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Biological insights from quantitative analysis of RTK signaling networks

F. White[1]
[1]*Massachusetts Institute of Technology, Department of Biological Engineering, MA Cambridge, USA*

Signal transduction mediated by protein phosphorylation regulates many cellular biological processes. Aberrations in protein phosphorylation due to kinase (or phosphatase) mutation or overexpression lead to dysregulation of cellular signaling and has been linked to a variety of pathologies, including cancer, autoimmune, and metabolic disorders. Quantification of specific phosphorylation sites regulating signaling pathways involved in these pathological disorders will enable a better understanding of the genesis and progression of the disease state, providing targets for more effective therapeutic intervention. The combination of mass spectrometry-based analysis of protein phosphorylation with phenotypic measurements and computational modeling has enabled the identification of sections of the signaling network that correlate strongly with biological response to cell perturbation. This approach should yield novel insights into the regulation of biological decisions on the network scale.

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Interactome networks

M. Vidal[1]
[1]*Dana-Farber Cancer Institute / Harvard Medical School, Cancer Biology, Boston MA, USA*

For over half a century it has been conjectured that macromolecules form complex networks of functionally interacting components, and that the molecular mechanisms underlying most biological processes correspond to particular steady states adopted by such cellular networks. However, until recently, systems-level theoretical conjectures remained largely unappreciated, mainly because of lack of supporting experimental data.

To generate the information necessary to eventually address how complex cellular networks relate to biology, we initiated, at the scale of the whole proteome, an integrated approach for modeling protein-protein interaction or "interactome" networks. Our main questions are: How are interactome networks organized at the scale of the whole cell? How can we uncover local and global features underlying this organization, and how are interactome networks modified in human disease, such as cancer?

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06 July 2008 10:15 - 12:15

SYMPOSIUM

Mouse models

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Mouse models of cancer

A. Berns[1]
[1]*The Netherlands Cancer Institute, Department of molecular genetics, Amsterdam, The Netherlands*

1. Infection of newborn mice with replication-competent Moloney Murine Leukemia Virus gives rise to T and B cell lymphomas. The underlying mechanism is proviral activation of proto-oncogenes and inactivation of tumor suppressor genes. We have used retroviral insertional mutagenesis in over 1000 tumor-predisposed KO and control mice to identify new cancer causing genes. The largest specific cohort consisted of p53 and p19Arf KO mice. The resulting dataset with close to 600 common insertion sites marking known and unknown proto-oncogenes, (haploinsuffcient) tumor suppressor genes, and microRNAs, also permitted us to score for genotype-specific common insertion sites and highly significant co-occurrence of mutations and hits in (haploinsufficient) tumor suppressor genes. Clearly, this large dataset provides new information that could not have been extracted from smaller datasets collected previously, illustrating the "added value" of performing these studies on a large scale in a defined genetic background. The approach is complementary to and can confirm the cancer-causing nature of genes identified by other approaches such as SNP analysis and high throughput sequencing of cancer genomes. Illustrating examples will be presented. 2 NSCLC and SCLC can be efficiently induced by Adeno-Cre mediated switching of a floxed mutant Ki-Ras allele or by inactivation of Rb and p53 floxed alleles, respectively. Interestingly, while the same subset of cells are infected by the Adeno-Cre virus, activation of Ki-Ras or inactivation of Rb and p53 results in very different tumors. mSCLC are heterogeneous carrying clonally related cells with neuroendocrine and a progenitor-like marker profile, respectively. We have established a series of cell lines from the mSCLC tumors representing these lineages and performed array CGH analyses using a 1 Mb BAC array. Orthotopic transplantation of both lineages gave rise to tumors although with very different characteristics. We are focusing on these models both as a system to study the consecutive events in tumor development and to test intervention strategies.

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Dissecting tumor suppressor gene networks in vivo

S. Lowe[1]
[1]*Cold Spring Harbor Laboratory, Howard Hughes Medical Institute, Cold Spring Harbor, USA*

Apoptosis is a regulated form of cell death that is important for normal development and tissue homeostasis. Senescence produces "genetic death" in that the senescent cell is incapable of further propagation. Both processes are frequently disrupted in cancer cells, and each act as potent barriers to tumorigenesis. Since radiation and many chemotherapeutic agents induce apoptosis or senescence, the integrity of these programs can influence the outcome of cancer therapy. Our laboratory strives to understand how cancer genes control apoptosis and senescence in normal cells, and how mutations that disrupt these processes impact tumor development and therapy. The goal of these efforts is develop therapeutic strategies based on an understanding of drug action and cancer genotype. We currently are using genetically engineered mouse models to understand how apoptosis and senescence are controlled in tumor cells, as well as the response of tumors to conventional and targeted therapeutics. Recent work from our laboratory has incorporated stable and conditional RNA interference to probe tumor suppressor network components in vivo and used integrated oncogenomics approaches and in vivo RNAi interference to identify and characterize new tumor suppressor genes.

