A Matter of Life and Cell Death

Gerard Evan and Trevor Littlewood

REVIEW

In multicellular organisms, mutations in somatic cells affecting critical genes that regulate cell proliferation and survival cause fatal cancers. Repair of the damage is one obvious option, although the relative inconsequence of individual cells in metazoans means that it is often a "safer" strategy to ablate the offending cell. Not surprisingly, corruption of the machinery that senses or implements DNA damage greatly predisposes to cancer. Nonetheless, even when oncogenic mutations do occur, there exist potent mechanisms that limit the expansion of affected cells by suppressing their proliferation or triggering their suicide. Growing understanding of these innate mechanisms is suggesting novel therapeutic strategies for cancer.

For a unicellular organism, repair of damaged DNA is the only sensible way to ensure survival. In metazoans, however, the optimal strategy for dealing with cells incurring DNA damage is less clear because certain mutations, those in genes regulating aspects of cell proliferation, adhesion, and apoptosis, are the cause of fatal neoplasms. Accordingly, in many instances, it may be "safer" for the organism as a whole to ablate or incapacitate a genetically damaged cell rather than risk its acquiring neoplastic autonomy. Repair, growth arrest, and cell suicide (apoptosis) are therefore all legitimate metazoan responses to DNA damage, although the choice of fate in each instance will depend on cell type, location, environment, and extent of damage. For example, apoptosis may be the prudent option in damaged cells that retain substantial replicative potential and therefore constitute a neoplastic risk. In contrast, it may be relatively "safe" to attempt repair of postmitotic cells or cells destined for imminent disposal, such as those riding the epithelial conveyer belt of gut or skin. Whatever, an effective response to DNA damage is critical to the long-term survival of multicellular organisms. Accordingly, lesions either in the machinery that senses DNA damage or in the machinery that implements responses to DNA damage greatly predispose to cancer (*1*) (Fig. 1).

A major arm of the mammalian DNA damage response involves a suite of protein kinases, distantly related to the intracellular signaling molecule phosphatidylinositol 3-kinase (PI 3-kinase) (*2*), of which the prototypes are ATM, mutated in the human autosomal recessive disorder ataxia talangiectasia, and DNA-dependent protein kinase (DNA-PK), first identified through its role in V(D)J recombination of immunoglobulin genes and absent in mice with severe combined immune deficiency. ATM and DNA-PK are the mammalian homologs of the yeast Rad-3/MEC1 kinases that likewise mediate DNA damage responses. Both ATM and DNA-PK trigger a plethora of cellular responses that include activation of cell cycle checkpoints and growth arrest, repair, and apoptosis.

p53: The Terminator

One pivotal target of ATM (*3*), and possibly DNA-PK (*4*), is the tumor suppressor p53, a transcription factor normally maintained in abeyance at low levels through interaction with the Mdm-2 protein that signals its degradation. Mdm-2 is itself a target for DNA-PK (*5*). DNA damage–induced phosphorylation of either p53 or Mdm-2 prevents the two proteins from interacting, thus stabilizing and activating p53. The high frequency with which p53 is functionally inactivated in human cancers attests to its pivotal role as a bulwark against expansion of mutated somatic cells. In many cases, p53 itself is mutated or deleted. However, lesions leading to elevated Mdm-2 also lead to p53 inactivation. Mdm-2 is amplified in certain tumors, and the Mdm-2 protein is a target for $p19^{ARF}$, the product encoded by the alternative reading frame within the *Ink4a* tumor suppressor gene locus—a site frequently deleted in human malignancies (*6*). Two cellular responses to p53 activation are well described—growth arrest (in cell cycle stages G_1 and G_2) and apoptosis (see Fig. 1). Which of these two responses prevails seems to depend on cell type, cell environment, and factors such as oncogene expression (discussed below). However, the end points of each of these two processes probably amount to the same thing. Evidence indicates that p53-induced growth arrest after certain types of DNA damage is irreversible (*7*); although alive, such cells are genetically dead and thus constitute no further neoplastic risk.

Substantial evidence suggests that a major part of p53-mediated growth arrest proceeds through induction of the cyclin-dependent kinase (Cdk) inhibitor p21 (*8*). In contrast, the mechanism by which p53 promotes apoptosis is more obscure, although many studies indicate that it involves induction of specific target genes (*9*) that differ from those implementing growth arrest (*10*). Examples of p53 targets implicated in apoptosis are the Bcl-2 antagonist Bax (*11*), the insulin-like growth factor–I (IGF-I) receptor (*12*) and the binding protein IGF-BP3 (*13*), components of the renin-angiotensin system (*14*), and proteins regulating angiogenesis (*15*, *16*). Moreover, in certain circumstances, transrepression of antiapoptotic genes has been implicated (*17*, *18*), and even nontranscriptional mechanisms may be involved (*15*, *19*).

