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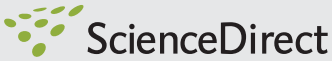

Tuesday 29 June 2010

Tuesday 29 June 2010 07:00–08:00

After Sunrise: Meet the Expert

606 Evolution of genetic and gene expression networks during tumour development

M. To[1], D. Quigley[2], J. Sjolund[2], A. Balmain[2]. [1]*UCSF Comprehensive Cancer Center, Surgery, San Francisco, USA,* [2]*UCSF Comprehensive Cancer Center, CRI, San Francisco, USA*

Background: Quantitative genetics has identified polymorphisms within gene regulatory factors or their target genes which cause variation in phenotypes, such as inflammation and obesity, that influence disease susceptibility. Expression levels individual genes can also be regarded as "phenotypes" under genetic control, some of which may influence cancer susceptibility. Applying expression Quantitative Trait Locus (eQTL) approaches to mouse strains with differing susceptibility to diseases, such as obesity and cancer, has identified signaling hubs that may be important targets for drug development.
Methods: We used network construction methods to analyze the genetic architecture of gene expression in normal mouse skin in a cross between tumour-susceptible *Mus musculus* and tumour-resistant *Mus spretus*.
Results: We demonstrate that gene expression motifs representing different constituent cell types within the skin such as hair follicle cells, haematopoietic cells, and melanocytes are under separate genetic control. Motifs associated with inflammation, epidermal barrier function, cell cycle control and proliferation are differentially regulated in mice susceptible or resistant to tumour development. The intestinal stem cell marker *Lgr5* is identified as a candidate master regulator of hair follicle gene expression, and the Vitamin D receptor (*Vdr)* links epidermal barrier function, inflammation, and tumour susceptibility. These gene expression networks undergo substantial rewiring during development of benign and malignant skin tumours. The Lgr5 stem cell marker is expressed in skin tumours but is no longer linked to the expression of hair follicle genes, while the individual components of the susceptibility network associated with the *Vdr* locus are independently rewired in tumours.
Conclusions: The combination of genetics and gene expression approaches offers substantially greater power than classical methods for identification of genetic factors that contribute to cancer susceptibility and progression.

607 Exploring chemoresistance in vivo; are we making progress

P. Lønning[1]. [1]*Haukeland University Hospital, Section of Oncology Department of Medicine, Bergen, Norway*

Chemoresistance remains the main obstacle to cancer cure. Contrasting prognostic factors, for which many are known in breast cancer as well as with respect to other solid tumours, few factors predicting sensitivity to chemotherapy are known. While HER-2/topo-II over expression is associated with enhanced anthracycline-sensitivity and recent evidence has linked KI67 levels to benefit of taxanes in ER+ breast cancer, so far predictive factors have not been implemented selecting patients for breast cancer chemotherapy. Notably, while gene expression signatures like the OncotypeDX has been associated with sensitivity to chemotherapy, it confers to a large extent the same information as has been covered by traditional parameters like histological grade and Ki67. Identification of the so-called basal cell-like breast cancer type has revealed a tumour sub-group with a gene expression profile resembling the bulk of tumours occurring in individuals harbouring BRCA1 mutations. Thus, current evidence propose these tumours may harbour defects in DNA double-strand repair resembling what has been revealed in BRCA1 and BRCA2 mutated tumours, and there is preliminary evidence suggesting these tumours like BRCA1/2 mutated ones may benefit from treatment with PARP inhibitors. Harbouring such defects, the potential exists that these tumours, similar to BRCA1 mutated ones may be sensitive to chemotherapeutic compounds like cis-platin, a drug not commonly used for breast cancer therapy in general. A major goal is to explore and understand the complete biology of chemoresistance. The fact that a limited number of genes seems to be critical to tumour development as well as growth (so-called "drivers", contrasting mutations in the bulk of genes considered to be "passengers") suggest chemoresistance may be due to defects affecting critical biological pathways executing processes like apoptosis or DNA repair. Now, with novel gene sequencing technologies open for complete genome sequencing of individual tumours we are facing an era allowing us, for the first time, to explore genetic alterations leading toward a full understanding of the processes guiding therapy response in breast cancer as well as in all forms of malignant disease.

