prognostic and predictive indicators. Unfortunately, our current understanding of the optimal adjuvant therapy for the individual patient is still very limited, with many being over- or under-treated, or treated inefficiently.
During the last years, gene expression profiles have been used to re-define standard biomarkers in breast cancer and to identify new prognostic and predictive biomarkers. We will illustrate this based on some examples.
Our group for example tried to refine the well-established histological grade. Indeed, clinicians face a huge problem with respect to patients who have intermediate-grade tumours (grade 2), as these tumours, which represent 30% to 60% of cases, are the major source of inter-observer discrepancy and may display intermediate phenotype and survival, making treatment decisions for these patients poses a great challenge. By comparing expression profiles of low and high grade tumours, we identified the genomic grade index (GGI), which was able to refine the reproducibility and prognostic value of the histological grading (Sotiriou et al. 2006). Several independent groups have also identified prognostic gene expression signatures. We demonstrated in a large meta-analysis of publicly available gene expression data that proliferation genes appear to be the common driving force of these different 1st generation prognostic signatures (Wirapati et al. 2008, Desmedt et al. 2008).
Another example concerns the refinement of the predictive biomarkers used in the clinic: the hormone receptors and the HER2 receptor. Although these biomarkers have optimal negative predictive values, their positive predictive value is rather limited. Also, their determination shows substantial variation both within and between laboratories. Several attempts have been done to provide a more quantitative and reproducible evaluation of ER and HER2, as well as a better representation of their corresponding phenotype (Paik et al. 2004, Desmedt et al. 2008).
Additionally, several studies, which will be further developed during this presentation, have also applied a genome wide approach to identify gene expression signatures that could predict drug sensitivity in breast cancer.

640 Predicting response to therapy in breast cancer

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In breast cancer, predicting the response to specific systemic treatments is an increasingly important step in guiidng therapy. Estrogren receptor status has been used to guide hormonal therapy for several decades; in the last decade, HER2 status has been used to guide HER2 targeted therapy. Additional therapy predicting tests would be of great clinical benefit.
To guide the choice of chemotherapy, hormonal therapy and targeted therapy, neoadjuvant studies are well suited to identify predictive factors for therapy response. For this purpose, we have analysed gene expression profiles in pre-treatment biopsies of 191 patients treated with neoadjuvant chemotherapy; and patients with HER2 positive breast cancer treated with the combination of chemotherapy and trastuzumab. Our results and studies from various other groups show that basal type/triple negative tumours show a pathological complete remission in 30−40% of cases; as compared to <5% in luminal type tumours. It has been more difficult to identify gene expression profiles associated with response to chemotherapy and response to trastuzumab using supervised classfication techniques. Research aimed at the identification of genetic classifiers for responsiveness to specific systemic therapies is expanding rapidly and should lead to clinically useful tests in the coming years.
At present, there are several ongoing randomised clinical trials investigating genetic profiling in guiding adjuvant systemic therapy; and in neoadjuvant systemic therapy. These studies will enable us to better understand differences between genetic sets; and allow us to develop our preferences based on results obtained in large well-controlled trials.

Tuesday 29 June 2010 17:30−18:20

Mike Price Lecture

641 Tumour metabolism: back to the future

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Tumours arise and eventually metastasize due to the cumulative effects of multiple mutations on multiple key genes. Oncogenes undergo mutations that cause them to become active when they shouldn't, and tumour suppressor genes (TSGs) sustain damaging alterations that obliterate their protective functions. TSGs include genes that normally control cellular differentiation, regulate cell growth and the cell cycle, participate in DNA repair, and govern pathways leading to programmed cell death or survival. Knowledge of the roles of these genes in preventing or promoting tumour formation has enabled molecular oncologists to seek mechanistically-based drugs for cancer treatment. Originally, the "Oncogene Revolution" prompted these investigators to concentrate on the development of agents that block cell growth and cell cycle progression. Although therapeutics based on this approach have had some success in the clinic, it has become increasingly clear that to be effective, anti-cancer agents must also target molecules involved in the metabolism, metastasis and death of tumour cells as well as proteins crucial for tumour angiogenesis. Our laboratory has spent much of the last decade identifying molecular pathways in cancer cells that can potentially be targetted. Our work has reached a fundamental level in that we are now turning our sights on molecules that prevent cancer cells from dying. Several major intracellular signaling pathways involving a plethora of known and unknown genes promote tumour cell survival. One of the most important of these pathways is driven by PI3′-kinase. In addition to its role in cellular survival, this lipid kinase activates a diverse array of signaling pathways affecting cell mobility, protein synthesis, proliferation, metabolism and hypoxia. Our laboratory identified DJ-1 (PARK7) as an important regulator of this pathway. More recently, mutations have also been found in the isocitrate-dehydrogenase genes in brain cancers and leukemias. In this presentation, I will discuss recent data from our and other laboratories suggesting that PI3′-kinase-mediated signaling in tumour cells is also intimately involved in mediating the Warburg Effect, the phenomenon whereby a cancer cell produces much of its energy through glycolysis rather than mitochondrial oxidation of pyruvate. In addition, I will describe our laboratory's efforts to identify non-glucose energy sources in tumours.

