pathway responds by initiating a process of cell cycle arrest, senescence or apoptosis which either permits repair of these errors or kills the clone of cells that contain these mutations. Central to this pathway are a series of proteins that respond to the stress signals and regulate the levels and activity of the p53 pathway. We have identified a number of single nucleotide polymorphisms (SNP) in those genes that regulate p53 activity and functions and these SNPs can play a role in the incidence of cancers in a population, the age of onset of cancers and the response to therapy. Some of the haplotypes containing these SNPs appear to be under positive evolutionary selection pressures in some human populations. The reason for this appears to be the role of p53 in the implantation of embryos into the uterus and the impact of some of these SNPs upon the fecundity of mice and humans. This process is mediated by the p53 regulated gene, Leukemia Inhibitory Factor or LIF, a cytokine that is essential for the implantation of embryos. SNPs in the p53 gene, the MDM-4 gene and the LIF gene regulate the efficiency of implantation of embryos in humans. It could well be that the p53 protein is also involved in the surveillance of developmental abnormalities.
The p53 transcription factor also regulates the synthesis of glutaminase-2, an enzyme that converts glutamine to glutamate in the mitochondria of both the liver and the brain. In the liver glutamate is converted to alpha-keto glutamate and this helps promote oxidative phosphorylation. In liver cancers glutaminase-2 is not produced and these cancers produce energy via aerobic glycolysis. Returning the glutaminase-2 gene to liver tumour cells increases glutamate levels and inhibits the growth of these cells. It appears that a metabolic regulator that restores oxidative phosphorylation can inhibit this type of cancer. In the brain glutamate is a neurotransmitter and five different glutamate receptors are also regulated by p53 in response to stress signals. In the brain the stress signals that activate p53 are communicated throughout the body by these glutamate receptors. Interestingly three of the genes that can cause Parkinsons Disease and the Huntington gene are also regulated by p53 in the brain. The role of p53 in the central nervous system remains to be explored.

Monday 28 June 2010 10:20–12:20

Symposium
Epigenetics: from DNA methylation to stem cell differentiation

322 Mechanisms of DNA methylation in mammals

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DNA methylation plays an important role in cancer and is associated with gene silencing of tumour suppressor genes. The methylation of CpG sites is established by the DNA methyltransferases – the DNMTs. One main interest in our lab is to better decipher the mechanisms by which these enzymes function and participate to cancerogenesis.
In recent years, we have contributed to show that the DNA methylation machinery brings about transcriptional repression through recruitment of histone modifying enzymes. In particular, a close connection was found between DNMTs and histone methyltransferases (1–5), with for exemple an impact on PML-RAR-mediated leukemia.
A key question still poorly understood is how are the DNMTs, and in particular their enzymatic activity, regulated. Data will be presented that suggest a new mechanism for the regulation of DNA methylation by post-translational modification.

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323 Human cancer epigenetics: from DNA methylation to microRNAs

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An altered pattern of epigenetic modifications is central to many common human diseases, including cancer. Many studies have explored the mosaic patterns of DNA methylation and histone modifications in cancer cells on a gene-by-gene basis, among them the seminal finding of transcriptional silencing of tumour suppressor genes by CpG island promoter hypermethylation. Epigenetic gene inactivation in transformed cells involves many "belts of silencing". We are in the process of completing the molecular dissection of the entire epigenetic machinery involved in methylation-associated silencing, such as DNA methyltransferases, methyl-CpG binding domain proteins, histone deacetylases, histone methyltransferases, histone demethylases and Polycomb proteins. The first indications are also starting to emerge about how the combination of cellular selection and targeted pathways leads to abnormal DNA methylation. In addition to classical tumour-suppressor and DNA repair genes, epigenetic gene silencing includes genes involved in premature aging and microRNAs with growth inhibitory functions. Recent technological advances are now enabling cancer epigenetics to be studied genome-wide. It is time to "upgrade" cancer epigenetics research and put together an ambitious plan to tackle the many unanswered questions in this field using genomics approaches to unravel the epigenome.

324 Understanding the origins of aberrant DNA methylation in cancer

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The conversion of normal cells to cancerous cells typically involves several steps resulting in the acquisition of unlimited growth potential (immortality). Both genetic and epigenetic changes have been detected in a number of different cancer cell types. Generally, these changes lead to the activation of oncogenes and the inactivation of tumour suppressor and pro-apoptotic genes. Although a number of tumour suppressor genes have been shown to be silenced by promoter DNA methylation, the following questions still remain: Do epigenetic changes contribute directly to cancer and if so when, where and how do they co-operate with genetic changes during the transformation process? To try to address these questions we have generated a human cancer cell model with defined genetic elements to study the global epigenetic changes associated with cellular immortalisation and transformation. We will describe the generation and characterisation of this cancer cell model and will provide preliminary evidence for progressive changes in promoter DNA methylation.

