317 Is epigenetics the holy grail of cancer research?

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It is now widely accepted that cancer is also epigenetic disorder and that epigenetic changes play key roles in cancer development and progression. The fact that epigenetic alterations are, in contrast to genetic changes, reversible has an important implication for cancer treatment and prevention. Epigenetic inheritance include DNA methylation, histone modifications and RNA-mediate silencing all of which are essential mechanisms that allow the stable propagation of gene activity states from one generation of cells to the next. Epigenetic states are profoundly altered in human cancer and epigenetic deregulation have been observed in virtually all types of human cancers, although the precise underlying mechanisms remain poorly understood. Recent years have witnessed a remarkable pace of discoveries in epigenetics and epigenomics which will revolutionize our understanding of cancer and other complex diseases. This should help to elucidate the mechanism underlying tumourigenesis, identify specific epigenetic targets and the critical windows of vulnerability. The intrinsic reversibility of epigenetic changes represents a tremendous opportunity for the development of novel strategies for cancer treatment and prevention. Recent conceptual and technological advances in epigenetics and ongoing efforts aiming to identify epigenetic targets that could be exploited in cancer prevention and therapy as well as molecular epidemiology will be discussed.

Monday 28 June 2010 08:00–08:50

Educational Lecture
DNA damage response & novel targets

318 The ATM-mediated DNA damage response: the system and the pathways

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The cellular DNA damage response (DDR) – a central axis in maintenance of genomic stability – is a complex system that is turned on most vigorously by critical DNA lesions such as double strand breaks (DSBs). The DSB response affects numerous cellular processes and involves marked changes in signaling pathways, in gene expression and RNA metabolism, and in protein turnover, location and post-translational modifications (PTMs). Following DSB induction, the nuclear protein kinase ATM mobilizes this intricate signaling web by phosphorylating many key players in its various branches. This complex mechanism requires many cellular resources and is prone to severe genetic defects that lead to genomic instability, tissue degeneration and cancer predisposition. For example, ATM loss or inactivation leads to the genomic instability syndrome ataxia-telangiectasia (A-T), characterized by neuronal degeneration, immunodeficiency, genomic instability, extreme radiation sensitivity, and cancer predisposition. The complexity of this system is, however, worth its cost: the system takes the cell safely through the DNA damage crisis and, within a few hours, leads it back to normal life cycle once the damage has been repaired. We are studying individual pathways in this network using an experimental approach aimed at isolating the effect of a single process against the noisy background of the entire network. Recently identified pathways will be presented. In parallel, high-throughput strategies to gain an overview of different DDR layers are being employed. One layer is the cellular transcriptome, which we explore by obtaining gene expression profiles and analyzing them using algorithms and software developed in our labs. This analysis has recently pointed out new transcription factors that are involved in the ATM-mediated DDR. Another layer of the DDR is protein PTMs. A major PTM that shows profound damage-induced dynamics is protein phosphorylation. We combined phosphopeptide isolation, advanced mass spectrometry and label-free quantitation to explore nuclear phosphoproteome dynamics following DSB induction and to determine quantitative changes in phosphorylation levels of specific sites. Hundreds of novel damage-induced phosphorylations and dephosphorylations were identified. Importantly, about 40% of damage-induced phosphorylations were ATM-independent. ATM was required not only for the initial phosphorylation of the ATM-dependent sites, but also for the maintenance of theses phosphorylations over time. We connected many of the phosphorylated and dephosphorylated proteins into functional networks. The data attest to the breadth of the cellular DDR and will aid in elucidation of novel signaling events in this ever expanding network.

Monday 28 June 2010 08:00–08:50

Educational Lecture
Statistical analysis

319 Analysis of complex datasets with descriptive and predictive models – application to biomolecular pathways

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Background: Datasets produced in today's molecular biology laboratories provide a bewildering amount of data and a wealth of opportunity to probe the underlying biological systems. This instructional talk will discuss various approaches for tackling biological questions that can be approached with some high throughput datasets. The focus will be on proteomic data but the approaches presented are applicable more generally in various biomolecular contexts.
Materials and Methods: We will handle a classic experimental scenario in which various states or experimental conditions are subjected to high throughput measurement techniques, e.g. protein measurements following cytokine stimulus, potentially with an outcome measurement as well (e.g. percent apoptosis). For these, we will discuss the types of questions that can be asked, including predictive tasks ('Can we predict the degree of apoptosis') and mechanistic questions ('By what molecular pathway does TNF induce apoptosis'). We will briefly discuss regression models and classifiers, followed by a more detailed discussion of Bayesian network models.
Results: Applications in molecular biology and examples from a clinical setting will be presented.
Conclusions: Some remaining challenges and future directions for algorithmic development will be discussed.

