Journal of Cellular Biochemistry 60:1–3 (1996)

VIEWPOINT

This series of articles summarizes the current understanding of the increasingly recognized importance of apoptosis in the neoplastic process. The remarkable relationship of apoptosis to many other physiological processes, including development, viral resistance, cachexia, immune responses, cellular generation of reactive oxygen species, and neurodegeneration, is touched on in these reports, as well. An improved understanding of the control of apoptosis therefore has many potential rewards.

The first two articles, by Junying Yuan and David Hockenbery, describe the basics of the cellular control of apoptosis. Junying Yuan describes the central finding that two of the three key genes controlling the programmed cell death pathway in nematodes have been found to be similar to mammalian genes that demonstrate important effects on apoptosis. In each case, a single gene from *C. elegans* has shown similarity to multiple related mammalian genes, suggesting that evolution, while conserving the lynchpins of the program, has enhanced the overall complexity. *Ced-9* is an inhibitor of developmental cell death in nematodes, and a family of related genes, which includes the prototype *bcl-2*, inhibits apoptosis in mammalian cells. However, although *bcl-2* can substitute for *ced-9* in nematodes, they are not completely functionally equivalent, since one specific point mutant of *ced-9* results in a gain of function, whereas the corresponding mutation in *bcl-2* results in a loss of function.

Ced-3 is required for developmental cell death in nematodes, and, as with *ced-9*, similar mammalian genes with similar effects on cell death have been identified. In mammals, these genes form the interleukin-1β-converting enzyme (ICE) family of cysteine proteases, which cleave substrates following aspartate residues. The importance of this family of cysteine proteases in mammalian cell death is suggested by the findings that (1) expression leads to cell death; (2) inhibition by a specific protease inhibitor, crm A, inhibits apoptosis in a standard paradigm of apoptosis, that of the withdrawal of nerve growth factor from dorsal root ganglion neurons in culture; and (3) during apoptosis, there is indirect evidence for ICE activation.

David Hockenbery extends the discussion of *bcl-2* by describing its targeting, homo- and hetero-multimerization, and possible mechanism of action. The Bcl-2 protein does not include a signal sequence, but its hydrophobic tail argues for membrane targeting. Several different approaches have converged on the notion that Bcl-2 is targeted to contact points of the inner and outer mitochondrial membranes, protruding into the cytosol; and to the endoplasmic reticulum. Bcl-2 undergoes homodimerization as well as heterodimerization with other family members, such as Bax. Some of these, such as Bax and Bcl-xs, have antagonistic effects on Bcl-2, leading to an increase in apoptosis. Furthermore, a mutant of Bcl-2 that abolishes heterodimerization but not homodimerization destroys the anti-apoptotic effect of Bcl-2, arguing for the importance of heterodimer formation in the action of Bcl-2.

The mechanism of action of Bcl-2 is unknown, but Hockenbery describes evidence that Bcl-2 may play a role in oxidative metabolism: its localization to sites of oxidative species production, its inhibition of oxidant-induced cell death, the induction of apoptosis by oxidants, the inhibition of apoptosis by antioxidants (both small molecules and proteins), and the development of lipid peroxidation during apoptosis, all argue for this possibility. However, no decrease in the generation of reactive oxygen species was detected, arguing that Bcl-2 may function downstream in an antioxidant pathway to prevent lipid peroxidation and potentially other secondary effects of oxidative stress.

Barbara Osborne follows with a developmental perspective on thymic lymphocyte apoptosis. Self-reactive T lymphocytes are eliminated during development by a process termed negative selection, which induces apoptosis. In order to identify genes involved in T cell apoptosis, Osborne and her colleagues employed a transgenic T-cell receptor mouse whose T cells undergo negative selection following exposure to the ovalbumin peptide 323–336. Following the induction of apoptosis, differential hybridization was

Received October 1, 1995; accepted October 3, 1995.

Address reprint requests to Dale E. Bredesen, Program on Aging, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

used to identify two clones whose expression was induced during apoptosis and two clones whose expression was repressed. *Apt-2,* whose expression was increased during apoptosis, was found to be a previously cloned gene, *nur77,* which encodes an orphan member of the steroid hormone receptor superfamily of zinc finger DNA binding transcription factors. Antisense inhibition of *nur77* expression inhibited apoptosis induced by T-cell receptor crosslinking, suggesting that *nur77* expression is required for this event. A second gene, *apt-4,* was found to be induced later in apoptosis than *nur77.* Sequencing of the cDNA for *apt-4* did not reveal similarity to any sequence in the Genbank database.

