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Laboratory of Cytokinetics focuses on the research in the field of cellular signalling and physiology relevant to cancer and potential role of lipid membrane elements and their derivatives in these processes. In particular, the effects of environmental substances, such as lipid nutrition components (essential polyunsaturated fatty acids and butyrate) and xenobiotics (cytostatics and environmental organic pollutants) on regulation of cytokinetics, i. e. cell proliferation, differentiation and apoptosis are studied. Using both tumor and non-tumorigenic cells, new types of interactions of lipid dietary components, anticancer drugs (non-steroidal anti-inflammatory drugs-NSAIDs, cytostatics) or selected environmental pollutants (polycyclic aromatic hydrocarbons, PCBs, dioxins) with physiological regulators of cytokinetics are being investigated. Attention is being paid especially to tumor necrosis factor (TNF) family, tumor growth factor (TGF) family, fibroblast growth factor (FGF) and Wnt/beta-

catenin pathway signalling. The results are exploited in cancer prevention/therapy and in ecotoxicology.

Cellular and molecular physiology of lipids

Among lipid nutrients, ω-6 and ω-3 essential polyunsaturated fatty acids (PUFAs) and short-chain fatty acid produced from dietary fiber - butyrate (NaBt) are required for a proper functioning of colon epithelium and they play a role in the colon carcinogenesis. Our previous studies have documented that lipid dietary components may interact both mutually and with endogenous regulators operating in the colon, such as apoptotic inducers of tumor necrosis factor (TNF) family. Moreover, we suggest that the response of colon cells is altered during colon cancer development.
Using human colonic cell lines derived from fetal tissue (FHC) and colon adenocarcinoma (HT-29, HCT-116) our attention is focused on interaction of i/ NaBt with arachidonic (AA) and docosahexaenoic acid (DHA), ii/ NaBt with TNF family molecules, and iii/ DHA with TRAIL. Different effects of NaBt, PUFAs, and their combination on FHC and adenocarcinoma cell lines.

We showed significant differences in cytokinetic response between these cell lines. While in FHC and HT-29 cells NaBt induced G0/G1 arrest, differentiation and low level of apoptosis, in HCT-116 cells G2/M arrest, no differentiation and high level of apoptosis were detected. Moreover, in FHC (and partially in HT-29 cells), a significant potentiation of apoptosis accompanied with an increased cell cycle arrest, cell detachment, and decreased differentiation were detected after combined treatment with NaBt and PUFAs, especially with DHA.

Using previously established models of non-adherent cellular cultivation of colon epithelial cells, we studied the effects of NaBt on the expression of proteins mediating cell-ECM and cell-cell interactions during both adherent and non-adherent cultivation of FHC and HT-29 cells. We have found changes in the expression and activation of two key protein kinases (focal adhesion kinase – FAK and integrin-linked kinase – ILK) involved in cell adhesion. NaBt induced the expression of both cell-ECM and cell-cell interaction molecules, but decreased the activation of the kinases involved in cell adhesion.

Mechanisms of TRAIL effects

TRAIL is a promising inducer of tumor-specific cell death, but in some cases it can also stimulate signalling pathways leading to proliferation and survival of cancer cells of different origin. We detected higher but limited sensitivity of cancer HT-29 then fetal FHC cells to TRAIL treatment. The TRAIL-induced apoptosis of HT-29, but not of FHC cells, was significantly enhanced by U0126, which inhibits MEK/ERK pathway. The most significant differences

between these two cell lines were observed with regard to the involvement of the mitochondrial apoptotic pathway.

In our previous work we showed that TRAIL is capable of triggering a response leading to significant early transient ERK-mediated transcriptional up-regulation of anti-apoptotic Mcl-1 mRNA and protein in colon cancer cells responsive to its apoptotic effects, but not in TRAIL-resistant colon cells. In 2007 we continued our studies focused especially on the anti-apoptotic action of Mcl-1 protein. Using an siRNA approach, we demonstrated that the up-regulation of Mcl-1 protein exerted by TRAIL could be an important mechanism attenuating/delaying its apoptosis-inducing effects.

Furthermore, we found an increase in FAK activation and Akt phosphorylation during non-adherent cell cultivation compared to adherent cultivation. Based on this, we have proposed an association between adhesion kinase and PI3/Akt pro-survival pathways, which might be responsible for the decreased sensitivity of the colon cells to the TRAIL action during the non-adherent type of cell cultivation.