Monday 28 June 2010 08:00–08:50

Educational Lecture
Novel diagnostics

320 Elucidation of pathomechanisms in human brain tumours by molecular profiling

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Identification of genomic and transcriptomic alterations have greatly contributed to revisions of tumour classification schemes and the identification of pathogenically relevant molecular pathways. We performed comprehensive molecular profiling on the level of the genome, the transcriptome and the epigenome, employed to the same samples of human brain tumours. Subsequently, these data were integrated and related to clinical parameters. Emerging candidate genes have been subjected to functional tests in dedicated cellular systems by means of ectopic expression as well as gene knock-down strategies and subsequent assays for cell viability, proliferation, apoptosis and cell migration. This approach allowed us to further elucidate pathomechanisms in human astrocytoma and oligodendroglioma and to uncover novel factors relevant to cell cycle control and cell migration. Notably, we identified (i) signatures distingiuishing two subgroups of primary glioblastoma, (ii) a pathogenic pathway downstream of TP53 regulated by DNA methylation in astrocytoma, (iii) selective pathway activations in glioblastoma of long term survivors, and (iv) genetic alterations in paediatric low grade astrocytoma that affect targeted molecular therapy procedures. Furthermore, novel algorithms to classify and to stratify paediatric and adult medulloblastoma patients will be presented. Possible consequences of these findings for the management of brain tumour patient will be discussed.

Monday 28 June 2010 09:00–09:50

Pezcoller – EACR Lecture

321 The p53 pathway: cancer, fertility, metabolic control and the central nervous system

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The p53 protein and the signal transduction pathway controlled by that protein responds to a wide variety of stress signals which can disrupt the fidelity of DNA replication and cell division. To prevent these errors or mutations the p53

pathway responds by initiating a process of cell cycle arrest, senescence or apoptosis which either permits repair of these errors or kills the clone of cells that contain these mutations. Central to this pathway are a series of proteins that respond to the stress signals and regulate the levels and activity of the p53 pathway. We have identified a number of single nucleotide polymorphisms (SNP) in those genes that regulate p53 activity and functions and these SNPs can play a role in the incidence of cancers in a population, the age of onset of cancers and the response to therapy. Some of the haplotypes containing these SNPs appear to be under positive evolutionary selection pressures in some human populations. The reason for this appears to be the role of p53 in the implantation of embryos into the uterus and the impact of some of these SNPs upon the fecundity of mice and humans. This process is mediated by the p53 regulated gene, Leukemia Inhibitory Factor or LIF, a cytokine that is essential for the implantation of embryos. SNPs in the p53 gene, the MDM-4 gene and the LIF gene regulate the efficiency of implantation of embryos in humans. It could well be that the p53 protein is also involved in the surveillance of developmental abnormalities.
The p53 transcription factor also regulates the synthesis of glutaminase-2, an enzyme that converts glutamine to glutamate in the mitochondria of both the liver and the brain. In the liver glutamate is converted to alpha-keto glutamate and this helps promote oxidative phosphorylation. In liver cancers glutaminase-2 is not produced and these cancers produce energy via aerobic glycolysis. Returning the glutaminase-2 gene to liver tumour cells increases glutamate levels and inhibits the growth of these cells. It appears that a metabolic regulator that restores oxidative phosphorylation can inhibit this type of cancer. In the brain glutamate is a neurotransmitter and five different glutamate receptors are also regulated by p53 in response to stress signals. In the brain the stress signals that activate p53 are communicated throughout the body by these glutamate receptors. Interestingly three of the genes that can cause Parkinsons Disease and the Huntington gene are also regulated by p53 in the brain. The role of p53 in the central nervous system remains to be explored.