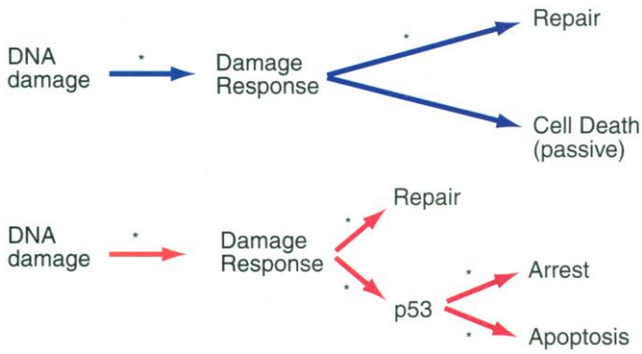

Fig. 1. Classical (**top**) and contemporary (**bottom**) views of DNA damage response. Until relatively recently, severe DNA damage was commonly thought to result in cell death by directly causing intracellular mayhem. Damage was sensed, and death then occurred passively unless the damage was repaired. In this paradigm, the only processes subject to mutation (marked with *) were DNA damage sensing and repair. It is now clear that there is a range of responses to DNA damage, none of which is passive: All involve active responses that can be subverted by mutation. Damage is sensed by a dedicated machinery, and the responses include DNA repair or arrest and apoptosis (here shown mediated by p53, although non–p53-dependent arrest and apoptotic responses probably exist). Mutations can arise in the DNA damage sensors, the repair mechanisms, p53 itself, or the growth arrest or apoptotic response pathways. Each type of mutation may have differing consequences in different cell types.

The authors are at the Imperial Cancer Research Fund Laboratories, 44, Lincoln's Inn Fields, London WC2A 3PX, UK.

p53 is also implicated in cell responses to a variety of insults that do not involve obvious DNA damage, for example, metabolite deprivation, physical damage, heat shock, hypoxia, and expression of oncogenes such as Myc and E1A (*20–22*). The signaling pathways by which such insults activate p53 are mostly unknown, although p19ARF, the protein encoded by the alternate reading frame of the *p16INK4* gene (see above), has recently been implicated in activation of p53 by E1A and Myc (*23*).

Cell Proliferation and Apoptosis

Although the processes of cell renewal and cell death appear to be opposing and mutually contradictory, substantial evidence now indicates that the two are linked. Largely apocryphal data from many early studies indicated that cells harboring activated oncogenes exhibited a high amount of cell death. With the advent of the acceptance of apoptosis into orthodox cell biology, the curious lethality of certain oncoproteins began to receive renewed attention. Early studies concentrated on the apoptotic properties of c-Myc and the adenovirus oncoprotein E1A, both potent inducers of cell proliferation. Since then, however, many differing promoters of cell proliferation have been found to possess proapoptotic activity.

Why should lesions that deregulate cell proliferation induce apoptosis? One early notion was that oncogenes forced cells into "unprepared" cell cycles by overriding cell cycle checkpoints and inducing "mitotic catastrophe." However, this simple idea foundered when elucidation of the genetic and molecular basis of apoptosis indicated that it shared no obvious mechanistic basis with that of cell division. A more enduring explanation of the link between cell proliferation and cell death is that the tendency of cells to undergo apoptosis is a normal consequence of engaging the cell's proliferative machinery—cell proliferative and apoptotic pathways are coupled.

