Regulation of Cell Death in Oncogenesis

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Introduction

An AACR special meeting entitled "Regulation of Cell Death in Oncogenesis" was organized by Drs. Eileen White and Stanley Korsmeyer and held from January 26 to 31, 2005. This meeting brought together the top scientists in the field to discuss new developments in the field of programmed cell death and alternate pathways of cell death, autophagy, and necrosis.

Key Effectors of Programmed Cell Death

Nagata gave the Keynote Address, clarifying the role of different DNases in the endonucleolytic cleavage of DNA during apoptosis. Nagata cloned the apoptotic DNase caspase-activated DNase (CAD) in 1998 and generated mice deficient in this enzyme using conventional gene-targeting techniques. Studies on these mice indicated that there are two different mechanisms for the degradation of DNA in dying cells. In the first, DNA is fragmented by CAD; in the second, fragmentation is mediated by the lysosomal DNase II of the engulfing cell. Nagata used cells from these mice to devise a clever screen for molecular components of a downstream event in apoptosis, engulfment. He combined CAD−/− apoptotic thymocytes with macrophages from wild-type mice and screened for monoclonal antibodies that inhibited the appearance of terminal deoxynucleotidyl transferase–mediated nick end labeling–positive cells (which would occur only following engulfment). Two recovered antibodies were detected against the cell surface of apoptotic–positive cells (which would occur only following engulfment).

A key molecule in apoptosis is cytochrome c. Assessment of the overall contribution of cytochrome c to apoptosis has been hindered by the fact that this protein has an essential role in respiration. Using information from yeast indicating that cytochrome c is nonfunctional in apoptosis due to Lys72 methylation, Mak created a knock-in mouse where Lys72 of murine cytochrome c is replaced with alanine. The K72A protein is active in cellular respiration but fails to bind to Apaf-1, and Apaf-1 remains monomeric. Studies on these mice revealed that cytochrome c−/− mediated apoptosis plays a particularly critical role in brain development. Interestingly, Mak finds that thymocytes from these mice still die by apoptosis in response to γ radiation, whereas thymocytes from Apaf-1−/− mice do not. These findings revealed a novel, Apaf-1-dependent, cytochrome c−/− independent pathway of cell death.

The essential role of cytochrome c in apoptosis has been disputed because this molecule seemed dispensable for programmed cell death in the fly. New data from the Stellar lab indicate that cytochrome c−d is required for the normal developmental apoptosis of the Drosophila eye. Stellar also reported on a dual function in death for reaper, which binds and inhibits inhibitors of apoptosis (IAP); reaper not only liberates caspases from IAP inhibition but also causes IAPs to redirect ubiquitylation from caspases to the IAP itself, thereby stimulating their own degradation. Similarly, Meier found that IAPs, in addition to binding to the active site pocket of caspases, can also bind to an IAP-binding motif (IBM) on some caspases that is exposed following processing of the "pro" domain. When IAPs bind to the IBM, the caspase cleaves the bound IAP to expose a free asparagine residue, which is then the target for an E3 ligase that then ubiquitylates and degrades the combined IAP/caspase complex.

Alternate Pathways of Cell Death

Driscoll presented her work on the necrosis pathway in Caenorhabditis elegans. She finds that nectric corpses in the worm are eliminated using the same phagocytic mechanism of engulfment and degradation that eliminates apoptotic corpses; however, the initiation and execution of necrosis is fully independent of genes that carry out apoptosis, and the pathway seems caspase independent. Driscoll finds that necrosis can be induced in the worm by hyperactivation of specific plasma

Note: This report is dedicated to the memory of our colleague, Dr. Stanley Korsmeyer.

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membrane calcium channels, which activate calpains and cathepsin proteases, whereas a calreticulin null mutation or partial loss of function mutations in endoplasmic reticulum Ca$^{2+}$ release channels can suppress necrosis.

Autophagy is a lysosome-dependent membrane-trafficking process involved in the degradation of cytosolic components. Jin presented studies showing that mice with heterozygous deletion of an essential autophagy gene (beclin-1) develop tumors. Interestingly, Jin finds that autophagy-deficient yeast strains and mammalian cells have defects in mitochondrial function and turnover. Jin proposes that the mitochondrial defects in cells with defects in the autophagy pathway may contribute to the increased cancer incidence in beclin-1-deficient mice.

Lieberman identified a novel complex of proteins, resident in the endoplasmic reticulum, that are key targets for proteolysis by granzymes. Interestingly, she finds that cell death by Granzyme A is accompanied by a rapid increase in reactive oxygen species and loss of mitochondrial transmembrane potential; notably, this is insensitive to bcl2 proteins or caspase inhibition. Ley reported on his efforts to determine the basis for cell death mediated by granzymes, using a technique called two-dimensional difference in gel electrophoresis (2D-DIGE). By identifying rapidly cleaved proteins using tandem mass spectrometry, he found that granzymes activate some previously defined caspase cascades and mitochondrial pathways, but they also rapidly target the destruction of cytoskeletal proteins, ribonuclear proteins, and proteins involved in stress responses. The combined data indicate that granzymes use a unique pathway that may be overlapping but is clearly distinct from programmed cell death.

