UV as well as to chemotherapeutics. In the present study, we examined the role of Myh1 in cisplatin-treated lung carcinoma cells.
Methods: A panel of small cell lung carcinoma (SCLC: H82, H69, U1285 and U1906) and non small cell lung carcinoma (NSCLC: A549, U1810, H23, H125 and H661) cell lines with different cisplatin sensitivity were analyzed for their basal level of Myh1 using western blotting. Myh1 localization and expression after cisplatin treatment in NSCLC cells was analyzed in cytosolic and nuclear fractions using western blotting, and was confirmed by immunofluorescence analysis. To assess the role of Myh1 in cisplatin-induced apoptotic signaling, siRNA to Myh1 was used and caspase-3 activity examined prior and post cisplatin treatment using flow cytometry.
Results: Western blot analysis of Myh1 in the lung cancer cell line panel revealed heterogenous Myh1 expression. In the NSCLC cell line U1810, a relatively cisplatin resistant NSCLC, cisplatin treatment was found to cause an increase in Myh1 both in cytosol and nucleus. This was evident already at 30 min post cisplatin addition and still evident at 2h. Moreover, immunofluorescence analysis of Myh1 after cisplatin treatment of U1810 cells revealed relocalization of Myh1 into nuclear foci. Finally, siRNA to Myh1 was found to increase cisplatin-induced caspase-3 activity in NSCLC U1810 cells.
Conclusion: Our data suggest that Myh1 is stabilized by cisplatin treatment in NSCLC cells and can act as a negative regulator of cisplatin-induced apoptotic signaling in this tumour type.

528 Sarcoma cell lines express stem-cell associated features

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Although soft tissue sarcomas comprise about 1% of human malignant tumours, they are a life-threatening cancer and pose a significant diagnostic and therapeutic challenge. Cancer initiating cells (CIC), that display stem-like features, have recently been identified in several malignancies as the major responsible for tumour growth and chemoresistance. Therefore, clarify the role of CIC in sarcomas might help in the setting of more efficient therapeutic approaches.
To assess whether a "stemness" component exists in sarcomas a series of 18 sarcoma-derived cell lines were investigated for the expression of genes known to be involved in the stem phenotype (OCT3/4-POU5F1, NANOG, SOX2 and the NOTCH1 pathway). The study was carried out by RT-PCR, qRT-PCR and by immunofluorescence.
Stem-like cells are reported to grow as spheroids in medium enriched of EGF and bFGF but devoid of serum. On this ground we compared the expression pattern of cells grown as adherent cells vs cells grown as spheroids in this medium. This analysis was conducted in 5 cell lines (SKUT-1, MG63, RD, RMS13 and RH28).
Preliminary results indicate that all but one (RD) cell lines cultured in stem medium were able to give rise to spheroids, suggesting that sarcoma cell lines might do have a component of CIC.
NOTCH pathway was activated in 10 out of 18 sarcoma cell lines grown in standard conditions, as demonstrated by the expression of the NOTCH targets HES1, HEY1 and HEY2. NOTCH targets was further upregulated in the spheroids of 3 out of 4 cell lines, but was also expressed at high levels in RD floating cells grown in stem medium.
No expression of OCT3/4 and NANOG was observed in any of the cell lines investigated, irrespective of growth conditions. SOX2 was expressed in the leiomyosarcoma cell line SK-LMS-1 in standard condition and was activated in all sarcoma-derived sheroids.
Our results suggest that a CIC component may actually exist in sarcoma cells and that SOX2 could be an important regulator of CIC in this tumour setting.

529 The role of aromatase and epidermal growth factor receptor in non-small cell lung cancer

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Background: Targeted therapy provides an exciting project for treatment of non small cell lung cancer (NSCLC). Aromatase catalyses the final step of estrogen synthesis in several tissues including lung. EGFR signaling is implicated in cell proliferation and metastasis. Cross-talking between these pathways has been reported. The aim of this study is to evaluate the antitumour effect of the combined inhibition of aromatase and EGFR.
Material and Methods: *In vitro* experiments were performed on H23, H358 and A549 NSCLC cell lines. Exemestane and erlotinib were applied. Cell proliferation was measured by MTT assay and cell death was detected using annexin V/propidium iodide assay. Cell migration was determined by boyden chamber assay. pEGFR status was estimated using an appropriate ELISA kit and EGFR location was detected by immunofluorescence assay using confocal microscopy.
Results: Exemestane and erlotinib, either alone or in combination, inhibited cell proliferation, through an increase in cell apoptosis. However, the combination of the agents had a synergistic effect only on H23 cell lines. The tested agents and their combination inhibited the migration of H23 cells. Exemestane inhibited H358 cell migration whereas erlotinib reversed this effect. No change was found on cell migration of A549. Further, pEGFR levels were increased by exemestane in H23 cells and decreased in A549 cells. These experiments are in progress for H358 cells. Moreover, it was found that EGFR translocated in mitochondria after exemestane application in H23 cells while erlotinib reversed this effect. These experiments are ongoing for H358 and A549 cells.
Conclusions: Although each agent alone exerted an antitumour effect on the proliferation of all cell lines, their combination had a synergistic effect on H23 cells. Exemestane activated EGFR pathway in H23 cell line suggesting the treatment of these cells should include an anti-EGFR agent.

