and establishes heterochromatic features at the rDNA promoter, including specific histone modifications, de novo DNA methylation and recruitment of HP1. Association with nucleolar chromatin and transcriptional repression requires the interaction of NoRC with RNA that originates from a promoter located ~2 kb upstream of the pre-rRNA transcription start site. These intergenic transcripts are processed into 150-300 nt RNAs, dubbed pRNA ('promoter RNA'), as their sequence matches the rDNA promoter. Depletion of pRNA leads to displacement of NoRC from nucleoli, decrease in rDNA methylation and activation of Pol I transcription. In malignant cells, the level of pRNA is strongly decreased, demonstrating that rDNA silencing and heterochromatin formation is abrogated in cancer cells. The data uncover noncoding RNAs as a key regulator in chromatin-based processes and link RNA-based silencing mechanisms to genomic stability and the control of cell proliferation.

7
RNA polymerase III transcription and cancer

L. Marshall[1], N.S. Kenneth[1], R.J. White[1]
[1]*Beatson Institute for Cancer Research, Beatson Labs, Glasgow, United Kingdom*

A feature of transformed and tumour cells is the elevated expression of RNA polymerase (pol) III products. This can arise through direct transcriptional activation by oncogenic factors such as c-Myc, as well as loss of direct repression by the tumour suppressors RB and p53, which restrain pol III activity in untransformed cells. To address the phenotypic consequences of pol III activation, we constructed cell lines in which synthesis of tRNA and 5S rRNA by pol III can be selectively stimulated in the absence of the complex genetic and epigenetic changes that normally accompany cell transformation. Induction of the pol III-specific transcription factor Brf1 was found to increase cell proliferation and cause oncogenic transformation. This response depends on the ability of Brf1 to activate pol III transcription. Amongst the gene products induced by Brf1 is the tRNAiMet that initiates polypeptide synthesis. Overexpression of this tRNA is sufficient to stimulate cell proliferation and allow immortalised fibroblasts to form tumours in mice. The data indicate that elevated tRNA synthesis by pol III can have a dramatic impact on tumourigenesis.

8
eIF4E and post-transcriptional gene regulation in cancer

K. Borden[1], B. Culjkovic[1], S. Vukosavic[1]
[1]*Universite de Montreal, Institute for Research in Immunology and Cancer, Montreal, Canada*

The eukaryotic translation initiation factor eIF4E is a potent modulator of gene expression. eIF4E overexpression leads to oncogenic transformation in cell culture and animal models. Further, eIF4E is highly elevated in several human cancers including breast, head & neck squamous cell carcinomas as well as in a subset of leukemias and lymphomas. Elevated eIF4E levels are correlated with poor prognosis. Thus, it is imperative to have a full understanding of the molecular activities of eIF4E in order to understand how it impacts on proliferation and survival, and to develop new therapeutic modalities for these cancers.

Traditionally, eIF4E modulates gene expression only at the level of cap dependent translation. It is well established that eIF4E does not modulate translation of all transcripts equally, preferentially affecting growth promoting mRNAs. Interestingly, eIF4E is found in both the nucleus and cytoplasm. Recent studies reveal that eIF4E mediated oncogenic transformation depends on its functions in both nuclear mRNA export as well as translation. Here, eIF4E overexpression leads to the nuclear export of a subset of growth promoting transcripts. Some of these mRNAs are also sensitive to eIF4E at the level of translation. In this way, eIF4E coordinately modulates the mRNA export and translation of a subset of transcripts involved in proliferation and survival. Sensitivity to eIF4E at the level of mRNA export is due to the presence of a 50 nucleotide sequence in the 3' untranslated region (UTR) of these mRNAs known as the eIF4E sensitivity element (4E-SE). We hypothesize that eIF4E is a central node in an RNA regulon which governs both proliferation and survival.

