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

tumour formation and progression. We are now combining new technologies of intra-vital dynamic molecular imaging, and mouse cancer modelling, to address the important question of whether agents that target adhesion regulators have anti-invasive or anti-metastatic activity, for example how E-cadherin dynamics are affected, and if so how such activity can be monitored in cancer models and tested clinically. We will present some new findings on on a novel 'direction sensing' polarization pathway regulated by FAK at nascent integrin adhesions and on the monitoring of E-cadherin dynamics in vivo. We seek a full understanding of the molecular mechanisms by which these adhesion regulated kinases promote the malignant phenotype, and how the biological properties they perturb may be targeted for therapy.

614 Mechanisms of cell signalling in metastasis

C. Marshall[1]. [1]*Institute of Cancer Research, Section of Cell & Molecular Biology, Oncogene Team, Chester Beatty Laboratories, London, United Kingdom*

Rho-family GTPase signalling underlies the cytoskeletal changes that are required for cell migration. In order to delineate which Rho-family GTPases are involved in cell migration and how they are controlled we are carrying out a systematic analysis of Rho-family GTPases and their regulators. We have used RNAis targeting 22 Rho-family GTPases, 80 guanine nucleotide exchange factors (GEFs) and 73 GTPase activating proteins (GAPs) to study the requirement for cell motility and morphology in a number of different systems. Using these approaches to study migration and invasion of melanoma cells we have described pathways controlling two distinct forms of movement. Elongated, mesenchymal-type movement is driven by Rac activation mediated by a pathway containing the adaptor protein NEDD9 and the exchange factor DOCK3. Interestingly, NEDD9 has been found by others to be over-expressed in malignant melanoma. In contrast amoeboid movement, an alternative form of cell migration, is suppressed by Rac activation but driven by Rho and Cdc42 activation. In amoeboid movement of melanoma cells Cdc42 activation is driven by the GEF DOCK10 and acts through the Cdc42 effectors NWASP and Pak2. Since Rac activity suppresses amoeboid movement we have investigated how Rac activity is down regulated in melanoma cells undergoing amoeboid movement. Our studies show that Rho and Cdc42 signalling in amoeboid cells drives high levels of actomyosin contractility that activates a RAC GAP ARHGAP22, which then inactivates Rac to permit amoeboid movement. These studies therefore demonstrate a tight interplay between Rho and Rac signalling in determining modes of cell movement.

615 TGF-beta regulation of the inflammatory tumour microenvironment

H. Moses[1]. [1]*Vanderbilt-Ingram Cancer Center, Nashville, USA*

Accumulating data indicate that an inflammatory microenvironment can play a critical role in cancer initiation and progression. TGF-beta signaling in both epithelial and stromal cells appears to be a key regulator of this microenvironment. There is now compelling evidence from transgenic mouse studies and analyses of mutations in human carcinomas indicating that the TGF-beta signal transduction pathway is tumour suppressive. However, there is evidence that TGF-beta signaling can promote tumour progression in the later stages. In order to examine the roles of TGF-beta signaling in cancer more closely, we have generated mice with *loxP* sites flanking exon 2 of the type II receptor gene, *Tgfbr2,* and crossed them with mice expressing Cre driven by different epithelial specific promoters. Loss of TGF-beta signaling in six different epithelial cells gave a minimal phenotype. However, when challenged with oncogene expression or tumour suppressor gene impairment, there was rapid development of invasive and metastatic carcinomas. In an effort to address mechanisms, we have now identified gene expression signatures associated with the TGF-beta signaling pathway in mammary carcinoma cells. The results strongly suggest that TGF-beta signaling mediates intrinsic, stromal-epithelial and host-tumour interactions during breast cancer progression, at least in part, by regulating induced *Cxcl1*, *Cxcl5* and *Ccl20* chemokine expression. To determine the clinical relevance of our results, we queried our TGF-beta associated gene expression signatures in four human breast cancer data sets containing a total of 1,319 gene expression profiles and associated clinical outcome data. The signature representing complete abrogation of TGF-beta signaling correlated with reduced relapse-free survival in all patients, particularly in patients with estrogen receptor positive tumours. The functional significance of increased chemokine expression in the knockout carcinoma cells in the mouse model is recruitment of immature bone marrow derived cells that express abundant TGF-beta and MMPs in the tumour microenvironment and promote invasion and metastasis. The data indicate that TGF-beta signaling is a major regulator of chemokine secretion and resultant bone marrow cell infiltration creating the inflammatory microenvironment and suggest that targeting pathways, which inhibit bone marrow cell differentiation or chemokine receptors, could be useful in both therapy and prevention of cancer.