Tuesday 29 June 2010 09:45−17:30

Poster Session
Molecular Biology

642 Implications of Calpain-Calmodulin association in colon cancer

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Calpains are Ca2+-dependent proteolytic enzymes which are overexpressed in colon cancer and may contribute to metastasis. Calpain 4, a subunit of m-Calpain, is required for enzyme activity. We attempted to identify role of calcium sensing receptor CaSR om regulation of calpain activity and its influence on calmodulin (CaM) homeostasis. Bioinformatics analysis, biophysical tools like circular dichroism, isothermal titration calorimetry, fluorescence anisotropy, 1D-NMR, cell biology experiments including western blotting and pharmacological interventions to elucidate signaling mechanisms were employed. Results suggest that in low extracellular Ca2+ (0.005 mM), HT-29 cells had 400±75 Units of Calpain activity which was reduced following 18 h incubation in 3 mM Ca2+ (200±14 Units, $p < 0.05$ n = 4). Other polyvalent CaSR agonists, GdCl3 (25 ?M), neomycin sulfate (350 ?M), polyarginine (1.5 ?M) and spermine (2 mM) in low Ca2+ medium, reduced Calpain activity 40−55% ($p < 0.05$, n = 4). Transient transfection of siRNA (200 nM) duplex against CaSR reduced CaSR protein expression and prevented reduction of Calpain activity after 3 mM Ca2+ challenge (340±24 Units, $p < 0.05$ n = 4). Western blotting of HT-29 cell lysates after 3 mM Ca2+ challenge demonstrated no change in Calpain-4 but a 6 fold increase in CAM. Bioinformatic analysis of Calpain-4 revealed a putative CaM binding site. A synthetic peptide of Calpain-4 containing this site was generated [PEP6]. CD spectra demonstrated binding of CaM (150?M) to PEP6 at Ca2+ of 1 mM, consistent with PEP6 being a random coil but after binding CAM becomes an ?-helix with 1 mM Ca2+. 1D-NMR analysis confirmed PEP6 binding to CaM with Ca2+. Isothermal titration calorimetry demonstrated PEP6 interaction of CaM with Kd of 5?M. Western blotting of CAM with Calpain after 30 min incubation demonstrated a 3 fold increase in autolysed Calpain and 5 fold reduction in 75 kDa subunit of Calpain. W7 or W13 (100 ?M) prevented CaSR-mediated decrease in Calpain activity ($p < 0.05$, n = 4). We hereby conclude that CaSR activation by Ca2+ or other agonists will increase CaM in colonic adenocarcinoma cells to reduce Calpain activity. CaM can bind Calpain which will trigger Calpain autolysis. Stimulation of Calpain autolysis will reduce Calpain activity. We speculate that CaSR-mediated reduction in Calpain activity may be an important determinant of calcium chemoprevention of colon cancer.

643 Degradation of C/EBPalpha by Trib proteins correlates with Trib mediated acute myeloid leukemia

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Background: Tribbles encode an evolutionarily conserved protein family that influences proliferation, motility, metabolism and oncogenic transformation. All three mammalian Trib homologues, Trib1, Trib2 and Trib3, are characterized by a central serine/threonine kinase-like domain (KD) and a C-terminal binding site for COP1 E3 ubiquitin ligase. Trib1 and Trib2 are associated with hematopoietic malignancies whereas Trib3 is not. Trib1 is elevated in AML and MDS patient samples with gene amplifications and Trib2 is elevated in a subset of AML patient samples.

