THE MOLECULAR MECHANISMS OF SELECTED PATHOLOGICAL PROCESSES IN THE CELL

Dear Readers,
I would like to present, for the third and unfortunately the last time, conference abstracts of 16 student scientific talks that are a part of a project 303/09/H048 entitled „Molecular mechanisms of selected pathological processes in the cell." The project was formed around 19 doctoral student positions in two institutions: the Faculty of Medicine and Dentistry, Palacký University in Olomouc, and Institute of Biophysics in Brno. The real number of students participating in the project during the four years of its life was almost double the number of positions. All student members of the team conducted their research in five principal fields of biomedicine: medical biology, medical biophysics, biochemistry, pharmacology and pathology. Although apparently broad in scope the actual topics where not that distant. You may want to compare the abstracts published last year. Primary goal of the project was to support individual endeavors of the students and provide a communication platform for building interdepartmental teams. Such a platform was the annual two-day meeting of the entire team where the students had a nice opportunity to present their successes also in the form of oral presentations.

Martin Modriansky

Study of epigenetic 5-azacytidine nucleosides and their derivatives

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Aberrant DNA methylation is a major hallmark of cancer. In cancer cells, global hypomethylation is accompanied by hypermethylated and transcriptionally silenced tumor suppressor and other cancer related genes, and contribute to the loss of proliferation control. The reversion of these epimutations can therefore restore proliferation control and apoptosis sensitivity. The cytosine analogues 5-azacytosine (azacytidine) and 2'-deoxy-5-azacytidine (decitabine) function as DNA methyltransferase inhibitors and have shown substantial potency in reactivating epigenetically silenced tumor suppressor genes *in vitro* and are currently most advanced drugs for epigenetic cancer therapies.

To characterize the demethylating activity of 5-azacytidine nucleosides and their derivatives we established a fluorescence detection system with high sensitivity that involves an endogenous promoter CpG island (CGI). Promoter CGI of FLJ32130 gene was found to be methylated in HCT116 cells and respond sensitively to decitabine by abundantly re-expressing its mRNA, therefore FLJ32130 gene was selected as a targeting site. Exon 3 of the FLJ32130 gene was more than 2 kb apart from the promoter CGI and it was expected that targeting of the exon would not affect the methylation status of the promoter CGI; therefore a targeting vector was constructed to replace exon 3 of the FLJ32130 gene with a fusion of IRES, Hygromycin and EGFP. To construct the reporter cell we transfected HCT116 cells with the linearized vector and isolated one clone that had the expected homologus recombination outcomes. It was expected that the Hygr'-EGFP fusion protein would not be expressed as long as the promoter CGI was kept methylated, but that it would be expressed when the CGI was demethylated. Under the fluorescence microscope, we examined the appearance of green fluorescence before and after addition of decitabine and the anticipated fluorescence was readily confirmed after addition of 1 μM decitabine.

Furthermore, the DNA methylation inhibitor 2'-deoxy-5-azacytidine develops resistance during treatment and mechanisms of resistance remain unknown, therefore we are currently investigating the mechanism of resistance to decitabine.

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Enhancement of TRAIL-induced apoptosis by platinum-based chemotherapeutic drugs in human colon cancer cells

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Application of platinum-based chemotherapy that is currently used for treatment of malignant tumors is accompanied by several problems such as acquired resistance of cancer cells and negative side effects on normal tissues. Therefore, there is a need for introduction and

testing of new drugs with more efficient and selective action. Platinum (IV) adamantylamine ligand-containing complex LA-12 is able to induce death in colon cancer cells and sensitize them to other apoptosis-inducing agents, which results in more effective cancer cell elimination. We investigated whether LA-12 can modulate the response of colon cancer cells to TRAIL (tumor necrosis factor-related apoptosis inducing ligand), a unique cytokine that selectively promotes apoptosis in tumor but not in normal cells.