Growth factors in cancer cell signalling

Pathophysiological conditions reflected in deregulation of differentiation, proliferation and apoptosis modify homeostasis and function of prostate epithelia and can lead to diseases such as benign hyperplasia and cancer. Transforming growth factor-beta, interleukin-6 (IL-6) and Wnt family members represent highly biologically active molecules, secreted to the tissue microenvironment, where they can induce different signalling pathways in paracrine and/or autocrine manner. These autocrine/paracrine factors have been shown to change microenvironment in prostate gland and modulate growth and survival of cancer cells. Defects in functions of components of these pathways have been observed in various human cancers. In 2007 we continued with studies focused on (1) functional role of GDF-15 (member of transforming growth beta family) in the effects of cyclooxygenase inhibitors (non-steroidal anti-inflammatory drugs (NSAIDs), (2) role of PUFAs in modulation of signalling pathway of pro-inflammatory cytokine IL-6, (3) modulation of neuroendocrine differentiation (NED) of prostate cancer.

Studies investigating anti-cancer effects of NSAIDs have shown modulation of the PI3K/Akt pathway. We observed significant differences in the sensitivity of prostate and colon cancer cell lines to antiproliferative effects of NSAIDs. The prostate cancer cell line LNCaP, which is PTEN and SHIP2 negative, was the most sensitive to these effects. Knockdown of SHIP2 by RNA interference in PTEN negative prostate and colon cancer cell lines resulted in higher sensitivity to antiproliferative effects of NSAIDs, which was assessed by analyzing the cell

cycle profile and expression of cell cycle regulating proteins. Using RNAi we have also disclosed that NSAIDs-induced early cell cycle arrest in LNCaP cells is not dependent on increased expression of GDF-15. Our data suggest that multiple defects in negative regulation of the PI3K/Akt pathway may contribute to increased sensitivity to chemopreventive effects of widely used drugs. The mechanisms driving this process are currently under investigation.

Neuroendocrine differentiation of epithelial prostate cancer cells is a phenomenon clearly associated with cancer progression. However, mechanisms controlling differentiation of prostate epithelial cells have remained poorly characterized. We have established experimental model of neuroendocrine differentiation of prostate epithelial cells in our laboratory. This model is currently used for studies focused on describing of mechanism driving neuroendocrine differentiation. The successful completion of these studies will reveal innovative strategies for treating prostate cancer in terminal stages.

Molecular mechanisms of Wnt signalling

Wnt signalling represents one of the most conserved means of intercellular communications, which regulates both development and diseases, such as cancer. Although it is generally accepted that Wnts are crucial regulators of both homeostatic and disease conditions, it is unknown how Wnt signal is modulated and transduced in the cytoplasm via phosphoprotein Dishevelled (Dvl).

Our results showed that all Wnts can induce activation of casein kinase 1, which in turn phosphorylates Dvl. These data identified a possible common module, which can be utilized by both canonical and non-canonical Wnt signalling (REF: JCS). When we studied in detail the effects of endocytosis on cytoplasmic Wnt signal transduction, we have surprisingly discovered that Dvl is unstable protein, which can be quickly and potently degraded when endocytosis is blocked (REF: Acta). These data suggested that level of Dvl is physiologically regulated and decides the sensitivity of cell to Wnt ligand. One of the possible partners of Dvl in endocytotic process is beta-arrestin. When we studied the interaction of these two proteins in detail we found out that beta-arrestin is required component for canonical Wnt signal transduction and may be a missing link between Dvl and downstream effectors such as axin (REF: PNAS). We have also critically reviewed this and other recently published data in the summarizing article about signal transduction between Frizzled receptors and downstream effectors such as Dvl.

Mechanisms of fibroblast growth factor 3 (FGFR3) signalling

In 2007 our main focus was on mechanisms of FGFR3 signalling in cartilage.

We have discovered several novel features of FGFR3 signalling in chondrocyte environment that are briefly outlined bellow.

(1) Description and analysis of FGFR3-mediated matrix metalloproteinase (MMP) induction and activation that underlines the novel phenotype of FGFR3 signalling in cartilage, i.e. the loss of chondrocyte extracellular matrix.
(2) Molecular analysis, for the first time, of the Frs2, Shc and Gab1 adaptor complexes proximal to FGFR3 and their contribution to the FGFR3-mediated sustained activation of Erk MAP kinase pathway in chondrocytes.
(3) Discovery of a critical role of protein kinase C in FGFR3-mediated activation of Erk MAP kinase pathway in chondrocytes.
(4) Development of the chondrocyte-based high-throughput screening assay for identification of novel pathways of FGFR3 signalling in chondrocyte environment.
(5) Exclusion of STAT1 and STAT3 transcription factors from their direct participation in FGFR3-mediated chondrocyte growth arrest.

We have also studied the FGF/FGFR signalling in B-cell chronic lymphoid leukemia (BCLL), resulting in a discovery of marked upregulation of a potential novel oncogene in BCLL, the apoptosis-inhibitor 5 (Api5).

