Cox proportional hazard regression analysis revealed that rs11614913 variant homozygous genotype CC was a significantly unfavorable prognostic factor of NSCLC [Hazard ratio (HR) =1.76, 95% CI=1.34-2.32)]. In the genotype-phenotype correlation analysis using 23 tumor tissue samples, rs11614913 variant homozygote CC was associated with a significantly increased mature hsa-mir-196-a expression in the recessive model (P = 0.037), which might be due to an enhanced processing of pre-hsa-mir-196-a to its mature form. Conclusions: rs11614913 might be an independent prognostic biomarker for NSCLC. Further characterization of miRNA SNPs may open new avenues for cancer biological studies and therapeutic interventions.

42 Oral

ATM in breast cancer susceptibility - results of a pooled analysis of case-control mutation screening data

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The susceptibility gene for Ataxia telengiectasia, ATM, has been established as an intermediate-risk breast cancer susceptibility gene. However, the answer to the question "what sort of sequence variation in ATM confers increased risk of breast cancer" has been controversial. To address this question, we have pooled available ATM mutation screening data and then carried out a joint analysis of truncating variants, splice junction variants, and rare missense substitutions. A total of 1,729 breast cancer cases and 941 controls from 13 published studies were included in our pooled analysis. The analysis of rare missense substitutions was accomplished using the missense analysis program Align-GVGD with our improved classifier and a ATM protein multiple sequence alignments containing ATM sequences from human to sea urchin. We found that a trend test, incorporating both truncating plus splice junction variants and several grades of rare missense substitutions outperformed simple consideration of truncating plus splice junction variants alone. We also found significant evidence of risk both in truncating plus splice junction variants and in the in silico predicted highest-risk grade of missense substitutions. Taken together, these results led us to two conclusions: (1) careful analysis of missense substitutions will have real utility in case-control mutation screening projects, and (2) the attributable fraction, for risk of breast cancer, of rare missense substitutions in ATM is approximately equivalent to that of truncating and splice junction variants. Resequencing the entire coding sequence of ATM in 650 familial cases and 650 controls from 4 different populations is currently ongoing in the Genetic Susceptibility Group at IARC. Combined analysis of truncating and splice junction variants, and rare missense substitutions will be performed on our sample set, as a validation step of this approach.

06 July 2008 17:30 - 18:30

PLENARY LECTURE

AICR Lecture

43

Rho GTPases, actomyosin contractility and cell migration

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Cell movement plays a central role in tumour metastasis and in the generation of tumour blood supply and other stromal functions. We seek to determine how signalling pathways determine movement of tumour cells and of associated non-neoplastic cells such as endothelial cells. We have shown that tumour cells move in a 3-dimensional environment in two very different ways termed "elongated-mesenchymal" and "rounded-amoeboid". While the "elongated-mesenchymal" mode of movement in 3D is akin to movement of mesenchymal cells in 2D, "rounded-amoeboid" movement is only seen in 3D. These different modes of movement have very different requirements for small GTPase signalling pathways. The "rounded-amoeboid" form of movement has an absolute requirement for Rho-Rho-kinase signalling to drive high levels of actomyosin contractility, whereas Rho-kinase is not essential for mesenchymal movement. A major focus of our current studies is to determine how different signalling pathways interact to determine cell movement. A key determinant of mode of cell movement is the degree of actomyosin contractility. We are studying how actomyosin contractility is regulated by GTPase and kinase signalling pathways, and how the degree of contractility influences cell behaviour.

POSTER SESSION

Cell and tumour biology 1

44 Poster

Reactivation of telomerase activity in telomerase-deficient human cells by the pseudourydine synthase domain of dyskerin

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A dyskerin internal fragment, isolated in a genetic suppressor element (GSE) screening for cisplatin resistance, named GSE24.2, increases telomerase activity in different cellular systems. GSEs are biologically active gene fragments that encode either peptides or inhibitory antisense RNAs and act dominantly upon expression in mammalian cells. The GSE24.2 contains the pseudourydine synthase domain of the dyskerin, a protein that forms part of the telomerase complex. This protein is mutated in patients with X-linked dyskeratosis congenita (X-DC) resulting in greatly reduced levels of telomerase activity. GSE24.2 expressing cells showed impaired telomerase inhibition after cisplatin or chemical telomerase inhibitors treatment. The promoter of telomerase component hTERT was constitutively activated in GSE24.2 cells in a c-MYC expression-dependent manner. Furthermore, expression of GSE24.2 in cell lines derived from X-DC patients and VA13 cells increases hTERT and hTR RNA levels and recovers telomerase activity. Finally, expression of GSE24-2 was able to rescue X-DC fibroblasts from premature senescence. These data demonstrate that this internal domain of dyskerin plays an important role in telomerase maintenance after cell insults, such as cisplatin treatment and in telomerase-defective cell lines. The expression of the dyskerin fragment showed a dominant function in X-DC cells, providing the basis for a therapeutic approach to this disease.