530 Isolation and functional characterization of cancer ctem cell-derived exosomes

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Cancer stem cells (CSCs) represent a small subpopulation of highly malignant tumour cells within the mass of solid tumours. CSCs are thought to be responsible for tumour initiation, growth and distant spread. Here we describe the isolation and functional characterization of lung cancer stem cell (LCSC)-derived exosomes. Exosomes are microvesicles of endosomal origin, which are secreted by various cell types. However, the biological significance of exosome secretion by tumour cells and the presence of exosomes in malignant effusions is not entirely clear yet.
We cultivated LCSC lines isolated from different histotypes of primary lung tumours including adenocarcinoma, squamous cell carcinoma and large-cell carcinoma, in a defined serum-free medium. These conditions allow for the propagation of undifferentiated, CD133-positive stem cell-like cells in spheroid cultures. Here we describe the isolation procedure to obtain exosomal particles from the supernatant of these cultures. The isolated exosomes were analysed for the expression of a number of exosomal proteins such as tetraspanins (CD9 and CD81) and transferrin receptor (CD71) by western blot analysis. In addition, we were able to demonstrate that exosomes derived from LCSCs induce migration of several lung cancer cell lines, such as A549 and NCI-H460. Moreover, we found that LCSC-derived microvesicles enhanced the matrix metalloproteinase (MMP) activity of stimulated target cells. Since MMP expression is induced by Wnt signaling, we investigated the presence of Wnt proteins in our exosomal preparations. We found that exosomes obtained from different LCSC lines contained a considerable amount of Wnt3a protein.
The presence of Wnt proteins suggest a tumour enhancing property of LCSC exosomes, which confer an enhanced migration and proliferative potential of target cells. A better knowledge of the exosomal-cellular mode of communication could lay the basis for the development of diagnostic and therapeutic anti-cancer strategies.

531 Synergy between HIF1a and LOX is critical for tumour progression

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The microenvironment of solid tumours is exposed to hypoxic conditions which lead to the activation of Hypoxia-Inducible Factor 1 (HIF1), a key transcription factor involved in cellular adaptation to changes in oxygen level. HIF1 plays a critical role in various cellular and physiological events, inducing the expression of several transcriptional targets such as Lysyl Oxidase (LOX). LOX is an amine oxidase that catalyzes crosslinking of fibrillar collagens and elastin in the extracellular matrix. Furthermore, LOX expression in tumour cells lines correlates with tumour progression and metastatic potential.
Using three different human colorectal carcinoma cell lines, LOX was stably overexpressed or knocked down by lentiviral transduction. In these models, we pointed out that besides HIF1-dependant regulation of LOX, LOX can also act on the HIF1 pathway under hypoxic conditions. Indeed, LOX enzymatic activity up-regulates HIF1a protein synthesis, and this action is mediated by the PI3K/AKT pathway. Thus, these results emphasize the existence of a mutual regulation between two main actors of tumoural progression: HIF-1a and LOX. To further determine the implication of both proteins in tumour progression, we generated human colorectal carcinoma cell lines modulating LOX and/or HIF1a expression. Our results show that LOX enzymatic activity increase cell proliferation and clonogenic potential *in vitro* and this role is partly dependant of HIF1a. Subcutaneous inoculation into the flank of Balb/c nude mice strongly reinforced these datas. Indeed, the tumours resulting from LOX overexpressing Hct116 cells were notably larger. HIF-1a silencing in LOX overexpressing cells strongly but not fully reduced the tumour development due to LOX forced expression. It suggests that LOX and HIF-1a act in synergy to favor tumour formation.

