irradiation indicating an important role for wild-type *MLL* in the control of cellular apoptosis.
Conclusions: The data suggests that reduced expression of wild-type *MLL* can contribute to GC resistance in ALL patients both with and without *MLL*-translocations.

507 In vitro analysis of population specific BRCA1 splicing variants

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Background: The BRCA1 is multifunctional tumour suppressor, important for DNA repair. Mutations in this gene are responsible for the majority of hereditary breast and ovarian cancer cases in the Czech Republic. Beside genetic alterations numerous splicing variants of unknown clinical significance have been detected. In order to test their biological importance, we have developed new, cell line based, *in vitro* system.
Methods: Stable clones of breast adenocarcinoma cell lines expressing population-specific *BRCA1* splicing variants, with/without co-expression of shRNAs that target the wtBRCA1 were prepared. Functionality of used expression systems was confirmed both at the mRNA and protein level by qPCR and Western blotting respectively. Proliferation of cells following g-irradiation (GI) was determined by real time cell analyzer. The kinetic of DNA double-strand break (DSB) repair was quantified by counting γH2AX foci colocalizing with 53BP1 protein (IRIF, Ionizing Radiation Induced Foci) during the post-irradiation time.
Results: We have established stable clones (1) expressing *BRCA1* splicing variants (14+15 del; 17−19 del), (2) down regulating *BRCA1* to <10% relative to control cells, and (3) stably expressing BRCA1 splicing variants 14+15 del and 17−19 del alongside to down-regulated wtBRCA1.
The proliferation of cells examined in relation to GI-induced DNA damage showed that clones up-regulating variant 17−19 del exert increased radio-resistance, contrary to the clones with up-regulated variant 14+15 del that were significantly more radio-sensitive. In accordance, formation and persistence of GI-induced IRIFs was markedly prolonged in clones with shRNA-mediated down-regulation of wtBRCA1 expression as well as in clones with up-regulated expression of BRCA1 17−19 del variant.
Conclusions: Our current *in vitro* results indicate that studied splicing variants of *BRCA*, with affected phosphorylation or BRCT domains, differentially influence growth properties of cells in relation to GI-induced DSB damage in the established model system and may alter DSB repair capacity on the level of IRIF dynamics.
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508 Alternative splicing of Lysyl Oxidase-Like 4 in ovarian carcinoma

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Introduction: Lysyl oxidase (LOX) is an amine oxidase that is usually synthesized and secreted by fibrogenic cells. Four LOX-like (LOXL) genes have been identified so far in mammalian genomes, encoding four different LOX-like proteins: LOXL1, LOXL2, LOXL3 and LOXL4. All members of the LOX family show a highly conserved C-terminus region that contains the catalytic domain. The N-terminus of the LOX isoforms is less conserved among the different members and is thought to determine the individual role and tissue distribution of each isoenzyme. LOXL4, the least studied member of the LOX-like family enzymes, undergoes a process of alternative splicing in cancer, in a site- and stage-specific manner that we have previously shown. The purpose of the current study was to uncover the splicing mechanism that is responsible for this process.
Experimental procedures: I. ShRNAs for four splicing factors: SF2/ASF, SRp55, hnRNP-A1 and hnRNP-A2, were transfected in two cell lines: U-87 MG cell line (human glioblastoma) and NCI-H460 (human large-cell lung carcinoma). II. Over-expression of SF2 was performed in MST0-211H cell line (human malignant mesothelioma), HeLa cell line (human epithelial cervical cancer) and MCF10A cell line (human mammary epithelial line). III. Western blotting for SF2/ASF and tubulin. IV. RT-PCR for LOXL4 full length, splice-variant1 (splv1) and splice-variant2 (splv2) mRNA expression.
Results: We examined LOXL4 expression in U-87 MG cells. When untreated, these cells express the full length and splv2, almost equally. The silencing of two factors, SF2/ASF and hnRNP-A1, resulted in a dramatic changes in the expression pattern of LOXL4. For both silenced factors, LOXL4 full-length mRNA expression was much stronger, while the shortest variant, splv2, completely vanished. The silencing of hnRNP-A2 led to a smaller decrease in splv2, while SRp55 silencing did not seem to change LOXL4 splicing. In NCI-H460 cells, which normally express small amounts of all variants, no significant changes were found following silencing. In an attempt to further establish the splicing factor responsible for LOXL4 splicing, we over-expressed SF2/ASF in MST0-211H cells, which normally express only the full length LOXL4. Expression of SF2/ASF resulted in the appearance of splv2, while dramatically reducing the expression of the full length. Similar results were seen in HeLa cells. Over-expression of SF2/ASF in MCF10A cells, which untreated, have the unique quality of expressing splv2 alone, caused only a slight increase in the expression of splv2.
Conclusions: These results demonstrate for the first time, that LOXL4 is a direct target of the splicing factor SF2 SF2/ASF. Furthermore, in concordance with our previous in-vivo findings, it can be concluded that LOXL4 splicing occurs similarly in other epithelial cancer types, such as breast cancer and mesothelioma.

