EACR Young Cancer Researcher Award

The Young Cancer Researcher Award winner Simon Boulton from the DNA damage Response Laboratory, London Research Institute, UK presented his Award Lecture at EACR-20 Lyon France.

The EACR Young Cancer Researcher Award Lecture was given in Lyon by Simon Boulton

RTEL1 is an anti-recombinase that impacts on genome stability and cancer

is based on his EACR-20 Lecture

RTEL1 is an anti-recombinase that impacts on genome stability and cancer

Homologous recombination (HR) is an essential conserved process for dividing cells. In mitosis HR is required not only for the accurate repair of DNA double-strand breaks (DSBs), but also for the restart of stalled replication forks. Furthermore HR is crucial for meiotic DSB repair, which is required for accurate chromosome segregation at the first meiotic division. However, inappropriate HR can give rise to genome instability and cancer as a result of erroneous chromosomal rearrangements and the persistence of intermediate recombination structures that cannot be resolved. Hence HR must be tightly regulated and temporally coordinated with cell cycle progression and replication.

Current models of eukaryotic HR propose that a DSB is resected to produce 3'-single stranded DNA tails that are bound by the DNA strand exchange protein RAD51 to form a nucleoprotein filament.

These filaments are the catalyst for strand invasion into homologous duplex DNA, resulting in the formation of a D loop structure. The invading 3' end provides a primer for DNA synthesis and D loop extension, which can be resolved either through displacement of the invading strand from the D loop and annealing to the other DSB end (synthesis-dependent strand annealing), or by the capture of the other resected end by the extruded strand of the D loop to form a double Holliday junction (dHJ). HR can be completed by endonucleolytic cleavage of the two HJs, which may result in a crossover.

In yeast, the initiation of strand invasion is antagonised by Srs2 to ensure that HR occurs at the appropriate time and place. Srs2, first identified 30 years ago, is a 3'-5' SF1 helicase related both by sequence and function to bacterial UvrD. *S. cerevisiae srs2* and *E. coli uvrD* mutants exhibit elevated rates of spontaneous recombination. Yeast *srs2* mutants are synthetic lethal with deletion of the yeast RecQ helicase, *sgs1*. It was subsequently found that the inviability of *srs2 sgs1* mutants results from the accumulation of toxic HR intermediates, as viability can be restored by loss of *RAD51* or *RAD54*, which are essential for the formation of the nucleoprotein filament and extension of the invading strand. Loss of *srs2* also results in significant sensitivity to a range of DNA damaging agents. Biochemical studies have shown that both UvrD and Srs2 act to inhibit strand exchange by disrupting RecA/Rad51 filaments. This has led to the model that UvrD and Srs2 negatively regulate HR by disassembling the nucleoprotein filament. Although Srs2 is critical for counteracting inappropriate recombination in yeast, functional homologues are not obviously conserved in higher eukaryotes.

In an attempt to identify a functional analogue of Srs2 in higher eukaryotes we performed a genetic screen in *C. elegans* to identify uncharacterised helicases that are synthetic lethal in combination with *C. elegans BLM* mutants, based on the *srs2 sgs1 (BLM)* synthetic lethality observed in yeast. This screen identified a novel helicase, RTEL-1 that is conserved from *C. elegans* to humans and exhibits many of the genetic and biochemical hallmarks of yeast Srs2. Genetic analysis has revealed that *C. elegans rtel-1* mutants are also synthetic lethal with *mus-81* and a distinct group

of non-replicative helicases: BLM, FANCJ and RECQ5, but not with WRN. Additionally, the lethality in all four double mutant combinations results from an accumulation of toxic recombination intermediates. *C. elegans rtel-1* mutants and *RTEL1* deficient human cells are also hyper-recombinogenic and exhibit exquisite sensitivity to interstrand cross-links that block replication forks. Rtel knockout mice die between days 10 and 11.5 due to dramatic genome instability and rapid telomere loss and Human RTEL1 is over-expressed in gastric tumours. Collectively, our work suggests that RTEL1 acts as suppressor of aberrant recombination.

Further support for an anti-recombinogenic function for RTEL1 has come from biochemical studies. Purified Human RTEL1 can actively disassemble D-loop recombination intermediates in an ATP-dependent manner. Collectively, our data indicate that the phenotypes observed in *C. elegans,* mice and human cells are likely caused by a failure to correctly regulate HR. Promiscuous disassembly of recombination intermediates is the likely underlying cause of genome instability in RTEL1 over-expressing cancers (Barber *et al*., Cell 2008). Current work is exploiting various experimental systems to investigate the role of RTEL1 during meiotic recombination, DNA replication, telomere maintenance and tumourigenesis.

Simon Boulton with Conference Chair Marco A Pierotti

Jorge S Reis-Filho and Qiuyu Wang

Young Cancer Researcher Award: Highly Commended

EACR-20, Lyon

Molecular pathology of breast cancer

Jorge S Reis-Filho

Breast cancer is a heterogeneous disease comprising a plethora of entities characterised not only by distinct histological features, but also different clinical behaviour and response to therapies. The aims of our laboratory are i) to refine the current taxonomy for breast cancers, moving from a largely descriptive morphological classification to one that is more biologically and clinically meaningful and predictive in nature; and ii) to identify targets for tailored therapies for specific subgroups of breast cancers.