325 The role histone methyl transferases and demethylases in stem cell differentiation and cancer

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A major goal of our research is to identify and characterize genes involved in the regulation of normal proliferation and differentiation that also contribute to the development of human disease. Consistent with an essential role of histone modifying enzymes in controlling cell-fate decisions, several of these are causally linked to the development of diseases, such as cancer and CNS-associated disorders.
Histone methylation regulates chromatin structure and gene regulation. Histone methylation patterns define the state of chromatin and it is regulated by histone methyl transferases and demethylases. The Polycomb group proteins (PcGs) were until few years ago best known for their essential role in development, however, several reports have established that PcGs are frequently deregulated in human tumours. Others and we have demonstrated that the PcG protein and histone methyl transferase EZH2 is an oncogene, which regulates the expression of a large number of genes dictating cell-fate decisions.
Recently, others and we have discovered a group of proteins that catalyze the demethylation of methylated lysines. Members of this Jumonji demethylase family are overexpressed in human cancer and mutated in neurological disorders. At the meeting, results will be presented describing the functional characterization of some of these very exciting proteins – that also present strong candidate targets for drug development.

Monday 28 June 2010 10:20–12:20

Symposium
Hypoxia & angiogenesis

326 Targets of "angioprevention": from inflammatory angiogenesis to hypoxia

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Background: The complex cancer microenvironment cooperates with tumour and endothelial cells to promote malignancy. We propose to identify molecules and pathways involved in cancer progression in order to prevent tumour development by targeting the microenvironment and inflammatory angiogenesis.

Material and Methods: *In vivo* we performed matrigel sponge assay to evaluate the anti-angiogenic effects of compounds. Then we tested molecule efficacy in Kaposi's sarcoma xenograft to follow their effects on tumour growth. *In vitro* we evaluated HUVECs (Human Umbilical Vein Endothelial Cells) ability to organize in capillary-like structures in matrigel, in presence of molecules or vehicle. By immunofluorescence we investigated whether these compounds affect NF-kB pathway in HUVECs.
Results: We have shown that various molecules, such as flavonoids, antioxidants and retinoids, act in the tumour micro-environment inhibiting the recruitment and/or activation of endothelial cells and innate immune cells. N-acetyl-cysteine, the green tea flavonoid epigallocatechin-3-gallate, and alpha lipoic acid prevent angiogenesis in the matrigel sponge assay *in vivo* and inhibit the growth of the highly angiogenic Kaposi's sarcoma tumour cells in nude mice. The synthetic retinoid 4-hydroxyfenretinide also showed anti-angiogenic effects. Recently we have added to the angiopreventive molecules also CDDO triterpenoids, hyperforin and beer hop isoflavon Xantohumol. We also identified overlapping sets of genes regulated by the anti-oxidants. The ROS-producing 4HPR induced members of the TGFβ-ligand superfamily, which, at least in part, explains its anti-angiogenic activity. NAC and the flavonoids all suppressed the IkB/NF-kB signalling pathway and showed reduced expression of many NF-kB target genes. We also investigated the anti-angiogenic proprieties of a synthetic peptide mimicking the intracellular Met-tail conjugated to cell-penetrating peptides (Antennapedia and Tat). Our observations indicated that this peptide inhibited ligand-dependent cell motility and morphogenesis *in vitro* and interfered with HGF-dependent downstream signaling and in vivo inhibited angiogenesis.
Conclusions: These data indicate that angiogenesis is a common and key target of most chemopreventive. The repression of the NF-kB pathway suggests anti-inflammatory effects for the anti-angiogenic compounds that may also have an indirect role in angiogenesis inhibition, by targeting cells in the tumour microenvironment.

327 Hypoxia-inducible factor-2alpha regulates macrophage function in mouse models of acute and tumour inflammation

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Hypoxia-inducible factor (HIF)-1α and -2α display unique and sometimes opposing activities in regulating cellular energy homeostasis, cell fate decisions and oncogenesis. To fully characterize hypoxic adaptations, distinct functions of HIF-1α versus HIF-2α must be elucidated. Macrophages accumulate both HIF-1α's under hypoxia, but HIF-2α overexpression in tumour-associated macrophages (TAMs) is specifically correlated with high-grade human tumours and poor prognosis. HIF-1α regulates myeloid-mediated inflammatory and antibacterial activities, in part through control of glycolysis and ATP production. However, the precise role of HIF-2α during macrophage-mediated inflammatory responses remained unclear. We demonstrate here that mice lacking myeloid HIF-2α are resistant to lipopolysaccharide-induced endotoxemia and display a marked inability to mount inflammatory responses to cutaneous and peritoneal irritants. Furthermore, HIF-2α directly regulates pro-inflammatory cytokine/chemokine expression in macrophages activated in vitro. Using independent murine hepatocellular and colitis-associated colon carcinoma models, we show that HIF-2α-deficient macrophages exhibit migratory defects associated with reduced tumour cell proliferation and progression. Of note, HIF-2α modulates macrophage migration by regulating the expression of chemotactic receptors M-SCFR and CXCR4, without altering intracellular ATP levels. Collectively, our data identify HIF-2α as an important regulator of innate immunity, suggesting it may be a useful therapeutic target for treating inflammatory disorders and cancer.