We report that LA-12 mediates effective sensitization of colon cancer cells to apoptosis induced by TRAIL. A functional role of TRAIL DR5 in LA-12-mediated potentiation of TRAIL-induced apoptosis was verified previously by us using specific siRNA. Here we show that the cytotoxic effects of LA-12+TRAIL are p53-independent, as similar cytotoxic response to this combination can be observed in model cell lines with wild type, mutant or lacking p53 protein. During colon cancer development sequential accumulation of characteristic mutations occurs. Biological behavior of cells may differ in diverse stages of the disease, as well as their response to therapy. Combination of LA-12 and TRAIL effectively promoted apoptosis of colon cancer cells derived from carcinoma (HCT116, SW480) and metastasis (SW620). Interestingly, it has also been shown by us to exert its cytotoxicity in human colon adenoma cells (AA/C1). In contrast, normal human colon epithelial cell line (NCM460) was not susceptible to LA-12 and TRAIL when applied in concentrations harmful to cancer cells.

Our results suggest that combination of LA-12 and TRAIL may be a promising strategy for anticancer treatment even in tumors with non-functional p53 and in various stages of colon cancer development.

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Novel fluoroquinolone-derived compound inhibits tubulin polymerization

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Newly synthetised fluoroquinolone derivative inhibited growth of tumor cell lines of different origin at the lowest IC50 value 1.3 μM in K562. Moreover, high sensitivity is maintained in Pgp-expressing paclitaxel-resistant subline K562-tax. Similarly daunorubicine-resistant CCRF-CEM DNR subline show comparable sensitivity as its parental cell line CCRF-CEM, suggesting activity against cancer cell lines with different mechanisms of drug-resistance. Cytotoxicity was significantly lower in normal human lung and skin fibroblast cell lines. Following treatment with 6 μM concentration of derivative, CCRF-CEM cells were arrested in G2/M phase. Elevated levels of phospho-histone H3, a mitotic marker, and cyclin B1 indicated block in mitosis, with their consequent decrease after 24 and 36 h due to apoptosis. Process of apoptosis is tightly regulated by Bcl-2 family proteins and we found overexpression of its proapoptotic member Bax 6 hours after treatment. *In vitro* turbidometric tubulin assay (99% tubulin purity) showed, that derivative acts as tubulin polymerization inhibitor. Using immunofluorescence technique, we detected shortened microtubules in fixed U2OS interphase cells. Furthermore, an aberrant mitotic spindle morphology was observed in α tubulin-GFP expressing cells, therefore we conclude that the main target of this new fluoroquinolone-derived compound is dynamic mitotic spindle of rapidly dividing cells.

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The effect of histone deacetylase and DNA methyltransferase inhibitors on expression of androgen receptor gene in androgen-independent prostate cancer cell lines

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Introduction. Androgen receptor (AR) expression in prostate cancer (CaP) cells varies due to multiple changes including epigenetic modifications such as DNA methylation and histone deacetylation. DNA methyltransferases and histone deacetylases inhibitors are promising for the treatment of CaP patients. Aim of our study was to analyze the 5-Aza-2´-deoxycytidine (Aza-dC) and sodium butyrate (NaB) effects on prostate cancer cells with modified AR gene expression.

Methods. Androgen-independent human prostate cancer cell lines PC3 (lacking a functional AR) and DU145 (strongly limited expression due to methylations in the AR gene) were used. PCR of bisulfite modified DNA and RT-PCR with bisulfite-sequencing were used for AR gene analysis of DU145 and PC3 cells after their treatment with Aza-dC and/or NaB. Re-acetylated histones around the AR gene were detected by conventional PCR of immunoprecipitated DNA obtained from treated cells.

Results. In both cell lines without AR expression, the combined treatment was followed with significant decrease of cell viability. The co-treatment of DU145 cells caused site-specific demethylation in the AR promoter region followed with gene re-expression and increased acetylation in histones H3 and H4.

Conclusion. The co-treatment with Aza-dC and NaB was the most effective in demethylation and re-expression of the AR gene. In the AR gene promoter, the location and density of demethylated CpGs implies an existence of distinct promoter hot spot that could be a target of AR gene activation therapy of prostate cancer patients during androgen deprivation.