An RNA regulon is a theoretical construct developed to explain how post-transcriptional regulation of gene expression can be coordinated in eukaryotes. In the regulon model, mRNAs involved in the same biochemical process, such as Akt signalling, are coordinately exported and translated, in order to ensure that all the proteins involved in this pathway would be produced in a coordinated manner. Coordinated expression of these mRNAs is achieved via the presence of USER codes in their UTRs. USER codes, such as the 4E-SE, permit regulation of the RNA at a particular level (i.e. translation, stability, mRNA export etc). If a set of mRNAs has the appropriate combination of USER codes, these will be coordinately expressed and thus the relevant proteins will be produced for the given pathway. We demonstrate here that eIF4E coordinately regulates the expression of proteins involved in the Akt signaling pathway and that this is linked to the survival activity of eIF4E. A potent inhibitor of eIF4E, the promyelocytic leukemia protein PML, inhibits the regulon and its survival functions. We also will discuss the design of new therapeutic modalities based on our findings.

9
A transcriptional module initiates and maintains mesenchymal transformation in the brain

A. Iavarone[1]
[1]*College of Physicians and Surgeons of Columbia University, Institute for Cancer Genetics, New York, USA*

Using a novel combination of cellular-network reverse engineering algorithms and experimental validation assays, we identified a small transcriptional module, including six transcription factors (TFs), that synergistically regulates the mesenchymal signature of malignant glioma. This is a poorly understood molecular phenotype, never observed in normal neural tissue1-3. It represents the hallmark of tumor aggressiveness in high-grade glioma, and its upstream regulation is so far unknown1. Overall, the newly discovered transcriptional module regulates >74% of the signature genes, while two of its TFs (Stat3 and C/EBP) display features of initiators and master regulators of mesenchymal transformation. Ectopic co-expression of Stat3 and C/EBP is sufficient to reprogram neural stem cells along the aberrant mesenchymal lineage, while simultaneously suppressing genes associated with the normal neuronal state (pro-neural signature). These effects promote tumor formation in the mouse and endow neural stem cells with the phenotypic hallmarks of the mesenchymal state (migration and invasion). Remarkably, silencing the two TFs in human high grade glioma-derived stem cells and glioma cell lines leads to the collapse of the mesenchymal signature with corresponding reduction in tumor aggressiveness. In human tumor samples, combined expression of Stat3 and C/EBP correlates with mesenchymal differentiation of primary glioma and it is a powerful predictor of poor clinical outcome. Taken together, these results reveal that synergistic activation of a small transcriptional module, inferred using a systems biology approach, is necessary and sufficient to reprogram neural stem cells towards a transformed mesenchymal state. This provides the first experimentally validated computational approach to infer master transcriptional regulators from signatures of human cancer.

05 July 2008 15:00 - 17:00

SYMPOSIUM

Diagnostic and predictive molecular markers

10
Blood expression profiles as early diagnosis of breast cancer

A. Børresen-Dale[1]
[1]*Institute for Cancer Research, Department of Genetics, Oslo, Norway*

Existing methods to detect breast cancer (BC) in asymptomatic patients have limitations, and there is a need to develop more accurate and convenient methods. There is growing evidence that analyzing changes in gene activity in sensor cells (like peripheral blood cells, PBCs) might possibly provide information on whether tumour cells are present elsewhere in the body, for instance in the breast. The rationale for using blood cells as monitors for a malignant disease is based on the hypothesis that a malignant growth will cause characteristic changes in the bio-chemical environment of blood. These changes will affect the expression pattern of certain genes in blood cells.

Previous reports from 3 separate studies (Sharma et al BCR 2005, Aarøe et al 97thAACR 2006, Aarøe et al 19thEACR, 2006) have shown potential use of gene expression profiling of PBCs for early detection of BC. In a recent study an RT-PCR based 96 gene assay was developed and used for classification of Caucasian BC patients with 82% accuracy, 87% sensitivity and 76% specificity (Børresen-Dale AL et al 97thAACR 2007). In a current study to investigate the efficacies of the blood based 96 assay test in another ethnic population, 720 subjects with or without BC from diverse areas of India are recruited; healthy includes women with benign lesions and women with no mammographic findings; cases include early and late stage BC patients. The results of interim analyses of approximately 350 cases indicate that the 96 assay set efficiently discriminates BC and non-BC samples obtained, providing evidence for a gene expression signature as a potential additional tool in BC diagnostic work-up.

Adjusting the analysis of gene expression profiles for confounders may increase the sensitivity of the diagnostic profile since exposure of risk