(Pol I). These observations lead us to hypothesise that cMyc's regulation of rRNA synthesis may contribute to its oncogenic properties. We have tested this hypothesis in a mouse model of Myc-driven lymphoma, the EμMyc transgenic mouse.

Methods and Results: B-cells purified from EμMyc mice display an increased growth rate in comparison to their wild-type littermates, with increased cell volume, total RNA and protein per cell. This phenotype is characterised by higher rates of 45S rRNA transcription and increased expression of factors specific for Pol I transcription. Knockdown of one of these factors, UBF, by RNAi in EμMyc lymphoma cell lines results in a selective proliferative disadvantage of cells *in vitro*, in a competition assay, and *in vivo,* in a transplant model. This phenotype is driven by an increased rate of apoptosis associated with a reduction in 45S rRNA transcription.

Based on these findings we explored the potential therapeutic effectiveness in this model of a novel specific small molecule inhibitor of Pol I, CX-5461, currently in preclinical development. Transplanted EμMyc tumours showed marked sensitivity to CX-5461 *in vivo*, with a dramatic reduction in tumour burden in the peripheral blood (97.54%±0.56) and lymph nodes (94.96%±0.90) due to induction of apoptosis 24hrs following a a single oral dose at 75 mg/kg. Importantly a normal B-cell population was preferentially maintained in treated mice (13%±1.39 wt B220+ cells versus 1.04%±0.24 tumour B220+ cells, as a percentage of total WBC) indicating specificity of the compound for tumour cells. Four doses of CX-5461, 75 mg/kg orally every third day, significantly delayed time to endpoint by 9.5 days ($P < 0.0001$) compared to untreated animals This delay was accompanied by a period of complete remission with normal white blood cell counts ($6.76 \pm 0.48 \times 10^9$cells/L) and no identifiable tumour cells in the peripheral blood. Interestingly, *in vitro* dose curves indicate a dependence of CX-5461 sensitivity on wild-type p53 function (p53 wt and ARF−/− cell line $IC_{50} = 9.28$ nM±1.53 in comparison to p53 mutant and p53−/− $IC_{50} = 1.70$ uM±0.03), which can be reduced with over expression of Bcl2 (Bcl2 $IC_{50} = 2.33$ uM±1.3). Notably even in the more resistant p53 mutant and p53−/− cell lines, cell death also occurred via apoptosis, suggesting p53-dependant and independent mechanisms to be involved in CX-5461 mediated cell death.

Conclusions: In summary, this work with UBF RNAi and CX-5461 identifies inhibition of RNA Pol I transcription as a novel and effective target in the treatment of cMyc-driven malignancies and for the first time establishes that dysregulation of rDNA transcription can directly contribute to malignant transformation.

634 Regulation of alternative splicing by the Ewing Sarcoma Protein (EWS) and DNA damage

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Background: The Ewing sarcoma protein EWS belongs to the FET family (FUS, EWS, TAF15) of polypeptides, which can bind RNA as well as DNA and are implicated in transcription, splicing, RNA transport, signalling and maintenance of genomic integrity. Translocations of the EWS gene are a landmark of Ewing sarcomas and are common in other tumours. EWS interacts with several core splicing factors like U1C and SF1 as well as with several splicing regulators of the SR and hnRNP families. Our goal was to identify alternative splicing events regulated by EWS, whose alteration could contribute to the biology of cancers in which expression of EWS is altered.

Materials and Methods: To identify alternative splicing events regulated by EWS, RNA from HeLa cells depleted of EWS or the corresponding controls were hybridized to a splicing sensitive microarray developed in our laboratory, which analyzes 1804 events in 482 genes relevant for cancer progression and RNA processing. RNA immunoprecipitation (RIP), Crosslinking-Immunoprecipitation (CLIP) and Chromatin-immunoprecipitation (ChIP) assays were used to assess the association of EWS with DNA and RNA of regulated genes.