328 Tumour metabolic adaptation to hypoxic and acidic stress

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Oxygen and nutrient sensing is a fundamental process of life. In its absence, fast growing cells of the developing embryo and of expanding tumours rapidly die. In fact, cell growth signaling is integrated with the capacity to sense availability of key nutrients and therefore to allow cells to rapidly respond to nutrient fluctuations in the microenvironment. Early on in evolution, oxygen sensing emerged, as a central control mechanism of energy metabolism and vasculogenesis. At the heart of this regulatory system is the Hypoxia-Inducible Factor, HIF-1, which controls the expression of, among other gene products, VEGF-A and Angiopoïetin-2, two key angiogenic factors in vertebrates. This finding has placed the hypoxia-signaling pathway at the forefront of nutritional control. HIF can induce a vast array of gene products controlling glycolysis, intracellular pH (pHi), angiogenesis, cell migration and invasion, and so has become recognized as a strong promoter of tumour growth. The pro-invasion feature of HIF-1, measured by stimulation of Epithelial-Mesenchyme-Transition, could be seen as an integrated program 'designed' for migration-induced nutrient-search, as in microorganisms. It is therefore not surprising that HIF-1 also promotes access to another source of nutrients by inducing macro-autophagy.
In this presentation, we will highlight some of the HIF1-induced gene products – carbonic anhydrases IX and XII (CAs) and monocarboxylate transporters (MCTs) − which regulate pHi by controlling export of metabolically-generated acids (carbonic and lactic acids). We report that targeting pHi-regulated processes in several human tumour models severely restricts tumour growth, a process that entails glycolysis-generated ATP levels.
We propose that membrane-bound carbonic anhydrases (CAIX, CAXII), monocarboxylate transporters (MCT1 and MCT4) as well as their chaperon Basigin/EMMPRIN/CD147), which are associated with exacerbated tumour metabolism represent new potential targets for anticancer therapy.

329 The von Hippel–Lindau tumour suppressor protein: oxygen sensing pathways and cancer

No abstract received.

Monday 28 June 2010 10:20−12:20

Symposium
Signalling & cancer

330 BRAF and RAS signalling in human melanoma

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BRAF is a protein kinase that is mutated in about half of human melanomas. Its upstream activator, the small G-protein NRAS, is mutated in a furthre 20% of cases. Oncogenic BRAF and RAS transform melanocytes and stimulate melanoma cell proliferation and survival in vitro. We have developed mouse models of melanoma driven by these oncogenes expressed at physiological levels. Oncogenic BRAF induces melanocyte hyperproliferation, senescence and ultimately melanoma, whereas oncogenic RAS does not induce any of these responses. Surprisingly however, kinase-dead BRAF cooperates with oncogenic RAS to induce melanoma through a mechanism that appears to involve paradoxical activation of CRAF. We have found that this activation occurs through direct binding of the drugs to BRAF, which stimulates BRAF binding to CRAF, leading to CRAF hyperactivation. In this complex BRAF does not appear to signal directly − rather it appears to act as a scaffold that supports CRAF hyper-activation, leading to hyper-activation of the pathway. This result explains the observation that whereas highly oncogenic version of BRAF such as ^{V600E}BRAF never occur coincident with mutations in RAS in cancer, kinase-dead mutations in BRAF do occur coincident with RAS mutations. These results have clinical implications as they suggest that BRAF-selective drugs could have unexpected side-effects in melanoma patients.

331 Inhibition of tumour suppressor protein phosphatase 2A (PP2A) in cancer

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As a disease entity, cancer is composed of numerous phenotypically heterogenous disease types. However, it has been recently established that regardless of the phenotypic variability between different cancer types, perturbation of limited number of genetic elements is sufficient to induce cellular transformation in many different human cell types. Experimentally, it was demonstrated that activation of Ras and telomerase (TERT), along with inactivation of the tumour suppressor proteins p53 and Retinoblastoma protein (Rb) can immortalize a variety of human cell types, which can subsequently transform to a tumourigenic state in response to inhibition of protein phosphatase 2A (PP2A). Therefore, these common genetic elements could be considered as master regulators of cancer development. Accordingly, it is obvious that further understanding of these genetic elements would be important in order to develop therapies against malignant diseases.
PP2A is a widely conserved protein serine/threonine phosphatase (PSP) that functions as a trimeric protein complex. As described above, recent experimental evidence has firmly established that inhibition of PP2A activity is a prerequisite for human cell transformation. Moreover, target molecules for which dephosphorylation is important for the tumour suppressor activity of PP2A have been recently identified. However, as the majority of evidence supporting the role of PP2A as a critical tumour suppressor, has been obtained by using viral antigens or chemical inhibitors, the *in vivo* mechanisms by which PP2A tumour suppressor activity is inhibited in spontaneously transformed human cancer cells have been unclear.
We have recently identified a novel protein as an endogenous interaction partner for PP2A complex. Our results show, that the protein, designated as Cancerous Inhibitor of PP2A (CIP2A), inhibits PP2A activity towards c-Myc