Sunday 27 June 2010 08:00–08:50

Educational Lecture
Molecular imaging

17 Intravital microscopy of cancer progression, regression and resistance to therapy

P. Friedl[1]. [1]*Radboud University Nijmegen Medical Centre, Department of Cell Biology (283) NCMLS, HB Nijmegen, The Netherlands*

Molecular programs controlling metastatic cancer progression are diverse in different cancers as well as within the microenvironment of a single lesion. These include amoeboid, mesenchymal and collective invasion processes, followed by different mechanisms to seed and condition the metastatic site for secondary survival and growth. Cancer plasticity may further be supported as side-effect of therapeutic interference, prompting cellular and molecular adaptation programs. Examples for unexpected adaptation programs suited to overcome molecular interference are the mesenchymal-amoeboid transition after the interference with surface proteases or surface integrins, the amoeboid-mesenchymal transition after interference with Rho/ROCK pathways, and the collective-to-amoeboid transition leading to the dissociation of multicellular lesions followed by amoeboid single-cell dissemination. Thus, an understanding of cellular plasticity of invasion programs will be important to better target cancer progression.
Multiphoton microscopy (MPM) has become the method of choice for investigating cell structure and function in tissues and organs, including the invasion and progression of cancer lesions. Using a novel approach of infrared-excited (IR-)MPM at wavelengths above 1080 nm that enhances deep tissue microscopy in orthotopic fibrosarcoma xenografts, we here show deep collective invasion strands of several hundred connected cells. These multicellular units proliferate and simultaneously move with velocities of up to 200 μm per day along pre-existing blood vessels but not tumour-induced neovessels and proliferate ("invasive growth"). These perivascular tumour cell strands further maintain invasion and robust survival during otherwise regression-inducing experimental radiation therapy. Both, primary growth and survival and increased resistance to experimental therapy were ablated by interfering with beta1 and beta3 integrins, implicating integrin-mediated signals as microenvironmental denoinators of response to therapy. In conclusion, intravital deep tumour imaging by IR-MPM identifies the tumour-vessel interface as preferred niche of invasive growth, radioresistance and enhance preclinical anti-cancer therapy.

Sunday 27 June 2010 08:00–08:50

Educational Lecture
Bioinformatics

18 Analyzing functional genomics data and networks to understand disease

O. Troyanskaya[1]. [1]*Princeton University, Lewis-Sigler Institute for Integrative Genomics Carl Icahn Laboratory, Princeton NJ, USA*

Background: Understanding complex disease at the molecular level requires cataloguing and modeling specific molecular-level changes that lead to disease. Modern genome-scale experimental techniques (including expression, sequencing, interaction, SNP data) enable monitoring of these molecular events, and their integrative analysis holds the promise of generation of specific, experimentally testable hypotheses, paving the way for a systems-level molecular view of carcinogenesis. The complexity and scale of human biology make it challenging to integrate such a large body of data, model them on a systems level, and use these models to study specific pathways, genetic disorders, acquired disease, and therapeutic responses.
Results: I will describe how these challenges can be addressed through the development of integrative bioinformatics frameworks that enable cancer researchers to effectively explore and analyze the entirety of functional genomics data in a way driven by their biological question of interest. Specifically, I will present HEFalMP, a regularized Bayesian integration system we developed that provides maps of functional activity and interactions in over 200 areas of human cellular biology and disease, each including information from ~30,000 genome-scale experiments pertaining to ~25,000 human genes. HEFalMP allows prediction of protein function and functional modules, cross-talk among biological processes, and association of novel genes and pathways with known genetic disorders. I will also describe our work in starting to model these systems-level processes in a cell-type/tissue specific context, starting with accurate predictions (and experimental confirmation) of tissue-specific expression. Gene expression and high throughput copy number data are perhaps the most widely used functional genomics approaches for the study of cancer. I will describe a software package we developed for probabilistic analysis of these data to identify phenotypically/clinically relevant aneuploidies.
Conclusions: The presentation will be accessible to those with biology or computational background and will be focused on using these methods to analyze functional genomics data and discovery novel biology. For more information, please explore: http://function.princeton.edu/ and function.princeton.edu/hefalmp