Tuesday 29 June 2010 08:00–08:50

Educational Lecture
Developing targeted therapy

608 Systems approach to personalized molecular medicine

G. Mills[1]. [1]*University of Texas, Department of Systems Biology, Houston Texas, USA*

The realization of the promise of personalized molecular medicine will require the efficient development and implementation of novel targeted therapeutics. The overall likelihood of response to particular drugs represents the interaction between predictors of sensitivity with predictors of resistance. The phosphatidylinositol 3′kinase (PI3K) pathway is aberrant at multiple levels across a wide variety of tumours making it the most common activating aberration in cancer. This has led to the development and now early clinical testing of drugs targeting multiple components of the pathway. The efficient utilization of these drugs will require the ability to accurately determine mutation and activation status in tumours as well as determining the interaction between the PI3K pathway and other pathways in driving tumour pathophysiology. The PI3K pathway is critically important to cellular function and is thus under exquisite homeostatic control. The feedforward and feedback loops in the pathway determine the response to perturbation of the pathway by mutation or therapeutic intervention. Strikingly inhibition of the pathway at the level of mTOR or AKT results in the activation of potent feedback loops resulting in activation of multiple cell surface tyrosine kinases, PI3K itself and AKT. This may contribute to the observation that mTOR inhibitors appear to make some patient tumours grow more rapidly an unexpected and disappointing consequence. Our preliminary systems biology-based mathematical and experimental models of the PI3K signaling network accurately predict these consequences as well as the biochemical processes involved. Further, the models suggest combinations of targeted therapeutics likely to reverse the negative effects of the mTOR inhibitors converting the outcome from negative to positive in terms of tumour growth.

Tuesday 29 June 2010 08:00–08:50

Educational Lecture
Cell cycle

609 In and out of G1

S. Mittnacht[1], E. Runnacles[1], H. Li[1], S. Stockwell[1]. [1]*The Institute of Cancer Research, Cancer Research UK Programme on Tumour Cell Signalling Networks, London, United Kingdom*

The cell division cycle is a series of events that takes place in a cell leading to cell duplication. Checkpoints act at different points throughout the cell cycle overseeing that events in the proceeding cell cycle phase have been accurately completed.

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Checkpoint control of progression from G1 (G for gap) phase during which a cell grows, into S (S for synthesis) phase where its DNA is replicated is an important process in determining proliferation activity and cell fate. Key components controlling exit from G1 and onset of S phase are the cyclin D-dependent kinases CDK4 and CDK6, and the cyclin E-associated kinase CDK2, which phosphorylate and through this inactivate the retinoblastoma tumour suppressor protein (pRB). Genetic alterations that weaken or disable G1/S checkpoint activation are extremely frequent in cancers and presumed to promote cancer development by permitting unlicenced proliferation. Conversely, G1/S checkpoint activation ensues in response to stress, including genotoxic insult and in such contexts is thought to provide resistance to therapy- and cancer inherent adversities. Thus G1/S checkpoint activation as well as its prevention could be a desirable strategy for the treatment of cancer.
Although the key components of G1/S checkpoint execution are recognised, our understanding how G1 inherent signalling and stresses operate to modulate checkpoint function is far from complete. We developed a high throughput assay format allowing quantification of the phosphorylated, inactive form of pRB in fixed cells seeded in a 96 well format. This assay in combination with targeted knockdown using siRNA libraries is identifying known and unexpected signaling required for checkpoint modulation in the different contexts. Results from these screens and their implications will be discussed.

Tuesday 29 June 2010 08:00–08:50

Educational Lecture

RNA editing

610 RNA editing meets cancer

G. Rechavi[1]. [1]*The Sheba Cancer Center, Israel*

Deregulation of epigenetic mechanisms collaborates with genetic alterations in the development and progression of cancer. DNA methylation and histone modifications are the best studied epigenetic control mechanisms shown to be altered in cancer.
Adenosine to inosine (A-to-I) RNA editing is a site-specific modification in stem-loop structures within precursor mRNAs, catalyzed by members of the ADAR (adenosine deaminase acting on RNA) enzyme family. ADAR-mediated RNA editing is essential for the normal development of both invertebrates and vertebrates. A number of editing sites occur in coding regions and may result in amino acid substitutions affecting the protein structure and activity. In recent years, bioinformatics and experimental studies revealed that the extent of editing in humans is large, affecting several thousand genes. The majority of these A-to-I editing events occur in noncoding repetitive sequences, mostly *Alu* elements which account for about ten per cent of the human genome. Editing in noncoding sequences was proposed to be involved in a variety of cellular functions such as RNA stabilization, nuclear retention and splicing. In addition, RNA editing was shown to be involved in RNA interference and in the regulation of biogenesis and expression control of microRNAs.
A growing body of evidence indicates that the extent A-to-I RNA editing of both coding and noncoding sequences differs between tumours and normal samples derived from the same tissues and represent a novel type of global epigenetic regulation. Abnormal editing is expected to affect malignant transformation and tumour progression by several avenues including recoding and control of mRNA and microRNA structure and expression. There are indications that manipulation of the editing-mediating machinery affects cancer cell properties. New methodologies were developed that allow the high throughput analysis of multiple editing sites. It is suggested that specific editing patterns may serve as diagnostic and prognostic tumour markers. The unraveling of the regulatory mechanisms that affect editing levels and specificity may lead to the development of new therapeutic interventions.

