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Department of Cytokinetics focuses on the research in the field of cellular signaling and physiology relevant to cancer and potential role of lipids 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 signaling. The results are exploited in cancer prevention/therapy and in ecotoxicology.

Cellular and molecular physiology of lipids (Jiřina Hofmanová)

In the self-renewing tissue of the colon the, a strict control of the balance between proliferation, differentiation, and apoptosis in the crypts is highly important. These processes are affected by many exogenous as well as endogenous agents, which may operate together in the colonic lumen. Among nutritional compounds, especially essential polyunsaturated fatty acids (PUFAs) of ω-6 and ω-3 types, and short-chain fatty acid butyrate produced from fiber were shown to affect the behavior of both normal and cancer colon cell populations. Moreover, these fatty acids may influence the effects of endogenous factors regulating cell growth, differentiation and apoptosis (cytokines, growth factors, apoptotic inducers). Nutritionally induced changes in cellular and tissue fatty acid composition may also result in altered sensitivity to chemo- and radiotherapy. Thus, lipids are capable of influencing a number of pathophysiological processes and modifying the effects of the drugs. Undoubtedly, the action of these compounds is complex and it involves a number of integrated signaling pathways that need to be elucidated.

In our group we investigated the effects of model PUFAs such as arachidonic acid (AA, 20:4, ω-6) and docosahexaenoic acid (DHA, 22:6, ω-3), sodium butyrate (NaBt), and apoptotic inducers of tumor necrosis factor (TNF) family (especially TNF related apoptosis-inducing ligand - TRAIL) during colon carcinogenesis using epithelial cell lines derived from human fetal colon (FHC), adenoma (AA/C1, RG/C2), carcinoma (HT-29, HCT-116), and lymph node metastasis (SW620). Importantly, we studied the mechanisms of the effects of these compounds either alone or in mutual combinations.

i/ Interaction of PUFAs and butyrate

We verified the hypothesis suggesting modulation of the effects of sodium butyrate (NaBt) by ω-3 or ω-6 polyunsaturated fatty acids (PUFAs). Comparing the responses of human colon epithelial cell lines of fetal (FHC) and adenocarcinoma (HT-29, HCT-116) origin, we detected significant differences in proliferation, differentiation, and apoptotic response to the treatment of NaBt, AA or DHA, and their combinations. While in FHC and HT-29 cells NaBt induced G0/G1 arrest, differentiation, and low level of apoptosis, a G2/M arrest, no differentiation, and a high degree of apoptosis were detected in HCT-116 cells. Moreover, in FHC cells, a significant potentiation of apoptosis accompanied with an increased arrest in the cell cycle, cell detachment, and decrease of differentiation was detected after combined treatment with NaBt and both PUFAs. Changes in cytokinetics induced by fatty acids were accompanied with membrane lipid unpacking, reactive oxygen species production, and decrease of mitochondrial membrane potential. Detection of caspase-3 activation and dynamic modulation of Mcl-1 protein expression imply their possible role in both cell differentiation and apoptotic response. Our results support the concept of modulation of NaBt effects by PUFAs, especially of ω-3 type, in colonic cells in vitro with diverse impact in cell lines derived from normal or neoplastic epithelium.

ii/ The role of PUFAs in modulation of TRAIL-induced apoptosis in colon cancer cells

We continued our studies focused on elucidation of the possible role of DHA in sensitization of colon cancer cells to the apoptotic effects of TRAIL. As our previous results demonstrated the ability of DHA to increase the sensitivity of HT-29 human colon cancer cells to TRAIL-induced apoptosis, we also aimed to investigate the response to these agents in other colon cancer cell lines that are otherwise relatively resistant to TRAIL (e.g. SW620). Moreover, selected combinations of TRAIL and DHA were tested using non-tumor human colon adenoma cell lines. In these cells, we did not observe any stimulating effect of DHA on TRAIL-induced cell death.