Sunday 27 June 2010 09:00–09:50

Radium Hospital Foundation Lecture: Epigenetics

19 Epigenetic therapy

P.A. Jones[1]. [1]*University of Southern California, Norris Comprehensive Cancer Center, Keck School of Medicine MC-9181, Los Angeles, USA*

The abnormal methylation of CpG islands located near the transcriptional start sites of human genes plays a major role in carcinogenesis. The methylation of cytosine residues in these regions is associated with alterations in chromatin structure including changes in the state of modification of histone residues in nucleosomes and the positioning of these nucleosomes with respect to the transcription start site. These alterations serve to reinforce each other and may lead to the heritable silencing of genes which can have profound implications for human cancer development. Unlike mutational changes, epigenetic alterations are acquired in a gradual process which is associated with cellular division. Thus, these progressive alterations are potentially susceptible to interventions to reverse silencing. Epigenetic changes can be observed in premalignant tissues so that understanding what causes the alterations and development of potential strategies to reverse them could have an impact on carcinogenesis. The mechanisms underlying progressive methylation of CpG islands leading to altered chromatin configuration are now beginning to be understood. Drugs such as 5-aza-2′-deoxycytidine can reverse DNA methylation changes and reactivate gene expression by changing not only the methylation, but also the nucleosomal occupancy of the promoter. Further understanding of epigenetic changes in cancer and the mechanisms by which they are acquired may therefore help in the search for new therapeutics.

Sunday 27 June 2010 10:20–12:20

Symposium
Senescence & aging

20 Roles and regulation of cellular senescence

S.W. Lowe[1]. [1]*Cold Spring Harbor Laboratory, Lowe Laboratory, New York, USA*

Cellular senescence was originally described as the process that accompanies replicative exhaustion in cultured human fibroblasts, and is characterized by a series of poorly understood markers. Our laboratory previously showed that deregulated mitogenic oncogenes could drive cells into a senescent state thereby preventing transformation, and that senescence could contribute to the outcome of chemotherapy *in vivo*. Based on the hypothesis that senescence is an important tumour suppressive mechanism, we continue to study the roles and regulation of senescence during cancer and other types of pathologies. Recently, we performed a series of genome wide gene expression profiling and chromatin binding experiments to identify genes controlled by the retinoblastoma tumour suppressor (RB) that are uniquely targeted as cells exist the cell cycle into senescence. These studies imply that RB acts, primarily, to suppress genes involved in DNA replication, particularly in cells undergoing cell cycle exit into senescence. These same processes are redundantly controlled by the RB family as cells exit cell cycle into quiescence. We believe this may be crucial to its tumour suppressive role and continue to work on the mechanism of this effect. We also study the biology of senescence and, recently, have shown that senescent cells can be targeted and cleared by the immune system in vivo. These results have implications for the role of senescence in tumour suppression and other human pathologies, and hence we continue to explore the factors underlying senescent cell clearance. Recent studies support a role for inflammatory programs, as well as the perforin/granzyme B pathway, in this processes. Current efforts to characterize molecular mechanisms of senescence the behavior of senescent cells in vivo will be discussed.

21 Molecular mechanisms of cellular senescence

F. d'Adda di Fagagna[1]. [1]*IFOM, Telomeres and Senescence Group, Milano, Italy*

Early tumourigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced

by oncogene activation are restrained by cellular senescence. We have previously shown that expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Senescence-associated heterochromatic foci (SAHFs) are enriched in heterochromatin markers and they have been proposed to enforce cellular senescence by suppressing the expression of proliferative genes.
We will discuss our most recent results on the interplay between DDR and heterochromatin formation, the differential repair of the human genome and the regulation of DDR in stem cells and its impact on their proliferation and viability.