509 Evaluation of Human Epididymis Protein 4 in endometrial cancer patients

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Background: Endometrial cancer (EC) is the most common gynaecologic malignancy in Western world. To date, no good marker for screening or disease monitoring for this cancer is available. The aim of this study was to investigate HE4 gene and protein expression in a panel of ECs and normal endometria (NE) and to test the potential utility of HE4 as serum marker for early EC detection. Moreover we correlated HE4 serum levels with clinicopathologic characteristics of the patients.
Material and Methods: Using quantitative real-time PCR we tested a total of 46 ECs and 20 NEs for HE4 gene expression. Protein expression was analyzed by immunohistochemical staining (IHC) in tissue sections from 155 EC patients (40 well-differentiated (G1), 60 moderately-differentiated (G2), 55 poorly-differentiated (G3)) and 33 NE controls. Finally, pre-operative serum samples from 141 EC patients (25 G1, 60 G2, 56 G3) were analysed for HE4 (HE4 EIA-assay, Fujirebio-Diagnostics) and CA125 (Architect-CA125II assay, Abbott-Diagnostics) levels. Serum control samples were obtained from 76 NE patients.
Results: EC patients showed a significant HE4 gene overexpression compared with controls (t-test, $p < 0.0001$), as measured by qRT-PCR. Using IHC, HE4 protein expression was found higher in ECs compared to NEs, with a significant difference between the 2 groups (Mann-Whitney, $p = 0.028$); HE4 immunoreactivity was stronger in G1 ECs and decreased with higher grade (Anova test, G3 vs G1, $p < 0.0001$; G3 vs G2, $p = 0.0062$). Finally, HE4 serum levels (sHE4) were significantly higher in EC patients compared with controls (t-test, $p < 0.0001$), as measured by ELISA. Setting the specificity at 95%, the sensitivities in detecting EC were 67% for HE4, 30% for CA125 and 68% for the combination of both markers. HE4 serum levels significantly increased with higher stage (<IIB vs ⩾IIB, $p < 0.001$), higher grade (G1 vs G2/G3, $p < 0.0001$) and deeper myometrial invasion (M0/M1 vs M2, $p < 0.001$). No significant difference in sHE4 levels was found among different tumour histotypes.
Conclusions: This study highlights that HE4 is overexpressed both at mRNA and protein level in EC and that it is secreted at higher levels in serum of EC patients compared with NE controls. HE4 serum levels are more sensitive and specific compared to serum CA125 levels in distinguishing healthy subjects from malignant disease, regardless of tumour stage and grade. sHE4 levels are positively correlated with high stage and grade, deeper myometrial invasion, lymph node positivity, therefore they could be associated with a more aggressive tumour phenotype.

510 Effects of anti-VEGFR and anti-EGFR agents in glioblastoma

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Background: Malignant gliomas are the most common and aggressive primary brain tumours. Sunitinib is an oral, multi-targeting receptor tyrosine kinase inhibitor (TKI), including platelet-derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR). Lapatinib is an ATP-competitive dual TKI for epidermal growth factor receptor (EGFR) and HER2/neu (ErbB-2). The aim of the current *in vitro* study was to assess the effect of sunitinib and lapatinib applied either alone or in combination on proliferation, apoptosis, invasion and release of MMPs into the culture medium of U87 and M059K human glioblastoma cell lines. Other parameters analyzed were the effect of lapatinib on the formation of EGFR-integrin b1 complex, as well as the effect of sunitinib on the VEGFR-integrin b3 and PDGFR-integrinb3 complexes formation on U87 cells.
Material and Methods: U87 and M059K cells were treated with Sunitinib and Lapatinib at several concentrations. The proliferation of cells was determined by MTT assay. Apoptosis was evaluated with Annexin binding assay. Migration assays were performed in 24-well microchemotaxis chambers. The release of MMPs into the culture medium of cells was measured by zymography.

