to increase the fitness of the host, modulate the host immune response, and favour colonization. Furthermore, our model will be useful to elucidate the relationship between bacterial genotoxin chronic infection effects and cancer development.

Sustainability and robustness of mined biological knowledge: solutions using network-based pathway analysis

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The characterization of molecular landscapes benefits from elevating analyses at the pathway and network level, i.e. quantifying alterations in functional modules (pathways) rather than those of individual genes (Alexeyenko and Sonnhammer, 2009). Our algorithm of network enrichment analysis (NEA) detects activation of such modules in a robust and unbiased way. Molecular features of cancer tumors are functionally interpreted via their network positions. This enables distinguishing driver mutations and discovering network-anchored biomarkers for specific phenotypes.

The NEA (Alexeyenko et al., 2012; Merid et al., 2014) is characterized by a simple and statistically sound way of using gene annotations and topological information from the network. It transforms the original space of altered genes into a space of pathways by using the global gene interaction network – as a reflection of the idea of individually rare alterations leading to the same cancer phenotype. Compared to original variables, pathway profiles are closer to the underlying biology and - potentially - more informative and robust predictors of clinical outcomes.

The main principle of the NEA algorithm can be understood through comparison with the gene set enrichment analysis (GSEA). An experimental or clinical state can be characterized by a set of altered genes (AGS), such as top ranking differentially expressed genes or a set of somatic mutations. The other component of the analysis is a collection of functional gene sets (FGS): pathways, ontology terms, or custom sets of biological importance. In GSEA, enrichment is determined by how many genes FGS and AGS share. NEA considers the network environment by counting network edges that connect any genes of AGS with any genes of FGS. In the both approaches, significance can be evaluated with appropriate statistical tests; in the case of NEA this is done by considering topological properties of the network nodes (Ashwini and Alexeyenko, in press).

I will present latest results of application of the method to finding sensitive and robust cancer biomarkers at the pathway level: scores from network enrichment analysis transform the original space of altered genes into a lower-dimensional space of pathways. These dimensions are then correlated with clinical phenotypes. We tested it using both public and novel experimental data: first on three anti-cancer drug screens and then on patient treatment using the same drugs. It proved to be superior to the traditional single-gene analysis in terms of 1) correlation with drug sensitivity in cancer cell lines, 2) consistency of the discovered correlates on an independent drug screen, 3) ability to explain differential survival of patients, and 4) relevance of the in vitro correlates to survival of patients who received the same drug.

POSTER PRESENTATIONS

Genotoxic sensitivity of peripheral blood lymphocytes in endometrial cancer patients to 4-hydroxyestradiol

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The endometrial cancer (EC) pathogenesis to a large extent may be determined by genotoxic effects of estrogen metabolites, among which the 4-hydroxyestradiol (4OHE2) is characterized by the most prominent DNA-damaging properties. Genomic instability and sensitivity to genotoxic estrogen metabolites in cells of EC patients depend on the DNA repair efficiency.

The aim of the study was to analyze the level of DNA damage in peripheral blood lymphocytes (PBL) of EC patients after treatment with the 4OHE2 and to evaluate the repair efficiency of induced DNA damage.

Total 33 EC patients (mean age 60.2±1.5) and 20 healthy women (mean age 55.7±3.1) were included in the study. The level of DNA damage in PBL was measured, using comet assay and expressed by % tail DNA (%TDNA). Estradiol (E2) level in blood serum was assessed by ELISA (pg/ml).

Large variability in basal level of DNA damage was observed in PBL of EC patients (1.8 — 20.5 %TDNA) as well as healthy donors (1.1 — 9.2 %TDNA). In average, EC patients had significantly higher basal level of DNA damage (7.9 ± 0.9 %TDNA) compared to healthy women (3.5 ± 0.5 %TDNA) ($p<0.05$). After incubation with 4OHE2 there was an increase in the %TDNA in PBL of EC patients (8.0 — 57.7 %TDNA) as well as of healthy donors (7.9 — 46.8 %TDNA). However, the average level of induced DNA damage in EC patients was significantly higher (38.9 ± 2.7% TDNA) compared to healthy women (25.8 ± 2.2 %TDNA) ($p<0.05$). To investigate the association between the sensitivity of PBL to 4OHE2 and estradiol level in the blood, we measured the concentration of E2 in the blood serum. We found that the blood level of E2 was higher (54.9 ± 4.1 pg/ml) in EC patients with high sensitivity of PBL to 4OHE2 than in patients with low sensitivity (38.2 ± 4.3 pg/ml). The higher level of unrepaired DNA damage in PBL was observed in EC patients (18.6 ± 2.6 %TDNA) compared to control group (13.1 ± 1.4 %TDNA). Furthermore, it was found that patients with high sensitivity of PBL to 4OHE2 had greater amount of unrepaired DNA damage (24.1 ± 4.4 %TDNA) than women with low sensitivity (13.1 ± 1.8 %TDNA).

These results indicate that PBL of EC patients are characterized by hypersensitivity to 4OHE2 and impaired DNA damage repair that is associated with hormonal status of women. It can be assumed that increased level of 4OHE2-induced genome instability may contribute to endometrial cancer pathogenesis.

Dendrimers as carriers in anticancer gene delivery

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This presentation examines a perspective to use newly engineered nanomaterials as effective and safe carriers for gene therapy of cancer. Different groups of cationic dendrimers were complexed with anticancer siRNA and the biophysical properties of the dendriplexes created were analyzed. The potential of the dendrimers as nanocarriers for anticancer siRNAs and additionally a scrambled sequence siRNA has been explored. Dendrimer/siRNA complexes were characterised by various methods including fluorescence, zeta potential, dynamic light scattering, circular dichroism, gel electrophoresis and transmission electron microscopy. In this part of study, the transfection of complexes in different types of cancer cells was analyzed using single apoptotic siRNAs. The dendrimers were compared as siRNA carriers. However, some of them were cytotoxic on their own, so that in this regard the application of all dendrimers in anticancer therapy will be discussed.

Pharmacological re-activation of p53 protein family affects proliferation and migration of cancer cells with TP53 gene mutations

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In the majority of all human cancer cases, the cancer suppressor protein p53 is lost or mutated. p53 belongs to a family of proteins that includes p73 and p63. The latter two can be categorized into two main groups: TA isoforms, which, like p53, act as tumor suppressors; and ΔN isoforms, which act like oncogenes and affect the functions of p53, TAp63, and TAp73.

TP53 mutations lead to gain of function, which promotes more metastatic and fatal disease that are extremely difficult to cure. As reconstitution of p53 suppresses established tumors in vivo, current targeted therapies focus on the activation of p53 but this strategy is still difficult to implement therapeutically. A promising strategy to overcome p53 deficiency and restore p53 pathway function is to manipulate the p53 family members, TAp63 and TAp73. In our on-going efforts we identified porphyrins, clinically approved compounds used in photodynamic therapy of cancer, to restore the p53 pathway and inhibit proliferation of cancer cells in tumors with various p53 mutations. Activation of TAp73 has emerged as a mechanism by which porphyrins activate p53 target genes, engage pro-apoptotic signaling and inhibit migration of metastatic cancer cells.

We propose a hypothesis that targeting interactions of TAp73 with its inhibitors with simultaneous inhibition of synthetic sick factors to those inhibitors can provide a therapeutic advantage over currently developed strategies to target tumors with TP53 loss or mutations.