45 Poster

A Twist – Snail axis critical for TrkB-induced metastasis

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Metastasis corresponds to the biggest cause of death of cancer patients. A better understanding of the molecular mechanisms mediating metastasis may help to tailor better drugs for anticancer therapy in the future. To identify novel metastasis-associated genes, we previously set up a functional, genome-wide screen for genes that could suppress anoikis (cell-detachment induced apoptosis). Anoikis is thought to provide a physiological barrier against the metastatic spread of tumor cells. In this screen, we identified the neurotrophic receptor kinase TrkB as a potent suppressor of anoikis. Consistent with our hypothesis, TrkB-expressing cells formed highly invasive and metastatic tumors when injected into nude mice. A structure-function analysis indicated that all of the oncogenic and metastatic properties strictly depended on TrkB's kinase activity. As TrkB is overexpressed in various human malignancies, it may represent a potential target for anticancer therapy.

Expression of TrkB in epithelial cells induced loss of intercellular adhesion and a striking change in cell morphology, reminiscent of epithelial to mesenchymal transition (EMT). In line with the hallmarks of EMT, we observed a downregulation of E-cadherin upon TrkB expression and an induction of the basic helix-loop-helix transcription factor Twist. Twist is a known mediator of EMT and a metastasis gene. We show by RNAi that Twist is required for TrkB-induced loss of E-cadherin and anoikis suppression, as well as for the growth of subcutaneous tumors in nude mice. To further investigate the function of Twist, we searched for potential downstream effectors. Studies in Drosophila suggested that Twist induces the zinc finger transcription factor Snail, a developmental gene associated with poor prognosis in human breast and other cancers. Indeed, Twist and TrkB each induced Snail. Further functional studies showed that Snail is acting downstream of Twist also in mammalian cells. Furthermore, RNAi-mediated knockdown of Snail impaired the loss of E-cadherin and anoikis suppression by TrkB. Snail knockdown did not affect tumor growth of TrkB expressing cells in nude mice. Instead, it specifically impaired the formation of lung metastases. In conclusion, our data suggest that TrkB signaling activates a Twist – Snail axis that is critically required for metastasis. This

knowledge increases our understanding of how TrkB could function in aggressive human cancers, and provides important insight into the functional relationship of two key transcriptional regulators of metastasis.

46 Poster

Myc and Mnt in lymphomagenesis

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The aim of this work was to investigate whether Mnt is a "master" controller of Myc complexes. Myc plays a central role in controlling cell growth, proliferation, transformation and apoptosis is deregulated in over 70% of human tumors. Mnt is thought to antagonize Myc by competitively binding to its binding partner Max and repressing gene expression. Consistent with this view, knockdown of Mnt has been shown to mimic Myc over-expression (1) and conditional Mnt deletion in mice results in mammary adenocarcinoma and T-cell lymphoma (2,3).

Recent work from our laboratory using transgenic mice emphasizes the importance of Myc levels in the development of haemopoietic malignancy (4,5). We are currently evaluating the impact of loss of Mnt in these VavP-myc10 transgenic mice. Mice bearing one copy of the VavP-myc10 transgene succumb to lymphomas of monocytic origin with a median of 41 weeks (5), while those bred to have two copies develop lethal early-onset T-cell lymphomas (median 13 weeks) (5). As the level of Myc determines both tumor kinetics and phenotype, we hypothesised that a decrease in Mnt would increase the functional level of Myc. In VavP-myc10 mice we expected this to result in acceleration of tumourigenesis and a switch of phenotype to T-cell lymphomas in mice carrying only one copy of the VavP-myc10 transgene.

Surprisingly, we found that heterozygous deletion of Mnt did not advance the onset of tumourigenesis or promote T-cell lymphomagenesis in VavP-myc10 mice. As Mnt null mice are not viable, we have now crossed Vav-Cre deleter mice with mice carrying floxed Mnt alleles to inactivate Mnt in all haemopoietic cells. These mice are viable and will be used to investigate if complete loss of Mnt disturbs haemopoiesis and results in lymphomagenesis in a manner similar to Myc over-expresson.

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47 Poster

A novel gene downstream of Pax2 is overexpressed in Wilms' tumors and encodes for a Calcineurin A binding protein

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Background: Wilms' tumor (WT) accounts for approximately 85% of childhood kidney cancer and occurs at a frequency of 1 in 10,000 live births. WT is a classical cancer type arising from abnormal differentiation of kidney progenitor cells. Pax2 (Paired-Box) is recognized as a critical regulator of kidney development. Pax2 expression normally attenuates as the kidney matures; however, abnormally high PAX2 levels have been observed in both WT and renal cell carcinoma. PAX2 expression in kidney cancer cells correlates with proliferation and increased invasive phenotype. Based on the above evidence, we believe that the misexpression of PAX2 and its target genes play an important role in tumor initiation and/or progression.