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Analysis of cell proteome after application of photodynamic therapy

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Photodynamic therapy (PDT) is a promising approach for fighting selected cancers. The PDT is based on two important steps: 1. accumulation of sensitizer in a cancer tissue, 2. site specific irradiation of a cancer tissue with accumulated sensitiser with light of suitable wavelength. After irradiation the photochemical reaction is provoked between a sensitiser, a light and a substrate and reactive forms of radical oxygen are developed. Damage of various subcelullar components (proteins, nucleic acids) and cellular structures are occurs as a consequence of oxidative stress.

The aim of presented work was to analyse the changes in a proteome of animal cell after the photodynamic therapy by method of quantitative proteomic based on an application of stable isotope labelling by amino acids of cell culture (SILAC). The experimental conditions were as follows: a sensitiser of phtalocyanine type ($ClAlPcS_2$), Hela S3 cell, source of light with wavelength of 660 nm. The project was divided into several steps. First step was the estimation of suitable concentration of the sensitiser at light dose of 5J/cm^2 (660nm) by viability assay (MTT). The second step was the preparation of two different Hela S3 cell fractions labelled with light and heavy isotopic forms of arginine and lysine. Both labelled cell fractions were incubated with the sensitiser at concentration of 1 μmol/L for 24 h at 37 °C and 5% CO_2. After incubation the light fraction of Hela cells was irradiated by the dose of 5J/cm^2 of light (660 nm). Irradiated cells (light form) and control cells (heavy form, no irradiation) were incubated for three different time points (2 h, 4 h, 6 h) in a culture incubator. After extended incubation the cells of both forms and all time points were mechanically harvested, washed with buffer and the cell pellets were stored at -80 °C until next analysis. The each cell pellet was thawed on ice, lyzed and the total concentration of proteins was determined. For the next proteomic analysis the samples were prepared by mixing of lyzed pellets at protein concentration in ratio 1:1 of light form (irradiated) and heavy form (control) at selected time point. As a result the three separated samples were prepared related to three time points. Proteins in the prepared samples (2 h, 4 h, 6 h) were separated on a SDS-PAGE electrophoresis, stainned by Commassie BB. Each gel line was cut into 13 slices, transferred into separated tubes, cut to small pieces and destained. Disulfide bridges of proteins in the destained gel pieces were reduced using dithiothreitol and alkylated by iodoacetamide. After alkylation the rest of chemicals was removed by washing, the gel pieces were shrinked by an acetonitrile and submitted in a process of in-gel digestion by trypsin. Digestion was performed at 37 °C over night. Released peptides were extracted from gel pieces, purified by solid phase extraction on a reverse phase microcolumn and submitted to a nanoLC-MS/MS analysis. Collected raw MS a MS/MS data were processed and submitted to Mascot search for peptide and protein identification and quantitation.

Summary of results. Under selected experimental conditions (Hela S3 cell, $ClAlPcS_2$ sensitiser, concentration 1 umol/L, incubation for 24 h, light dose 5J/cm^2, incubation after irradiation for 2 h, 4 h, 6 h) at least 746 proteins were identified and quantified for each time point. The largest number of up-regulated protein (20.3%) was detected after 2 h incubation. The number of upregulated proteins rapidly decreases to 4.3% after 6 h incubation. The opposite trend is observable in a number of down-regulated protein where were detected only 3.4% down-regulated proteins at 2 h incubation but 12.7% and 20.7% at 4 h and 6 h incubation, respectively. Basic bioinformatic analysis were carried out on the lists of identified proteins and a distribution into diferent GO terms categories were performed. The analysis of list of up-regulated proteins after 2 h incubation after induction of PDT showed significant enrichment of protein regulated processes of DNA repair. To confirm the DNA damage under selected experimental conditions the comet assay for detection of DNA fragmentation was performed. The results showed that the DNA fragmentation is already detected after 2 h incubation. With prolonged incubation the extensive DNA fragmentation was observed.