22 DNA repair and cancer

H.E. Krokan[1], B. Kavli[1], M. Otterlei[1], M. Akbari[1], G. Slupphaug[1], P.A. Aas[1]. [1]*Norwegian University of Science and Technology, Department of Cancer Research and Molecular Medicine, Trondheim, Norway*

DNA is continuously being damaged by spontaneous decay and exposure to carcinogens. Such damage is cytotoxic and mutagenic. Spontaneous depurination alone accounts for more than 10,000 events per human cell per day, whereas some 100–200 DNA-cytosines are deaminated to mutagenic U:G mismatches per day. DNA repair processes eliminate cytotoxicity that would otherwise kill the organism, probably within few days, while preventing mutations is important to avoid cancer development. Inherited DNA repair deficiency is associated with strongly increased cancer risk, e.g. rare syndromes like Xeroderma pigmentosum and ataxia teleangiectasia and more common forms of cancer e.g. early onset breast cancer and hereditary nonpolyposis colorectal cancer (HNPCC). In addition, there is evidence that more common single nucleotide polymorphisms (SNPs) in DNA repair genes may increase cancer risk, e.g. lung cancer development, although relative risk increases are generally low. The degree of contribution of DNA repair deficiency arising in the life of somatic cells is less clear, but there is evidence that mutations, epigenetic silencing and imbalanced expression of DNA repair proteins may increase cancer risk. Using mice with targeted mutations in DNA repair genes, defects in each of the excision repair pathways have been found to increase cancer risk. However, in some repair pathways, e.g. base excision repair (BER) some defects do not increase cancer risk possibly due to overlapping functions of some of the repair proteins. Importantly, many DNA repair proteins, such as uracil-DNA glycosylase (UNG) and mismatch repair proteins are also essential for the adaptive immune responses somatic hypermutation (SHM) and class switch recombination (CSR) in B-cells. In mice, UNG-deficiency increases the risk of developing B-cell lymphoma ~20-fold. SHM is essential to generate high affinity antibodies. However, it is a risky process and dysregulated SHM may be an important contributor to B-cell lymphoma. In conclusion, DNA repair proteins may contribute to cancer prevention both via DNA repair and adaptive immunity.

23 Aging and tumour suppression: the double-edged sword of cellular senescence

J. Campisi[1]. [1]*The Buck Institute for Age Research, Lawrence Berkeley National Laboratory, Novato California, USA*

Background: Aging is the largest single risk factor for a host of chronic diseases, most of which are degenerative in nature. These degenerative diseases include cardiac failure, vascular degeneration, macular degeneration, sarcopenia, type II diabetes-associated disability, osteoporosis and others. The exception is hyperproliferative disease, of which cancer is the most important. Cancer is indubitably an age-related disease, but hardly degenerative in nature. For many mammalian species, including humans, both hyperproliferative diseases and degenerative diseases increase with approximately exponential kinetics after about the mid-point of the life span. Is there a common biology that links cancer to the other diseases of aging?
Results: Our research suggests the answer to this question is yes. Aging is most likely driven by somatic damage, which is also a major cause of cancer. Damage occurs to virtually all cellular components, but the genome is particularly vulnerable. Cells respond to severe genomic damage by undergoing cell death or permanent loss of proliferative capacity (cell senescence). These responses are tumour suppressive, and are required to prevent the development of cancer in young mammalian organisms. We find that genomic damage, when severe enough to cause cell senescence, also induces the secretion of a large number of cytokines and other proteins that promote inflammation. Inflammation underlies virtually all age-related diseases, including cancer. We now have molecular evidence to suggest a model by which somatic damage elicits an inflammatory response that drives many age-related pathologies, both degenerative and hyperproliferative.
Conclusions: Our findings not only provide insights into how diverse age-related pathologies might arise, but also provide strategies for rational interventions into the basic aging process, and hence multiple age-related diseases.