**A Hierarchy among Death Programs?**

White presented her studies on genetically defined epithelial tumors in mice, focusing on tumors that lack the key apoptotic effectors Bak and Bax. White found that Bak/Bax null tumors are highly resistant to the antimicrotubule drug paclitaxel and also that normal cells could be made resistant to paclitaxel by expression of the activated oncogene Ha-ras. White's group found that the Bcl2 family member Bim is profoundly induced by paclitaxel treatment and further that cells with activated Ha-ras have a constitutively phosphorylated form of Bim that is degraded by the proteasome. White then reported that activation of the Akt pathway can induce massive necrosis in Bak/Bax-deficient tumors. She suggests that there is a hierarchy among death programs in the body's defense against tumor formation, with apoptosis as plan A, autophagy as plan B, and necrosis as plan C. This hypothesis may explain why, in genetically defined tumors, apoptosis seems the major form of cell death, but in spontaneous tumors, necrosis predominates because the apoptotic pathway must be disabled during tumor development. Tsujimoto reported that the autophagy genes (Atg5 and Atg6) are required for etoposide and staurosporine-induced death in Bak/Bax null cells, thereby supporting a role for autophagy in the death of cells containing defective apoptotic pathways. Interestingly, he finds that several genes with roles in autophagy are transcriptionally induced during such treatments, suggesting that up-regulation of Atg genes may be a marker for autophagocytic cell death.

**Programmed Cell Death in Tumor Suppression**

Using a myc chimeric protein that can be finely controlled pharmacologically, Evan overexpressed myc in the pancreas of mice and found that myc's apoptotic function dominates over its expansion function. Inhibition of this apoptotic function by overexpression of bcl-xL or knock-out of Bax produces highly aggressive tumors in these mice. Evan found that deletion of the p14ARF protein (part of the p53 pathway) enhances the proliferation burst induced by c-myc, suggesting that there is a p14ARF/p53 growth arrest program that suppresses myc tumorigenesis. Evan finds that this p53/p14ARF growth arrest program can be efficiently blocked by bcl-xL. With his finding that bcl-xL blocks up-regulation of p14ARF by myc, Evan made the interesting finding that the bc2 family member Bim is an early and specific myc target gene and that knock-down of Bim inhibits myc-dependent up-regulation of p14ARF.

Whereas p53 is known to potently induce apoptosis in many cell types, this protein has also been shown to have a protective or survival role in some tumors; this may explain why p53 induces growth arrest in one cell type but cell death in another. Vousden reported that p53 can transactivate the gene JAVA, whose sequence resembles a critical bisphosphatase of the glycolytic pathway. Vousden finds that up-regulation of JAVA by p53 leads to decreased glycolytic rate in cells, along with decreased production of reactive oxygen species, which may ordinarily play a positive role in apoptosis. Consistent with its prosurvival role, she finds that silencing of endogenous JAVA enhances p53-mediated apoptosis.

Two talks highlighted a transcription-independent role for p53 in programmed cell death. Codon 72 of p53 can encode either proline (P72) or arginine (R72). Murphy found that cells with inducible or endogenous R72 undergo a 10- to 15-fold increase in programmed cell death compared with those with P72; this is due to enhanced mitochondrial localization of the R72 variant. At the mitochondria, Murphy finds that p53 interacts with the mitochondrial protein Bak and that p53 can directly oligomerize Bak and induce cytochrome c release. Green reports that p53 can directly oligomerize Bax, both in cells and in cell-free extracts using artificial liposomes loaded with Bax. Because the knock-out mouse for the p53 target gene PUMA has a severe apoptotic defect, Green hypothesized that PUMA may also play a role in the direct mitochondrial pathway of death by p53. He reports that in the absence of PUMA, most of p53 is complexed to the antiapoptotic protein bcl-xL; added PUMA can displace p53, which is then able to oligomerize Bax and initiate cell death. In cells from the PUMA null mouse, Green finds that all of the cytosolic p53 induced by DNA damage is completely complexed with bcl-xL, resulting in an impaired mitochondrial pathway of death.