Cellular and Molecular Toxicology

Diverse environmental organic pollutants are known interfere with physiological regulatory mechanisms controlling cell proliferation, apoptosis or cell-to-cell communication, thus leading to disruption of homeostasis and development of diseases, including cancer. The principal aim of our studies is to contribute to understanding of their effects at molecular and cellular level, which might be linked to carcinogenesis, reproductive or developmental impairment. In 2007, our work concentrated on the AhR-dependent gene transcription, deregulation of cell proliferation and cell-to-cell communication in cells affected by various group of organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins. The results published in 2007 can be divided into three principal groups. First, we characterized the impact of some less-known groups of PAHs on model liver cells, including methylated derivatives of phenanthrene, anthracene, naphthalene and benz[a]anthracene, or PAHs with higher molecular weight, including dibenzoanthracenes and benzochrysenes. We have characterized some structural determinants of their AhR-mediated activity and/or ability to inhibit gap-junctional intercellular communication. We found that given their environmental levels, many of these compounds should be considered to be important contributors to toxic effects related to carcinogenesis, including activation of AhR.

Second, we have further characterized rat liver progenitor cells as a valuable in vitro model, which enables insight into toxic modes of action of environmental carcinogens. We have shown that rat liver 'stem-like' cells are a useful tools for studies on genotoxic effects of PAHs, harboring high levels of inducible enzymes involved in metabolic activation of PAHs and providing material for analysis of formation of DNA adducts and related genotoxic events. Moreover, we have used this cellular model to characterize impact of different classes of AhR ligands (agonists vs. partial antagonists) on deregulation of cell proliferation or expression of xenobiotic-metabolizing enzymes.

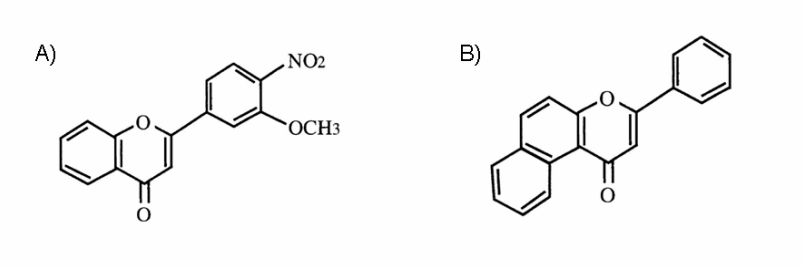

Fig. 1. Chemical structures of model flavones. A) 3´-methoxy-4´-nitroflavone was designed as AhR antagonist with basic flavone structure; B) beta-naphthoflavone, so-called 5,6-benzoflavone, was shown to act as potent AhR agonist.

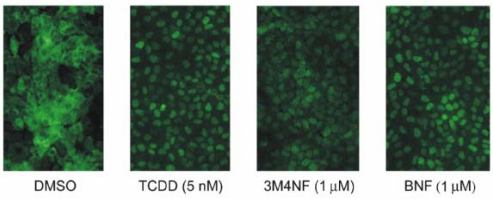

Fig. 2. Cellular localization of AhR. Localization of AhR was assessed by indirect immunofluorescence using staining with anti-AhR antibody in confluent WB-F344 cells treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3´-methoxy-4´-nitroflavone (3M4NF), beta-naphthoflavone (BNF); DMSO (0.1%) was used as a control.

Finally, we have analyzed interactive effects of AhR ligands and a principle

proinflammatory cytokine, tumor necrosis factor- alpha (TNF). We have observed that TNF is able to synergistically enhance the ability of dioxins or PCBs to disrupt contact inhibition in rat liver epithelial cells, which associated with enhanced induction of cyclin A expression. Moreover, TNF- alpha was found to differentially affect expression of cytochrome P450 (CYP) enzymes involved in metabolic activation of promutagens, such as benzo[a]pyrene, leading to enhanced expression of CYP1B1, increased formation of DNA adducts and related genotoxic events. These results suggest that conditions of chronic inflammation might potentiate toxic effects of environmental pollutants related to both tumor initiation and promotion. Our present results thus have implications for understanding of the process of chemical carcinogenesis and evaluation of risks associated with exposure to carcinogenic compounds.

The effects of cytostatic compounds

LA-12 is a novel platinum(IV) compound with adamantylamine, which is able to overcome intrinsic and acquired resistance to widely used platinum compound cisplatin in ovarian cancer cell lines. We intensively examin the mechanisms of LA-12 effects and compare its effects with cisplatin and oxaliplatin (III. generation of platinum drug). Modulation of cell cycle machinery reflects complexity of the cellular response to platinum-compound caused DNA damage and leads to the cell cycle arrest and/or apoptosis. Analysis of the cell cycle of ovarian cancer cell line A2780 revealed that equitoxic concentration of cisplatin and LA-12 caused accumulation of cells in S-phase of the cell cycle, but only in case of LA-12, persistent arrest was achieved. Furthermore, various components of cell cycle machinery were studied with regard to regulation of passage through the S-phase to G2-phase, e.g. associated kinase activities and protein-protein interactions between CDK inhibitor p21 and cyclin depentent kinase 2 (Cdk2), cyclins A and B1. Additionally, expressions of various proteins involved in DNA-damage-responsive signalling were assesed. Simultaneously, LA-12 toxicity was characterized in colorectal cancer cell lines HCT116 and HT-29. Compared to oxaliplatin, LA-12 associated cytotoxicity was superior to oxaliplatin in both cell lines tested.

