(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

J. Valcárcel[1], M.P. Paronetto[2], B. Miñana[2], E. Becchara[2], C. Ben-Dov[2]. [1]*ICREA and Centre de Regulacio Genomica, Gene Regulation, Barcelona, Spain,* [2]*Centre de Regulacio Genomica, Gene Regulation, Barcelona, Spain*

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

J.L. Manley[1]. [1]*Columbia University, Department of Biological Sciences, New York, USA*

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

C. Prives[1], A.M. Barsotti[1], R. Beckerman[1], L. Biderman[1], O. Laptenko[1], M. Mattia[1], T. Tanaka[1], S. Singer[1], A. Zupnick[1]. [1]*Columbia University, Biological Sciences, New York, USA*

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

C. Desmedt[1]. [1]*Institut Jules Bordet, Brussels, Belgium*

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