Taken together, our results demonstrate a direct crosstalk between HIF1a and LOX in the tumour microenvironment and underline a critical role of this mutal regulation in tumour progression *in vitro* and *in vivo*.

532 NG2 expression identifies a tumour competent population in glioblastoma with distinct molecular signature

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Introduction: We previously demonstrated that NG2 expressing ($NG2^+$) cells in glioblastoma (GBM) exhibits robust proliferative and tumourigenic activity and share phenotypic and functional similarities with NG2 expressing glial progenitors (NGP). Here we conducted comparative studies to address the difference of the molecular signature of GBM-$NG2^+$ and GBM-$NG2^-$ cells.
Methods: GBM cell lines (GLs) were derived from clinical samples under serum-free conditions according to our Cambridge Protocol (Fael Al-Mayhani et al.; 2009). GBM-$NG2^+$ Cells were sorted using FACS. Comparative molecular studies on GBM-$NG2^+$ and GBM-$NG2^-$ cells were conducted using microarray, comparative genomic hybridization (CGH) and western blot.
Results: Microarray data indicated that $NG2^+$ cells over-expressed a group of genes previously recognized by Cancer Genome Atlas within the Mitosis and Cell-Cycle Module (MCM). KEGG, Transpath and Transfac studies showed over-expression of unique set of proliferative pathways and transcription factors (TFs) by GBM-$NG2^+$ cells. Gene Ontology enrichment identified more than 200 gene categories that were enriched in the GBM-$NG2^+$ cells. The top 10 categories were related to cell cycling, M phase and DNA replication. Similarly, array CGH demonstrated subtle molecular structural differences between the cytogenetic profile of GBM-$NG2^+$ and GBM-$NG2^-$ cells as unique chromosomal abnormalities were found in GBM-$NG2^+$ cells. Finally, we demonstrated that MAPK and Akt pathways were significantly over-activated in GBM-$NG2^+$ cells compared to $NG2^-$.
Conclusion: We previously showed the robust proliferative activity and tumourigeneity of GBM-$NG2^+$ cells. Here, we provide evidence that our previous observations are linked to the distinct molecular signature of GBM-$NG2^+$ cells. This signature includes notable structural chromosomal abnormalities, unique enrichment of TFs and MCM genes and over activation of MAPK and Akt pathways.

533 New cell culture models of bladder carcinoma

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Background: Although there are numerous bladder cancer cell lines in current use, they do not cover the whole array of disease phenotypes and participating cell types. The majority of routinely used cell lines were derived from invasive or metastatic urothelial cancers, whereas papillary urothelial carcinomas, which represent the clinically prevailing tumour type, are grossly underrepresented. Moreover, although there is ample evidence for crucial role of stromal cells in tumour development and progression, there is to our knowledge no single established cell line of bladder cancer stromal cells. We report establishment and initial characterization of a new bladder cancer cell line (BC61) and a pair of carcinoma (BC44) and carcinoma-associated fibroblast (BC44Fibr) – cell lines derived from the same tumour.
Materials and Methods: Cell lines were established following our published protocoll (Seifert et al., World J Urol. 2007; 25:297–302). Carcinoma associated fibroblasts were immortalized by retroviral transduction of the hTERT gene. The Fibroblast Growth Factor Receptor 3 gene was analysed by DNA sequencing, CDKN2A by PCR, protein expression by indirect immunofluorescence. Karyotyping followed a standard protocol.
Results: Both carcinoma cell lines retained an epithelial phenotype, as revealed by morphology, cytokeratin and E-cadherin expression. Both contain CDKN2A/p16 deletions frequent in urothelial cancers. BC61 has a pseudotriploid karyotype with changes typical of early progression stages such as loss of chromosomes 9 and 11. The cell lines displays a functional p53-response to genotoxic stress and constitutive expression of DNA damage checkpoint pathway genes. It bears an activating mutation in FGFR3 (S249C), although the receptor is expressed at a relatively low level. BC44 has an complex aneuploid karyotype and lacks p53 expression. The corresponding diploid BC44Fibr cells display typical attributes of carcinoma-associated fibroblasts like ubiquitous expression of Vimentin and Smooth Muscle α-Actin, prevalent expression of Fibroblast Activation Protein. Focal expression of CD13 reveals their bladder stroma origin.
Conclusion: We believe that these new cell lines will be valuable models for numerous aspects of urothelial carcinogenesis.
Supported by the project no. MSM 0021620819 “Replacement of and support to some vital organs” of the Ministry of Education of the Czech Republic.

