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

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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