**Programmed Cell Death in Development**

In C. elegans, neurons of the CEM die during normal development in hermaphrodites but survive in males. Schwartz did a genetic screen for mutations that cause CEM neurons to inappropriately survive in males. He reported on one gene, ceh-30, which belongs to a class of BarH homeobox transcription factors; Schwartz finds that ceh-30 plays an evolutionarily conserved role in survival, and its antiapoptotic function relies on its ability to transcriptionally repress BH3-only genes like egl-1. Bouillet spoke on the role of the proapoptotic protein Bim in programmed cell death and development. It is known that the phenotype of the bc2 knock-out mouse includes polycystic kidney disease, lymphopenia, premature graying, and early death. Bouillet crossed his Bim knock-out mouse with the bc2 knock-out mouse and...
found that loss of a single allele of Bim was sufficient to reverse the polycystic kidney disease of these mice and that loss of both alleles completely abrogated the lymphopenia and graying phenotypes. Interestingly however, in other mouse crosses, he finds that bcl2 has antiapoptotic functions clearly separable from Bim inactivation.

New Therapeutic Modalities

Using a genetically controlled model of myc-induced lymphoma in mice, Lowe showed that overexpression of either bcl2 or Akt greatly enhances tumorigenesis in mice, producing tumors that are pathologically similar. However, whereas both tumor types fail to respond to single agent treatment with either Adriamycin (DNA-damaging agent) or rapamycin [inhibits the Akt/mammalian target of rapamycin (mTOR) pathway], tumors that overexpress Akt are markedly sensitive to combination treatment. Lowe’s finding that rapamycin, which specifically inhibits the translational regulator mTOR, sensitizes tumors reliant on the Akt pathway to Adriamycin suggested that the control of translation may be critical. In efforts to test this hypothesis, Lowe overexpressed either Akt or one of downstream targets of this pathway, the cap-dependent translation protein eIF-4e in myc-driven lymphomas. Whereas he finds that overexpression of eIF-4e approximates that of Akt in tumorigenesis, only the Akt expressors respond to rapamycin used in combination with Adriamycin thus highlighting the importance of tailoring therapy based upon the molecular signature of a tumor.

Rosenberg presented efforts toward the design and implementation of pharmacologic inhibitors of the bcl2 family of antiapoptotic proteins. One such compound is ABT-737, which blocks the intracellular interaction of proteins containing BH3 domains with bcl-xL. Rosenberg reported that ABT-737 potentiates chemotherapy and has single-agent activity against primary lymphoid malignancies, which are historically known to activate the bcl2 pathway. Ashkenazi presented data on the use of tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), a key ligand in the death receptor pathway, as an anticancer drug. Like ABT-737, TRAIL is shown to exhibit single-agent cytotoxicity and to synergize with chemotherapeutic drugs. Wang presented his efforts to create small molecule mimics of the protein SMAC/Diablo, which binds to IAPs and blocks their survival function(s). With the knowledge generated from his laboratory that one mechanism whereby SMAC induces apoptosis is via binding to the tetrapeptide sequence AVPI and displacing caspases, he created a small molecule mimic of this compound in dimer form. This compound, which he dubs compound 32, kills cells in low nanomolar range and synergizes with TRAIL to induce apoptosis.

Energy Requirements of Tumor Cells

In addition to overcoming proliferative and survival restraints, Thompson argues that cancer cells must activate the nutrient uptake pathway for long-term survival; he finds that nutrient uptake can be regulated by the Akt/mTOR pathway, which directly stimulates glycolysis, as well as by Jak/Stat and Pim1. Interestingly, Thompson finds that some cancer cells are more sensitive to certain chemotherapeutic drugs because of their increased glycolytic requirement. Specifically, he states that cancer cells highly reliant on glycolysis are also highly dependent on NAD⁺ and that alkylating agents, which cause activation of the DNA repair protein poly(ADP-ribose) polymerase (PARP), can preferentially kill some tumor cells because PARP activation causes NAD depletion. Danial presented data indicating that the proapoptotic protein Bad integrates glycolysis and apoptosis. Danial purified a complex that phosphorylates Bad at the mitochondria. This complex contains glucokinase, a key component of the glucose-sensing machinery in mammalian cells. Interestingly, Bad is required for this complex to form, and Danial finds that in cells from Bad knock-out mice, there is decreased glucokinase activity and glucose-driven mitochondrial respiration.

Synopsis and Future Research

Significant questions arose during the course of this meeting: What is the nature of the cytochrome c–independent role of Apaf-1 in programmed cell death? Do other genes in the autophagy or necrosis pathway play roles in tumor suppression? With the emergence of autophagy as a pathway that may be tumor suppressive, and potentially overlapping with programmed cell death, comes a dire need for reliable markers for autophagy. The fact that programmed cell death molecules like bcl2 family members also play roles in the control of glycolysis and that the altered fuel requirements of tumor cells may be their Trojan horse, suggests that we need to pay careful attention to this pathway. We are only just beginning to see the fruits of years of research on cell death, with the development of efficacious new molecules that hijack the apoptosis pathway to kill tumor cells, along with efforts to tailor chemotherapy based upon the molecular signature of tumors. It is critical that efforts continue in this regard.

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