Meeting report

New insights into tumor suppressors

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A report of the Cold Spring Harbor Laboratory meeting 'Mechanisms and Models of Cancer', Cold Spring Harbor, USA, 13-17 August 2008.

The Mechanisms and Models of Cancer meeting held at the Cold Spring Harbor Laboratory this year brought together researchers from all over the world to discuss new advances in the field. Several distinct themes emerged: the use of genetic screens designed to integrate mouse and human cancer data; new insights into the mechanisms of well-studied oncogenes and tumor suppressors; and advances in the area of experimental therapeutics. This report presents a few of the highlights.

Genetic screens to identify novel oncogenes and tumor suppressors

Lynda Chin (Dana-Farber Cancer Institute, Boston, USA) described a strategy for using cross-species comparisons of mouse-human genomic data to search for melanoma tumor suppressors and metastasis mediators. First, gene-expression profiling was used to compare two mouse models of melanoma that differ in their metastatic potential. The list of differentially expressed genes was then used to interrogate human genomic data from primary and metastatic melanomas. Using the human data as a filter, Chin and her colleagues identified a metastatic melanoma signature of 360 genes. Testing these genes in an *in vitro* functional assay of invasion narrowed the list down to 20, most of which had not been implicated in metastasis previously. These 20 genes were shown to be correlated with progression in a variety of human tumor types, and the expression patterns of 12 genes showed high correlation with breast cancer progression as well as being predictive of survival. Lawrence Kwong (Dana-Faber Cancer Institute, Boston, USA) from Chin's group described a related study on the use of comparative genomic hybridization (CGH) data from primary and metastatic melanoma samples to identify chromosomal regions lost in metastatic disease. RNA interference with pooled small hairpin RNAs (shRNAs) was then used to target the genes in these regions and test their ability to decrease tumor latency in an *in vivo* mouse melanoma model. Seven candidate genes were identified and are currently being tested to determine whether they are indeed novel tumor suppressors for melanoma or other tumors. These studies very nicely illustrated the power of cross-species comparisons for novel gene discovery.

Genetic screens can also be designed to investigate particular gene families. Michael Hemann (Massachusetts Institute of Technology, Cambridge, USA) presented work from his lab using a pooled shRNA screen *in vivo* to determine the importance of BCL2 family members in responses to chemotherapy. Using a transplantable B-cell lymphoma mouse model, they compared pre- and post-chemotherapy levels of different shRNAs_in a pooled screen to identify genes involved in chemotherapy resistance. *Bid*, a gene whose protein product participates in the extrinsic death pathway, was identified as a critical mediator of chemotherapy resistance *in vivo*. This effect was not seen *in vitro*, underscoring the importance of *in vivo* studies. Karen Cichowski (Harvard Medical School, Boston, USA) presented an *in vitro* shRNA screen investigating whether members of the Ras-GAP family other than the negative Ras regulator NF1 show tumor-suppressive functions. The family member DAB2IP was identified as a novel tumor suppressor, and in an orthotopic (transformed human cells introduced into the mouse prostate) transplant mouse model for prostate cancer, knockdown of DAB2IP expression was more potent in inducing tumors than was expression of the *H-Ras* oncogene. Loss of DAB2IP also resulted in tumor metastasis in this model. This effect could be attributed to the fact that DAB2IP is a much more potent inducer of the epithelial-to-mesenchymal transition than is Ras.

Novel roles for oncogenes and tumor suppressors

Several talks described novel roles for a variety of oncogenes and tumor suppressors. Gigi Lozano (University of Texas MD Anderson Cancer Center, Houston, USA) presented work using a mouse model to study the function of the mutant tumor suppressor protein p53175H, which carries a mutation frequently found in human cancer. In p53175H mutant mice, which have a gain-of-function phenotype characterized by increased metastasis that is not seen in p53 null mice, p53 was unstable in normal tissues, and only some, but not all, tumors showed p53 stability. To understand the molecular basis of this lack of stability, the mutant mice were crossed with either $Mdm2^{-/-}$ or $p16^{INK4A}$-null mice. In both cases, p53 was stabilized in the progeny. This confirmed that the point mutant p53 is regulated by mdm2 in a similar fashion to wild-type p53. These results demonstrate that p53 stabilization is not synonymous with mutation. Lozano pointed out that this has important clinical implications, as it suggests that loss of the mdm2-p53 interaction may actually help to stabilize the mutant form of p53 and make tumors more aggressive. Importantly in this regard, mdm2-null, p53172H mice have increased metastasis and decreased survival compared to p53172H mdm2 wild-type mice.

