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I am a member of the Cancer Genome Project group, directed by Prof. Mike Stratton, FRS and Dr. Andy Futreal, at the Wellcome Trust Sanger Institute, UK. Our aim is to study somatic changes to cancer genomes and I currently focus on uncovering somatic mutations in renal cell carcinoma (RCC).

Around 190,000 new cases of renal cancer are diagnosed worldwide each year. Despite the frequency of this cancer, little is known about the genetic events involved in sporadic RCC. One notable exception is the VHL gene which has a deleterious somatic mutation in around two thirds of all clear cell RCC. Our group screened RCC samples for somatic mutations in protein kinase genes but failed to identify any with substantial evidence for involvement in the disease.

It is the aim of the current mutation screen to find other genes somatically mutated in RCC. We sequenced the coding exons of 3,726 genes in a collection of 101 DNA samples from 96 primary cancers and 5 RCC lines, each with a matched normal DNA sample. The gene set was derived from several sources including gene families where one member has previously been shown to be mutated in human cancer, genes resident in amplified regions of human cancer genomes, and genes found to be targeted in mouse mutagenesis screens for cancer. We have a further 300 primary renal tumours, each with a matched normal sample, for use in follow up work.

This screen is being carried out using capillary sequencing (6Mb DNA per sample) which requires a large team to carry out the lab work and data analysis. It is my role to carry out analysis to confirm all of the mutations and try to interpret their relevance to RCC development.

To date we have discovered over 500 somatic mutations which are located in over 40 different genes (http://www.sanger.ac.uk/genetics/CGP/Studies/Renal/). One of the genes which we found deleterious somatic mutations in is UTX. This gene, located on chromosome X, codes for a Jmjc domain containing histone 3 lysine 27 (H3K27) demethylase. Removal of H3K27 methylation leads to activation of gene expression. UTX is also found in a complex with MLL2/3 which promotes H3K4 methylation - a mark associated with active gene expression. After screening 419 RCC samples, 7 truncating mutations were discovered. We expanded analysis to tumours from other tissues and found a total of 39 deleterious UTX mutations in 1390 tumours. Loss of UTX in these tumours is expected to lead to deregulation of H3 methylation.

Current work is focusing on further analysis of other genes (around 40 in total) highlighted during the initial RCC mutation screen. Hopefully this work will provide a much deeper insight into RCC development.

I would like to thank Applied Biosystems and the EACR for the 40th anniversary research award I received in Lyon and also for the opportunity to speak about this work during the conference. It was a great honour to receive this reward and participate in a conference which I found both stimulating and educational.

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Tumour cells invading into a tissue can display these two different modes of individual cell movement. The mesenchymal/elongated movement requires extra-cellular proteolysis but does not have an obligate requirement for Rho-ROCK signalling. In contrast, in the amoeboid/rounded mode, movement is independent of proteases but requires high Rho-ROCK signalling that drives the elevated levels of actomyosin contractility associated with amoeboid movement. Importantly these two modes of tumour cell movement are inter-convertible depending on environmental conditions; providing a mechanism whereby tumour cells can adapt their mode of movement depending on their environment. Amoeboid movement is characterized by high levels of actomyosin contractility driven by ROCK, consistent with a mechanism whereby cells moving in an amoeboid manner squeeze through voids and deform the matrix. In contrast, mesenchymal movement is characterized by Rac
dependent actin rearrangements that push protrusions through channels in the extracellular matrix created by protease action.

To investigate mechanisms that underlie different modes of tumour cell movement we studied how regulation of the activity of the Rho family GTPases determines the mode of tumour cell movement. Guanine nucleotide exchange factors (GEFs) and GTPase accelerating proteins (GAPs) are key regulators of the activity of small GTPases with GEFs promoting activation to the GTP bound state and GAPs promoting inactivation by stimulating GTP hydrolysis. To identify GEFs and GAPs determining the mode of tumour cell movement we used siRNA to screen for regulators of Rho-family GTPases affecting modes of tumour cell movement. Using this strategy we identified two important signalling pathways regulating amoeboid and mesenchymal types of motility. We showed how mesenchymal movement is controlled by activation and inactivation control plasticity of tumour cell movement. Cell, 135, 510-23.