In cooperation with UPJS Košice, Slovak Republic we have developed the study of hypericin photocytotoxicity mediated by photodynamic therapy (PDT) in colon cancer cells.

Hypericin, one of the promising photosensitisers, is known to induce apoptosis with high efficiency in various cell lines. However, we reported the prevalence of necrosis accompanied by suppression of caspase-3 activation in colon adenocarcinoma HT-29 cells exposed to an extensive range of PDT doses evoked by various hypericin concentrations and light doses. Introduction of Bcl-

2 into HT-29 cells invoked caspase-3 activation, changed the Bcl-XL expression pattern, increased apoptosis and arrested cells in G2/M phase of the cell cycle.

We evaluated the effectiveness of a combined modality approach using pretreatment of HT-29 cells with various inhibitors of lipoxygenase (LOX), cyclooxygenase (COX) and cytochrome P450-monooxygenase pathways followed by hypericin-mediated PDT. We demonstrated that pretreatment of cells with 5-LOX inhibitor MK-886 as well as 5-, 12-LOX and 12-LOX inhibitors (esculetin and baicalein, respectively) significantly and dose-dependently altered hypericin-mediated PDT effects on cell proliferation and viability. Pretreatment of cells with various COX inhibitors promoted PDT therapy, but these effects are probably COX independent. These results imply that some of inhibitors used could be considered for potentiation of PDT.

Granted projects

GA CR 301/07/1557, Novel anticancer platinum complexes – mechanisms of their action and innovative chemotherapy. Principal investigator: A. Kozubík, 2007 - 2011

GA CR 524/07/1178, Importance of cell lipid changes during differentiation and apoptosis of colon epithelial cells. Principal investigator: J. Hofmanová, 2007 - 2011

GA CR 204/07/0834, Role of transforming growth factor-beta in regulation of proliferation, differentiation and apoptosis in prostate and colon cancer. Principal investigator: K. Souček, 2007 - 2009

GA CR 310/07/0961, The role of environmental pollutants in mechanisms regulating development of prostate carcinoma. Principal co-investigator: K. Souček, 2007 - 2010

GA CR 524/05/0595, Interactions of physiological growth regulators, arachidonic acid and xenobiotics. Principal investigator: A. Kozubík, 2005 - 2007

GA AS CR KJB500040508, Cell adhesion and anoikis of intestinal cells - role of TNF family members, AA metabolism, and differentiation. Principal investigator: M. Hýžďalová, 2005 - 2007

GA AS CR 1QS500040507, Lipid nutrition compounds-modulation of their effects and possibilities of practical application. Principal investigator: A. Kozubík, 2005 - 2009

GA CR 524/06/0517, Mechanisms of disruption of cell-to-cell communi-cation

and regulation of cell proliferation in liver cells. Principal investigator: J. Vondráček, 2006 - 2008

GA CR 524/06/P345, Activity of inflammatory regulator NF-kappaB modulated by alteration of arachidonic acid metabolism. Principal investigator: J. Procházková, 2006 - 2008

Programme KONTAKT, International Scientific and Technological Cooperation (Czech Republic - Hungary), Role of lipid rafts in regulation of cell signalling leading to modulation of cytokinetics of cancer cells. Principal investigator: A. Kozubík, 2007 - 2008

PLIVA-LACHEMA, a. s. - Contract (LA-12 programme). Principal investigator: A. Kozubík, 2006 - 2008

European Social Fund (ESF) - Ministry of Education, Youth and Sports Improvement of qualification and flexibility of Ph.D. students of the Faculty of medicine, UP. Principal co-investigator: A. Kozubík, 2006 - 2007

Programme KONTAKT, International Scientific and Technological Cooperation (Czech Republic – Slovak Republic). The effects of COX-2 inhibition in both tumour and non-tumour colon epithelial cells exposed to phototoxic hypericin effects. Principal investigator: A. Kozubík, 2006 - 2007

MU Rector's Programme for Students´ Creative Activity Support 20061431C0007, Effects of cytostatics and xenobiotics on deregulation of cell proliferation and apoptosis. Principal investigator: J. Zatloukalová, 2006 - 2007

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