iii/ The molecular mechanisms of TRAIL effects in colon epithelial cells

In order to determine the role of particular molecules in regulation of colon cancer cell resistance to TRAIL-induced cell death, mechanistic studies were performed in different colon cancer cell lines, especially focused on the role of anti-apoptotic Mcl-1 protein, and selected pro-survival pathways such as MEK/ERK and PI3K/Akt. We examined the changes of anti-apoptotic Mcl-1 protein level following TRAIL treatment in human cell lines representing different stages of colon carcinogenesis-adenocarcinoma (HT-29, HCT116) or secondary metastasis (SW620), or cell line derived from fetal colon (FHC). While TRAIL was capable of triggering anti-apoptotic signaling leading to significant early ERK-mediated transcriptional up-regulation of Mcl-1 in selected colon adenocarcinoma cell lines, none or minimal effects were demonstrated in cell lines derived from colon lymph node metastasis or fetal colon, respectively. We showed that it is essential to consider the dynamics of modulation of Mcl-1 level and the balance between TRAIL-induced pro- and anti-apoptotic pathways when predicting the response of cells in different stages of cancer development, and designing the anticancer therapy using TRAIL.

The mechanisms of the decreased sensitivity to TRAIL observed in HT-29 colon adenocarcinoma cell line and FHC fetal cell line, during non-adherent cell cultivation as compared to adherent one were investigated in detail. We focused on the pro-survival (PI3/Akt, MAPK/ERK and NF-κB) pathways, their activation and connection with increased phosphorylation of focal adhesion kinase (FAK) during non-adherent cultivation using inhibitors of particular kinases. Our results suggested that the downstream activation of PI3/Akt pathway by phosphorylated FAK kinase appears to be responsible for the decrease in TRAIL-induced apoptosis during non-adherent cell cultivation.

Growth factors in cancer cell signaling (Karel Souček)

Multifunctional cytokines from Transforming Growth Factor-β family play crucial role in regulation of cytokinetics (proliferation, differentiation and apoptosis) in various types of normal and transformed cells. One of the divergent members of TGF-β family is Growth/Differentiation Factor -15 (GDF-15). Functional role of GDF-15 is not known in all details. On one hand its increased expression is often associated with effects of various chemopreventive compounds; on the other hand it also follows progression of some cancer diseases. Our recent study demonstrated that expression of GDF-15 is not essential for anti-proliferative effects of nonsteroidal anti-inflammatory drugs (NSAIDs). However we were able to demonstrate that sensitivity of the cells to the antiproliferative effects of NSAIDs depends on deregulation of PKB/Akt signaling pathway.

In 2009 we continued with studies focused on (1) functional role of GDF-15, (2) role of TGF- β in pathological plasticity of epithelial cells, (3) mechanisms of neuroendocrine differentiation (NED) of prostate cancer. Studies

investigating GDF-15 have shown modulation of differentiation of osteoclasts. Our studies proved novel properties of GDF-15 which can help to clarify its role in cancer progression. TGF-β cytokines belongs to the important inductors of epithelial-mesenchymal transition (EMT). This process is crucial for dissemination of cancer cells and metastasis development. In our work, we established novel application of cellular impedance analysis for dynamic, non-invasive monitoring of EMT in real-time. Application of such an approach opens a new opportunity for study of this important process in vitro. NED of epithelial prostate cancer cells is phenomena clearly associated with cancer progression. However, mechanisms controlling differentiation of prostate epithelial cells have remained poorly characterized. Surprisingly we described that induction of neuroendocrine phenotype by androgen ablation is associated with acquisition of senescent phenotype. Increase of SA-β-gal activity (general marker of senescence) by cell density is reversible process, but when induced by androgen ablation, the increased activity is permanent. Mechanisms driving this process are currently under investigation. The successful completion of these studies will help to understand mechanisms of cancer progression and reveal innovative strategies for treating prostate cancer in terminal stages.