Role of the extracellular matrix molecule tenascin-C in tumorigenesis

The extracellular matrix component tenascin-C is highly expressed in most solid tumors. Its high expression correlates with a bad survival prognosis in patients with several cancers. Results from several experimental systems support a role of tenascin-C in enhancing tumor cell proliferation, promoting angiogenesis, invasion and metastasis.

We showed that tenascin-C induces cell rounding, which may enhance proliferation and migration, by two mechanisms. Tenascin-C counteracts the tumor cell proliferation-suppressing effect of fibronectin by blocking the syndecan-4/integrin alpha 5/beta 1.complex. This causes cell rounding (Orend et al., 2003, Oncogene 22, 3917) and stimulates tumor cell proliferation (Huang et al., 2001, Cancer Res. 61, 8586) by activation of oncogenic Wnt and MAPkinase signaling (Ruiz et al., 2004, Cancer Res. 64, 7377). Tenascin-C also stimulated endothelin receptor type A (EDNRA) expression, and signaling through EDNRA maintains cell rounding. By using knockdown and overexpression studies, we identified paxillin, RhoA and tropomyosin-1 as critical targets of cell rounding by tenascin-C downstream of syndecan-4 and EDNRA (Lange et al., 2007, Cancer Res. 67, 6163). An anti-adhesive tenascin-C substratum can promote tumor cell spreading and migration upon stimulation with promigratory lysophosphatidic acid in combination with platelet derived growth factor-BB (Lange et al., 2008, 68, 6942) or upon signaling by endothelin receptor type B (Lange et al., 2007, Cancer Res. 67, 6163) which restored the function of paxillin, FAK, RhoA and tropomyosin-1. To determine a potential tumorigenesis-promoting effect of tenascin-C in vivo, we generated transgenic mice that ectopically express human tenascin-C in the pancreatic islets. Tenascin-C-transgenic mice were crossed with insulinoma-prone Rip1Tag2 mice, that develop insulinomas due to ectopic expression of the SV40T-antigen. Double transgenic mice exhibited an enhanced tumor progression due to several mechanisms. This knowledge could be important to design specific strategies to combat tenascin-C actions in cancer.

High - intermediate - and low-risk genes in breast cancer susceptibility

Familial clustering of breast cancer occurs in specific inherited breast-cancer syndromes in which single genes including BRCA1, BRCA2, PTEN and TP53 confer a high risk. Family-based linkage studies confirmed the existence of rare BRCA1 and BRCA2 high
perform joint analyses of different type of genetic variations, compare the contribution of the KConFab study and Melbourne and through the from Northern California, Ontario Breast Cancer Family Registry subjects are selected through the matched population controls. These cases (~1500) with a family history candidates in a large series of the coding sequence of about 15 Our aim is to mutation screen that are not easily classified as intermediate- or high-risk genes revelation rare missense substitutions, we are using a strategy that might to a first approximation, measure risk attributable to rare missense variants in susceptibility genes. This strategy allows for the resolution of evolutionarily unlikely from more likely missense variants along a graded trend based on complete mutation screening of the gene of interest in a suitably ascertained set of cases and controls and a protein multiple sequence alignment of variants and rare missense substitutions identified in candidate genes using a case-control mutation screening design. For the analysis of rare missense substitutions, we are using a strategy that might to a first approximation, measure risk attributable to rare missense variants in susceptibility genes. This strategy allows for the resolution of evolutionarily unlikely from more likely missense variants along a graded trend based on complete mutation screening of the gene of interest in a suitably ascertained set of cases and controls and a protein multiple sequence alignment of sufficient phylogenetic depth using the program Align-GVGD.

By combining cases-control genotyping of known low-risk SNPs, case-control mutation screening of candidate genes, genotype-based tests of differential expression and bioinformatics, our laboratory can carry out integrated analyses of known and/or candidate susceptibility genes for breast cancer. The final goal of our research is to compare the population attributable fraction and the familial relative risk of intermediate-risk genes to that of the ensemble of common modest-risk alleles in our studied population.

References