

Meeting report

Revealing the intricacies of cancer

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A report on the 14th Lorne Cancer Conference, Lorne, Victoria, Australia, 14-17 February 2002.

The 'guardian of the genome', the p53 protein, a key regulator of cell cycle arrest and apoptosis, featured prominently at this year's Lorne Cancer Conference. The plenary seminar was presented by David Lane (University of Dundee, UK), whose research has been central to the transformation of p53 from being considered a weak oncogene to its now clearly demonstrated role as a powerful tumor suppressor. There were also several reports on pathways mediated by another tumor suppressor, the retinoblastoma protein (pRb) as well as updates on an ever-increasing array of proteins and signaling pathways implicated in cancer. We learned of several animal models for cancer and how they may be used to screen for as-yet unidentified oncogenes or tumor suppressors; and we heard about attempts to develop cancer vaccines, p53-targeted tumor therapy, and the use of metallo-proteinase inhibitors in cancer treatment. The following are some of the highlights.

Upstream and downstream of p53 and pRb

The activation of p53 in response to DNA-damaging agents and other forms of stress is part of a protective mechanism that ensures cell-cycle arrest and DNA repair or, alternatively, apoptosis. Many tumor cells lack p53 function, and it seems there is enormous potential for therapy based on restoring p53 function, either directly or by inhibiting the E3 ubiquitin ligase MDM2 that regulates p53 degradation. By switching on p53, therapies would selectively assist tumor cells - but not healthy cells - to undergo apoptosis, because the tumor cells are less responsive to normal environmental survival signals and are generally exposed to more apoptotic stress (for example, hypoxia and oncogenic activation). The importance

of establishing the exact p53 status of the tumor before considering p53-based tumor therapies was emphasized several times at the conference, however. For example, high levels of mutant p53 in cancerous cells could impede the function of introduced wild-type p53 protein, if the mutant protein proves able to act in a dominant-negative fashion.

The plenary talk by Lane focused on how p53 function might be restored to tumor cells, emphasizing that in many cases the p53 gene is not mutated but there is a block in a pathway(s) affecting p53. The MDM2 protein featured as a key target for therapy; in this instance, small peptides that block the p53-MDM2 interaction or that block MDM2 activity could serve to activate wild-type p53. Indeed, only the first 16 amino acids of a natural inhibitor of MDM2, the p14^{ARF} protein, are necessary for p14^{ARF} to interact with MDM2. In cervical cancer, p53 is targeted for degradation by the E3 ligase activity of the human papillomavirus (HPV) 16/18 E6 protein. The drug leptomycin B protects p53 from degradation by E6 or MDM2, probably by blocking nuclear export of p53 and, when used in combination with a second drug, actinomycin D (to inhibit E6 mRNA expression), has the potential to increase p53 levels further. Although leptomycin B has been found to be toxic in clinical trials, lower doses could still prove to be effective in the treatment of tumors that have wild-type p53, in particular in HPV-associated cervical cancer, where topical application could be used. For treatment of tumor cells lacking wild-type p53, Lane proposed mimicking the activity of p53 target genes (for example, p21) as a more appropriate approach.

The p53 protein acts as a transcription factor - but is that all it does? Karen Vousden (National Cancer Institute, Fredrick, USA) revealed what she thinks are the capabilities of p53. Although everyone agrees that p53 can induce the transcription of proteins involved in DNA repair (for example, p53R2 and Gadd 45), in cell-cycle arrest (for

example, p21^{CIP} and 14-3-3) and in apoptosis (for example, PUMA, Noxa and p53AIP1), its apparent ability to induce apoptosis independent of transcription was not expected. The evidence for this independence comes from experiments using a truncation mutant of p53 (Tr210) that is missing the carboxy-terminal oligomerization domain and much of the DNA-binding domain. Tr210 is unable to induce the transcription of several known p53-inducible genes but can, nevertheless, induce apoptosis. Because Tr210 lacks the oligomerization domain, it cannot be overcome by the dominant-negative effect of p53 mutant proteins, that are often highly expressed in cancer cells, and it is also not targeted for degradation by either of the E3 ubiquitin ligases, MDM2 or the viral protein E6. The pro-apoptotic function of Tr210 was mapped to a 37 amino-acid region, raising the possibility of peptide-based tumor therapy. Given that many tumors have lost their ability to stabilize p53, Vousden has begun screening for specific inhibitors that would target the E3 ubiquitin-ligase activity of MDM2 but not that of other E3 ligases.