534 In situ immobilization in alginate foams – a novel 3D in vitro cell culture system

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The use of 3-dimensional (3D) matrices for cell culture is gaining popularity as a substitute for traditional 2D cell culture as it can approximate cell architecture and cell-cell contact found in tissues, organs and tumours. Alginate-based foams for culturing cells in 3D have been developed in which cells can be immobilized within the foam structure using an *in situ* gelation technology. The principle of the novel immobilization technique is: Cells suspended in a Na-alginate solution are applied to the alginate foam and induces *in situ* gelation as calcium ions are donated from the foam and cross-link the added alginate, effectively entrapping the cells within the pores throughout the foam.
Mouse myoblasts C2C12 (ATCC CRL-1772) were cultured in 2D and prepared as a suspension of 1.0% sodium alginate in DMEM medium. 125 µl cell suspensions were added to g-sterilized NovaMatrix-3D™ foams fitted to 24-well culture plates at cell densities of 10 000 or 25 000 cells/foam. Cell localization within the foam was visualized using a confocal microscope to identify cells fluorescently labeled cells using a carboxyfluorescein marker.
At different time points cell proliferation was measured. The foams were first de-gelled by incubating in 50 mM sodium citrate solution, then cells were counted after centrifugation and resuspension.
C2C12 myoblasts proliferated slower when immobilized within the foam compared to the standard 2D culture plate. Despite the reduced proliferation rate, the cells remained viable over extended periods of culturing (trypanblue staining).
As alginate does not provide attachment factors necessary for some cells to retain a high proliferation rate, the use of the cell attachment peptide RGD coupled to the immobilizing alginate was investigated. After two days of culture, the foam with RGD-alginate had three times as many myoblasts as the plain alginate matrix.
Use of alginate foams with concomitant *in situ* immobilization of cells results in a 3D model with the potential to approximate cell proliferation and architecture within tissues or tumours. The technology enables biomimetic approaches by varying e.g. matrix elasticity, gelling ions, attachment peptides and foam degradation. The foam may also be implanted as a xenograft, making the NovaMatrix-3D™ system truly versatile.

535 Tryptase up regulate VEGF and PDGF in squamous cell carcinoma cells (SCC)

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The interaction of tumour cells with their environment plays a crucial role for progression, metastasis and angiogenesis of tumours with growth factors such as VEGF, PDGF playing a crucial role. Several immunohistochemical studies indicated that mast cells, which are present in increased number in tumour-stroma, might regulate tumour progression and angiogenesis via secreted cytokines, growth factors or mediators like histamine or tryptase. In this context we were interested to study, how mast cells regulate VEGF and PDGF in cutaneous tumours.
Various squamous cell carcinoma (SCC) cell lines were cultivated for 24 h with or without conditioned medium derived from IgE-activated or non-activated primary, dermal mast cells. Protein expression of growth factors, VEGF and PDGF, was estimated by ELISA in each individual cell line in order to look for a potential modulation of these growth factors as a result of tumour-mast cell interaction. Furthermore, tumour cells were stimulated with the mast cells related mediators histamine or tryptase to examine how these mediators would influence expression of VEGF and PDGF in tumour cells.
In all cell lines examined, PDGF was released in increased amounts after co-cultivation of SCC cell lines with mast cell supernatant. Interestingly, the modulatory effect of supernatant derived from activated versus none activated mast cells on PDGF release from tumour cells differed only slightly. Stimulation of SCC cell lines with low concentrations of tryptase (0.1 µg/ml) led to a strong increase in PDGF release (in SCC-12 cells from 32 pg/ml to 150 pg/ml and in SCC-13 from 18 pg/ml to 92 pg/ml). This result suggests that mast cell derived tryptase may be a major inducer of PDGF release from SCC cells. In addition, application of the tryptase inhibitors LDTI but not of the chymase inhibitor SBTI to conditioned medium led to an attenuation of PDGF release. Unlike PDGF, SCC cells release VEGF constitutively. This continuous release was further augmented by incubation of SCC in mast cell conditioned medium. In contrast, addition of chymase or cathepsin G inhibitors SBTI elevated VEGF level. Our results indicate that mast cell-descendent mediators like tryptase and chymase have contrary effects on the release on PDGF and VEGF from SCC. Our results indicate that the two known different phenotypes of mast cells, which are defined according to their different expression of proteases ($M_{Tryptase}$ or $M_{TryptaseChymase}$), may differ with regard to their impact on the progression of cutaneous tumours.