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Photodynamic therapy applied on cell lines and bacterial strains

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In the last decade photodynamic therapy (PDT) has been approved and used to treat malignant and non-malignant tumors. Photodynamic therapy is based on the delivery of a photosensitizers towards struck tissues followed the interaction of visible light and accompanied by the production of reactive oxygen species. Our study of PDT is focused on region of interest of bacterial resistance to antibiotics. In this study we compared effectiveness of two photosensitizers: TMPyP (5,10,15,20-tetrakis(N-methylpyridium-4-yl) and $ZnTPPS_4$ (zinc-5,10,15,20-tetrakis(4-sulfonaphenyl) porphyrin) porphyrin) on two cell lines – NIH3T3 (Mouse embryonic fibroblast cell line) and HeLa (Human epithelial carcinoma cell line) and for three bacterial strains: Gram – positive *S. aureus* and *MRSA* and Gram – negative *E. coli*. We applied photosensitizers alone and bound in the complex created with hp-β-cyclodextrin (CD) and we compared their effectiveness to the cell lines and bacteria. The light emitting diodes (LEDs 414 nm) were used as a source in PDT at the doses 0, 1, 5 and 10 $J.cm^{-2}$ for cell lines and 0 and 150 $J.cm^{-2}$ for bacterial strains. Up to now our results suggest that TMPyP and ZnTPPS4 are efficient alone and the efficiency even more increases in complex created with hp-β-cyclodextrin (ratio photosensitizer:CD - 1:4, 1:1, 2:1) for cell lines and Gram – positive bacteria strains. Viability of *E. coli* was increased only in the highest concentrations s with CD.

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Different effect of natural inhibitor of histone deacetylases on cancer and normal prostate cells

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Sodium butyrate (NaB), as a natural inhibitor of histone deacetylases (HDACI), is a non-toxic agent, with an ability to modify histone acetylation and affect the expression large number of genes, including also coregulators of androgen receptor (AR). Coregulators, including coactivators and corepressors play an important role in AR-mediated growth and progresion of prostate cancer. Transcription activity of AR can be increased by coactivators or decreased by corepressors. It is known that between corepressors and coactivators, there is competition for binding to the AR in the presence of ligands and therefore the changes in coregulators expression and ratio could be important for prostate cancer survival. Examples of important AR coregulators are nuclear corepressor SMRT (silencing mediator for retinoid and thyroid hormone receptors) and coactivator p300.

In our study we used two prostate cancer cell lines (LNCaP, C4-2) and one normal prostate cell line (RWPE-1). All cell lines were affected by different concentrations of NaB for 24 and/or 48 h. To investigate the effect of NaB on cell survival was used MTT assay. The changes in gene expression of AR, PSA, SMRT and p300 after NaB treatment were assessed by RT real-time PCR and Western blot analysis was used for detect the protein level of AR, PSA and acetylated H4K8 and H4K12.

This study shows different effect of sodium butyrate on normal and cancer prostate cells. Compared with RWPE-1 cell line, in LNCaP and C4-2 cell lines treated with NaB particularly after 48 h, there was significantly visible decrease in cell viability. Real-time PCR analysis showed that NaB treatment in prostate cancer cells has an effect on increase of both coregulators expression - but the level of SMRT expression was almost double that of p300. On the other side, NaB treatment decreased expression level of AR and PSA. By Western blot analysis was detected gradually decreasing level of AR and PSA, but increasing level of acetylated H4K8 and H4K12.

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Role of asporin in breast cancer cell lines and their invasivity

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Asporin (ASPN) belongs to a small leucine-rich repeat protein family class I and is known to be related to the development and progression of osteoarthritis. Increased asporin expression has also been found by our laboratory and others in different types of cancer (i.e. breast, prostate and pancreatic cancer)

To determine whether asporin influences cell invasion through the 3D collagen scaffold, we performed cell invasion assays using collagen coated CIM plates. To validate commercially available anti-ASPN antibodies, we did a western blot analysis.

Breast cancer cell lines BT 549, MDA MB 231, Hs 578t and gingival fibroblasts were used for antibody validation. Four commercially available anti human ASPN antibodies were validated on selected breast cancer cell lines, human gingival fibroblasts and series of samples derived from human dental pulp stem cells after odontogenic differentiation. The same set of commercial cell lines with gingival fibroblasts was induced to invade collagen I plug in the two chambers CIM plate (xCELLigence System, Roche). Collagen I plug was prepared with or without recombinant asporin.

