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.
Acknowledgement: Supported by MSM0021620808, GAAV IAA500040802.

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.