Methods and Results: To test this hypothesis, we screened for target genes of Pax2 by cDNA microarray in the embryonic kidney. From this, we identified a novel gene, called CnABP for Calcineurin A Binding Partner, under Pax2 regulation. In situ hybridization indicates that CnABP coexpresses with Pax2 in the condensing mesenchyme, progenitor cells of Wilms' tumor. Expression analysis by quantitative PCR indicates that CnABP is overexpressed in more than 70% of Wilms' tumors. Interestingly, in the proportion of tumors with upregulated Pax2 expression, more than 80% also overexpress CnABP. CnABP is a highly conserved protein containing a N-myristoylation signal and a Calcineurin binding motif. Functional analysis indicates that CnABP is a membrane-anchored protein that primarily promotes cell migration. Yeast-two-hybrid and immuno-precipitation identify an interaction between CnABP and Calcineurin A, the catalytic subunit of a calcium-responsive serine/threonine phosphatase. Importantly, we showed that CnABP modulates phosphatase activities of Calcineurin.

Conclusions: Recently, components of the Calcineurin complex have been implicated as signature genes for recurrent Wilms' tumor. This is in line with the evidence we presented, as CnABP is upregulated in Wilms' tumors and is shown to promote migration. Together these data identify a new promigratory protein regulated by Pax2 that potentially play a role in tumor progression.

48 Poster

Tissue-specific ablation of Prkar1a causes schwannomas by suppressing neurofibromatosis protein production

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Background: Signaling events leading to Schwann cell tumor initiation have been extensively characterized, owing to their association with the well-studied inherited tumor syndromes Neurofibromatosis (NF) Types 1 and 2. Similar tumors are also observed in patients with the endocrine neoplasia syndrome, Carney Complex (CNC), which results from inactivating mutations in PRKAR1A, the gene encoding the Type 1A regulatory subunit of the cAMP-dependent protein kinase (PKA). Loss of PRKAR1A leads to enhanced PKA activity, although the pathways leading to tumorigenesis are not well characterized. Materials and methods: We previously reported that Prkar1a$^{+/-}$ mice are tumor prone, and approximately 33% of these mice develop schwannomas. Similar tumors are observed in a limited neural crest knock-out of Prkar1a (TEC3KO) with nearly 80% penetrance by 10 months. These heterogeneous neoplasms occurred either uni- or bilaterally, and were clinically characterized as genetically engineered mouse (GEM) schwannomas, grades II and III. The TEC3KO tumors were further studied for the molecular mechanisms by which PKA dysregulation may affect NF signaling. Results: At the molecular level, analysis of the TEC3KO tumors revealed almost a complete loss of both NF proteins, whereas transcript levels were increased; indicating post-transcriptional regulation. Although Erk and Akt signaling are typically increased in NF-associated tumors, we observed no activation of either of these pathways in our tumors. Furthermore, the small G-proteins Ras, Rac1, and RhoA are all known to be involved with NF signaling. In TEC3KO tumors, all three molecules showed modest increases in total protein, but only Rac1 showed significant activation. Conclusions: These data suggest that dysregulated PKA activation causes tumorigenesis via pathways that overlap but are distinct from those described in NF tumorigenesis.

49 Poster

Triterpenoids affect angiogenesis and prevent neoplastic progression

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INTRODUCTION: Angiogenesis is the base for solid tumor growth and dissemination; recently anti-angiogenic drugs have begun to show promise in clinical trials. We propose to identify molecules and pathways involved in cancer progression in order to prevent neoplastic development and metastatic dissemination. Chemoprevention focuses on the primary or secondary prevention of cancer using natural or synthetic agents usually showing mild or no collateral effects.

MATERIALS: We have recently assessed the activity of novel compounds, CDDO-Me and CDDO-Im, derived from the olanoleic acid triterpenoid, that have shown a potent antiangiogenic activity at really low dosages. In vivo we performed matrigel sponge assay; on day +4 day from matrigel injection, CDDO-treated and CTRL nu/nu CD1 mice were sacrificed to measure Hb content in matrigel sponges. Then we tested CDDO analogues efficacy in Kaposi's sarcoma xenograft; after KS-Imm cells injection in C57BL mice, we administered CDDO or vehicle and monitored tumor growth. On day of sacrifice tumors were isolated and processed for morphological and IHC analysis.In vitro we evaluated HUVECs ability to organize in capillary-like structures in matrigel, in presence of CDDO analogues or vehicle. Then we treated HUVECs and quantified proliferation. By immunofluorescence we investigated whether these compounds affect NF-kB pathway in HUVECs.