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Epidermal stem cells in tissue homeostasis and cancer. Role of Rac1 and Myc

S. Aznar-Benitah[1]
[1]*Center for Genomic Regulation, Differentiation and Cancer, Barcelona, Spain*

Adult stem cells are potentially the only long-term tissue residents that can accumulate enough oncogenic mutations resulting in the development of neoplasias. Upon transformation, adult stem cells retain hallmarks of stemness such as self-renewal, high proliferation potential, and tissue-remodelling activities, among others, but loose the ability to follow the organizational cues that restrain uncontrolled growth and invasion in healthy tissues. We have previously shown that Rac1 GTPase is required to maintain epidermal stem cells quiescent and located within their niche. Upon deletion of Rac1, epidermal stem cells exit the niche through a by-functional mechanism, cell cycle entry and egression from the stem cell niche that ultimately results in loss of self-renewal of the entire epidermal unit. Mechanistically, Rac1 exerts some of its epidermal effects via PAK2-mediated phosphorylation of the transcription factor c-Myc. Here we propose a novel function of Rac1 and c-Myc in epidermal stem cells and squamous tumours. Phosphorylation of Myc, downstream of PAK2, regulates quiescence and self-renewal of skin progenitors, affects the onset of differentiation, and modulates homing to the stem cell niche. In addition, phospho-Myc changes the invading and tissue remodelling potential of epidermal progenitors, and squamous cell carcinomas in 3D and 2D assays. Thus, we propose that the Rac/PAK2/Myc axis is required to sustain epidermal stem cell homeostasis and that deregulation of this pathway might play a role in epidermal neoplasias.

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Integrative comparative oncogenomics of mouse and human tumors

L. Chin[1]
[1]*M.D. Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA*

An increasing compendium of mutant genes and their tumor biological impact has informed the rational development of effective targeted therapies and the use of such agents in appropriate patient populations. This genetic paradigm now motivates intensive efforts in cancer gene discovery and validation. High-resolution genome scanning technologies, such as array-based comparative genome hybridization (array-CGH), have uncovered highly re-arranged human cancer genomes harboring strikingly large numbers of recurrent copy number alterations (CNAs). The challenge of deciphering this complexity is furthered by the presence of 'causal' genomic events targeting cancer-relevant genes as well as bystander "genomic noise". Cross-species triangulation with tumor-associated alterations in refined genetically engineered mouse (GEM) models is a powerful filter to

prioritize and focus on evolutionarily conserved events that are more likely to be biologically important. Examples of such approach will be described.

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YOUNG CANCER RESEARCHER'S WORKSHOPS

How to be effective in applying for fellowships

06 July 2008 13:45 - 14:35

AWARD LECTURE

Young Cancer Researcher's Award

37 Oral

SPAR1 is an anti-recombinase that impacts on genome stability and cancer

S. Boulton[1]
[1]*Cancer Research UK London Research Institute, Clare Hall Laboratories, South Mimms, United Kingdom*

DNA double-strand breaks represent a major threat to genome integrity and are predominantly repaired by homologous recombination (HR). Unscheduled or excessive HR can also lead to gross chromosomal rearrangements characteristic of cancer cells, but the mechanisms that restrain HR remain poorly understood.

Yeast Srs2 and E. coli UvrD are related helicases that suppress aberrant recombination by disrupting a specific step in HR, however functional homologues are not obviously conserved in higher eukaryotes. We therefore performed a genetic screen in C. elegans to identify uncharacterised helicases that are synthetic lethal in combination with C. elegans BLM mutants, based on the srs2 sgs1 (BLM) synthetic lethality observed in yeast. This screen identified a novel helicase, SPAR-1 that is conserved from C. elegans to humans and exhibits many of the genetic and biochemical hallmarks of yeast Srs2. Genetic analysis has revealed that C. elegans spar-1 mutants are also synthetic lethal with mus-81 and a distinct group of non-replicative helicases: BLM, FANCJ and RECQ5, but not with WRN. Additionally, the lethality in all four double mutant combinations results from an accumulation of toxic recombination intermediates. C. elegans spar-1 mutants and SPAR1 deficient human cells are also hyper-recombinogenic and exhibit exquisite sensitivity to interstrand cross-links (ICL) that block replication forks. SPAR1 knockout mice die between days 10 and 11.5 due to dramatic genome instability and rapid telomere loss and Human SPAR1 is over-expressed in gastric tumours. Collectively, our work suggest that SPAR1 acts as suppressor of aberrant recombination.

Further support for an anti-recombinogenic function for SPAR1 has come from biochemical studies. Human SPAR1 co-purifies with the critical recombinase Rad51, and is recruited to replication forks via interaction with PCNA. Purified Human SPAR1 can also actively disassemble post-synaptic recombination intermediates in an ATP-dependent manner. Our data indicate that the phenotypes observed in C. elegans, mice and human cells are caused by a failure to counteract inappropriate or persistent recombination intermediates. Furthermore, we suggest that promiscuous disassembly of recombination intermediates is the underlying cause of the genome instability of SPAR1 over-expressing cancers and propose a potential therapy for treating these cancers with a drug currently in clinical trials.