Next, Douglas Green and John Reed describe the role of apoptosis in the pathogenesis of cancer. Douglas Green notes that tumor aggressiveness correlates inversely with the degree of apoptosis but directly with the degree of necrosis. Surprisingly, however, no tumor cells that lack the ability to undergo apoptosis have been identified. This is counter-intuitive, given the effects of anti-apoptotic genes such as *bcl-2* on tumor development and resistance to chemotherapy. Green makes the provocative suggestion that molecular players in the apoptosis program may also be required for normal cell survival and proliferation; in this way, the loss of the program, instead of leading to cancer, may lead to an inability of cells to proliferate, or in some cases, to survive. Another possible interpretation is that the program has enough redundancy that it is very rare to inactivate it completely. Whether or not it is capable of complete inactivation with preservation of cellular survival and proliferation, it is clear that key parts of the program can be inhibited by the expression of genes such as *bcl-2* and *v-abl.* This offers potential targets for cancer chemotherapy.

John Reed returns to the action of Bcl-2, addressing it from the standpoint of its induction of resistance to cancer chemotherapy. Traditionally, resistance to cancer chemotherapy has been attributed to effects in one, or a combination of, four areas: drug delivery to the cancer cell, modification of the drug target, increased rate of DNA repair, and diminished rate of DNA (or other macromolecular) damage. Interestingly, *bcl-2* does not appear to affect any of these areas; rather, it defines a new category of chemoresistance genes that regulate the apoptotic response to chemotherapy rather than the initial physical cellular alteration, thus converting cytotoxic drugs to merely cytostatic.

Reed also demonstrates that the expression of *bcl-2* and its antagonistic relative *bax* are regulated by the transcription factor p53, which binds upstream of both open reading frames, leading to enhanced transcription of *bax* but binding to a negative response element upstream of *bcl-2.* He then discusses potential mechanisms of action of Bcl-2, noting effects on mitochondrial calcium accumulation, and two Bcl-2-interacting proteins, Nip-2 and Nip-3, with sequences that have potential calcium-binding sites or have similarity to calbindin-D. Other potential mechanisms, including effects on protease regulation, are discussed.

The final section, which includes contributions from David Lynch, Grace Wong, and Carl Ware, considers apoptosis from the point of receptor initiation, focusing on the tumor necrosis factor receptor superfamily. David Lynch notes the unexpected complexity of Fas-Fas_L (Fas ligand) signalling: apoptosis induction is dependent on cellular activation state, and, far from being involved only in apoptosis, Fas may also play a role in cellular activation and proliferation (this description is reminiscent of the notion that apoptosis mediators are critically involved in cellular proliferation and integrity, from the contribution by Douglas Green summarized above). Decreased activity of this pathway may lead to autoimmune disease due to a paucity of activation-induced cell death, whereas increased activity of this pathway may play a role in the enhanced apoptosis characteristic of T cells isolated from patients infected with the human immunodeficiency virus.

Grace Wong demonstrates that, although tumor necrosis factor receptor I (TNFR I) and Fas are members of the same superfamily of receptors, they signal apoptosis by different, synergistic pathways. Furthermore, stimulation through the TNFRs results in two, apparently opposing results: a decreased sensitivity to radiation and additional TNF, which is mediated by the upregulation of genes such as MnSOD (manganous superoxide dismutase), and an increased sensitivity to radiation, which may be mediated by the production of reactive oxygen species such as the superoxide radical. Stimulation of both the TNFR I and Fas concurrently may offer an approach to apoptosis induction in a large number of tumor cells.

Carl Ware details the evolving complexity of signaling by TNF-related cytokines. In addition to diffusible TNF and lymphotoxin (LT), there are cell-associated forms of LT consisting of heterotrimers of LTα and LTβ subunits, the latter of which anchors the complexes. The most abundant form expressed on the surface of activated T cells is α1β2, which binds not to the TNFRs, but rather to lymphotoxin β-receptor (LTβR). Signalling via the LTβR appears to be required for normal development of the peripheral lymphoid tissue. Studies of signaling via this family have implicated a number of systems, such as NFκB, acidic and neutral sphingomyelinases, serine/threonine protein kinases, phospholipase A_2, G proteins, and others. In the case of TNFR II, the initial events that follow receptor multimerization may involve the TRAF family; since TRAF-2 includes a ring finger domain, the possibility of an essentially direct link from receptor to DNA has been considered. However, the role(s) that the TRAF members play in TNF signaling remain(s) unproven.

In summary, these reports shed light on the role of apoptosis in cancer, from the initiation of neoplastic transformation to the maintenance of the neoplastic state, and ultimately to what is hoped will be effective approaches to eliminate malignant cells.

Dale E. Bredesen

Program on Aging
La Jolla Cancer Research Foundation
La Jolla, CA 92037