Materials and Methods: Retroviral mediated expression of wild type and mutant Trib proteins and in vivo bone marrow cell transduction and transplantation used to assay AML. Protein expression analysis of C/EBPalpha following knockdown of COP1 E3 ligase. Binding interaction assays performed using immunofluorescence, subcellular fractionation, GST pulldown and peptide array technology.
Results: In a murine bone marrow transplant model, mice reconstituted with hematopoietic stem cells (HSC) retrovirally expressing Trib1 or Trib2 but not Trib3, uniformly developed fatal transplantable AML. Investigation of the structural domains of Trib2 showed that the C-terminal COP1 E3 ligase binding site and the kinase domain are required for its oncogenic activity. Trib2 contains variant catalytic loop sequences compared to conventional kinases that we show are necessary for Trib2 function. Trib2 (and Trib1) associated with and led to the proteasomal-dependent degradation of C/EBPalpha, a critical nuclear transcription factor frequently dysregulated in AML. Trib2 localizes to both the cytoplasm and the nucleus, but interaction with C/EBPalpha is exclusively nuclear. Trib2 binding to C/EBPalpha results in COP1 E3 ligase mediated degradation of C/EBPalpha and is essential for Trib2-induced AML.
Conclusion: This works highlights Trib proteins as potent AML oncogenes, and identify the structural domains that are required for oncogenic function in AML. These data strengthen the correlation between Trib2 mediated C/EBPalpha degradation and leukaemia which may have prognostic and therapeutic implications.

644 A screen for cellular senescence reveals candidate tumour suppressor genes

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Background: Of interest to cancer researchers, cellular senescence upon prolonged oncogene activation or loss of tumour suppression is an emerging paradigm, and is detected in early stage human tumours. Since *in vivo* senescence thus acts as a tumour suppression mechanism, we reasoned that a senescence screen should reveal novel cancer genes.
Materials and Methods: We developed a high-content screening assay applying cell morphology (increased nuclear size) and proliferation (decreased Ki67) parameters to identify senescence-like siRNAs in a human kinome library. The screen was performed in hTERT-immortalised human Retinal Pigment Epithelial (RPE) cells expressing an inducible p53shRNA. Data mining was readily achieved using the Acuity Express software package. Actual senescence was quantitated by staining for senescence-associated β-galactosidase marker (SA-βGal), followed by measurement of typical senescence gene expression signatures and genomic profiling analysis.
Results: A high-content screen for senescence-like kinome siRNAs yielded 17 candidate genes. Of these, 14 genes were confirmed to be senescence regulators by SA-βGal staining. Senescence was predominantly p53-dependent and often correlated with DNA damage and p16INK4A induction. Interestingly, genomic profiling of tumour samples revealed that a significant proportion of the genes showed copy number alterations, and suggests that we may have identified novel tumour suppressor genes. To link senescence gene functions back to replication stress and/or DNA damage, we are characterising molecular components of novel senescence pathways through proteomic approaches. Special focus lies on the EPHA3 receptor tyrosine kinase, a gene found to be frequently mutated in human lung and colorectal cancers in recent genome sequencing efforts.
Conclusion: We successfully applied a high-content cellular senescence siRNA screen to identify a defined set of candidate tumour suppressor genes.

