

EACR - 20 Research Awards

Applied Biosystems is an established Sustaining Member of the European Association for Cancer Research and gave additional support to EACR-20 by Sponsoring the EACR Research Awards to speakers selected to present in the Presidential Sessions.

Gillian Dalglish

Cancer
Genome
Project
Wellcome
Trust
Sanger Inst.
Hinxton, UK



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.

Victoria Sanz-Moreno
Cancer Research UK Centre for Cell and Molecular Biology
Institute of Cancer Research
London UK

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 characterised 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 of Rac (1) and amoeboid movement is in part controlled by activation of Cdc42 (2).

There are several ways to promote tumour cell inter-convertibility. Among them, we showed that decreasing contractility of amoeboid moving cells either with ROCK inhibitors or low doses of ATPase inhibitor blebbistatin, promotes interconversion into a more elongated Rac dependent type of motility (1). On the other hand, cells do not convert from amoeboid to mesenchymal in the presence of protein synthesis inhibitors (VSM unpublished data). Acto-myosin contractility is necessary but not sufficient to control cell morphology and type of movement as new protein synthesis is required. I am currently studying how differences in cell morphology and mode of migration are reflected in changes in gene expression. Affymetrix gene array analysis has been performed in conditions of actomyosin contractility inhibition. This analysis has revealed a series of genes that are upregulated and downregulated

when promoting inter-conversion by contractility inhibition. I am focusing on the functional analysis of those genes found to be important in maintaining cell morphology and controlling cell movement.

1-Sanz-Moreno.V., Gadea, G., Ahn, J., Paterson, H. Marra, P. Pinner, S., Sahai, E. and Marshall C.J. (2008). Rac activation and inactivation control plasticity of tumour cell movement. *Cell*, 135, 510-23.

2- Gadea, G., Sanz-Moreno.V., Self, A., Godi, A. and Marshall C.J. (2008) DOCK10-Mediated Cdc42 activation is necessary for tumour cell invasion *Curr Biol*,18,1456-65.



Gertraud Orend
INSERM
Strasbourg
France

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.

Fabienne Lesueur
Genetic Susceptibility Group
International Agency for Research on Cancer,
Lyon, France



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

penetrance alleles that account for more than 80% of families with 6 or more cases of early onset cancers^{1,2}. However, population studies have demonstrated that these 2 major genes account for a minority of the overall familial risk of breast cancer³ and that other genes conferring a risk equivalent to that of *BRCA1* and *BRCA2* (Relative risk (RR) > 10) are unlikely to exist⁴. It has been proposed that breast cancer susceptibility is largely polygenic, and could be explained by a model in which a large number of low-penetrance genes that confer small risks individually act in combination to cause wide variation in risk in the population⁵. Recently, empirical genomewide association studies have identified six new modest-risk SNPs alleles (within genes *CASP8*, *FGFR2*, *TNRC9*, *MAP3K1* and *LSP1*, and at locus 8q24) that are common in the population⁶⁻⁸ (RR<1.5). Inheritance of rarer genetic variants that clearly damage protein function has also been unequivocally associated with intermediate risk of breast cancer, with OR of 2 to 3, in *CHEK2*⁹, *ATM*¹⁰, *BRIP1*¹¹ and *PALB2*¹². These intermediate-risk alleles were identified in family-based studies by sequencing candidate genes in women from families with multiple cases of breast cancer that are not due to mutations in *BRCA1* and *BRCA2*. In addition to truncating or splice junction mutations that clearly alter the function of the protein, this latest approach often reveals rare missense substitutions in intermediate- or high-risk genes that are not easily classified as pathogenic or neutral.

Our aim is to mutation screen the coding sequence of about 15 candidates in a large series of cases (~1500) with a family history of breast cancer / early onset and matched population controls. These subjects are selected through the Breast Cancer Family Registry¹³ from Northern California, Ontario and Melbourne and through the KConFab study¹⁴. In order to compare the contribution of the different type of genetic variations, we perform joint analyses of truncating variants, splice junction

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

References

1. Easton, D.F., Bishop, D.T., Ford, D. & Crockford, G.P. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. *Am J Hum Genet* 52, 678-701 (1993).
2. Wooster, R. et al. Localization of a breast cancer susceptibility gene, *BRCA2*, to chromosome 13q12-13. *Science* 265, 2088-90 (1994).
3. Peto, J. et al. Prevalence of *BRCA1* and *BRCA2* gene mutations in patients with early-onset breast cancer. *J Natl Cancer Inst* 91, 943-9 (1999).
4. Smith, P. et al. A genome wide linkage search for breast cancer susceptibility genes. *Genes Chromosomes Cancer* 45, 646-55 (2006).
5. Pharoah, P.D. et al. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet* 31, 33-6 (2002).
6. Easton, D.F. et al. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* 447, 1087-93 (2007).
7. Hunter, D.J. et al. A genome-wide association study identifies alleles in *FGFR2* associated with risk of sporadic postmenopausal breast cancer. *Nat Genet* 39, 870-4 (2007).
8. Stacey, S.N. et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet* 39, 865-9 (2007).

sufficient phylogenetic depth using the program Align-GVGD^{15,16}.

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.

9. Meijers-Heijboer, H. et al. Low-penetrance susceptibility to breast cancer due to *CHEK2*(*)1100delC in noncarriers of *BRCA1* or *BRCA2* mutations. *Nat Genet* 31, 55-9 (2002).
10. Renwick, A. et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet* 38, 873-5 (2006).
11. Seal, S. et al. Truncating mutations in the Fanconi anemia J gene *BRIP1* are low-penetrance breast cancer susceptibility alleles. *Nat Genet* 38, 1239-41 (2006).
12. Rahman, N. et al. *PALB2*, which encodes a *BRCA2*-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* 39, 165-7 (2007).
13. John, E.M. et al. The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer. *Breast Cancer Res* 6, R375-89 (2004).
14. Mann, G.J. et al. Analysis of cancer risk and *BRCA1* and *BRCA2* mutation prevalence in the kConFab familial breast cancer resource. *Breast Cancer Res* 8, R12 (2006).
15. Tavtigian, S.V. et al. Comprehensive statistical study of 452 *BRCA1* missense substitutions with classification of eight recurrent substitutions as neutral. *J Med Genet* 43, 295-305 (2006).
16. Tavtigian, S.V., Byrnes, G.B., Goldgar, D.E. & Thomas, A. Classification of rare missense substitutions, using risk surfaces, with genetic- and molecular-epidemiology

The EACR-20
Research Awards
Sponsored by
Applied
Biosystems

Victoria Sanz-Moreno, UK
Gertraud Orend, France
Vanja Sisirak, France
Hege Russnes, Norway
Anthony Kong, UK
Beatrice Borgia, Switzerland
Gillian Dalglish, UK
Rinske Drost, Netherlands
Luis Carvajal-Carmona, UK
Hongbing Shen, China
Fabienne Lesueur, France