MINIREVIEW

Regulation of Apoptosis by the Transforming Genes of the DNA Tumor Virus Adenovirus (43631)

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The human DNA tumor virus adenovirus encodes two transforming genes, the E1A and E1B oncogenes, which cooperate to oncogenically transform primary rodent cells. The E1A products efficiently stimulate cell proliferation, but fail to transform cells due to the induction of programmed cell death (apoptosis). Expression of either the E1B oncogene, or the human *bcl-2* proto-oncogene, blocks E1A-associated apoptosis to produce transformation with high frequency. Thus, induction of proliferation by E1A must be coupled to suppression of an intrinsic cell suicide pathway for the efficient transformation of primary cells.

The E1B oncogene encodes the unique 19-kDa and 55-kDa proteins, both of which independently suppress apoptosis and greatly enhance transformation by E1A. Suppression of apoptosis by the E1B 19-kDa protein is required not only in transformation of rodent cells with E1A, but also in adenovirus-infected human cells, where it sustains cell viability to maximize virus production. The E1B 19-kDa protein has the additional ability to block apoptosis induced by tumor necrosis factor (TNF)-α, and anti-Fas antibodies, potentially contributing to escape from antiviral and anticancer immune surveillance.

What E1A does to trigger apoptosis and the mechanism utilized by E1B 19-kDa and 55-kDa proteins and Bcl-2 to suppress apoptosis is of fundamental importance to understand how programmed cell death is regulated. It is now clear that the p53 tumor suppressor gene product mediates apoptosis by E1A, and that the E1B gene encodes independent, overlapping functions to disable p53-mediated apoptosis. The E1B 55-kDa protein binds to and inhibits p53 directly, but the mechanism by which the 19-kDa protein interferes with p53 function is not yet known.

In this review, I will discuss the means by which these conclusions were derived and how the transforming genes of adenovirus can be utilized to ascertain the molecular basis by which apoptosis is regulated. It is becoming increasingly apparent that intracellular defense against virus infection and cancer requires regulating both cell death and proliferation. Loss of p53 function is the most frequent event in human cancer. Determining how the transforming proteins of DNA tumor viruses subvert p53 function will provide insight into the cause and prevention of cancer and for developing new strategies in anticancer therapy.

E1A and E1B Transforming Genes of Adenovirus

The DNA tumor virus adenovirus normally infects and replicates in human cells by recruiting the host cell transcription, translation, and DNA replication machinery for the purpose of synthesizing viral proteins and DNA (reviewed in [1]). With the exception of laboratory growth conditions, adenovirus normally encounters and infects quiescent or nondividing cells. To facilitate the production of viral products, the host cell is recruited from the quiescent state to one of proliferation so that the cellular synthetic machinery is available to be used by the virus. The viral early genes encoded within Early Region 1 (E1) are largely respon-

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sible (reviewed in [2, 3]). In rodent cells that are semipermissive for adenovirus replication, oncogenic transformation is a byproduct of this deregulated growth control. The viral genes required for transformation by adenovirus are the E1A and E1B genes encoded within E1. These adenovirus transforming genes represent an excellent model system for determining which cellular processes are essential for normal growth regulation and how their deregulation leads to oncogenic transformation.

The products of the E1A gene recruit cells into a proliferative state by intervening in host cell growth control pathways. The E1A proteins comprise three conserved functional regions, Cr1, Cr2, and Cr3 encoded by 13S and 12S mRNA species (reviewed in [4, 5]). Cr1 and Cr2 are required for transformation and encode the binding sites for a number of cellular proteins (6, 7). Cr2 serves as a binding site for the product of the retinoblastoma (p105 Rb) susceptibility gene, a tumor suppressor gene whose loss of function is oncogenic (8). Another Cr2 binding protein is Rb related (p107) and is likely to function similarly. Rb is normally found in association with the transcription factor E2F (reviewed in [9]). The association of E1A with Rb displaces and activates the transcriptional activity of E2F, suggesting that E2F may act positively to turn on the expression of cellular genes required for cell proliferation. Still other cellular E1A-associated proteins, such as p60-cyclin A and associated kinases, are regulators of cell cycle control (10). Cr1 overlaps the binding site for the p300 cellular DNA binding protein, whose function has not yet been determined (11). Both Cr2 and Cr1 possess the capacity to induce cellular DNA synthesis in quiescent cells and can modulate transcription, both negatively and positively (reviewed in [1, 12]). Therefore, E1A likely activates cell proliferation by modulating transcription and cell cycle progression.

