examinations using 1H MRS together with MRI for in vivo metabolic evaluation of EOC.
Conclusions: Abnormal PC metabolism has implications in cancer biology and provide an avenue to the development of non-invasive clinical methods for diagnosis and treatment follow-up. PC-plc and ChoK may represent a novel target for the design of therapeutic strategies in ovary cancer.

379 The role of translesion DNA polymerase eta in p53 activation and DNA damage response

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Background: DNA polymerase eta (PolH), a Y-family translesion polymerase, is required for repairing UV-induced DNA damage, and loss of PolH is responsible for early onset of malignant skin cancers in patients with Xeroderma Pigmentosum Variant, an autosomal recessive disorder.
Materials and Methods: Both molecular and Cellular techniques, including transcriptional studies, gene expression, and protein stability and degradation, were used to define how PolH is regulated in vitro and in vivo.
Results: We found that PolH is induced by DNA damage and necessary for DNA damage-induced apoptosis in a p53-dependent manner. Interestingly, we also found that PolH is necessary for DNA damage-induced activation of p53. Due to the critical role of PolH in maintaining genome stability, PolH expression is subject to regulation by multiple mechanisms. Here, we found that PolH is degraded by proteasome, which is enhanced upon UV irradiation. We also found that PolH interacts with Pirh2 E3 ligase, a target of p53, via the polymerase associated domain in PolH and the RING finger domain in Pirh2. In addition, we showed that overexpression of Pirh2 decreases, whereas knockdown of Pirh2 increases, PolH protein stability. Interestingly, we found that PolH is recruited by Pirh2 to, and degraded by, 20S proteasome in an ubiquitin-independent manner. Finally, we observed that Pirh2 knockdown leads to accumulation of PolH and subsequently enhances the survival of UV-irradiated cells.
Conclusion: We uncovered a novel function for PolH: modulating the DNA damage checkpoint and p53 activation. In addition, we postulate that UV irradiation promotes cancer formation in part by destabilizing PolH via Pirh2-mediated 20S proteasomal degradation.

380 Combined analysis of SNPs in IL-6/IL-6R pathway predict prostate cancer aggressiveness and development of resistance to hormonal castration

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The IL-6/IL-6R pathway is involved in Prostate Cancer (PCa) progression and in the development of resistance to hormonal castration (RHC). We hypothesized that functional variants in *IL6*, *IL6R* and *gp130* genes, which influence transcriptional rate and protein function, can influence susceptibility, aggressiveness and response to treatment in PCa.
We conducted a case-control study in biopsy-proven PCa (n = 1263) and benign prostatic disease (n = 472) patients. Genotyping was performed in *IL6*, *IL6R* and *gp130* genes, through PCR-RFLP and Real Time-PCR allelic discrimination. Genotypes from *IL6*, *IL6R* and *gp130* polymorphisms were functionally combined according to the cell signalling activation potential attributed to genetic profile.
When analysed individually, results showed an increased risk for TT carriers in the *IL6* polymorphism at locus −6331 to present a PSA level $\geqslant$20 ng.mL-1 at the time of diagnosis ($P = 0.004$). Furthermore, in the polymorphism at locus −174 of *IL6* gene, C carriers had increased risk for aggressive disease (PSA $\geqslant$ 20 ng.mL^{-1}, $P = 0.012$; distant metastasis, $P = 0.089$ and poorer overall survival, $P = 0.033$). Univariate Kaplan–Meier function plots analysis evidenced shorter time to the development of resistance to hormonal castration in TT carriers of *IL6* −6331 T>C polymorphism ($P = 0.027$).
Moreover, when polymorphisms in the receptor were functionally combined according to the cell signaling genetic profile, an increased risk for developing PCa was observed for the high signallers ($P = 0.010$). Combined analysis of polymorphisms in the *IL6* promoter region showed association of a high/intermediate *IL6* expression genetic profile with aggressive disease (advanced stage, $P = 0.040$; distant metastasis, $P = 0.023$ and PSA $\geqslant$ 20 ng.mL^{-1}, $P = 0.001$). The functional combination between polymorphisms in *IL6*, *IL6R* and *gp130* genes, evidenced that IL-6/IL-6R high activation genetic profile carriers are overrepresented in the group with distant metastasis ($P = 0.017$) and earlier development of RHC ($P = 0.021$).
Combination of *IL6*, *IL6R* and *gp130* genetic polymorphisms according to the functional profile revealed its relevance in susceptibility, aggressiveness and in progression-free interval in patients submitted to hormonal castration. Accordingly, these results support IL-6/IL-6R pathway as a targetable mechanism in PCa. The *IL6*, *IL6R* and *gp130* functional polymorphism might be useful molecular markers for PCa aggressiveness and as a predictive factor for the RHC.