645 Array comparative genomic hybridization (aCGH) differentiates the major intrinsic subgroups of breast carcinomas

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Introduction: Breast Cancer is a heterogeneous disease as reflected by histopathology, molecular alterations and clinical behavior. Gene expression based classification as proposed by Perou et al in 2000 has proven to give important biological insight into the diversity of breast cancer. The intrinsic subtypes includes Luminal A, Luminal B, Normal-like, Basal-like and ERBB2-enriched subtype and have been shown to have clinical relevance and to be robust in several datasets. Due to strict tissue requirements and technical complexity, whole genome gene expression profiling is not likely to be applicable to a routine use in the clinical setting. Immunohistochemistry (IHC) for selected markers has been proposed as a surrogate for expression data, but a consistent system for scoring of intrinsic subtypes has been challenging. A limitation of IHC is the lack of a consistent scoring system and the subjectivity in the scoring. It has been shown that the intrinsic subtypes harbor major differences in copy number change, but a classifier based on genomic changes alone is yet to be defined.
Material and Methods: Tumour material from two clinical cohorts (MicMa and Ull) was included in this study. (1) MicMa: Fresh frozen biopsies were collected from 130 of the 920 patients included in the "Oslo Micrometastasis Project" from 1995 to 1998, data previously reported. Tumour DNA from 49 of these samples was available for aCGH-analysis. (2) Ull: Fresh frozen tumour specimens from 212 patients with primary breast cancer were collected at Oslo University Hospital from 1990 to 1994, and aCGH-analysis was performed on the total cohort. Expression data from 73 of these samples were available.
DNA was isolated and analyzed using the Agilent Human-Genome-CGH Microarray 244k platform. A piecewise constant regression function were fitted to the log transformed aCGH data, using the algorithm multi-PCF (Piecewise Constant Fit) on each subgroup separately. This resulted in a combined matrix with 1303 segments. Both ANOVA and multiclass SAM analyses were performed with the intrinsic subgroups as response variable.
Results: The ANOVA analysis gave 89 significant segments after Bonferroni correction. This included major regions on 1p, 1q, 2p, 3p, 4p, 5q, 6p, 7q, 10p, 16p and 16q, and smaller segments on other chromosomal arms. Multiclass SAM gave similar significant aberrations in the same regions. A clustering analysis using the significant regions for the aCGH-data showed clearly a separation between a luminal and a non-luminal group of tumours. In the non-luminal branch of the dendrogram, an erbB2 and a basal cluster were evident.
Conclusion: Our analyses show that the aCGH-data alone may separate between the major intrinsic subclasses of breast cancer. A classifier for the intrinsic subtypes based on genomic changes alone is yet to be defined. Such a DNA-based scoring system for breast cancer would be a valuable clinical tool and will add to our biological understanding of breast cancer subtypes.

646 MeCP2 has no role in intestinal tumourigenesis but is required for normal homeostasis in the murine intestine

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Mutations of the methyl binding domain protein MeCP2 underlie RETT syndrome (RTT), a human neurodevelopmental disorder. Other methyl binding proteins like MBD2 and Kaiso, have been shown, when deficient, to delay onset of Apc ^{Min} tumourigenesis. Given the observation that spatial gene expression in the gut is regulated by MBD2, and the role of MBD2 and KAiso in tumourigenesis, we have addressed the hypothesis that mutation of *Mecp2* will directly impact upon intestinal physiology and intestinal tumourigenesis.
To investigate the consequences of MeCP2 deficiency in the intestine, we used a Cre-Lox strategy to delete *Mecp2* from the adult mouse small intestinal epithelium. Male mice bearing the cytochrome p450 inducible AhCre transgene and the LoxP flanked *Mecp2* allele were induced with b-naphthoflavone. Small intestine homeostasis, proliferation, cell death, differentiation and transcriptome were analysed. MeCP2 role in intestinal tumourigenesis was studied using 2 Apc^{Min} model: $Apc^{Min/+}$ where Apc loss is occurring after LOH; and $Rb9Apc^{Min/+}$ where the loss of Apc is favoured towards epigenetic silencing.
Although $Mecp2^{-/y}$ mice did not develop intestinal symptoms of disease, loss of MeCP2 altered intestinal homeostasis, increasing the proliferative compartment within the crypt, decreasing cell death in the crypt-villus axis, increasing cell migration rates onto the villus and leading to longer villi. Cell differentiation was not altered. Transcriptome analysis identified upregulation of the MeCP2 target *Igfbp3*. This provides a ready mechanism for our observations, as Igfbp3 is implicated in the control of villus length by altering cell proliferation and death rates in the crypt-villus. The Survival and tumour burden was unchanged in the two $Apc^{Min/+}$ models in presence or absence of Mecp2 despite the increase of the tumour size in absence of Mecp2.
We show here that *Mecp2* is required for normal intestinal homeostasis but not tumourigenesis. RTT is caused by *MECP2* mutation, a neurological disorder however, our results extend the role of *Mecp2* beyond its known neurological role to the intestinal epithelium.

647 Withdrawn