Dying to Proliferate

One of the first oncogenes demonstrated to have proapoptotic activity was c-*myc*. c-*myc* is one of a family of related mammalian genes that encode the Myc proteins, transcription factors of the bHLH-zip family. Deregulated expression of *myc* genes is frequent in cancer (*24*), and substantial evidence implicates Myc proteins in the control of cell proliferation: Myc proteins are expressed in proliferating cells but are absent in quiescent cells. Ectopic expression of Myc is sufficient to drive many cells into the cycle in the absence of external mitogens. However, in certain circumstances, Myc also promotes apoptosis (*25–27*). The mitogenic and proapoptotic properties of c-Myc are genetically inseparable. Both require an intact NH_2-terminal transcriptional activation domain, DNA binding and dimerization domains, and interaction with the Myc partner protein Max (*25*, *28*). However, the precise mechanism by which Myc promotes apoptosis remains obscure. Obvious candidate apoptotic effectors of Myc, given their activation during proliferation, are the Cdks. Indeed, Cdks are activated in apoptosis arising in factor-deprived neurons, during myocyte differentiation and during induction of apoptosis in lymphocytes by granzyme B, tumor necrosis factor (TNF), Fas, or human immunodeficiency virus Tat protein (*29*). However, Cdk activity appears not to be required for Myc-induced apoptosis (*30*). Other candidates include several potential c-Myc transcriptional targets such as ornithine decarboxylase, Cdc25a, and lactate dehydrogenase A (*31*, *32*). However, none of these has been unambiguously demonstrated as essential for Myc apoptosis, and it remains formally possible that Myc promotes apoptosis by a nontranscriptional mechanism.

E1A is the principal growth-promoting oncoprotein encoded by adenovirus. Early hints of the cytotoxicity of E1A arose from the study of adenovirus mutants with defects in E1B that caused rapid destruction of host cell DNA and concomitant cell death (*33*). Later studies revealed that E1A, like Myc, is a potent inducer of apoptosis whose lethal effects must be gainsaid by the antiapoptotic products of the E1B genes for viral replication to succeed (*34*). Both the growth-promoting and apoptotic functions of E1A map to the NH_2-terminal region (*35*) that is involved in binding the pocket proteins, most notably the retinoblastoma protein (Rb), a key regulator of cell cycle progression (discussed below), and the transcriptional corepressor p300 (CBP) (*36*). Interaction with either Rb or p300 or both is required for E1A-induced proliferation and apoptosis (*37*).

Myc and E1A are examples of dominant oncogenes whose deregulated expression drives cell proliferation. However, cancers also arise through inactivation of growth-suppressive pathways, in particular lesions affecting the activity of Rb, which restrains activation of genes necessary for progression through G_1 and into S phase. Phosphorylation of Rb, by a pathway involving cyclin D, Cdk 4, and the Cdk inhibitor p16INK4, eliminates its growth-restraining action, thus allowing cells to enter the cell cycle. Lesions within this Rb regulatory pathway are thought to be ubiquitous in cancer (*38*). A critical function of Rb is to bind and inactivate the E2F proteins, evolutionarily conserved transcription factors represented in mammals by a family of five related proteins. Together with their heterodimeric DP1 partners, E2F proteins regulate expression of genes required for G_1 phase cell cycle progression (*39*). Ectopic expression of E2F abrogates mitogen dependence and induces entry into S phase. As with Myc and E1A, however, this entry into S phase is usually accompanied by apoptosis (*40*) that typically overwhelms any increased proliferative potential.

Mutations in E2F that prevent its interaction with Rb accelerate S phase entry and apoptosis, attesting to the critical role of Rb in restraining E2F action (*41*). Not surprisingly, therefore, inactivation of Rb has broadly similar consequences to deregulation of E2F. Cells lacking Rb exhibit deregulated entry into the cell cycle (*42*), hence the targeting of Rb by viral oncoproteins such as adenovirus E1A, simian virus 40 (SV40) large T, and human papilloma virus (HPV) E7. However, such cells also undergo apoptosis. Rb knockout mice die in utero at embryonic days 12 to 13 and exhibit both excessive cell proliferation and massive apoptosis in various critical tissues, including the nervous system, liver, lens, and skeletal muscle (*43*), all of which express high levels of Rb at that stage of development. Codeletion of another pocket protein, p107, exacerbates this pathology (*44*), indicating a limited degree of functional redundancy among pocket proteins.

Oncogenes as Tumor Suppressors

The propensity for growth-deregulating mutations to induce cell death raises the possibility that apoptosis acts as a "fail-safe" to hinder expansion of potentially malignant cells. In effect, oncoproteins such as Myc, E1A, and E2F may, in certain circumstances, act as tumor suppressors. This dichotomous potential of oncoproteins is appositely illustrated by the phenotype of E2F-1 knockout mice. As expected from the role of E2F-1 in cell cycle progression, such mice show defects in cell proliferation in certain tissues, such as testis (*45*). The limited severity of phenotype probably reflects the redundancy of function among different members of the E2F family. Loss of E2F-1 also substantially suppresses the incidence of thyroid tumorigenesis that occurs in $Rb1^{+/-}$ mice (*46*). However, E2F-1 knockout mice develop dysplasias and lymphocytic hyperplasias (*45*, *47*), in part because of insufficient apoptosis. Most strikingly, E2F-1 knockout mice develop highly aggressive tumors (*45*), implying a bona fide role for the protein as a tumor suppressor.