Immunoprecipitation and western analysis were conducted in order to detect the formation of each growth factor-integrin complex. Confocal microscopy was used in order to perform immunofluorescence, detecting the above mentioned complex formations.
Results: The application of both agents either alone or in combination showed significant reduction in proliferation and chemotactism in both cell lines. There was also an induction of apoptosis in both cell lines. MMP levels were down regulated in M059K cells while there was no change of MMP levels in U87 cells. Lapatinib intercepted the formation of EGFR-integrinb1 complex, in both cell lines while sunitinib intercepted VEGFR-integrinb3 complex formation in U87 cells. Immunofluorescence revealed colocalisation of molecules in the above mentioned complexes and their disengagement after application of agents in a time course manner.
Conclusions: Lapatinib and Sunitinib have a strong inhibitory effect. Combinational dosing of these agents has a better and stronger effect in the above mentioned parameters than each one of them on its own. The current data showed an implication of the tested agents in the integrin – growth faxtor's pathway.

511 Caveolin-1, TGFβ/Smad2 and Alpha5 Beta1 integrins connection in human glioblastoma

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Background: Caveolin-1 (cav1) plays a crucial role in cancer development and progression. Although caveolin-1 expression is increased in glioma, cav1 negative ($cav1_{low}$) and positive ($cav1_{high}$) cells coexist in glioblastoma (GBM). We reported that $cav1_{low}$ GBM cells exerted a more aggressive phenotype than $cav1_{high}$ GBM cells, suggesting that cav1 is a tumour suppressor in brain tumours. Transcriptomic analysis showed that cav1 represses integrins especially $\alpha_5\beta_1$ integrin so that cav1 and $\alpha_5\beta_1$ integrin expressions were inversely correlated. We identified $\alpha_5\beta_1$ integrin as the mediator of cav1's effect in GBM. This study focused on the mechanisms by which cav1 regulate $\alpha_5\beta_1$ integrin expression.
Material and Methods: 6 different GBM cell lines were used. Some were silenced (using $si/shRNA_{cav1}$) or forced to express ($pEGFP_{cav1}$) cav1. TGFβ was quantified using a commercially available kit. Protein expression and activity was determined by western blot. Drugs used were SB431542, LY294002 and U0126 (inhibiting the TGFβ receptor, Pi3K and MEK1 respectively), K34c (a $\alpha_5\beta_1$ integrin antagonist), TGFβ and activin. Surviving fraction after drug treatment was determined by clonogenic assays. Gene expression was studied by qPCR.
Results: Cav1 affects the TGFβ/Smad2 pathway, previously identified as a regulator of integrin expression. Silencing cav1 increased the secretion of TGFβ, the expression of TGFβ receptor and the activity of its downstream effector Smad2. Conversely, forced expression of cav1 repressed the TGFβ/Smad2 pathway so that cav1 expression and TGFβ/Smad2 activity are inversely correlated. Using selective inhibitors, we showed that the TGFβ/Smad2 pathway was involved in the regulation of $\alpha_5\beta_1$ integrin expression by cav1. Two Smad2-dependent signaling pathways were involved; one independent on the TGFβRI (cav1 $\rightarrow$ ERK $\rightarrow$ Smad2 $\rightarrow$ $\alpha_5\beta_1$ integrin) and one dependent on the TGFβRI (cav1 $\rightarrow$ TGFβRI $\rightarrow$ Pi3K/Akt $\rightarrow$ Smad2 $\rightarrow$ $\alpha_5\beta_1$ integrin). Therefore, $cav1_{low}$ cells exert high level of TGFβRI/Smad and $\alpha_5\beta_1$ integrin and vice and versa. The reverse correlation between cav1 and $\alpha_5\beta_1$/TGFβ/Smad2 was confirmed in different GBM cell lines. Finally, we showed that $cav1_{low}/\alpha_5\beta_1$/TGFβ/$Smad_{high}$ cells (identified as being the most aggressive) are highly sensitive to SB431542 and K34c.
Conclusions: Cav1 controls $\alpha_5\beta_1$ integrin expression through the TGFβ/Smad2 pathway. The status of cav1/$\alpha_5\beta_1$/TGFβ/Smad2 might be a useful marker of the tumour behavior and a predictor of anti-TGFβ or anti-$\alpha_5\beta_1$ integrin therapies.

