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

R. Marais[1]. [1]*Institute of Cancer Research, Signal Transduction Team, London, United Kingdom*

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

serine 62, and thereby prevents c-Myc proteolytic degradation. Moreover, siRNA-mediated depletion of CIP2A, markedly increased PP2A activity in the c-Myc-PP2A complex. We also demonstrated that CIP2A is required for the malignant cellular growth and for *in vivo* tumour formation. In accordance with the oncogenic role of CIP2A, overexpression of CIP2A promotes Ras-elicited cell growth and transforms immortalized human cells (HEK-TERVs). In addition CIP2A was overexpressed in two common human malignancies, human head and neck squamous cell carcinoma (HNSCC) and colon cancer. Thus, our results demonstrated that CIP2A is a novel human oncoprotein that inhibits PP2A in human malignancies. More recently we have shown that in addition to its overexpression in HNSCC and colon cancer, CIP2A expression predicts poor prognosis in certain subtypes of human gastric cancers and correlates with breast cancer aggressivity. Together these results validate CIP2A as a clinically relevant human oncoprotein.

Reference(s)

Junttila et al., Cell, 130, 2007.
Khanna et al., J. Natl. Cancer Inst., 101, 2009.
Come et al., Clin. Cancer Res., 15, 2009.

332 Dual-specificity protein phosphatases and the regulation of MAP kinase signalling

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DUSP5 and DUSP6/MKP-3 are members of a structurally distinct subfamily of ten dual-specificity (Tyr/Thr) protein phosphatases, which are responsible for the regulated dephosphorylation and inactivation of mitogen-activated protein kinases (MAPKs) in mammalian cells and tissues. DUSP6/MKP-3 is a cytoplasmic phosphatase, which is inducible in response to fibroblast growth factor (FGF) signalling during early embryonic development where it acts in a negative feedback loop to regulate the activity of the classical ERK1 and ERK2 MAPKs. DUSP5 is also a specific ERK phosphatase. However, it is a mitogen-inducible nuclear enzyme and can also act as a nuclear anchor for ERK1 and ERK2. Despite extensive knowledge of the biochemical and structural basis of their catalytic activity, until recently relatively little was known about the regulation and physiological functions of DUSP5 and DUSP6. In particular, it is unclear if these phosphatases play any role in the regulation of ERK activation in tumours where activated oncogenes drive high levels of signalling through the Ras/ERK pathway. Recent reports have identified DUSP6 as a gene, which is up regulated during the early stages of tumour development in lung and pancreatic cancers. However, its expression is lost as tumours become more advanced, suggesting that DUSP6 may be a candidate tumour suppressor. In order to test this hypothesis we have generated mice with targeted deletions of the genes encoding DUSP5 and DUSP6 and studied the effects of gene loss in both cultured cells and in mouse models of cancer. Our preliminary data would indicate that DUSP6 is an important regulator of ERK activity in tumours initiated by mutant ras and that DUSP6 loss results in increased levels of ERK activity which is associated with both an increased frequency and more rapid progression of ras-induced carcinogenesis.

333 Hedgehog signalling in skin and pancreatic cancer

R. Toftgård[1]. [1]*Karolinska Institutet, Center for Biosciences, Stockholm, Sweden*

The Hedgehog (Hh) signalling pathway ending with activation of the GLI transcription factors is of central importance during embryo development and implicated in control of stem cell renewal and proliferation. When aberrantly activated the Hh pathway contributes to cancer development in several tissues such as skin and pancreas. To address the interaction with another key oncogenic pathway frequently activated in pancreatic cancer we have investigated the molecular crosstalk between RAS and Hh signalling and found that mutant RAS induces expression of the SHH ligand but at the same time potently inhibits autocrine Hh signal transduction. Mutant RAS functions in a cilium-independent manner upstream or at the level of Sufu and interferes with Gli2 activation and Gli3 processing. Mechanistically the cell-autonomous negative regulation of Hh signalling by mutant RAS is dependent on the dual specificity tyrosine kinase DYRK1B. Basal Cell Carcinomas (BCC) are the most common skin cancers and are dependent on deregulated Hh signalling. Using mouse models conditionally expressing GLI1 or with homozygous inactivation of the *Ptch1* gene we find that stem cells residing in the hair follicle represent a cell of origin. This stem cell population also contributes to wound healing and the wound environment alters stem cell lineage selection and accelerates BCC development providing a link between tissue injury and cancer risk.

Monday 28 June 2010 12:45–13:45

Young Cancer Researcher's Workshop

334-335 Career opportunities

No abstract received.

Monday 28 June 2010 13:45–14:35

Award Lecture: Anthony Dipple Carcinogenesis Award

336 Intricacies of BRCA1 genome integrity control and cancer suppression functions

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BRCA1 is a high penetrance breast and ovarian tumour suppressing gene. It encodes at least three, distinct products. The largest is p220, a multiphosphorylated, nuclear polypeptide that, when heterodimerized with a tightly binding partner protein, BARD1, operates as an E3 ubiquitin ligase. p220 has been much more extensively studied than the other known *BRCA1* products (BRCA1-IRIS and dl 11). It exerts tumour suppressing activity and is engaged in a variety of processes, each dedicated to the maintenance of genome integrity. These functions include: 1) support of error-free repair of DNA double strand breaks by homologous recombination (HR), a process performed coordinately with the major product of the other, known, high penetrance breast cancer gene, BRCA2; 2) suppression of illegitimate recombination between chromosomes; 3) support of key aspects of the process that leads to repair of bulky adduct-driven DNA damage; 4) participation in the building of mitotic spindle poles; and 5) a role in the regulation of centriole formation. Thus far, genetic studies strongly suggest that the contributions of p220 to HR and to mitosis control are linked to its breast cancer suppressing function, although how these two functions are linked to breast cancer suppression is not well understood. Moreover, whether the other functions operate in this regard is unknown.
Results are now emerging which suggest that a modicum of control is applied by certain BRCA1 binding proteins to the HR and illegitimate recombination suppression functions of p220. Breakdown in these functions can elicit major biological defects that have potential clinical significance. The nature of these controls, how they are executed, and the abnormal outcomes associated with failure of their function will be discussed.

Monday 28 June 2010 14:35–16:35

Symposium

Discovery based translational research

337 Biomarkers in early clinical development

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Current attrition rate of new oncology drugs is very high, this leading not only to an unsustainable budget in the pharmaceutical industry but a sense of great disappointment in the oncology community level. To curtail this rate of attrition, investigation leaders need to make confident decisions as early as possible during the drug development process and to ensure that only those drugs with an optimal safety/efficacy profile move to phase III development and only patients most likely to benefit from the drug are enrolled into the pivotal regulatory trials. A greater understanding of the biology of cancer coupled with major advances in biotechnology has resulted in the identification of rationally-designed targeted agents. Proof of principle and robust antitumour activity may be most efficiently demonstrated in phase II studies involving patients bearing tumours that are principally driven by aberrations of the specific target or dysregulation of related signal transduction pathways. The hope is that by identifying tumours that are dependent upon the targeted pathway and by demonstrating that the drug modulates the pathway (either abrogating or promoting the signal) it will be possible to identify the right population of patients for the pivotal trials and hence significantly increase the probability of success. Once a biomarker has been identified, the assay validated and its intended use defined, the challenge becomes how to incorporate the biomarker assay into the drug development program and design of the phase II and phase III development. At this time point several decisions have to be made regarding whether to evaluate the biomarker prospectively or retrospectively,