Molecular mechanisms of Wnt signaling (Vítězslav Bryja)

We studied biochemical and biological properties of canonical and non-canonical Wnt signaling. We have identified novel and surprising role of the Lrp6 receptor in the non-canonical Wnt signaling pathway. Using in vitro techniques, and Xenopus and mouse in vivo models, we identified Lrp6 as physiologically relevant negative regulator of non-canonical Wnt signaling. Loss of Lrp6 leads to the overactivation of non-canonical Wnt signaling pathway, which then results in the developmental defects associated with non-canonical Wnt signaling such as defects in the neural tube closure or in the gastrulation movements. In collaboration with other groups we studied the role of Wnt signaling in the differentiation of stem cells. We were able to show (using Wnt5a-deficient embryonic stem cells) that Wnt5a is required for endothelial differentiation of ES cells and that Wnt/β-catenin signaling acts as a negative regulator of the neural differentiation of ES cells (using Wnt1- and Lrp6-deficient ES cells). Further we focused on the role of Wnt signaling in neural stem cells. We were able to show that canonical Wnt signaling controls the decision between glial and neuronal fates, and that the inhibition of the canonical Wnt signaling promotes gliogenesis in P0 neural stem cells. In neural stem cells we have identified a novel protein CXXC5, which can bind Dishevelled, a key mediator of Wnt signaling, and inhibit its function in the Wnt cascade.

Mechanisms of fibroblast growth factor signaling (Pavel Krejčí)

Fibroblast growth factor receptor 3 (FGFR3) is a transmembrane tyrosine kinase that serves as a receptor for the members of fibroblast growth factor (FGF) family and functions in many biological processes including cell proliferation, differentiation, migration and survival. To date, activating mutations in FGFR3 have been associated with several human disorders such as skeletal dysplasias, multiple myeloma, and cervical and bladder carcinomas. There is no treatment available for achondroplasia at present, thus inciting the development of novel approaches to target FGFR3. Therefore the research was focused in this direction.

Experiments aimed on discovery of novel small molecule inhibitors of FGFR3 signaling: We recently developed a chondrocyte-based high-throughput screening assay for identification of novel inhibitors of FGFR3 signaling in a chondrocyte environment. Using this assay, we identified a novel, potentially therapeutic inhibitor of FGFR3. This work was also a subject of recently approved patent application (PCT/US09/54340: Methods of Inhibiting FGFR3 Signaling).

Experiments aimed to determine yet unknown phenotypes of FGFR3 signaling in disease: We characterized a novel cellular senescence-like phenotype induced by FGFR3 signaling in chondrocytes. For the first time we described the molecular and cellular features of a premature senescence induced by aberrant FGFR3 signaling in chondrocytes, and discussed similarities between the known effects of FGFR3 signaling in skeletal dysplasias and oncogene-induced cellular senescence.

During our analysis of FGF signaling in the proliferating chondrocytes we have summarized recent progress in the field of FGF biology and human disease. This work resulted in two reviews – one focusing on biology of height molecular weight FGF2 and second focusing on the involvement of individual members of the FGF family in human diseases.

Cellular and molecular toxicology (Jan Vondráček)