No *myc* gene has been shown to act directly as a tumor suppressor, probably because there is little redundancy of function among Myc family members in most adult tissues so that cells lacking c-Myc would be unable to proliferate anyway. However, there is much indirect evidence that apoptosis substantially limits Myc's oncogenic activity. Most notable is the acceleration of Myc transgene-induced lymphomagenesis by the apoptosis suppressor Bcl-2 (*48*), arising

specifically because Bcl-2 blocks Myc-induced apoptosis (*49*). Myc-induced apoptosis is also suppressed by survival signaling pathways such as that triggered by IGF-I in fibroblasts or interleukin-3 in myeloid cells (*50*, *51*). In the case of IGF-I, a discrete antiapoptotic pathway routes through Ras, PI 3-kinase, and the serine-threonine kinase Akt/protein kinase B (*50*, *52*), ultimately impacting on Bad, a key modulator of the Bcl-2 family (*53*).

The sensitization to apoptosis afforded by oncogenes explains the remarkable sensitivity of many primary tumors to anticancer agents when compared with their normal counterparts. In effect, cells with deregulated oncogenes are primed for apoptosis, a fact that remains the most substantial Achilles' heel of the cancer cell. Unfortunately, this therapeutic window is eventually eroded by further mutations, some of which disable the cell's apoptotic response. Given the intense focus on neoplastic mutations that deregulate cell proliferation, our parlous knowledge of antiapoptotic mechanisms in cancer is sobering. Such mechanisms would, after all, be superb therapeutic targets because the tumor cell is totally dependent on them for its survival.

Dial p53 for Murder?

An early interpretation of apoptosis induced by Myc, E1A, E2F, or loss of Rb was that it resulted from a conflict between the growth-promoting action of the oncoprotein and simultaneous growth-inhibitory signals (for example, low serum). This notion was somewhat reinforced by the apparent requirement for p53 in oncoprotein-induced apoptosis because p53 is widely accredited as "guardian" of replicative normalcy and general factotum of cellular cataclysm. During normal adenovirus infection, E1A causes accumulation of p53 (*54*), which would trigger apoptosis were it not for the actions of the two E1B proteins p19 and p55. E1B 19K acts as a functional homolog of Bcl-2 (*17*, *55*), whereas E1B 55K binds and inactivates p53 (*54*). If p53 is either absent or inhibited, E1A-induced apoptosis is severely compromised (*23*). This requirement of adenovirus to block p53 for productive infection has recently been exploited for its therapeutic potential (*56*). Normal cells with wild-type p53 are unable to support replication of E1B-defective adenovirus mutants. In contrast, the virus proceeds through a complete lytic cycle in p53-deficient tumor cells (*57*).

An obligate role for p53 in Myc-induced apoptosis is less clear-cut. Mouse embryo fibroblasts derived from p53 knockout mice are refractory to Myc-induced apoptosis (*21*, *58*), and, as with E1A, reintroduction of p53 into p53-negative cells expressing c-Myc rapidly triggers apoptosis (*59*). Furthermore, Myc transgenes induce tumors in mice more effectively in a p53-null background (*60*), implying that absence of p53 confers some advantage to cells expressing c-Myc. However, this advantage need not necessarily imply a direct function of p53 in Myc-induced apoptosis: Loss of p53 substantially increases mutation rates (*61*) that would facilitate the evolution of clonal variants with enhanced growth, irrespective of the underlying mechanism. Indeed, in certain situations, there is clear evidence for p53-independent c-Myc–induced apoptosis in lymphocytes and kidney epithelium (*26*, *62*), and, even in fibroblasts, loss of p53 may only delay, not prevent, apoptosis (*63*).

