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

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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

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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