Gerard Evan (University of California, San Francisco, USA) presented work from his laboratory showing that there is a threshold of expression that tips the balance between the tumor suppressor and oncogene activities of Myc. In a transgenic mouse model in which ubiquitous Myc expression can be induced by tamoxifen, the phenotype varied depending on the level of expression; mice homozygous for the Myc transgene showed massive cell proliferation in most organs on Myc induction, whereas the heterozygous mice did not. Using a different transgenic mouse in which even higher levels of Myc expression in the pancreas can be achieved, Myc activation resulted in widespread apoptosis in this organ. Evan also presented results suggesting that the level of expression of specific Myc targets may be the explanation for the different outcomes.

The protein kinase Aurora A is frequently found amplified or overexpressed in human cancers, and TerryVan Dyke (National Cancer Institute, Bethesda, USA) presented work from her laboratory aimed at elucidating the roles of the Aurora A family. Using a variety of mouse models with different levels of Aurora A expression, they found that a threshold level of Aurora A is required for the mice to be viable. Upon deletion of Aurora A in serum-starved mouse embryonic fibroblasts, the cells enter, but cannot complete, mitosis. Roughly 30% of these cells had monopolar spindles with an activated spindle-checkpoint response. When Aurora-A-null embryos that did not survive beyond blastocyst stage were examined, monopolar spindles were also present, confirming the *in vitro* studies.

As solid tumors develop, they are subject to decreased oxygen availability (hypoxia). Celeste Simon (University of Pennsylvania School of Medicine, Philadelphia, USA) discussed her laboratory's efforts to understand the cellular response to reduced oxygen availability. This response is largely regulated at the transcriptional level by the hypoxia-inducible factors HIF-1α and HIF-2α. These two transcription factors have distinct effects on cellular self-renewal pathways: HIF-1α expression decreases cell proliferation whereas HIF-2α has the opposite effect; HIF-1α can form a complex with Notch whereas HIF-2α regulates Oct4. Simon's results suggest that these differences are at least partially due to opposing effects of the two HIFs on c-Myc: HIF-1α inhibits c-Myc activity whereas HIF-2α promotes it. She also reported that a hypoxic environment leads to increased Wnt/β-catenin signaling in several stem-cell populations, and that these hypoxia-inducible effects are attenuated upon differentiation.

To determine the relevance of the distinct roles of HIF-1α and HIF-2α in oncogenesis, Simon's group has focused on renal cell carcinoma (RCC). She reported that 30% of RCCs express HIF-2α but not HIF-1α, and that these tumors seem to have increased activation of c-Myc targets that correlates with increased cell proliferation. Furthermore, HIF-2α-positive RCCs also have increased expression of DNA damage response genes, and there is evidence of a decrease in genomic instability. Thus, RCCs can be separated into biologically distinct categories on the basis of VHL (Von Hippel Lindau) status and HIF-1α expression. Simon's results provide a beautiful example of how mouse models, *in vitro* mechanistic studies and analysis of human tumor specimens can be combined to garner new insight into the distinct mechanisms that drive tumorigenesis in histologically similar tumors.