Despite the capacity of E1A to recruit cells from G_0 into S phase of the cell cycle (13, 14), E1A function is insufficient to transform primary rodent cells. Transformation by E1A requires a second function supplied by a cooperating oncogene such as the E1B gene (15). E1B encodes two distinct polypeptides, the 19-kDa and 55-kDa proteins, in different but overlapping reading frames with independent transforming activities (16–18). Thus, E1B encodes two separate transforming functions, either of which is sufficient for transformation with E1A (19). The E1B 55-kDa protein functions in transformation by binding to and inhibiting the p53 tumor suppressor gene product (20, 21). Although the transforming activity associated with the E1B 19-kDa protein is formidable, no function can be extrapolated from its amino acid sequence, nor does it possess any known biochemical activity or associations with cellular proteins that might suggest a function. The contribution of the E1B 19-kDa protein to the transformation process is, therefore, likely to be unique.

Induction and Suppression of Apoptosis by E1A and E1B in Adenovirus-Infected Human Cells

The function of E1B 19-kDa protein was initially approached from the study of adenovirus mutants that express a nonfunctional E1B 19-kDa protein. The absence of the E1B 19-kDa protein during productive infection of human cells induces the degradation of host cell and viral DNA (*deg* phenotype) and enhanced cytopathic effect (*cyt* phenotype) (22–24). Since DNA fragmentation does not normally occur in uninfected cells, this suggests that another viral gene product is cytotoxic and that the E1B 19-kDa protein is required to prevent these cytotoxic effects. A genetic analysis of a series of adenovirus mutants mapped the functional region responsible for the induction of DNA fragmentation to E1A Cr1 (25, 26). Thus the E1B 19-kDa protein is required to block the induction of DNA fragmentation induced by E1A. Without this function, the infected cell dies prematurely and virus yield is severely compromised (23, 27, 28). This survival maintenance function of the E1B 19-kDa protein is advantageous to the virus because the host cell is kept alive as long as possible and virus production is maximized. More importantly, however, the requirement for the E1B 19-kDa protein to prevent DNA fragmentation and death of the host cell suggests that the 19-kDa protein functions as an inhibitor of apoptosis (26).

Cell death can occur either by necrosis or apoptosis. Necrotic cell death is usually associated with physical injury and cytoplasmic destruction predominates. Apoptosis has been proposed to be an active process whereupon a cell makes a decision to commit suicide in response to the presence or absence of environmental stimuli (reviewed in [29–32]). Destruction of the nucleus, particularly the fragmentation of chromosomal DNA into nucleosome-size fragments, and the condensation of chromatin are indicators of apoptosis (33). The observations that E1B 19-kDa mutant adenovirus-infected cells degrade their DNA and die inappropriately suggest that E1A expression was triggering apoptosis and that the function of the E1B 19-kDa protein may be to inhibit apoptosis (26). These functions are important for sustaining a normal productive infection by adenovirus since viruses that lack either of these activities are impaired (26). Furthermore, the requirement for both E1A and E1B function to produce oncogenic transformation indicates that regulation of apoptosis may be a component of that process as well.

E1B 19-kDa Protein Inhibits Apoptosis Mediated by TNF-α and Fas Antigen

Apoptosis or programmed cell death occurs in a variety of different circumstances, some essential for

development (34), for regulation of the immune system (reviewed in [30]), and, perhaps, for the selective elimination of abnormal, infected, or transformed cells. If the E1B 19-kDa protein functioned as an inhibitor of apoptosis, which was indicated from the study of adenovirus-infected cells, then it might inhibit apoptosis in other physiologic circumstances. TNF-α is a tumoricidal cytokine that will induce death of many transformed cell lines (reviewed in [35]). TNF-α exhibits antiviral activity and may represent an component of the immune system's response to viral infection. There are two TNF-α receptors that are members of the nerve growth factor, Fas antigen, and CD40 receptor family (36, 37). Both TNF-α and anti-Fas antibodies are potent inducers of apoptosis (36, 38). The function of Fas antigen is important for negative selection by apoptosis in the thymus, and the absence of this function causes autoimmune disease in mice (39). Interestingly, E1A expression confers supersensitivity to TNF-α (40–43), suggesting that both may function on a convergent pathway to induce apoptosis (reviewed in [44]). If TNF-α does act in the same apoptotic pathway as E1A, then the E1B 19-kDa protein might inhibit cytolysis by TNF-α, and perhaps Fas.

