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

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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

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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

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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

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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

factors for BC could partly explain the gene expression differences in the cases. In order to increase the repertoire of informative genes we are extending the analyses to a population based prospective study (NOWAC, Dumeaux et al 2008, BCR) with a larger number of BC and non-BC samples performing large-scale gene expression analysis. In this cohort changes in blood-derived gene expression profiles were observed for HRT users compared to non HRT users (Dumeaux et al 2006 Mol Cancer Ther). Thus, gene expression changes as a diagnostic test for BC may have to be adjusted for confounding factors related to different exposures (e.g. hormone exposure).

11
Gene methylation for the diagnosis and prognosis of cancer

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Alterations in DNA methylation, an epigenetic process present in mammalian cells, are a hallmark of human cancer. Aberrant hypermethylation of the promoter region of genes is associated with loss of function of the gene. A number of genes, many implicated in important biological pathways, are known to be methylated in cancer. Gene hypermethylation can occur frequently and early in the development of cancer. Sensitive methylation specific PCR technology exists that permits detection of gene methylation in tumor cells in tissue biopsies, urine, blood and other body fluids. Conceptually, tumor suppressor gene methylation is highly specific for neoplastic cells. For these reasons, methylation is a promising target for the detection and prognosis of cancer in tissue biopsies and body fluids.

Gene methylation has been identified by examination of individual candidate genes and, more recently, by global screens such as demethylating drug-based reactivation of expression in tumor cell lines. Bisulfite sequencing and MSP technology are used to assess methylation status. The effect of promoter methylation upon transcription is examined by Northern, RT-PCR, Western or immunohistochemical analysis. Studies of the frequency, timing and neoplastic cell specificity of individual genes by sequencing or MSP analysis have identified genes suitable as targets for early detection of cancer. The pattern of genes methylated in particular tumors has been studied for predictive power for stratification of a cancer patient's outcome or response to a certain therapy

Feasibility studies have demonstrated sensitive and specific detection of gene methylation in tissue biopsies and non-invasive body fluids from patients with cancer of an early stage when treatment can result in a better outcome. Global screens are leading to the elucidation of the cancer cell methylome. The average total number of genes methylated with functional significance in a tumor cell is unknown but might be reasonably estimated as several hundred. Mining of this data can improve current panels of genes used for early detection studies and extend such panels to provide signatures for differential diagnosis and prognosis. Correlative studies of gene methylation with clinicopathological parameters have highlighted potential markers of tumor behavior including response to therapy.

Challenges for methylation-based detection include: the likely need for larger panels of methylated genes in detection, optimization and standardization of specimen processing and technology for analysis, further study of gene methylation in normal or non-neoplastic cells, knowledge of timing of methylation of a gene in regard to clinically significant disease, and the ability for differential diagnosis of the anatomical site of origin of a tumor in a body fluid. Elucidation of the cancer cell methylome is accelerating and providing new candidate methylated genes for early detection and predictive classification of tumor behavior. In addition, new technologies are emerging that may allow more comprehensive and robust study of aberrant gene methylation in cancer cells.

12
Predictive gene profiles for breast cancer patients

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The use of gene expression assays in clinical medicine is a goal that will only be realized after validation of the technology and potential classification lists. Our previous gene expression profiling studies identified five major molecular intrinsic subtypes of breast cancer (Luminal A, Luminal B, Basal-like, HER2+/ER-, Normal Breast-like) that show significant differences in patient survival and metastatic potential. Mounting evidence also suggests that these subtypes vary in their responsiveness to chemotherapeutics and biologic agents. In fact, the expression of drug targets like HER1 and HER2, within selected subtypes only suggests that logical combinations of chemotherapeutics and biologic agents will be subtype-specific; however, this must be empirically evaluated. Nonetheless, selecting the right chemotherapeutic(s) and biologic agent combination for each subtype has yet to be experimentally or clinically determined. Therefore, I will present the available data concerning the responsiveness of the intrinsic subtypes relative to neoadjuvant chemotherapy and tamoxifen.

In addition to the prognostic and predictive abilities of the intrinsic subtype classification, a number of other groups have also identified gene sets and methods that predict outcomes in breast cancer patients including the 70-gene profile of van't Veer et al. (MammaPrint™), the Genomic Health Recurrence Score Assay (Oncotype DX™). Using a single data set of 295 patient/samples from the Netherlands Cancer Institute, each sample was assigned a class for each of these predictors. Within the Basal-like, Luminal B and HER2+/ER- tumor subtypes, great concordance across all predictors was observed. However, within the Luminal A group, there was heterogeneity in predictions, suggesting that further stratification with this largest group is still needed and could be provided by the MammaPrint or Oncotype DX assays. These data will be presented in more detail including a discussion of additional gene expression predictors and how these data can be used to guide current therapeutic decision making.

13
TP53 as an important molecular marker

No abstract received.

05 July 2008 17:00 - 18:00

PLENARY LECTURE

Cancer stem cells

14
Breast cancer stem cells

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Stem cells are defined as cells with self-renewal capacity and the ability to give rise to multiple differentiated cell types. Recent in vitro and in vivo clonality and tumorigenicity studies have demonstrated the existence of cells with stem cell like properties both in normal human breast tissue and breast carcinomas that are required for normal mammary gland development and tumorigenesis, respectively. However, the molecular basis underlying the maintenance and differentiation of normal mammary stem cells are largely unknown.

To characterize cells with stem-like characteristics, we determined the gene expression and genetic profiles of distinct cell populations purified from breast carcinomas and normal breast tissue using cell surface markers CD24 and CD44 that have been associated with stem cell-like properties. Gene expression profiles were analyzed using SAGE (Serial Analysis of Gene Expression), whereas genetic alterations were investigated using SNP (Single Nucleotide Polymorphism) arrays and FISH (Fluorescence In Situ Hybridization). SNP analysis suggested that CD24+/CD44– and CD24–/CD44+ cells from the same tumor are clonally related, but can be both genetically and epigenetically distinct. For example, CD44+ cells have an activated TGFb signaling pathway, while TGFβ-signaling specifically decreases in CD24+ cells due to increased TGFBR2 promoter methylation. This is consistent with the observation that CD44+ cells respond strongly to an inhibitor of TGFBR, acquire more differentiated epithelial cellular morphology and membrane localization of E-cadherin and β-catenin, while CD24+ cells do not respond to the inhibitor of TGFBR. Furthermore, gene expression profiling identified breast tumors with a higher or lower fraction of CD44+ cells and revealed that lymph node-negative breast cancer patients whose tumors were enriched for CD44+ cells had shorter overall survival as well as shorter distant metastasis-free survival. Interestingly, CD24+ cells appeared to be more prevalent in metastases to distant organs, even when the primary breast tumor was enriched for CD44+ cells. This suggests that the tumor cells may be altered during the metastatic process, or that CD24+ breast cancer cells are intrinsically more competent for metastasis. These studies demonstrate that cancer cell phenotype is subject to dynamic regulation by genetic and epigenetic mechanisms as well as by the tumor microenvironment. Thus, tumor progression is a dynamic and complex process that is influenced strongly by the intrinsic level of genetic instability in a given tumor at a given time and location.