BT549 and MDA MB 231 invaded faster through collagen I clot mixed with asporin than collagen barrier made without asporin. There was no significant difference in invasion of Hs578t or gingival fibroblasts (both having high ASPN expression). All tested antibodies showed non-specific bands, for this reason, no immunohistochemistry was done.

Asporin presence inhibited collagen I fibrillogenesis which led to significantly faster invasion of selected breast cancer cell lines. Abnormal asporin expression in breast cancer may affect collagen fibrillogenesis leading to changed tumour microenvironment and promoting cell motility and metastasis.

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The role of selected miRNAs in MDR caused by P-gp, MRP1 and LRP/MVP in NSCLC patients

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Lung cancer is the leading cause of cancer deaths worldwide. Therefore, there are still the efforts to find new biomarkers and therapeutic approaches. Currently, small non-coding miRNAs appear to be important in this field. They regulate protein levels in the cells through induction of translational repression and mRNA degradation. Hence, they are involved in a variety of biological processes, such as proliferation, differentiation and apoptosis. Dysregulation of miRNAs may promote tumorigenesis and the response to anti-cancer treatment. The aim of our study was to analyse the relationships between the expression of these molecules and multidrug resistance (MDR) phenotype in non-small cell lung carcinomas (NSCLC) patients.

We tested 62 NSCLC samples from patients treated at the University Hospital in Olomouc between years 1996-2000. After isolation of total RNA from these samples we detected miRNAs using TaqMan® MicroRNA Assays for miR-21, miR-126 and miR-205. We correlated the obtained results with disease free survival (DFS) and overall survival (OS), histological subtypes of NSCLC and with the expression of P-glycoprotein (P-gp), Multiple drug resistance protein 1 (MRP1) and Lung resistance-related protein/Major vault protein (LRP/MVP).

Our results suggest that miR-21, miR-126 and miR-205 levels are not significantly different in histological subtypes of NSCLC and they do not affect the length of DFS and OS. We have found higher miR-205 levels in squamous cell carcinoma compared to adenocarcinoma samples ($P<10^{-4}$). We also observed negative correlation between miR-205 levels and the expression of P-gp ($P<0.03$). Therefore, we can conclude that miR-205 may be an important diagnostic and predictive marker for NSCLC patients.

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miR-29 expression during imatinib treatment of chronic myeloid leukemia patients

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MicroRNAs (miRNA) are short non-coding oligonucleotides responsible for regulation of protein expression on post-transcriptional level. MiRNAs play significant role in the pathogenesis of many cancers including chronic myeloid leukemia (CML). CML is a hematopoietic disorder characterized by a translocation between chromosomes 9 and 22, known as the Philadelphia chromosome. It leads to generation of a specific fusion protein – tyrosine kinase BRC/ABL. CML is treated by tyrosine kinase inhibitor, imatinib. Successful treatment with imatinib is demonstrated by a significant decrease of BCR/ABL level in patient's blood, reaching undetectable level in the course of several months. In this work we determined the expression level of miR-29, which is known as a tumour suppressor, because it modulates the expression of several proteins involved in apoptosis, including Tcl-1, Mcl-1. Others shown that transfection of Philadelphia chromosome positive cells with miR-29b isoform causes apoptotic death of these cells. We found that the expression level of miR-29 is decreased in CML patients when compared to control group and another hematopoietic disorder, chronic lymphocytic leukemia. Increased expression level of miR-29 was found after 2-3 months treatment with imatinib. Statistical analysis showed significant negative correlation between alterations of BCR/ABL and alterations of miR-29b and c. The two miR-29 isoforms are located on chromosome 1 and their expression is regulated by c-Myc and Smad3. Our data suggest that BCR/ABL affects expression of miR-29 and increasing level of miR-29b by other means may aid CML treatment.

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Her2 evaluation in samples with inconclusive FISH

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Trastuzumab is the treatment of choice in breast cancer patients with *Her2* gene amplification/overexpression evaluated by *in situ* hybridization (ISH) and immunohistochemistry (IHC). In approximately 5% breast cancer cases however, ISH assay fails due to poor sample quality caused by inappropriate fixation method or other tissue damage. In these cases, *Her2* protein expression is evaluated by IHC only. Unfortunately, IHC false negativity/positivity was shown in poor quality samples. It is therefore necessary to evaluate *Her2* copy number by some other DNA-based method to avoid inappropriate therapy management.