E1B 19-kDa protein expression blocks DNA fragmentation and viability loss following treatment with TNF-α (45–47). Resistance to TNF-α was conferred by E1B 19-kDa expression by infection with virus, utilizing E1B 19-kDa plasmid expression vectors in transient expression assays, or in stable cell lines (47). Similar resistance to cytolysis by anti-Fas antibodies is also conferred by E1B 19-kDa expression (46). Therefore, the E1B 19-kDa protein functions as an inhibitor of apoptosis in multiple circumstances. By doing so, this function may provide an advantage to adenovirus by contributing to escape of host immune surveillance mechanisms mediated by cytotoxic cytokines such as TNF-α and the Fas antigen ligand.

Induction and Suppression of Apoptosis by E1A and E1B During Transformation of Primary Rodent Cells

In a standard primary baby rat kidney (BRK) cell transformation assay, E1A expression is efficient at initiating focus formation; however, proliferation fails to be sustained, causing foci to degenerate and die (19, 47). These observations suggest that the obstacle to transformation by E1A derives not from insufficient stimulation of proliferation, but from a failure to block death. With coinduction of proliferation and death, cell loss through apoptosis counteracts any net increase in cell number through cell division (47). Rare E1A-immortalized clones do arise after prolonged culture (47). The clones that survive have been selected to overcome cell death and may have sustained a mutation either in a cellular gene or E1A that would enable escape from the apoptosis program. Identification of these genetic events that suppress apoptosis should provide further insight into the molecular mechanism of cell death (see below).

Coexpression of either E1B protein with E1A in a BRK cell transformation assay does not substantially increase the frequency of initiation of transformation (19, 47). Rather, cell death that would normally occur is abrogated and transformants arise with high frequency (19). Thus, transformation can be divided into two distinct phases that can be distinguished based on differential requirements for E1A and E1B expression. Initially, E1A is exclusively required to initiate focus formation by stimulating proliferation. E1B has no measurable capacity to do so, nor does E1B expression substantially increase the frequency with which E1A stimulates focus formation. The second, subsequent event, which is exclusively E1B dependent, is required to sustain proliferation by suppression of cell death (19, 47). Both the E1B 19-kDa or 55-kDa proteins can provide this function, although the 19-kDa protein is, by far, more effective (19).

E1A and *bcl-2* Cooperate to Transform Rodent Cells

If failure to overcome apoptosis significantly impedes transformation with E1A alone, then specific inhibition of apoptosis by means other than E1B expression would be predicted to complement E1A in a transformation assay. The *bcl-2* proto-oncogene has been demonstrated to function by suppressing apoptosis in other systems. It is most commonly translocated in human B cell lymphomas, resulting in overexpression of the normal Bcl-2 protein (48–50). The apoptosis inhibiting function of the Bcl-2 protein was derived from the observations that its overexpression results in suppression of apoptosis in hematopoietic lines (51–53), extends the normal life span of B cells, and promotes transformation (54–56). Bcl-2 is expressed in proliferating or long-lived cells of tissues associated with apoptotic death, suggesting that Bcl-2 may normally function as an inhibitor of apoptosis in growth and differentiation (57).

Cooperation between E1A and Bcl-2 in transformation was assessed in primary BRK cells. Focus formation by E1A was substantially enhanced by cotransfection of E1A with *bcl-2* expression vectors (19). Furthermore, all transformed cell lines derived from transfection of E1A plus Bcl-2 plasmids overexpress the human Bcl-2 protein (19). Collectively, these results demonstrate that E1A expression is associated with the induction of apoptosis during transformation. E1B gene products, predominantly the 19-kDa protein, or overexpression of the Bcl-2 protein, can inhibit apoptosis and rescue transformation. Furthermore, this replacement of E1B 19-kDa protein function by Bcl-2 is not restricted to transformation. Bcl-2 will complement the

function of an E1B 19-kDa gene deletion in adenovirus-infected cells, providing further evidence for functional equivalency (S. K. Chiou and E. White, manuscript in preparation).