As it enhances p53 stability, p14^{ARF} is receiving much attention. Intriguingly, p14^{ARF} is encoded by a locus that also specifies p16^{INK4a}, a distinct polypeptide that has attracted equal attention, but this time by virtue of its ability to promote pRb function. Whereas p14^{ARF} acts by inhibiting MDM2-mediated ubiquitination and degradation of p53, p16^{INK4a} inhibits phosphorylation mediated by cyclin-dependent kinase and inactivation of pRb. Gordon Peters (Cancer Research UK, London, UK) has found that dermal fibroblasts from certain melanoma-prone individuals have inherited mutations in both alleles of the *INK4a/ARF* locus that result in loss of expression of p16^{INK4a} but no effect on p14^{ARF} function. The cells are insensitive to growth arrest mediated by the Ras GTPase (a proto-oncogene), and Ras expression allows cells immortalized by over-expression of telomerase to form anchorage-independent colonies in soft agar, although it does not facilitate the growth of these cells as tumors in nude mice, suggesting that additional genetic modifications are required. David Thomas (University of Melbourne, Australia) has found a role for pRb in osteoblast differentiation and cell-cycle exit that relies on the ability of pRb to interact with the osteoblast transcription factor CBFA1, shedding light on the observation that mutation in the *Rb* gene commonly leads to osteosarcoma formation. Further details of p53 and genetic susceptibility to cancer are available with the complete version of this article, online.

Cancer models

Our understanding of cancer has greatly benefited from the generation of mouse models, although they have their limitations. Tyler Jacks (Massachusetts Institute of Technology, Cambridge, USA) reported on a mouse conditional lung carcinoma model, in which adenoviral delivery of the Cre recombinase enzyme to the lungs of genetically targeted

animals carrying *loxP* insertion sites (recognized by Cre) results in the activation of an oncogenic *K-Ras* allele. Tumors develop four weeks after infection with adenoviral vector, even at the lowest doses of virus tested, and result in multiple tumors per mouse. Simultaneous K-Ras activation and expression of a dominant-negative mutant p53 resulted in metastatic tumors similar to those seen in man. Further details of cancer models are available with the complete version of this article, online.

Screening for new tumor suppressors and oncogenes

Reproducing Bloom's syndrome in mice by removal of one copy of the Bloom's syndrome (*BLM*) gene provides more than just a mouse model for a human condition. As Allan Bradley (The Wellcome Trust Sanger Institute, Hinxton, UK) reported, it also provides a very powerful tool for screening for tumor-suppressor genes and oncogenes. The loss of heterozygosity rates in Bloom's syndrome mice is much higher than in wild-type mice, resulting in a ten-fold increase in sister-chromatid exchange, an increase in mitotic recombination and an increased susceptibility to tumors. Combined with arrays of bacterial artificial chromosomes (BACs) that allow comparative genome hybridization (CGH), it is possible to establish where genomic regions are deleted or amplified in tumors. Another mouse model for screening involves Cre-*loxP*-mediated 'megabase deletion', to generate mice with large intra-chromosomal deletions. Although it is not possible to generate mice with deletions in both copies of a given chromosome (because embryonic lethality arises from the loss of too many genes), heterozygous mice can be examined over time to see if they have increased tumor susceptibility; such an increase might suggest the loss of a tumor suppressor in the deleted region that has also spontaneously been lost or mutated on the other chromosome. Once again, CGH can be used to establish which genes are deleted or amplified on the originally intact chromosome. These screens are in progress (in Bradley's group) and have so far detected known tumor suppressors such as *p53* and adenomatous polyposis coli (*APC*) genes.

Using a comprehensive set of screening approaches, including CGH, cDNA microarray technology and antibody- and peptide-based microarrays, Carlos Cordon-Cardo (Memorial Sloan-Kettering Cancer Center, New York, USA) identified several proteins that have altered expression in bladder cancer. Of these, elevated cyclin E resulting from gene amplification was identified as a predictive marker for bladder cancer. Altered expression of moesin, a cell-adhesion molecule, and of Her-2, a member of the epidermal growth factor (ErbB/EGF) receptor family of tyrosine kinase signaling molecules, were also associated with poor prognosis and significantly reduced survival in transgenic mouse models. Further sections discussing signaling pathways, immunosurveillance and cancer vaccines, other markers of cancer,

and metalloproteinases as therapeutic targets are available with the complete version of this article, online.

As we try to unravel the mysteries of tumorigenesis, it is becoming increasingly clear that for every type of cancer - and there are many - deregulation and/or mutation of a specific subset of genes is likely to be involved. The affected genes may include oncogenes that become activated or amplified leading to enhanced cell growth, or tumor suppressors that are lost and fail to oppose it. For every tumor we need to know the precise molecular basis of disease: which genes have been mutated or silenced, and what proteins are over-represented or absent? The importance of 'knowing your tumor' so that individually tailored tumor therapy can be put into place was an emerging theme at the Lorne Cancer Conference and one that seems likely to have an impact in the clinic in the not too distant future.

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The complete version of this article, available online at <http://genomebiology.com/2002/3/6/reports/4015>, includes the following additional information:

Additional discussion of p53, genetic susceptibility to cancer and cancer models.

Additional sections discussing signaling pathways, immunosurveillance and cancer vaccines, other markers of cancer, and metalloproteinases as therapeutic targets.