512 Tetraoxanes induced ROS production and activation of caspase 3 in HeLa cells

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Background: It was demonstrated that mixed steroidal tetraoxanes inhibit cancer cell proliferation at micromolar level through an apoptotic mechanism. It will be interesting to see if these compounds may possibly induce oxidative stress, which could lead to induction of apoptosis in tumour cells. As tumour cells contain more iron than other normal tissues it is reasonable to suggest that tetraoxanes could react with iron, generating alkoxy radicals or even highly reactive hydroxyl radicals in a Fenton-like reaction. To gain further insight into the mechanism of cell death induced by tetraoxane endoperoxides, we tested production of reactive oxygen species (ROS) and level of activation of caspase 3 in tumour cells treated with several newly synthesized tetraoxanes.
Material and Methods: Stock solutions of investigated tetraoxanes, were prepared in DMSO at concentrations of 10 mM and afterwards they were diluted with complete nutrient medium to various final concentrations. Target cells used were malignant cervix carcinoma HeLa cells.
Production of intracellular ROS was measured using the fluorescent dye 2′,7′-dichlorofluorescein diacetate, a nonpolar compound that readily diffuses into cells, where it is hydrolyzed to 2′,7′-dichlorofluorescein (H_2DCFDA), a nonfluorescent polar compound. In the presence of an oxidizing compound, H_2DCFDA is converted into highly fluorescent 2′,7′-dichlorofluorescein.
Level of active caspase 3 is measured using the caspase 3 fluorimetric assay kit (Sigma Chemicals), based on the hydrolysis of the peptide substrate by caspase 3, resulting in the release of the fluorescent 7-amino-4-methylcoumarin moiety.
Results: After treatment with investigated tetraoxanes ROS level in HeLa cells significantly increased indicating possible oxidative stress, maybe as a result of the production of reactive alkoxy or hydroxyl radicals. Significant increase in activity of caspase 3 was observeded after incubation of HeLa cells with all investigated tetraoxanes.
Conclusion: Taken together, these results demonstrate that tetraoxanes potently generates ROS, and strongly inhibits the growth of HeLa cells throughout apoptosis. Although the mechanisms by which mixed tetraoxanes activates caspase 3 in HeLa cells remains unclear, those results provide correlation between ROS production, caspase 3 activity and tetraoxanes-induced apoptosis.

513 DNA copy number changes in radiation-induced mammary carcinoma of (SD x COP) F1 hybrid rats

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Background: Epidemiological studies indicate that breast is one of the most susceptible organs to radiation-induced carcinogenesis. Most studies, however, have failed in identifying clear genetic alterations in radiation-induced breast/mammary cancers. The Copenhagen (COP) rats are completely resistant, whereas Sprague-Dawley (SD) rats are highly susceptible, to chemically-induced mammary carcinogenesis, and this resistance of the COP background is regarded as a dominant trait.
Material and Methods: Mammary cancer-prone SD, -resistant COP, and their hybrid (SD $\times$ COP) F1 rats were irradiated with gamma-rays at 4 Gy and underwent autopsy at the time of spontaneous death or at 1.5 years post-irradiation. Genomic DNA was extracted from mammary cancers and ear skins of corresponding individuals. Genome-wide DNA copy number was analyzed by array comparative genomic hybridization (aCGH).
Results: COP rats were resistant to radiation-induced mammary carcinogenesis. Interestingly, F1 rats showed a relatively susceptible trait, suggesting recessive inheritance of the resistance to radiation-induced mammary carcinogenesis. The preliminary results of aCGH analysis indicated that partial deletions of the proximal region of chromosome 2, where Mcs-1 (mammary cancer susceptibility gene-1 for chemical carcinogenesis) is mapped, were occasionally observed, suggesting a tumour suppressive role of Mcs-1 in radiation carcinogenesis. Other aberrations including small deletions and aneuploidy were also frequent but scattered throughout the genome.
Conclusions: These findings implicate that radiation-induced rat mammary cancers are rather heterogeneous with regard to copy number changes. The frequent deletion of COP alleles suggests that these tumour suppressive alleles may be readily targeted by radiation but not carcinogenic chemicals.

514 Co-expression of E- and P-cadherin in breast cancer: role as an invasion suppressor or as an invasion promoter?

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Background: Cadherins are cell-cell adhesion molecules. During tumour progression, their expression and/or function are frequently altered. E-cadherin down-regulation is often associated with tumour initiation and progression in breast cancer, whereas P-cadherin overexpression is associated with a worse patient survival, as well as with invasive breast cancer cells. In this study, we aimed to understand if P-cadherin overexpression could interfere with E-cadherin invasion suppressor role in breast cancer.
Materials and Methods: E- and P-cadherin expression was evaluated in a series of invasive breast carcinomas. The results were correlated with prognosis and clinico-pathological parameters. To study the functional value of E- and P-cadherin co-expression, we silenced the transcription of both cadherins in BT-20 breast cancer cells, and investigated the *in vitro* effects