Tuesday 29 June 2010 09:00–09:50

Award Lecture: Carcinogenesis Young Investigator's Award

611 Homologous recombination in cancer development, treatment and development of drug resistance

T. Helleday[1]. [1]*University of Oxford, Gray Institute for Radiation Oncology and Biology, Oxford, United Kingdom*

Although DNA double-strand breaks (DSBs) are substrates for homologous recombination (HR) repair it is becoming apparent that DNA lesions produced at replication forks, for instance by many anti-cancer drugs, are more significant substrates for HR repair. Cells defective in HR are hypersensitive to a wide variety of anti-cancer drugs, including those that do not produce DSBs. Several cancers have mutations in or epigenetically silenced HR genes, which explain the genetic instability that drives cancer development. There are an increasing number of reports suggesting that mutations or epigenetic silencing of HR genes explain the sensitivity of cancers to current chemotherapy treatments. Furthermore, there are also many examples of re-expression of HR genes in tumours to explain drug resistance. Emerging data suggest that there are several different sub-pathways of HR, which can compensate for each other. Unravelling the overlapping pathways in HR showed that BRCA1 and BRCA2 defective cells rely on the PARP protein for survival. This synthetic lethal interaction is now being exploited for selective treatment of BRCA1 and BRCA2 defective cancers with PARP inhibitors. Here, I discuss the diversity of HR and how it impacts on cancer with a particular focus on how HR can be exploited in future anti-cancer strategies.

Tuesday 29 June 2010 10:20–12:20

Symposium

Migration, invasion & metastases

612 Metastasis – gene expression differences associated with site and treatment sensitivity

O. Fodstad[1], S. Tveito[1], R. Kaaresen[2], T. Sauer[3]. [1]*Oslo University Hospital Radiumhospitalet, Tumour Biology, Oslo, Norway*, [2]*Oslo University Hospital Ullevaal, Breast and Endocrine Surgery, Oslo, Norway,* [3]*Oslo University Hospital Ullevaal, Pathology, Oslo, Norway*

It is a well known clinical experience that in many tumour types metastases in different organs differ in their sensitivity to treatment, and that in specific cancers metastases often appear in a certain order of organ involvement. To investigate these phenomena at the molecular level it is necessary to obtain tissue samples from metastases in different tissues, and preferably also from the primary tumour. To eliminate unwanted signals from normal cells it is necessary to isolate pure populations of tumour cells in the samples. In a comprehensive collaborative study on breast cancer, we are studying and comparing gene and protein expression levels in tumour cells obtained from sentinel lymph nodes (SLNs) and bone marrow (BM) aspirates from patients undergoing surgery for primary breast cancer, and also relating the results with similar findings in primary tumour tissue samples from the same patients. Cells from SLNs and BM are first enriched by immunomagnetic beads coated with an anti-EpCam antibody, and the expression of cell surface markers examined simultaneously by binding of fluorescent latex non-magnetic beads coated with antibodies to breast cancer-associated antibodies. The expression of intracellular markers is studied at the mRNA level with RT-PCR on cells isolated by picking individual target cells with bound immunobeads by means of CellEctor (MMI, Switzerland). Surprisingly, the correlation between results obtained with the different methods was poor. The anti-EpCam immunobeads isolated (IMS) positive cells in a high fraction of the SLNs, and a high percentage of these bound 1–3 additional immunobeads. In contrast, RT-PCR against mammaglobin and three other genes were positive only in about 50% of the IMS positives. By using RT-PCR array and hierarchial clustering analysis, we could group samples into EpCam positive/mammaglobin positive or negative, and found that in the cells in the latter group had epithelial/mesenchymal transition like signatures, including loss of E-cadherin, CK19 and EGFR expression. The data may indicate that these cells may be particularly aggressive, and that they might be missed by the most commonly used detection methods, including anti-cytokeratin immunohistochemistry. If so, the findings would have important clinical implications. The SLN data will be compared with results obtained on BM samples and primary tumours.

613 Targeting invasion and metastasis

M. Frame[1], B. Serrels[1], A. Serrels[1], M. Canel[1], E. Sandilands[1], V. Brunton[1]. [1]*Edinburgh Cancer Research Centre, University of Edinburgh, Edinburgh, United Kingdom*

One of the hallmarks of cancer cells is their ability to invade into adjacent tissue and spread to distant sites within the body. We have been studying invasion and metastasis, leading to findings that will take forward development of small molecule inhibitors of invasion for clinical use. Our basic work focuses on the role of the non-receptor tyrosine kinases Src and its substrate focal adhesion kinase (FAK). Src is the prototypical oncogene and we have established that is has an important role in controlling both cadherin-mediated cell-cell contacts and integrin-dependent cell-matrix adhesions, and the crosstalk between these that is perturbed in cancer during the epithelial to mesenchymal transition (EMT). Indeed, highly elevated Src activity in rarely required for the proliferation of advanced tumour cells, instead promoting cancer invasion and metastasis by perturbing cancer cell adhesions and polarity We also showed, via conditional deletion of FAK in the skin of mice, that FAK plays a key role in