Monday 28 June 2010 10:20–12:20

Symposium
Epigenetics: from DNA methylation to stem cell differentiation

322 Mechanisms of DNA methylation in mammals

F. Fuks[1]. [1]*Laboratory of Cancer Epigenetics, Faculty of Medicine, Free University of Brussels, Brussels, Belgium*

DNA methylation plays an important role in cancer and is associated with gene silencing of tumour suppressor genes. The methylation of CpG sites is established by the DNA methyltransferases – the DNMTs. One main interest in our lab is to better decipher the mechanisms by which these enzymes function and participate to cancerogenesis.
In recent years, we have contributed to show that the DNA methylation machinery brings about transcriptional repression through recruitment of histone modifying enzymes. In particular, a close connection was found between DNMTs and histone methyltransferases (1–5), with for exemple an impact on PML-RAR-mediated leukemia.
A key question still poorly understood is how are the DNMTs, and in particular their enzymatic activity, regulated. Data will be presented that suggest a new mechanism for the regulation of DNA methylation by post-translational modification.

Reference(s)

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[2] Brenner & Fuks. *Developmental Cell* (2007) 12(6):843–4.
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323 Human cancer epigenetics: from DNA methylation to microRNAs

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An altered pattern of epigenetic modifications is central to many common human diseases, including cancer. Many studies have explored the mosaic patterns of DNA methylation and histone modifications in cancer cells on a gene-by-gene basis, among them the seminal finding of transcriptional silencing of tumour suppressor genes by CpG island promoter hypermethylation. Epigenetic gene inactivation in transformed cells involves many "belts of silencing". We are in the process of completing the molecular dissection of the entire epigenetic machinery involved in methylation-associated silencing, such as DNA methyltransferases, methyl-CpG binding domain proteins, histone deacetylases, histone methyltransferases, histone demethylases and Polycomb proteins. The first indications are also starting to emerge about how the combination of cellular selection and targeted pathways leads to abnormal DNA methylation. In addition to classical tumour-suppressor and DNA repair genes, epigenetic gene silencing includes genes involved in premature aging and microRNAs with growth inhibitory functions. Recent technological advances are now enabling cancer epigenetics to be studied genome-wide. It is time to "upgrade" cancer epigenetics research and put together an ambitious plan to tackle the many unanswered questions in this field using genomics approaches to unravel the epigenome.

324 Understanding the origins of aberrant DNA methylation in cancer

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The conversion of normal cells to cancerous cells typically involves several steps resulting in the acquisition of unlimited growth potential (immortality). Both genetic and epigenetic changes have been detected in a number of different cancer cell types. Generally, these changes lead to the activation of oncogenes and the inactivation of tumour suppressor and pro-apoptotic genes. Although a number of tumour suppressor genes have been shown to be silenced by promoter DNA methylation, the following questions still remain: Do epigenetic changes contribute directly to cancer and if so when, where and how do they co-operate with genetic changes during the transformation process? To try to address these questions we have generated a human cancer cell model with defined genetic elements to study the global epigenetic changes associated with cellular immortalisation and transformation. We will describe the generation and characterisation of this cancer cell model and will provide preliminary evidence for progressive changes in promoter DNA methylation.

325 The role histone methyl transferases and demethylases in stem cell differentiation and cancer

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A major goal of our research is to identify and characterize genes involved in the regulation of normal proliferation and differentiation that also contribute to the development of human disease. Consistent with an essential role of histone modifying enzymes in controlling cell-fate decisions, several of these are causally linked to the development of diseases, such as cancer and CNS-associated disorders.
Histone methylation regulates chromatin structure and gene regulation. Histone methylation patterns define the state of chromatin and it is regulated by histone methyl transferases and demethylases. The Polycomb group proteins (PcGs) were until few years ago best known for their essential role in development, however, several reports have established that PcGs are frequently deregulated in human tumours. Others and we have demonstrated that the PcG protein and histone methyl transferase EZH2 is an oncogene, which regulates the expression of a large number of genes dictating cell-fate decisions.
Recently, others and we have discovered a group of proteins that catalyze the demethylation of methylated lysines. Members of this Jumonji demethylase family are overexpressed in human cancer and mutated in neurological disorders. At the meeting, results will be presented describing the functional characterization of some of these very exciting proteins – that also present strong candidate targets for drug development.

Monday 28 June 2010 10:20–12:20

Symposium
Hypoxia & angiogenesis

326 Targets of "angioprevention": from inflammatory angiogenesis to hypoxia

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Background: The complex cancer microenvironment cooperates with tumour and endothelial cells to promote malignancy. We propose to identify molecules and pathways involved in cancer progression in order to prevent tumour development by targeting the microenvironment and inflammatory angiogenesis.