Meeting report

Cancer and programmed cell death

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A report on the 15th Lorne Cancer Conference, Lorne, Australia, 13-16 February 2003.

The unifying theme of the 2003 Lorne cancer conference was set by Nobel Laureate H. Robert Horvitz (Howard Hughes Medical Institute and Massachusetts Institute of Technology, Cambridge, USA) in the plenary address describing his seminal work on the genetic control of programmed cell death in *Caenorhabditis elegans*. Apoptosis, as he pointed out, is a major feature of normal development, and disruption of this process results in a variety of human disorders, including cancer.

Cell death

The Nobel prize in physiology or medicine for 2002 was awarded to Sydney Brenner (The Salk Institute, La Jolla, USA), John Sulston (University of Cambridge, UK) and Horvitz for their combined work in establishing *C. elegans* as an experimental model organism and elucidating the genetic pathway for programmed cell death. Robert Horvitz extended the work of Brenner and Sulston by identifying the central genes of the cell death (*ced*) pathway, including *egl-1, ced-3, ced-4* and *ced-9*. Loss-of-function mutations in *egl-1, ced-3* and *ced-4* cause the 131 cells normally fated to die by programmed cell death to survive in the adult hermaphrodite. Current work in the Horvitz lab centers on understanding the signals between cells undergoing programmed cell death and the cells that will engulf them. In a typically simple yet powerful screen, *Ced-3* hypomorphic mutant worms have been made transgenic for a reporter construct comprising green fluorescent protein (GFP) under the *lin11* promoter (*lin11::GFP*), which marks ventral cord cells that are fated to undergo programmed cell death. A combination of mutagenesis and direct observation of GFP fluorescence in live worms has led to the identification of a number of mutations in known and novel genes that act in this pathway. Two mutations disrupting the engulfment process have been mapped to the *dpl1* and *MCD-1* genes.

Two major pathways control the caspase-dependent apoptosis of a cell, namely the extrinsic and intrinsic pathways. The dogma in the field has suggested that initiation of the intrinsic pathway of apoptosis, which can be activated in response to cytotoxic stress, such as DNA damage, requires permeabilization of the mitochondria and subsequent formation of the apoptosome, a protein complex made up of cytochrome c, Apaf-1 and caspase-9. Given that cytochrome c release in *C. elegans* and *Drosophila* is not required for apoptosis, however, several groups have hypothesized that some apoptotic pathways are independent of the mitochondria and the apoptosome, and that the mitochrondria serve to amplify but not to initiate the apoptotic caspase cascade.

Yuri Lazebnik (Cold Spring Harbor Laboratory, USA) described the use of the increasingly popular technology of small interfering RNA (siRNA) to inhibit the production of caspase-2. Using siRNA avoids the need to produce gene knockouts and does not appear to produce the anti-viral responses that are triggered by traditional antisense RNA methods. Lazebnik and his coworkers have demonstrated that inhibition of caspase-2 production using siRNA can inhibit the translocation of the pro-apoptotic protein Bax from the cytoplasm to the mitochondria following induction of DNA damage with the drug etoposide. When translocated to the mitochondria, Bax is involved in mitochondrial permeabilization and release of cytochrome c. Thus, caspase-2 activation precedes activation of the well-characterized apoptosome cell-death machinery. One obvious implication of this work is that a detailed study of caspase-2 activation in human tumors may provide insight into the failure of apoptosis *in vivo* and lead to the discovery of new therapeutic targets. Further evidence that caspase activation occurs upstream of the mitochondria and that the apoptosome

functions to amplify the caspase cascade was presented by Vanessa Marsden (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). In order to study the requirement for Apaf-1 and caspase-9 in apoptosis, given that deficiency of Apaf-1 and caspase-9 is lethal to mouse embryos, Marsden and colleagues engineered mice in which only the fetal liver cells are deficient in Apaf-1 and caspase-9 by transferring fetal liver cells deficient in these two proteins into lethally irradiated wild-type hosts. They found that apoptosis in lymphocytes in response to growth-factor withdrawal and stress was largely unaffected by the loss of Apaf-1 and caspase-9. Similarly, apoptosis in embryonic fibroblasts derived from embryos deficient in Apaf-1 and Caspase-9 appeared normal.

One of the most interesting observations made by Lazebnik was that the field of apoptosis research is afflicted with an overwhelming productivity. This is manifest in the more than 10,000 articles that have been published yearly in this field for the last few years. How, Lazebnik asks, are we to assimilate and conceptualize all this information? To answer his own question, he created an analogy of a broken transistor radio. The functional properties of cellular signal transduction can be likened to those of a transistor radio; both are made up of many components, which function together to receive, transduce and transmit specific signals. Could we as biologists, fix a broken radio? Firstly, we might obtain working examples of the radio and sooner or later find that we could remove the back to see inside. We would study it carefully and describe and categorize the size, morphology and coloring of all the components. Next, some scientists might begin a functional analysis by removing specific parts or cutting their connections to other parts. Finally we could arrive at a working model that might help us in diagnosing the problem with the original radio. If the radio is not working due to the lack of one component or a broken connection, or perhaps a fused and discolored component, no problem. We can replace the part and fix the radio. But what if the radio does not work as a result of a number of small changes in several tunable components not apparent in our analysis? We need an electrical engineer with a circuit diagram that describes the precise physical and functional relationships between the components that make up the transistor radio. Likewise, in order to understand cellular signal transduction, perhaps we need to develop a formal language to describe these relationships.