Results: We have identified 39 alternative splicing events in 31 genes affected by EWS knockdown. RIP, CLIP and ChIP data document direct association of EWS with at least some of the target genes. These changes are enriched in alternative acceptor choices and underrepresented in exon skipping events. Interestingly, alternative splicing changes were identified that affect key genes involved in the response to DNA damage. Remarkably, one third of these changes were also induced upon UV irradiation of control cells. We have also observed a remarkable change in subnuclear localization of the EWS protein upon UV irradiation. While ATM and p38 activities are apparently not required for this effect, inhibition of Erk and Jnk kinases partially impairs EWS translocation.

Conclusions: Some key genes important for response to DNA damage are regulated postranscriptionally by EWS. The EWS protein itself is re-localized upon UV irradiation. These results suggest the possibility that changes in EWS activity are part of the mechanisms underlying the changes in alternative splicing induced by genotoxic stress. They also offer a potential explanation for the observation that EWS knockout mice show hypersensitivity to ionizing radiation and premature senescence.

635 Transcription and RNA Processing: links to cancer

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Changes in gene expression patterns are characteristic of cancer cells and play important roles in facilitating cell proliferation. These changes occur at essentially all steps in the gene expression pathway. I will describe studies from my lab that examine molecular mechanisms by which changes in alternative splicing and polyadenylation of mRNA precursors occur in cancer. With respect to splicing, I will describe a pathway that is activated in cancer cells that results in alternative splicing of pyruvate kinase (PK) mRNA. This switch in splicing, which allows production of the embryonic PK isoform that is necessary for cancer cell proliferation, is mediated by the action of several hnRNP proteins that repress inclusion of an adult-specific exon while favoring inclusion of an exon specific to the embryonic form of the enzyme.

Alternative polyadenylation, which occurs in expression of over 50% of human genes, is also known to change in cancer. This results in shortening of the 3′UTRs of many proliferation-associated mRNAs, removing negative miRNA sites and contributing to their enhanced expression. I will describe studies from my lab that address how changes in both transcription efficiency and the make-up of the complex polyadenylation machinery can lead to changes in 3′ processing efficiency, and as a results the use of upstream, promoter-proximal polyadenylation sites.

Together, these studies illustrate how changes in mRNA processing can contribute to alterations in gene expression that contribute to enhanced proliferation of cancer cells.

636 Mechanisms by which the p53 tumour suppressor protein selects its target genes

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The p53 tumour suppressor protein mediates various cellular processes such as cell cycle arrest, senescence, DNA repair, cell death and even survival. Serving as a sequence specific transcriptional regulator, p53 has a large number of well validated transcriptional target genes that facilitate these outcomes. Yet some p53 outcomes are contradictory (eg. survival vs apoptosis) suggesting that not all target genes are equivalently induced by p53 under conditions that produce a specific and exclusive outcome. We have spent several years striving to elucidate the basis for target gene selectivity by p53. The experimental approaches we take include biochemical and cell-based assays. We and other have identified modifications, co-factors and cellular states that play roles in such selectivity. Recent work from our group continues to focus on this area. We have discovered that transcriptional activity of p53 is regulated at a surprising number of levels. These include (1) binding site recognition in the context of naked vs nucleosomal DNA, (2) the extent and sites of modification of the protein by acetylation, (3) selective repression by Mdm2 and MdmX (4) initiation vs. elongation of target gene RNA and (4) post-transcriptional mechanisms. We have also identified novel p53 target genes that play roles in cell cycle progression, energy metabolism, and even pro-oncogenic functions. Our work reveals the complexity of the p53 network and thereby poses challenges for future studies to deconstruct the key processes that are required for p53 tumour suppression.

Tuesday 29 June 2010 14:35–16:35

Joint European Pathology Society–EACR Symposium

637 Integrative high throughput analysis for the identification of novel therapeutic targets in breast cancer

No abstract received.

638 Tyrosine kinase inhibitor resistance mechanisms: molecular & histologic correlates

No abstract received.

639 Optimizing pathological diagnosis with new biological tools: examples in breast cancer

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In current clinical practice, the majority of patients with early breast cancer will receive some form of systemic adjuvant therapy (chemo- and/or endocrine therapy). A variety of clinical and pathological factors are being used as

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.