p53 is also implicated in apoptosis induced by lesions in the Rb pathway. E2F-induced apoptosis is inhibited by dominant interfering mutants of p53, by expression of the p53 inactivator Mdm2, and in cells derived from $p53^{null}$ mice (*64*). Inactivation of Rb by transgenic expression of HPV E7 in mouse lens or of SV40 LT in mouse retina triggers massive apoptosis that is inhibited by expression of the HPV p53 inactivator E6 or when the animals are crossed into a p53-negative background (*65*). Once again, however, the role of p53 in apoptosis resulting from Rb pathway lesions is not always unequivocal. Adenovirus-mediated delivery of E2F1 to postmitotic adult myocardium from p53-deficient mice (*66*) or human breast and ovarian carcinoma cells lacking functional p53 (*67*) results in effective apoptosis. Moreover, several recent studies indicate that mitogenic and apoptotic functions of E2F are separable. E2F-1, -2, and -3 are all effective at promoting cell cycle progression, but only E2F-1 induces apoptosis (*68*), implying that E2F-induced apoptosis is not merely a consequence of its mitogenic potential but a distinct property possessed by one member of the family. Furthermore, E2F-1 mutants lacking transactivation activity are defective for promoting proliferation but still induce apoptosis that is suppressable by Rb (*69*). Thus, deregulated cell proliferation is not a prerequisite for E2F-induced cell death, and, conversely, Rb suppression of apoptosis does not always depend on its ability to restrain cell proliferation. That Rb might be a generalized suppressor of apoptosis is consistent with its ability to suppress cell death induced by interferon, transforming growth factor–β, and p53 (*70*). Furthermore, Asp-X-X-Asp (where X is any amino acid) caspase-dependent degradation of Rb appears to be essential for effective TNF, CD95, and drug-induced apoptosis (*71*). This raises the possibility of an intriguing twist to the causal relation between Rb, p53, and apoptosis. In certain circumstances, loss of Rb function may trigger a cell cycle catastrophe that activates p53-dependent apoptosis. In other circumstances, Rb may serve to suppress default induction of cell death by p53 or other triggers, so that its loss exposes an underlying proapoptotic signal. This potential complexity in the causal relations between growth-regulatory lesions, p53, and apoptosis will be discussed further below.

A Matter of Some Sensitivity

It is difficult to reconcile the idea that oncogene-induced apoptosis results from an intracellular growth conflict with manifold observations that oncogenes also sensitize cells to a wide range of mechanistically different triggers of apoptosis including DNA damage, nutrient deprivation, interferon, protein synthesis inhibitors, hypoxia, TNF, and CD95 (also called Fas or Apo-1) (*25*, *31*, *72*–*74*), many of which exert no obvious direct effect on cell proliferation. Instead, oncoprotein-induced apoptosis may merely reflect the fact that the machineries mediating growth and apoptosis are coupled processes: the dual signal model (*75*). In this model, apoptosis is not caused by any conflict. Rather, activation of the cell proliferation necessarily primes the cellular apoptotic program that, unless countermanded by appropriate survival signals, automatically removes the affected cell. Survival signals are normally provided by neighboring cells, and this ensures that somatic cells remain mutually interdependent for survival and so limits the possible proliferative autonomy of any individual cell. Thus, it is the balance between proapoptotic growth processes and antiapoptotic survival signals that determines whether a cell proliferates or dies.

The idea that growth-deregulating mutations do not themselves induce apoptosis but merely sensitize cells to other apoptotic triggers offers an insight into one of the most curious and enduring features of apoptosis. Even in clonal, synchronized cell populations, apoptosis is asynchronous: What triggers the death of each individual cell is unknown. It is possible that oncoproteins sensitize cells to a variety of minor insults that normal cells resist. For example, low-level sporadic DNA damage, which in normal cells is presumably repaired before the p53 suicide program is implemented, might trigger apoptosis in an oncoprotein-sensitized cell. Another potential and ubiquitous apoptotic signal arises from autocrine and paracrine interactions between members of the CD95 death receptor family and their cognate ligands (*73*, *74*). Myc, for example, sensitizes cells to induction of apoptosis upon ligation of such receptors. In the case of Myc-induced apoptosis in fibroblasts, evidence suggests it is the specific autocrine interaction between CD95 and its ligand that provides the source of the apoptotic signal that Myc recruits (*74*). One further baroque twist may be the emerging relation between DNA-damaging agents, p53, and the CD95 pathway. Ultraviolet irradiation directly activates the CD95 receptor in the absence of ligand (*76*), and both CD95L and CD95 receptor are

induced after DNA damage (*77*), in part through a p53-dependent mechanism (*78*). This observation potentially stands the causal relation between Myc and p53 on its head. In place of the conventional idea that Myc causes p53 to trigger apoptosis (Fig. 2A), Myc acts to sensitize cells to a p53-induced CD95 death signal (Fig. 2B). Such a scheme potentially reconciles many inconsistencies, such as the disparity in data implicating p53 in Myc-induced apoptosis, by positing that the oncoprotein does not itself trigger apoptosis but acts as a sensitizer to whichever trigger is extant. Thus, in a cell harboring activated p53 (for example, because of innate DNA damage or E1A expression), c-Myc would cause apoptosis by raising sensitivity to the p53 trigger. In such cells, p53 would appear to be necessary for Myc-induced apoptosis. Similarly, in situations where CD95 signaling obtains, Myc-induced apoptosis would appear to be dependent on CD95.

