05 July 2008 13:30 - 14:30

Muhlbock Lecture

1
Identification of stem cells in small intestine and colon by a single marker gene Lgr5

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The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. Current models state that 4-6 crypt stem cells reside at the +4 position immediately above the Paneth cells in the small intestine; colon stem cells remain undefined. Lgr5/Gpr49 was selected from a panel of intestinal Wnt target genes for its restricted crypt expression. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. In addition, Lgr5 was expressed in rare cells in several other tissues. Using an inducible Cre knock-in allele and the Rosa26-LacZ reporter strain, lineage tracing experiments were performed in adult mice. The $Lgr5^{+ve}$ crypt base columnar cell (CBC) generated all epithelial lineages over a 60-day period, implying that it represents the stem cell of the small intestine and colon. The expression pattern of Lgr5 suggests that it marks stem cells in multiple adult tissues and cancers.

05 July 2008 15:00 - 17:00

SYMPOSIUM

Receptor signalling

2
Computational simulation of the EGF receptor system

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The Epidermal Growth Factor (EGF) family of receptors and ligands consists of four receptors and eleven ligands. The Neuregulin sub-group of ligands can be made in over thirty different forms due to alternative splicing. This complex system has evolved to process multiple inputs and produce an array of outputs which ultimately regulate some of the most important behaviours of cells including cell replication, differentiation, motility and survival.

Numerous reports have demonstrated that these factors are expressed at altered levels in cancer cells and they are now well established targets for different types of signal transduction inhibitor drugs. We have developed a computer simulation of the system using object orientated modelling in which the levels of the different receptors and ligands can be selected and the system run to equilibrium.

We have determined the level of expression of the four receptors and eleven ligands in 100 cases of breast cancer using immunohistochemical staining. We are inputting this information into the program to simulate the entire system. The output enables us to determine the level of activity of each receptor type in each case. This tool may aid in the future in selecting the correct choice of signal transduction inhibitor drug for individual patients making them more effective and economic to use.

3
The EGF receptor signalling system

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Growth factors and their transmembrane receptors contribute to all steps of tumor progression, from the initial phase of clonal expansion, through angiogenesis and metastasis. Hence, the information relay system involved in growth factor signalling provides potential site for signal interception and tumor inhibition. A relevant example comprises the epidermal growth factor (EGF) and the respective receptor tyrosine kinase, namely ErbB-1/EGFR, which belongs to a prototype signalling module that drives carcinoma development. The extended module includes two autonomous receptor, EGFR and ErbB-4, and two non-autonomous receptors, namely: a ligand-less oncogenic receptor, HER2/ErbB-2, and a kinase-dead receptor (ErbB-3). This signalling module is richly involved in human cancer and already serves as a target for several cancer drugs. Due to inherent complexity and a large amount of experimental data, we propose a systems approach to understanding ErbB signaling. EGF - to - ErbB signaling is envisioned as a bow-tie configured, evolvable network, sharing modularity, redundancy and control circuits with robust biological and engineered systems. Our work concentrates on system controls, a plethora of negative feedback loops, which include E3 ubiquitin ligases, receptor endocytosis and newly transcribed genes. Because network fragility is an inevitable tradeoff of robustness, systems level understanding is expected to identify therapeutic opportunities for targeting aberrant activation of the network in human pathologies. Specific examples include anti-receptor antibodies such as Trastuzumab and kinase inhibitors, such as Lapatinib. Mechanisms underlying response to drugs and evolvement of secondary resistance will be discussed.

4
The EGF receptor system as a target for therapy

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The epidermal growth factor receptor (EGFR) and its ligands that belong to the EGF family of peptides are involved in the pathogenesis of different types of carcinoma. These proteins form a complex system that regulates the proliferation and the survival of cancer cells and that represents a suitable target for novel therapeutic approaches. Two main classes of anti-EGFR agents are in advanced clinical development: monoclonal antibodies (MoAb) that compete with ligands in binding to the extracellular domain of the EGFR, and tyrosine kinase inhibitors (TKI), which bind and inactivate the EGFR TK domain. The anti-EGFR MoAbs cetuximab and panitumumab have been approved for treatment of metastatic colorectal cancer (mCRC). Cetuximab has been shown to improve the activity of irinotecan-based chemotherapy in EGFR-positive irinotecan-resistant or –refractory mCRC patients. Panitumumab was found to improve the survival of mCRC patients as compared with best supportive care. The EGFR-TKI erlotinib has been approved for 2nd and 3rd line treatment of advanced or metastastic non-small-cell lung cancer (NSCLC) following the results of the BR.21 study that showed a survival advantage for patients treated with erlotinib as compared with placebo. More recently, the TKI gefitinib was found to be non-inferior to docetaxel in the treatment of advanced NSCLC. The EGFR-TKIs failed to increase the efficacy of standard chemotherapy in the first line treatment of NSCLC. The major drawback in the clinical development of anti-EGFR agents is the lack of predictive markers. No correlation between the levels of expression of EGFR and response to anti-EGFR agents was found in both CRC and NSCLC. The response rate to EGFR-TKIs is higher in NSCLC patients carrying mutations of the EGFR TK domain. However, patients that do not carry such mutations can also benefit of treatment with EGFR-TKIs. Contrasting results on the role of EGFR gene amplification in determining the sensitivity to EGFR targeting agents have been reported. Recent findings suggest that both CRC and NSCLC patients carrying a mutated KRAS do not benefit from treatment with anti-EGFR agents. In this regard, panitumumab has been approved for treatment of mCRC patients that carry a wild type KRAS gene. In conclusion, anti-EGFR drugs have shown promising activity in different tumor types. However, biological markers to select patients that can benefit of treatment with anti-EGFR agents are definitely needed.

5
Mechanisms of resistance to EGFR inhibitors

No abstract received.

05 July 2008 15:00 - 17:00

SYMPOSIUM

Gene expression and cancer

6
Non-coding RNA and chromatin remodeling: intergenic transcripts regulate the epigenetic state of rRNA genes

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Epigenetic control mechanisms silence half of the ribosomal RNA genes (rDNA) in eukaryotes. This silencing is brought about by NoRC, a SNF2h-containing remodeling complex, that recruits chromatin modifying activities

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.

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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