Alicia Cole (Beatson Institute for Cancer Research, Glasgow, UK) presented an analysis in conditional-mutant *APC* mice, aimed at determining why loss of the tumor suppressor *APC* primarily affects the intestine and not other tissues in which it is deleted. Even though these mice showed high levels of loss of *APC* in the kidneys, only a small proportion developed renal cancers. Cole described how, unlike the situation in the intestine, *APC* loss in the kidney apparently induces cell senescence. When these mice were crossed with either p21-null or INK4a-null mice, renal cancers developed more frequently in the progeny because of the loss of the senescence response to *APC* loss. Johannes Zuber (Cold Spring Harbor Laboratory, New York, USA) presented work investigating the different responses to chemotherapy in acute myelogenous leukemias (AMLs) bearing different translocations. Using a mosaic mouse model of leukemia in which the mice expressed either the AML-ETO or MLL-ENL fusions, he and his colleagues found increased survival in the AML-ETO mice after treatment with cyclophosphamide, but no benefit of the same drug in MLL-ENL-induced leukemias.

Gene-expression analysis showed that the treatment resulted in an increase in the tumor suppressor proteins p53 and p21 in the AML-ETO mice, but not in the MLL-ENL mice.

Advances in understanding therapeutic responses

Several groups presented work aimed at increasing the clinical benefit of therapeutic treatments through new insights into how cells respond to such treatments. Seiko Ishida (University of California, San Francisco, USA) described how copper chelators synergize with the chemotherapeutic agent cisplatin in a mouse model of cervical cancer. Cells respond to the copper-chelating agent by increasing the levels of a copper transporter (to make up for the copper deficiency), which in turn allows the cells to take up more cisplatin. The double treatment enhanced the number of cisplatin adducts specifically in the tumors. This work suggests that copper chelators may enhance cisplatin's efficacy without an increase in its toxicity.

Precisely targeted cancer therapies are generally desirable, but some have not proved effective when tested clinically. As one example, inhibition of individual receptor tyrosine kinases is of limited benefit in glioblastoma. Jayne Stommel (Harvard Medical School, Boston, USA) presented work demonstrating that combinations of inhibitors may be more effective. She showed that one possible reason for the lack of benefit of inhibiting the epidermal growth factor receptor (EGFR) in glioblastoma is because other receptor tyrosine kinases are activated and can compensate. Other primary human cancers also showed multiple activated receptor kinases. Targeting of multiple receptor tyrosine kinases in the glioblastoma cells reduced cell viability and inhibited proliferation.

The inhibition of the BCR-Abl oncoprotein, for example by the drug imatinib (Gleevec), is beneficial in chronic myelogenous leukemia (CML) but less so in acute lymphocytic leukemia (ALL). Richard Williams (St Jude Children's Research Hospital, Memphis, USA) showed that the duration of BCR-Abl inhibition in a transplantable mouse model of ALL was dependent on the tumor burden as well as the intensity of treatment. On continuous treatment, most of the leukemic mice spontaneously relapsed, developing B-cell leukemia with point mutations in the kinase domain of BCR-Abl that are known to confer drug resistance. With shorter-duration treatment, however, relapse was primarily seen in the central nervous system, with a low level of the mutation known to confer the greatest drug resistance. James DeGregori (University of Colorado Denver School of Medicine, Aurora, USA) described a shRNA-based screen in CML and ALL cell lines to uncover synthetic lethal interactions in the context of imatinib treatment. Surprisingly, multiple members of the noncanonical Wnt/calcium pathway were implicated in sensitivity to imatinib. Knockdown of members of this pathway in either CML or ALL led to increased sensitivity to BCR-Abl inhibition.

Galina Selivanova (Karolinska Institute, Stockholm, Sweden) discussed a novel small-molecule inhibitor of p53 called RITA. In contrast to the inhibitor Nutlin, which binds directly to Mdm2, inhibiting its interaction with and the subsequent degradation of, p53, RITA directly binds p53, preventing its interaction with Mdm2. RITA was able to induce p53-dependent apoptosis in cell lines and suppress tumor formation. This is in contrast to the effects of Nutlin, which mainly induces a p21-dependent cell-cycle arrest. Interestingly, the authors found that RITA treatment actually results in less p21, both through a novel direct Mdm2-mediated degradation and through Mdm2 degradation of a p53 cofactor known to be important for expression of *p21* mRNA.

Participants at the 2008 Mechanisms and Models of Cancer meeting enjoyed hearing about much new and unpublished work and taking part in lively and informative discussion, and we look forward to the 2009 meeting, which will be held in San Diego.