Tuesday 29 June 2010 10:20–12:20

Symposium
CancerOmics

616 Sequencing cancer genomes

P. Stephens[1]. [1]*The Sanger Centre, Cancer Genome Project, Cambridge, United Kingdom*

Using massively parallel paired end sequencing, it is feasible to sequence the entire genome of cancer samples, allowing the generation of comprehensive catalogues of somatic mutations of all classes. We have developed bespoke algorithms to identify somatically acquired point mutations, small indels, copy number changes and genomic rearrangements, which have been extensively validated by confirmatory testing. The findings from our first handful of cancer genomes illustrate the potential for next-generation sequencing to provide unprecedented insights into mutational processes, cellular repair pathways and gene networks associated with cancer development.

617 Translational opportunities from genomic and chemical biology of cancer

O. Kallioniemi[1]. [1]*Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland*

Cancer genomics projects, such as the International Cancer Genomics Consortium (ICGC), will in the near future provide a complete catalogue of genomic, epigenomic and transcriptomic changes in up to 25,000 different cancer samples, from up to 50 different major cancer types and subtypes. With such a comprehensive catalogue in the horizon, scientists need to consider the implications that this will have for biological and translational cancer research. This presentation will highlight different aspects of cancer research that we believe will gain in importance in the years to come as the full cancer genomic profiles start to emerge. These include: bioinformatics, genome-scale cancer biology, chemical biology, and translational research into individualized cancer medicine.

First, the availability of this massive catalogue of cancer genomics information will emphasize the central importance of bioinformatics and data mining to generate knowledge and testable hypotheses. For example, on a scale of >10,000 samples, we have created developed a transcriptomics data mining capability for the rapid bioinformatic analysis of gene expression levels in vivo in thousands of clinical samples, available at www.genesapiens.org (Kilpinen et al.. Genome Biology, 2008). The transcriptomics data have been normalized, QC-checked and annotated with clinical information to generate a fully integrated, curated and searchable database to systematically explore gene functions across the body in different cells, tissues and diseases, cancer in particular.

Second, we have developed an ultra-high-density cell-microarray screening system for siRNAs and miRNAs. The cell array technology has up to 200-fold screening throughput as compared to 384-well-based assays. In this technology, siRNAs and transfection agents are first printed as a microarray with up to 10−20,000 cell spots at a time, resulting in a highly parallel reverse transfection of siRNAs into cells. Cell phenotypes resulting from the knockdown of specific genes are read with HTS and HCS instrumentation using up to 4 parameters at a time. This technology will facilitate the genome-scale biology and will create causal data from in vitro model systems that can be linked with the descriptive cancer genomics data from in vivo specimens.

Third, many of the breakthroughs in cancer therapeutics have arisen out of coincidence, such as drugs developed for one specific indication showing promise in another disease or cancer subtype. To explore such opportunities, we have been carrying chemical biology screens covering all known drugs. For example, in prostate cancer cell lines we identified the anti-alcoholism drug Disulfiram as a nanomolar inhibitor of prostate cancer growth (Iljin et al., 2009). By investigating molecular mechanisms of these inhibitory effects, it will be possible to establish the molecular basis of chemical vulnerabilities of cancer cells as well as to create pre-clinical and clinical study designs to validate these therapeutic opportunities.

Finally, the genomic and functional tools developed for cancer research can in the near future be tested in the clinical setting in facilitating therapeutic decisions. We have investigated such opportunities in individual cancer cases where clinical decisions have not been possible with evidence-based medical guidelines. The challenges and opportunities with such future clinical "canceromics" approaches will be discussed.