We developed a quantitative real-time polymerase chain reactions (qPCR) comparing *Her2* to *Gcs1*, *Dck* and *Epn2* reference genes usable for evaluation of *Her2* gene copy number in formalin-fixed, paraffin-embedded (FFPE) tissue samples. The detection limit of the method was determined using a concentration range of CALU3 and MDA-MB-231 cell lines to 5% of higly positive cells. The method was validated on a set of 181 breast cancer patient samples where the FISH (PathVysion), IHC (HercepTest) and qPCR were done in parallel. The levels of sensitivity/specificity comparing qPCR to FISH, resp. qPCR to IHC were 0.978/1.000 and 0.946/0.985. The overall concordance between qPCR and FISH/IHC was 98.7% and 96.2% respectively. To date, qPCR has been used for *Her2* evaluation of a prospective cohort of 198 breast cancer tissue samples with inconclusive FISH result and was able to determine *Her2* copy number in 69.2% cases. qPCR was shown to be highly concordant, specific and sensitive DNA-based alternative of *Her2* copy number evaluation when FISH assay fail.

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Occurrence of off-label and unlicensed drug use in pediatrics

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In the daily practice it is difficult to find registered drugs for children, because many of them are prescribed to be given in ways and for conditions not approved in the marketing authorization. Their prescription is often limited to certain age. Therefore, sometimes they are prescribed as unlicensed (drugs unregistered for children) or off-label (prescribed for younger children than those for which the drug is registered). In this study, we evaluated the incidence of this type of prescription for medicinal products prescribed by the Department of Pediatrics, University Hospital Olomouc in a period of 3 months. Total 4174 prescriptions for 2100 children were evaluated. Unlicensed or off-label drugs were found in 8.62 % of prescriptions. Unlicensed prescriptions were significantly more common in boys than in girls. The prescription of unlicensed drugs was significantly more frequent in school age children than in any other age group. The most commonly prescribed unlicensed drugs were ACE-inhibitors. Among off-label drugs, antihistamines and bronchodilators were most frequently prescribed. To avoid exposing children to unnecessary risks and to avoid depriving them of potentially effective pharmacotherapy, both enforcement of legislation and continued concepts are needed to encourage the development of pediatric medicines.

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Influence of probiotic *E. coli* strain Nissle 1917 on the pharmacokinetics of amiodarone in the rat

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Escherichia coli Nissle 1917 (EcN) of serotype O6:K5:H1 is a non-pathogenic and probiotic strain. However, it is not known whether it alters the pharmacokinetics of concomitantly taken drug (as the antiarrhythmic drug amiodarone (AMI)). In this study, live bacterial suspension of probiotic EcN (or non-probiotic *E. coli* strain ATCC 25922, or, saline solution as a control) was administered orally (gavage) to healthy male Wistar rats daily for seven days. On the eighth day, the amiodarone hydrochloride was applied as one single oral dose (50 mg/kg) to all rats (N = 30 in each group). After 0, 1, 2, 3, 4, 5.5, 7, 9, 14, 22, and 30 h, the blood samples were taken from rat abdominal aorta. The plasma levels of AMI and its metabolite N-desethylamiodarone (DEA) were measured by HPLC with UV detection. Results show that the administration of EcN caused an increased AUC_{0-30} of AMI and DEA (by 43% and 62%, respectively) in comparison with control samples. This effect was not found when EcN was replaced by a reference, non-probiotic strain *E. coli* ATCC 25922. Thus, EcN was most probably responsible for better AMI absorption from the gastrointestinal tract. Higher DEA levels in samples from EcN-treated rats may be explained either by better absorption of AMI and/or by an increased activity by CYP2C forms (known to participate in metabolism of this drug) caused by EcN application discovered in an earlier study. Concomitantly taken probiotic EcN may modulate AMI pharmacokinetics by increasing bioavailability of this drug; however, the simultaneous uptake of the EcN strain and this drug most probably pose no harm to the patient.