Through what effectors might oncoproteins generate sensitivity to apoptosis? A clue comes from the recent isolation of an E1A-induced caspase-activating activity (*79*). Although the constituents of this "oncogene-generated activity" have yet to be characterized, likely candidates are the components of the intracellular "apoptosome"—the complex whose assembly is central to the activation of apoptosis and that comprises the vertebrate homolog of the nematode Ced-4 protein, Apaf-1, holo-cytochrome c (released from mitochondria), deoxyadenosine triphosphate, and pro-caspase 9 (*80*). Activation or accumulation of any of these apoptosome components could generate the requisite oncogene-dependent sensitization.

Interlocking Oncogenes: Together We Stand, Divided We Fall

Proliferation promotes apoptosis, but, in addition, suppression of apoptosis appears, in certain circumstances, to suppress proliferation. One notable example of this suppression involves the Bcl-2 protein. Although elevated expression of Bcl-2 suppresses apoptosis, the affected cells have difficulty entering the cell cycle (*81*), which probably explains why Bcl-2 is by itself such a poor oncogene when transgenically expressed in lymphocytes. Conversely, the proapoptotic Bcl-2 antagonist Bax accelerates cell cycle progression (*82*). The mechanism by which Bcl-2 family members impact on cell proliferation is unclear; however, growth inhibition by Bcl-2 requires critical residues in the NH_2-terminal BH4 domain of the Bcl-2 protein that are dispensable for suppression of apoptosis and, in lymphocytes, correlates with suppression of activation of the NFAT transcription factor. Interference with the CD95 signaling pathway also inhibits cell proliferation. Interference with the action of FADD, a critical intermediary for at least some of the apoptotic signaling initiated by CD95, results in a profound impairment in cell proliferation (*83–85*) that is p53-dependent (*84*).

The Ras oncoproteins provide a particularly interesting example of how proliferative and apoptotic pathways have been entwined. Ras proteins are key transducers of mitogenic signals through their activation of Raf-MAP kinase pathways, a fact attested by the high frequency of Ras-activating mutations in human cancer. Ras proteins are also involved in transducing survival signals from a receptor such as IGF-I receptor to downstream effectors such as PI 3-kinase, Akt, and Bad (*50*, *52*). In principal, therefore, oncogenic mutation of Ras appears able to simultaneously activate cell proliferation and suppress the concomitant apoptosis—a potentially catastrophic combination. Paradoxically, however, when activated Ras is expressed in untransformed cells, it triggers precisely the opposite responses—a profound p53-dependent growth arrest (*86–88*), frequently accompanied by apoptosis (*50*), both of which

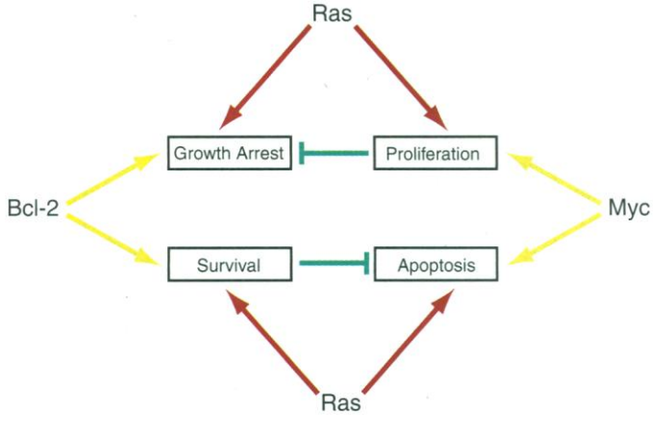

Fig. 3. Interlocking relations between oncoproteins Ras, Myc, and Bcl-2. The innate growth-inhibitory properties of individual oncoproteins mean that only in combinations can they result in productive cell proliferation. When the proteins are activated in a coordinated way, by various converging signals, their growth-inhibitory activities (death of arrest) are gated by the growth-promoting actions of the others. However, when triggered in isolation, as would occur after oncogenic mutation, the growth-inhibitory functions predominate and shut the offending cell down.