Cytokine signaling

In the Signaling and Cancer session, John O'Shea (National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Maryland, USA) described finding patients with mutations in the cold autoinflammatory syndrome 1 (*CIAS1*) gene; such mutations (of which 20 have been reported) result in a spectrum of diseases, including neonatal-onset multisystem inflammatory disease (NOMID, also known as chronic infantile neurologic, cutaneous, articular, or CINCA, syndrome). O'Shea found that patients with *CIAS1* mutations resulting in NOMID syndrome produce markedly elevated levels of the cytokines IL-3, IL-5, IL-6, IL-1 and the IL-1 receptor antagonist, IL-1Rα. Interestingly, mutations in *CIAS1*, which encodes the protein cryopyrin, a regulator of the transcription factor NF-κB and the processing of IL-1, were found only in approximately 50% of the cases clinically identified as NOMID/CINCA syndrome. O'Shea proposed that heterogeneity in the promoter or intron regions of cryopyrin or cryopyrin homologs may explain the spectrum of diseases in humans with this genetic abnormality. Furthermore, he suggested that it may be possible to alleviate some of the symptoms of this disease by using drugs that block IL-1 signaling.

Dendritic cells are specialized antigen-presenting cells that coordinate immune responses to bacterial and viral pathogens. The development of dendritic cells is controlled both by interactions with the stromal environment, including T lymphocytes, and by cytokines, including those that signal through receptors bearing the signal-transducing γ common chain, which is shared by receptors for IL-2, IL-4, IL-7, IL-9 and IL-15. The Janus kinase Jak3 is an essential signaling component immediately downstream of the γ common chain. Mice deficient in Jak3 show an impaired response to IL-2, IL-4, IL-7 and IL-15. Morgan Wallace (University of Massachusetts Medical School, Worcester, USA) and collaborators have shown that Jak3-deficient mice display a three-fold decrease in $CD8\alpha^+$ splenic dendritic cells whereas $CD11\beta^+$ splenic dendritic cell numbers were normal. $CD11\beta^+$ cells are normally found in the marginal zone of the spleen and are responsible for Th2 T-helper cell responses, whereas $CD8\alpha^+$ dendritic cells are found in T-cell areas of the spleen and are probably the only dendritic cells capable of cross-priming $CD8^+$ T cells. To test whether the decrease in $CD8\alpha^+$ dendritic cells was a haematopoietic-specific defect, bone-marrow chimeras were generated using Jak3-deficient cells. The $CD8\alpha^+$ dendritic cells isolated from these chimeric mice showed a decrease in number and an increase in the expression of the CD40, B7.1 and B7.2 activation markers. Several possibilities have been suggested to account for the observed alterations in dendritic cell populations: activated Jak3-deficient T cells may kill $CD8\alpha^+$ dendritic cells or may provide inappropriate dendritic cell maturation signals; disrupted splenic architecture may also prevent normal maturation. An alternative hypothesis revealed at the conference is that Jak3-deficient T cells produce a three- to five-fold increase in levels of IL-10, providing a possible negative feedback signal for $CD8\alpha^+$ dendritic cell development.

Cancer therapeutics

The conference included a number of notable presentations on current research into cancer therapeutics. The applications

of combinatorial library technology were outlined by Kit Lam (University of California, Davis, USA) in a talk that focused on anti-cancer drug development and cancer proteomics. Combinatorial peptide chemistry is a blossoming field that is increasingly being utilized for the identification of cell-surface ligands and lymphocyte epitopes, and for studies of peptides binding to the major histocompatability complex (MHC), as well as vaccine development. The one-bead one-compound library method was first described by Lam in 1991: each 80-100 μm bead expresses approximately 10^{13} copies of a unique peptide (of which there are up to 10^9 permutations). These peptide-coated beads are capable of binding a desired target, such as an antibody, protein, cell-surface receptor, tumor cell, virus or bacteria. Individual beads bearing a particular peptide can be isolated and sequenced by Edman degradation. This year, Lam described a peptide library screen designed to identify specific peptides that bind to tumor cells but not to normal cells. It is envisaged that these peptide agents could be used to target drugs to tumor cells. Tumor-specific peptides prepared using D-amino acids would be less likely to be rapidly degraded *in vivo* or to induce immune responses and might therefore be optimal as therapeutics.

The conference was the occasion of the inaugural Ashley Dunn oration. For the past decade, the Lorne cancer conference has been chaired by Ashley Dunn (Ludwig Institute for Cancer Research, Melbourne, Australia). The success of the conference during this time is undoubtedly a reflection of the patience, commitment and grace under pressure that has been a characteristic of Dunn's tenure. The Ashley Dunn oration acknowledges the contribution Dunn has made not only to the Lorne Cancer Conference but to fostering research excellence within the Australian research community. Mary-Claire King (University of Washington, Seattle, USA) delivered the inaugural Ashley Dunn oration on the genetic analysis of breast and ovarian cancer in the past, present and future. The first *BRCA1* mutations in families with inherited breast cancer were described by King and others in the early 1990s. These researchers have continued to define the spectrum of mutations present in the *BRCA1* and *BRCA2* genes in familial breast and ovarian cancer and to search for other genes linked to these cancers. Recently, King and co-workers observed that most of the *BRCA1* mutations cause truncation and loss of the carboxy-terminal transactivation domain. They have also identified a number of direct transcriptional targets of *BRCA1*, including the genes encoding Myc and cyclin D1, which are frequently overexpressed in breast tumors. In summary, the 15th annual Lorne cancer conference confirmed that breakthroughs in understanding cancer can be achieved by using the power of diverse yet complementary approaches.