06 July 2008 14:35 - 16:05

PRESIDENTIAL SESSION
APPLIED BIOSYSTEMS – EACR 40TH ANNIVERSARY RESEARCH AWARDS

Signalling and tumour environment

31 Oral

Rac activation and inactivation control plasticity of tumour cell movement

V. Sanz-Moreno[1], G. Gadea[1], H. Paterson[1], C.J. Marshall[1]
[1]*Institute of Cancer Research, Cellular and Molecular Biology, London, United Kingdom*

Background: One of the major discoveries of the last two decades has been the identification of Rho-family GTPases as key regulators of actin dynamics and cell movement. The activity of these GTPases is controlled by activators, guanine nucleotide exchange factors (GEFS) and inactivators, GTPase accelerating proteins (GAPs). How these GEFs and GAPs work together to regulate cell behaviour is a key issue in biology.

Materials and Methods: We carry out the first systematic screen of all known human GEFs and GAPs for Rho-family GTPases.

Results. We identify a GEF-GAP signalling module controlling Rac activity that controls the movement of metastatic melanoma cells. We show that a Rac-GEF interacts with an adaptor protein, that was recently shown to be up-regulated in human tumours and in metastases in a genetically engineered mouse model of melanoma. We show that the complex between the adaptor and the Rac GEF mediates the activation of Rac for cell movement. However, tumour cells can adopt two different modes of movement; a mesenchymal mode where cells have an elongated polarised morphology and an amoeboid mode where cells have a rounded morphology. We show that a series of human melanoma cell lines when cultured on a deformable collagen matrix consist of varying proportions of cells moving in mesenchymal and amoeboid fashions and importantly we show that individual cells within a culture convert between these two different modes. Significantly we show that this inter-convertibility is reciprocally controlled by Rac and Rho. Rac activation through the Rac GEF drives mesenchymal movement and suppresses amoeboid. Rho through activating Rho-kinase activates a Rac-GAP suppressing Rac activation and thereby permitting the high levels of actomyosin contractility required for amoeboid movement. Significantly we show that the expression of the GEF and the GAP determines the way in which different melanoma cells move.

Importantly the data we present is highly relevant to consideration of tumour cell movement in vivo. The biological properties of these different forms of movement provide cells with the ability to cope with different environments in vivo. Mesenchymal movement may be the most fit for rigid tissue environments that require extra-cellular proteolyisis while amoeboid movement in more deformable environments is rapid and its associated high actomyosin contractility provides cells with mechanical strength to deal with shear forces such as following entry in to the blood supply.

Conclusion: Our work leads to the important prediction that tumour cells that can exploit alternative modes of movement may be the most metastatic and that therapies targeting metastasis will have to block both forms of cell movement.

32 Oral

Tumorigenesis-promoting events and signaling by tenascin-C

K. Lange[1], M. Kammerer[1,2], Y. Jia[1,2], A.C. Feutz[1], G. Christofori[1], G. Orend[1,2]
[1]*Institute of Biochemistry and Genetics, University of Basel, Basel, Switzerland;* [2] *Inserm U682, Institute of Development & Pathophysiology of the Intestine and the Pancreas, Strasbourg, France*

BACKGROUND: The ECM component tenascin-C is highly expressed in most solid tumors. Its high expression correlates with a bad survival prognosis in patients with several cancers. Results from cell culture experiments support a role of tenascin-C in enhancing tumor cell proliferation, promoting angiogenesis, invasion and metastasis. We showed that tenascin-C induces cell rounding, which may enhance proliferation and migration, by two mechanisms. Tenascin-C counteracts the tumor cell proliferation-suppressing effect of fibronectin by blocking the integrin alpha 5 beta 1/syndecan-4 complex. This caused cell rounding (Orend et al., 2003, Oncogene 22, 3917) and stimulated tumor cell proliferation (Huang et al., 2001, Cancer Res. 61, 8586) by activation of oncogenic Wnt and MAPkinase signaling (Ruiz et al., 2004, Cancer Res. 64, 7377). Tenascin-C also stimulated endothelin receptor type A (EDNRA) expression, and signaling through EDNRA maintained cell rounding (Lange et al., 2007, Cancer Res. 67, 6163). By using knockdown and over-expression studies, we identified paxillin, RhoA and TM1 as critical targets of cell rounding by tenascin-C downstream of syndecan-4 and EDNRA (Lange et al., 2007, Cancer Res. 67, 6163).

MATERIAL & METHODS: To determine a potential tumorigenesis-promoting effect of tenascin-C in vivo, we generated transgenic mice that ectopically express human tenascin-C in the pancreatic islets. Tenascin-C-transgenic mice, that are apparently healthy and fertile, exhibit normal development of the pancreas, but showed enhanced angiogenesis in the pancreatic islets. Next, we crossed RipTNC mice with tumor-prone RipTag2 (RT2) mice, that develop insulinomas due to ectopic expression of the SV40T-antigen and compared tumorigenesis in RT2/TNC and RT2 mice.

RESULTS: Double transgenic RT2/TNC mice experience more frequent and earlier death incidences than RT2 mice. RT2/TNC mice exhibit several signs of enhanced tumor progression, such as the appearance of local and distant metastasis.