The principal aim of our studies is to contribute to understanding of effects of environmental organic pollutants at molecular and cellular level, which might be linked to carcinogenesis, reproductive or developmental impairment. At the same time, these toxicological data help us to understand the physiological role of key signaling proteins that are affected by environmental toxicants, and their functional interactions with other signaling pathways. A principal protein, which is responsible for the action of toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins, is the aryl hydrocarbon receptor (AhR). Nevertheless, many persistent organic pollutants that fail to activate this transcription factor may also exert significant toxic effects within an organism. Therefore, in 2009, we further concentrated both on the role of AhR in regulation of key metabolic enzymes responsible for the mutagenicity of PAHs and on the effects of non-dioxin-like compounds on intercellular communication. We characterized the impact of a model non-dioxin-like PCB 153 on key proteins involved in formation of gap junctions or adherents junctions. Moreover, we found that PCB 153 may affect not only degradation of gap and adherents junction constituents, such as connexin 43, E-cadherin or β-catenin, but also the signaling function of the latter one, which is an integral part of canonical Wnt signaling pathway. We characterized the role of AhR in the regulation of expression and activities of critical enzymes participating in metabolic activation of PAHs in rat liver progenitor cells, including both CYP1 family enzymes (CYP1A1, CYP1A2, CYP1B1), cytosolic aldo-keto reductases (e.g. AKR1C9) or NQO1. In collaboration with the Centre of Molecular Medicine (Institute of Virology, Slovak Academy of Sciences), we participated in a study that described previously unknown interactions of AhR and hypoxia-inducible factor-1 in regulation of carbonic anhydrase IX, a model target of HIF-1. The present results these results contribute to our understanding of the possible mechanisms of toxic actions of non-dioxin-like PCBs at cell membrane, which might be of relevance to their tumor promoting properties. The interactions of AhR with NRF2 or HIF-1 transcription factors shed further light on the multifaceted effects of AhR ligands on transcriptional regulation in vertebrates.

Mechanisms of the effects of platinum derivatives (Alois Kozubík)

The platinum(II) derivatives (e.g. cisplatin, oxaliplatin) are used in the therapy of many solid cancers, but their negative side effects and cell resistance limit their therapeutic application. Therefore, there is a need for introduction and characterization of the effects of new platinum-based compounds capable of overcoming at least some of these limitations. In this respect, LA-12, a novel Pt(IV) compound with bulky hydrophobic ligand adamantylamine, is a very promising candidate known to exert cytotoxic effect, and no cross-resistance in a panel of cisplatin-sensitive and resistant cell lines of different origin. We have previously shown the ability of LA-12 to overcome acquired and intrinsic resistance to conventionally used cisplatin in ovarian cancer cells. We also demonstrated the significantly higher efficiency of LA-12 compared to cisplatin and oxaliplatin in inhibition of proliferation, and triggering cell death in colorectal cancer cell lines. Therefore, we further focused our attention to more-detailed examination of mechanisms of LA-12-induced cell cycle modulation, and apoptosis in these cancer cell types. The LA-12 response was particularly studied in HT-29 human colon adenocarcinoma cell line, grown in various degrees of confluence, and we compared LA-12-induced effects with those exerted by cisplatin and oxaliplatin. Importantly, we showed that LA-12 is able to overcome confluence-dependent resistance of HT-29 cells, which was observed in other platinum compounds. Our results highlight the selective advantage of application of LA-12, compared with several platinum(II)-based drugs, in treatment of solid tumors, including the slowly growing types.

In addition to being used as a single agent, our further work supports the role of LA-12 or cisplatin as important candidates to be used in combination therapy with TRAIL. TRAIL is a cytokine which can selectively trigger apoptosis in various cancer cell types. However, the resistance of some cancer cells TRAIL may hamper the previous expectations resulting from the unique killing abilities of this cytokine. The platinum-based drugs have been suggested as interesting agents capable of sensitizing the resistant cancer cells to TRAIL-induced apoptosis. We showed that platinum(IV) complex LA-12 or cisplatin enhanced killing effects of TRAIL in human colon HCT-116 cells and prostate cancer cells PC-3, which was associated with stimulation of activity of initiator caspase-8 and effector caspase-3, and overall apoptosis. Our results highlight the striking ability of LA-12 to sensitize the cancer cells to TRAIL-induced apoptosis even when applied in significantly lower doses compared to cisplatin. Molecular mechanisms responsible for the effects observed are currently under investigation in our laboratory. Our observation will help to improve therapeutical approaches to cancer diseases in terms of more efficient killing of cancer cells, while minimizing the side effects of the therapy.