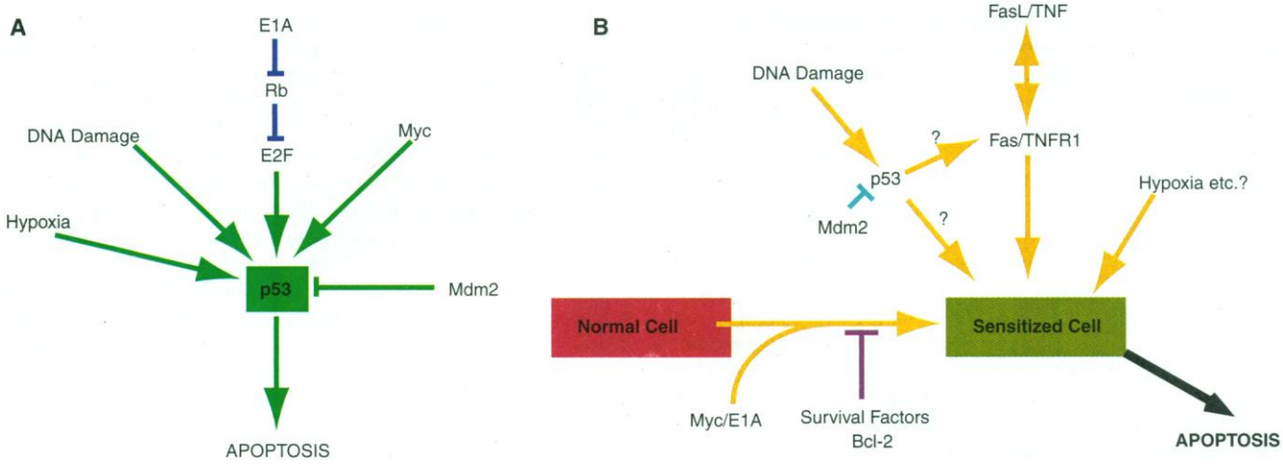

Fig. 2. Two models for the relations between oncogenes and various death signals. (**A**) A conventional model in which p53 acts as the transducer of a variety of diverse signals. (**B**) In this model, it is proposed that growth-deregulating mutations sensitize cells to apoptotic triggers. The models are simplified and not intended to encompass the total complexity of signaling pathways.

actions are mediated by the Ras kinase, Raf (*50*, *87*). Thus, only if these two dominating growth-inhibitory pathways are quenched, presumably through the agency of intersecting signals, can the growth-promoting activities of Ras rise to the fore. Like Myc, Ras bears the seeds of its own destruction.

The growth-inhibitory properties of oncoproteins such as Myc, Bcl-2 and Ras potentially provide an answer to one of the most important and curious medical questions: Why is cancer so rare? Cancer is a formidable and omnipresent threat to all metazoans but one that is counteracted with remarkable efficacy: Even in large, long-lived organisms such as man cancers arise on average less than once in every three lifetimes out of a total of some 10^{17} possible cellular targets. Although Myc, Ras, and Bcl-2 each by themselves have potent growth-promoting ability—cell proliferation for Myc and Ras and cell survival for Bcl-2 and Ras—these potentially neoplastic activities cannot be realized because of corresponding growth-inhibitory activities—apoptosis for Myc and Ras and growth arrest for Ras and Bcl-2. Thus, such proteins exhibit mutual interdependency; each is in some way dependent on the properties of one or both of the other two for its growth potential to be manifest (Fig. 3). A better understanding of the obligate interrelations of such important biological molecules may well provide the basis for more rational cancer therapies in the future.

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The Bcl-2 Protein Family: Arbiters of Cell Survival

Jerry M. Adams and Suzanne Cory

REVIEW

Bcl-2 and related cytoplasmic proteins are key regulators of apoptosis, the cell suicide program critical for development, tissue homeostasis, and protection against pathogens. Those most similar to Bcl-2 promote cell survival by inhibiting adapters needed for activation of the proteases (caspases) that dismantle the cell. More distant relatives instead promote apoptosis, apparently through mechanisms that include displacing the adapters from the pro-survival proteins. Thus, for many but not all apoptotic signals, the balance between these competing activities determines cell fate. Bcl-2 family members are essential for maintenance of major organ systems, and mutations affecting them are implicated in cancer.