By combining traditional histopathology with high throughput molecular genetics, we have devised an approach based on reducing the complexity of breast cancer a priori for the identification of biologically and clinically meaningful subgroups of breast cancer and of novel therapeutic targets. Based on this rationale, we have analysed classic lobular carcinomas (CLCs), tumours that are rather homogeneous and consistently express hormone receptors and lack HER2 gene amplification, using high-resolution comparative genomic hybridisation (aCGH) and in situ hybridisation. Gains of genomic material on 8p12-p11.2, the genomic location of FGFR1, were found in 46% of these cancers and FGFR1 gene amplification in approximately 10% of these lesions. Profiling of breast cancer cell lines with aCGH and microarray-based expression profiling led to the identification of a cell line model that harboured genetic and transcriptomic aberrations similar to those found in primary CLCs harbouring FGFR1 gene amplification. RNA interference-mediated silencing and chemical inhibition of FGFR1 revealed that FGFR1 expression was selectively required for the survival of cells harbouring amplification of 8p11.2-p12 and overexpression of this tyrosine kinase receptor, suggesting that FGFR1 is a potential therapeutic target in this subset of CLCs. In addition, analysis of a large cohort of breast cancer patients with chromogenic in situ hybridisation confirmed that FGFR1 amplification is present in approximately 10% of all breast cancers and is both a prognostic factor independent of size, grade and nodal stage, and an independent predictor of tumour recurrence following endocrine therapy in oestrogen receptor positive disease. By studying grade III invasive ductal carcinomas with aCGH, we have identified a subgroup of 20% of HER2 amplified breast cancers and 7% of luminal tumours harbouring amplification of 17q23.2, encompassing PPM1D. Using a combination of RNA interference-mediated silencing and chemical inhibition of PPM1D, we have demonstrated that PPM1D expression and phosphatase activity are selectively required for the survival of cancer cells harbouring PPM1D gene amplification, providing strong circumstantial evidence to suggest that PPM1D is a therapeutic target in tumours harbouring 17q23.2 amplification.

Our group has also contributed to the development of a refined taxonomy of breast cancer, by delineating the morphological and molecular features of basal-like breast cancers and breast cancer special histological types. We have demonstrated that metaplastic breast carcinomas are consistently of basal-like phenotype at the immunophenotypic and transcriptomic levels and harbour alterations of the BRCA1 pathway.

Jorge S Reis-Filho, Simon Boulton and Qiuyu Wang

In fact, BRCA1 gene promoter methylation and aberrations of p53 were found in >60% of metaplastic cancers. These findings were further corroborated by the analysis of tumours from the Blg-Cre: Brca1F/F/ Trp53+/- mouse model, which displayed metaplastic features in the vast majority of cases. Although metaplastic breast cancers pertain to the basal-like subgroup of breast cancers, when compared to ductal carcinomas of basal-like phenotype, metaplastic breast cancers displayed a down-regulation of BRCA1 DNA damage response pathway, PTEN, a gene whose loss of function is associated with resistance to chemotherapy, and TOP2A, the molecular target of anthracyclines. These findings may explain the reported poor responses to chemotherapy of this histological subtype.

Our group has also shown that pleomorphic lobular carcinomas are variants of classic lobular cancers. In addition, we have demonstrated that invasive micropapillary carcinomas and acinic cell carcinomas of the breast are distinct entities, providing strong circumstantial evidence for phenotypic-genotypic correlations in breast cancer.

Combining pathology and genetics can unravel the complexity of breast cancers and refine current classification systems. Molecular pathology is not merely an exercise of ‘tumour philately’! With the identification of more homogeneous subgroups of patients, the identification of molecular drivers of tumour growth and progression, and therapeutic targets in breast cancer will be expedited

Elucidating the roles of different isoforms of the transcription factor PAX3 in tumourigenesis

Qiuyu Wang

The paired box gene, PAX3 encodes a transcription factor involved in development of muscle, melanocytes and sympathetic neurones, with significant roles in cell migration, proliferation and survival in early embryonic development. Loss-of-function mutations of PAX3 result in Waardenburg’s syndrome in humans. Abnormal expression is also characteristic of the muscle tumour, rhabdomyosarcoma, as well as neuroblastomas and melanomas of neural crest origin.

Our research group is interested in the roles of PAX3 isoforms in embryogenesis and tumourigenesis. Human PAX3 contains 10 exons encoding a paired domain (PD), a paired-type homeodomain (HD), and a transactivation domain (TD). Seven alternatively spliced isoforms occur: PAX3a-h. PAX3a and PAX3b are truncated prematurely; each is composed of four exons and lack the HD and TD. PAX3c, d and e have eight, nine and ten exons respectively. All three isoforms posses intact PD, HD and TD. PAX3g and PAX3h are truncated isoforms of PAX3d and PAX3e respectively; both lack part of the TD encoded by exon 8. Various isoforms differ in their structures, as well as in binding and activation of target genes.

Our group has shown different expression patterns of PAX3 isoforms within tumours of the same type or cell line, and also variation among different neural crest tumours or cell line, for example, PAX3c and PAX3d are predominantly expressed in melanoma and PAX3g and PAX3h in neuroblastomas. It is tempting to speculate that various PAX3 isoforms have distinct roles in the regulation of embryogenesis and tumourigenesis. We have constructed stable transfectants of melanocytes, myoblasts and embryonic stem cells that express individual PAX3 isoforms to study specific functions of these isoforms during development and tumourigenesis. The effects of loss-of-function of PAX3 was investigated using siRNA to knockdown their expression in PAX3-expressing tumours. Microarrays analyses were used to screen for alterations in downstream gene expression of different PAX3 isoform transfectants.

Our data have shown that PAX3 isoforms regulate distinct but overlapping sets of genes in different transfectant systems in vitro. Thus, the isoforms show different transcriptional specificities and regulate the expression of genes involved in cell differentiation, proliferation, migration, adhesion, apoptosis and angiogenesis.

Our studies have enhanced knowledge about the effects of PAX3 isoform expression in different cell types. Further studies on the functions of the various isoforms of PAX3 in different transfectant systems will extend this understanding, particularly in their roles in embryogenesis and the manner in which their expression contributes to tumourigenesis.