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