Sunday 27 June 2010 10:20–12:20

Symposium
Noncoding RNA

24 Cancerous microRNAs and regulatory RNA binding proteins

R. Agami[1], M. Kedde[1], M. van Kouwenhove[1], W. Zwart[1], J. Oude Vrielink[1]. [1]*The Netherlands Cancer Institute, Department of Gene Regulation, Amsterdam, The Netherlands*

MicroRNAs (miRNAs) are genes involved in normal development and cancer. They inhibit gene expression through interaction with 3′-Untranslated regions (3′UTRs) of messenger RNAs (mRNAs), and are thought to regulate a large proportion of protein coding genes. Patterns of mis-expression of miRNAs in cancer suggest key functions of miRNAs in tumourigenesis. We performed in the past genetic screens to identify cancer functions of miRNAs. Using a library of vectors expressing human miRNAs and we identified miRNAs that cooperate with oncogenes in cellular transformation, which stimulate cellular migration, invasion and metastasis, as well as key regulators of tumour suppressor genes.
In recent years, it is becoming apparent that the miRNAs themselves are subjected to intense regulation at various levels. miRNA biogenesis and activity can be kept in pace by RNA-binding proteins (RBPs). We show that interplay between RBPs and miRNA exists that affects gene expression and processes such as development and cancer.

25 Non-coding RNA production by RNA polymerase III is implicated in cancer

R.J. White[1]. [1]*Beatson Institute for Cancer Research, Glasgow, United Kingdom*

RNA polymerase III is responsible for ~10% of nuclear transcription and makes a variety of short non-coding RNAs, including tRNA. Elevated expression of Pol III products has been observed in many types of transformed and tumour cells. This overexpression can be ascribed to three categories of molecular change [1].
(a) Release from repression by tumour suppressors. In untransformed cells the pol III-specific transcription factor TFIIIB is directly repressed by RB and p53 [2,3]. Inactivation of one or both of these tumour suppressors is frequent in cancer and releases TFIIIB from restraint, allowing pol III output to rise.
(b) Activation by oncogene products. Pol III transcription can be stimulated by many oncogene products. Perhaps the most important is c-Myc, which binds to TFIIIB and recruits GCN5 to pol III-transcribed genes [4,5].
(c) Pol III-specific transcription factors are produced at abnormally high levels in some types of tumour, such as prostate and ovarian carcinomas [6].
One of the key pol III products is the initator tRNAMet, which is required for production of new polypeptides. Levels of this tRNA are limiting for translation in fibroblasts. Mild overexpression of initator tRNAMet not only stimulates protein synthesis, but also promotes cell proliferation and oncogenic transformation [7]. Translational induction of c-Myc is implicated in this. Positive feedback may occur, with c-Myc stimulating pol III transcription of tRNA genes and then elevated tRNA selectively promoting translation of mRNA encoding c-Myc.

Reference(s)

[1] Marshall & White (2008) Nature Rev Cancer 8, 911–914.
[2] White et al. (1996) Nature 382, 88−90.
[3] Cairns & White (1998) EMBO J 17, 3112–3123.
[4] Gomez-Roman et al. (2003) Nature 421, 290–294.
[5] Kenneth et al. (2007) PNAS 104, 14917–14922.
[6] Winter et al. (2000) PNAS 97, 12619–12624.
[7] Marshall et al. (2008) Cell 133, 78−89.

26 Interweaving microRNA, inflammatory cytokine and p53 pathways in human cancer

C.C. Harris[1]. [1]*National Cancer Institute, Center for Cancer Research, Bethesda, USA*

We and others have identified specific microRNAs and changes in their expression in human lung, colon, and esophagus cancers that are associated with diagnosis, prognosis, and therapeutic outcome. We have also identified expression profiles of inflammation-related genes that can be combined by COX regression hazard analysis to be prognostic classifies, i.e., inflammatory risk score (IRS). For example, IRS and miR-21 expression are independent predictors of prognosis and together may be clinically useful in identifying patients with early stage cancer at high risk of metastases. As our