381 Functional studies of gene products and signal transduction mechanisms involved in cancer progression and metastasis – influence of extracellular S100A4

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The S100A4 protein is a small, multifunctional protein located in the cytoplasm, the nucleus and the extracellular space. S100A4 has been linked to the invasive and metastatic behavior of cancer cells, and its metastasis-promoting properties are mediated through both intracellular and extracellular functions, however the exact molecular mechanisms by which S100A4 exerts these effects are incompletely understood. Importantly, an association between exposure to, or expression of S100A4 and members of the matrix metalloproteinase (MMP) system is shown, and S100A4 is suggested to promote cancer progression by regulating remodeling of the extracellular matrix.
In a previous study we demonstrated that extracellular S100A4 induces phosphorylation of IkBa and activation of the classical NF-kB pathway in a subset of human cancer cell lines. In search for downstream effect molecules, we have demonstrated that S100A4 induces ephrin-A1 and the multifunctional chemokine osteopontin (OPN) through an NF-kB dependent mechanism. Interestingly, both ephrin-A1 and OPN are previously shown to influence cell migration, angiogenesis and metastasis.
In the present work we have investigated the S100A4-induced signal transduction pathways upstream of IkBa through the use of specific inhibitors and kinase activity assays with special focus on the involvement of the serine kinases MEKK1, AKT and NIK. Moreover, S100A4-induced effect of identified downstream target molecules on invasion and metastatic capacity is examined.
We have established that common signal transduction pathways (e.g.: PLC, PI3K and GPCR) are not involved in transmitting the signals from the plasma membrane to the ultimate activation of NF-kB, while Ser/Thr kinases seem to play a significant role. Importantly, in a transwell invasion chamber assay a significant increase in cell migration and invasion was observed upon exposure to S100A4 and induction of OPN. Furthermore, strong indications indicated that the proteases uPA and MMP-13 are responsible for these observed phenotypic effects. These novel findings are an important contribution to understand the molecular mechanisms underlying the biological effects induced by S100A4.
In conclusion, these results indicate that S100A4 through induction of Ser/Thr kinase-dependent NF-kB-activation of OPN initiates a metastatic cascade, which subsequently may enhance cellular dissemination and facilitate metastasis.

382 A simple and robust assay to study endothelial cell function in vivo

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Tumour growth depends on the ability to recruit blood vessels from the host tissue. Correspondingly, angiogenesis has become a major target for antitumour therapy. However, the complexity of the angiogenic cascade limits cellular approaches to studying angiogenic endothelial cells (EC). To overcome this limitation, we developed a reliable and robust *in vivo* angiogenesis assay that is based on the xenotransplantation of human EC in immunocompromized mice. Implantation of spheroidal endothelial aggregates in a Matrigel/fibrin matrix resulted in the formation of a complex three-dimensional network of human neovessels. Implants were dissected after 20 days and analyzed for microvessel density (MVD), mural cell recruitment and perfusion. The assay provides unique opportunities to perform studies in the field of vascular research. First, EC can be manipulated *ex vivo* prior to implantation for GOF and LOF studies to investigate vascular function. Downregulation of Ang-2 or PDGF-BB in HUVEC led to a significant reduction of MVD. Loss of Ang-2 or PDGF-BB in HUVEC resulted in the reduction of α-smooth muscle actin (α-SMA) coverage. However, Desmin coverage was only increased in Ang-2 silenced HUVECs. Second, EC can be manipulated to mimic pathological conditions. Towards this end we generated HUVECs lentivirally silenced for CCM1, one of the three genes causally involved in the formation of Cerebral Cavernous Malformations. CCM1 LOF led to the formation of hypervascularized vascular networks mimicking human CCM lesions. Third, HUVEC can