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The effect of gold and silver nanoparticles on HaCaT cell lines

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Skin wound healing is a highly regulated process, involving re-epithelization, matrix deposition and remodeling. The skin is an epithelium consisting of several cell types such as Langerhans cells, melanocytes and keratinocytes. Keratinocytes are the most abundant cell types, which synthesize major structural components of the epidermal barrier. The repair process is initiated immediately after injury by the release of various cytokines, matrix metalloproteinases and low-molecular weight compounds in the extracellular matrix. Antibacterial properties of silver and anti-inflammatory properties of gold nanoparticles are described in literature. In our work, we have studied two samples of silver (ionic silver and metallic silver) and one sample of gold (nanogold) on the biomarkers involved in the wound healing process such as collagen type I, interleukin-6 (IL-6), matrix metalloproteinase-1(MMP-1),

-3 (MMP-3) and -10 (MMP-10) on HaCaT cell lines. Our results have demonstrated that all the samples of metals decreased the production of IL-6. In addition, it was shown that ionic silver might have a positive effect on wound healing because of increased production of proteins that promote keratinocyte migration (collagen type I, MMP-1) and contraction of the wound (MMP-3). We are planning to study other effects of silver and gold on skin wound healing.

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Changes of lipid metabolism after DHA treatment in colon cancer cells

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Cancer cells can often be characterized by resistance to therapeutic drugs due to dysfunction of various intracellular signaling pathways, including those involved in regulation of lipid metabolism. Ceramides are the basic units of the sphingolipids and can play role in modulation of the cytotoxic action of various anticancer agents. Docosahexaenoic acid (DHA), n-3 polyunsaturated fatty acid, is an important component of cellular membranes, able to modulate processes such as proliferation, differentiation or apoptosis in various cancer cell types. It is documented that DHA and ceramides can modulate processes that are important for induction of apoptosis in cancer cells. We investigated the sensitizing effect of DHA on apoptosis triggered by TRAIL (TNF-related apoptosis inducing ligand) in human epithelial cell line derived from colon cancer metastasis, and its association with ceramide metabolism. TRAIL is a cytokine known for its ability to selectively induce apoptosis in cancer cells, but not in most normal cells. However, many cancer cells including colon are still resistant to cytotoxic effects of TRAIL.

In our colon cancer cell model we showed that DHA-mediated potentiation of TRAIL-induced apoptosis was associated with enhanced activation of caspases, cleavage of their substrates, and stimulation of mitochondrial pathway. These events were accompanied by significant changes of amount of selected lipid classes. Cellular lipid analysis (HPLC-MS-MS) showed that DHA increased amount of so-called proapoptotic ceramide classes (16:0). Moreover, DHA decreased amount of lipid classes (24:1) which were demonstrated to play a role in cancer cell resistence to TRAIL.

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Fatty acids modulate cell signaling and cytokinetics of colon cancer cells

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The disturbance of imbalance between proliferation, differentiation and apoptosis in the intestinal epithelium plays important role in colorectal carcinogenesis. The development of this disease can be significantly influenced by dietary lipids, especially by specific types of fatty acids. These compounds are important modulators of many aspects of intestinal epithelial cell behaviour. Their role in inflammation and carcinogenesis is assumed because of their ability to inhibit cell proliferation and induce differentiation and/or apoptosis. Thus, we investigated the response of human epithelial cell lines derived from fetal colon (FHC) and colon adenocarcinoma (HT-29) to essential ω-3 polyunsaturated docosahexaenoic acid (DHA), sodium butyrate (NaBt), short chain fatty acid produced by microbial fermentation of fibre in the colon, and their combination.