Life requires death. Multicellular organisms eliminate redundant, damaged, or infected cells by a stereotypic program of cell suicide termed apoptosis (*1*). Interest in the control of apoptosis has grown exponentially with the recognition of its vital roles in normal development, tissue homeostasis, and defense against pathogens (*2*), and the realization that disturbed apoptosis may contribute to cancer and to autoimmune and degenerative diseases (*3*, *4*). Penetrating genetic analysis of the nematode *Caenorhabditis elegans* revealed two loci, *ced-3* and *ced-4*, essential for programmed cell death during worm development, and a third, *ced-9*, that could prevent their action (*5*). The first mammalian regulator emerged when *bcl-2*, the gene activated by chromosome translocation in human follicular lymphoma (*6*), was unexpectedly found to permit the survival of cytokine-dependent hematopoietic cells, in a quiescent state, in the absence of cytokine (*7*). This discovery, verified in other cell lines and transgenic mice (*3*), established that cell survival and proliferation were under separate genetic control and that disturbances in both were likely to contribute to neoplasia.

The mechanism of apoptosis is remarkably conserved (Fig. 1), albeit with the expected greater complexity in mammals. CED-9 and Bcl-2 proved to be functional and structural homologs (*8*), and their survival function is opposed either by close relatives such as Bax (*9*) or by distant cousins such as mammalian Bik (also known as Nbk) (*10*) and nematode EGL-1 (*11*). The execution phase was illuminated when CED-3 proved to belong to a new family of proteases, now called caspases, whose sequential activation and cleavage of key target proteins dismantles the cell (*12*). Synthesis of caspases as minimally active precursors precludes their premature activation, and the long-mysterious CED-4 and its mammalian homolog Apaf-1 (*13*) are now recognized to be adapters for facilitating the autocatalysis that initiates the proteolytic cascade (*12*).

The growing Bcl-2 family can somehow register diverse forms of intracellular damage, gauge whether other cells have provided a positive or negative stimulus, and integrate these competing signals to determine whether the cell is "to be or not to be." Certain death signals, however, such as those from the CD95 "death receptor" (also known as Fas or APO-1) (*14*), seem largely to bypass the step controlled by Bcl-2 (Fig. 1 and below). Recent insights about the biochemical and biological functions of the Bcl-2 family and its role in neoplasia are the focus of this review. Related issues are addressed in previous reviews (*3*, *4*, *15*, *16*) and the accompanying articles (*12*, *14*, *17*, *18*).

Opposing Factions in the Family

At least 15 Bcl-2 family members have been identified in mammalian cells and several others in viruses (*3*). All members possess at least one of four conserved motifs known as Bcl-2 homology domains (BH1 to BH4) (Fig. 2). Most pro-survival members, which can inhibit apoptosis in the face of a wide variety of cytotoxic insults, contain at least BH1 and BH2, and those most similar to Bcl-2 have all four BH domains. The two pro-apoptotic subfamilies differ markedly in their relatedness to Bcl-2. Bax, Bak, and Bok (also called Mtd), which contain BH1, BH2, and BH3, resemble Bcl-2 fairly closely. In contrast, the seven other known mammalian "killers" possess only the central short (9 to 16 residue) BH3 domain; they are otherwise unrelated to any known protein, and only Bik and Blk are similar to each other. These "BH3 domain" proteins (*19*) may well represent the physiological antagonists of the pro-survival proteins, because programmed cell death in *C. elegans* requires EGL-1 (Fig. 1), which binds to and acts via CED-9 (*11*). BH3 is essential for the function of the "killers," including EGL-1 (*11*, *19*).

Pro- and anti-apoptotic family members can heterodimerize and seemingly titrate one another's function, suggesting that their relative concentration may act as a rheostat for the suicide program (*9*). Mutagenesis established that the BH1, BH2, and BH3 domains strongly influence homo- and hetero-dimerization (*19*, *20*), and the three-dimensional structure of Bcl-x_L provided the explanation (Fig. 3). Coalescence of the α helices in its BH1, BH2, and BH3 regions creates an elongated hydrophobic cleft, to which a BH3 amphipathic α helix can bind (*21*). BH3-cleft coupling, reminiscent of ligand-receptor engagement, may account for all dimerization within the family. Hence, Bax and its analogs may prove to have alternate conformations: one like Bcl-x_L and another with BH3 rotated outside to allow its insertion into the groove of a pro-survival protein (*21*).

Heterodimerization is not required for pro-survival function (*22*), contrary to early indications (*20*). For pro-apoptotic activity, heterodimerization is essential in the BH3 domain group (*19*), but less so for those of the Bax group, which may have an independent cytotoxic impact (below). Indeed whether Bax binds to Bcl-2 inside cells has become controversial, because the detergents used in cell lysis facilitate their association (*23*).

Some death agonists may preferentially target subsets of the death

The authors are at the Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria 3050, Australia.