The aim of our study was: a) to explain some mechanisms of combined NaBt and DHA action b) to determine the role of PI3K/Akt signaling pathway in the effects of studied fatty acids. Results obtained by flow cytometry, fluorimetry analysis and expression of specific proteins (western blot) showed that combination of these compounds increases their antiproliferative as well as apoptotic effects and modulates differentiation depending on cell line. These effects were associated with membrane lipid structure changes, increased lipid droplet accumulation, reduced mitochondrial membrane potential, increased production of reactive oxygen species and changes in the expression or activity of some regulatory molecules connected with apoptosis (caspases, Bax, Bak, Bid, Mcl-1, survivin, XIAP) and lipid metabolism such as fatty acid synthase, caveolin-1 and peroxisome proliferator-activated receptor (PPAR) γ. After Akt 1/2 inhibitor application we detected significant changes in cytokinetics and lipid metabolism suggesting the involvement of the PI3K/Akt pathway in the effects of fatty acids.

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The prognostic role of BRCA1 and Filamin A protein expression in patients with non-small cell lung cancer

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Introduction. BRCA1 is a 220kDa multifunctional protein which has recently gained a major scientific interest as a potential prognostic and/or predictive marker for various tumors, including non-small-cell lung cancer (NSCLC). Overexpression of BRCA1 mRNA has been associated with poor survival in chemonaive, stage IB and in advanced stage NSCLC patients after DNA damaging chemotherapy. These findings are not confirmed by immunohistochemistry which is more reliable and widely used tool for diagnostic purposes. On the other hand only mRNA expression does not reflect the presence of full length, functional protein. An actin-binding protein Filamin A (FLNA) serves as a scaffold in various signaling pathways. Recently, it has been reported that FLNA interacts with BRCA1 protein and is required for efficient regulation of early stages of DNA repair processes. We aimed to investigate the prognostic impact of BRCA1 immunohistochemical expression in NSCLC patients, using different antibodies, as well as the FLNA immunohistochemical expression and it's correlation with BRCA1 protein expression.

Material and Methods. We tested five antibodies (Abcam). Three of them against different parts of BRCA1 proten: MS110 against N-terminal (1-304aa), GLK2 against C-terminal (1832-1863aa) and 17F8 against central (762-1315aa) part of BRCA1. Also, two antibodies against phosphorilated forms of BRCA1 at Ser1423 (phosporilated during normal cellular functioning of BRCA1 and also in response to DNA damage) and Ser1524 (specifically phosphorylated by ATM in response to DNA damage). After testing several antigen retrieval methods GLK2 and 17F8 were excluded from the study. We performed BRCA1 immunohistochemistry on tissue microarrays (TMAs) composed of 104 early (I,II stage) and advanced (III, IV stage) NSCLCs. Patients with III and IV stage disease were treated by adjuvant cisplatin-based chemotherapy. Staining results were statistically analyzed in correlation with all available clinicopathological characteristics. Also, we performed a preliminary study of FNLA protein expression in 50 NSCLC patients. Formalin-fixed paraffin-embedded tissue sections were stained by immunohistochemistry, using antibody against FLNA C-terminus (EP2405Y,LSBio), Staining intensity was estimated semi-quantitatively and correlated with all available clinico-pathological factors.

Results. BRCA1 MS110 staining showed extremely weak nuclear positivity in 28% of NSCLC cases, 82% were positive for Ser1423 and only 27% for Ser1524 antibody. Statistical analysis of data showed no significant correlation between BRCA1 MS110 and Ser1423 expression with respect to clinicopathological data. Only BRCA1 Ser1524 nuclear positivity was significantly correlated with longer overall survival (OS) and disease free survival (DFS) in stage I and II patients (P<0.001), whilst OS and DFS were shorter in S1524 positive stage III and IV patients (P=0.001). FLNA expression was significantly higher in cancer, compared to normal lung tissue. Positive correlation has been revealed between FLNA and BRCA1 phospho-ser1423 protein expression. Also, 5 year overall survival rate was higher in patients with strong FLNA expression.

Conclusions. (1) The discrepancy between BRCA1 mRNA and protein study results might be due to the BRCA1 protein nature and inability to detect the full length, functional protein; (2) BRCA1 phosphorylaton, at least in ser1524, differently determines the prognosis of early and advanced NSCLC, supposedly due to its role in DNA repair. (3) The detection of phosphorilated forms of BRCA1 might serve as useful prognostic marker for patients with NSCLC. (According to our preliminary study results the prognostic role of FLNA protein expression deserves to be a subject for further studies in patients with NSCLC.)

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