Genetic Evidence for a Single Function of the E1B 19-kDa Protein

A mutational analysis of the E1B 19-kDa protein was initiated as a means for identifying discrete functional domains and for establishing the relatedness of functions. Comparison of the E1B 19-kDa sequence from 11 different adenovirus serotypes has revealed tripartite conservation. The protein consists of a highly conserved central region, a moderately conserved amino-terminal region, and a poorly conserved carboxy terminus (47). All amino acid substitutions through the highly conserved central region produced a loss of transforming activity (47; C. C. Tseng and E. White, manuscript in preparation). When the ability of the various E1B 19-kDa mutants to inhibit apoptosis by TNF-α and Fas was determined, mutations producing loss of transforming function also produced loss of ability to block apoptosis by TNF-α and Fas. Conversely, mutants that retain transforming function retain the ability to block apoptosis by TNF-α and Fas (47; C. C. Tseng and E. White, manuscript in preparation). This inability to genetically separate transforming activity from TNF-α and Fas resistance strongly argues that both result from the same function of the protein. This supports our proposal that the single act of blocking programmed cell death can account for the function of the E1B 19-kDa protein in infection and transformation (19, 26, 47).

Wild-type p53 Mediates Apoptosis by E1A

Analysis of E1A mutants has indicated that induction of apoptosis is probably an indirect consequence of an E1A function required for transformation (26). Although this analysis is not yet complete, deletion of an amino-terminal region of E1A that is required for induction of DNA synthesis and transforming activity is also required for induction of apoptosis (26). The cellular apoptotic machinery is activated by this E1A activity to kill the cell. There was some suggestive evidence that the tumor suppressor protein p53 may be responsible for directing apoptosis in response to growth deregulation by E1A. First, the E1B 55-kDa protein, which can conditionally overcome apoptosis by E1A, functions by direct physical interaction with and inactivation of p53 (20, 21). Creating a p53 "null" phenotype by expression of the E1B 55-kDa protein will, therefore, enable E1A to be expressed without the induction of apoptosis (19). Second, reintroduction of a wild-type p53 into a myeloid leukemic cell line that had lost both p53 alleles induced apoptosis (58). However, in similar experiments with other transformed cell lines, wild-type p53 induced growth arrest at G_1/S (59–61). This suggests that in some circumstances, p53 expression could result in cell death by apoptosis.

The requirement for wild-type p53 function in E1A-associated apoptosis was addressed with a dominant-interfering, temperature-sensitive mutant allele of p53. The p53(val135) protein has a single amino acid substitution and is predominantly in the mutant conformation at 38.5°C (restrictive temperature), and in the wild-type conformation at 32°C (permissive temperature) (61). By utilizing this dominant-negative mutant p53, the tumor suppressor activity of the endogenous wild-type p53 in the primary BRK cells would be rendered nonfunctional at the restrictive temperature. Transformation assays in primary BRK cells were performed with the expectation that if E1A-associated apoptosis was dependent on possessing functional p53, then E1A and mutant p53 should cooperate to produce transformation. When cotransfected into primary BRK cells with E1A at the restrictive temperature, p53(val135) suppresses all signs of apoptosis and enhances the transforming activity of E1A by an order of magnitude (62). This result demonstrates that by blocking p53 function, E1A-associated apoptosis could be suppressed during transformation of primary rodent cells (62).

If p53 function is required for apoptosis in transformed BRK cells, then induction of apoptosis might occur upon return of p53 to the wild-type conformation. When E1A plus p53(val135) BRK transformants are shifted from the restrictive to the permissive temperature, dramatic loss of viability occurs, accompanied by the fragmentation of DNA and chromatin condensation (62). Thus, p53 will inhibit apoptosis when in the mutant conformation and induce apoptosis in the wild-type conformation. This indicates that p53 is the molecular "switch" to initiate apoptosis upon E1A expression and transformation. Because wild-type p53 functions as a tumor suppressor and will suppress transformation by E1A and *ras* (63), it is tempting to speculate that all or part of the p53's tumor suppressor activity is related to the induction of apoptosis.

E1A Immortalization Is Accompanied by Mutations in p53

Transfection of primary BRK cells with E1A in the absence of a cooperating oncogene results in apoptosis, but after prolonged culture E1A-immortalized clones arise with low frequency (47). These transformants show no signs of apoptosis and most likely have sustained mutations in a cellular gene to disable the apoptotic program. As the affected genes are predicted to be mediators of apoptosis, the identification of these mutational events should provide insight into the mechanism of cell death. The suppression of apoptosis by a dominant-interfering mutant of p53 indicates that p53

mutations could be one mechanism to bypass the apoptosis program. Examination of the p53 status in a collection of E1A-immortalized BRK clones has revealed p53 mutations with high frequency (L. Rao and E. White, in preparation). The p53 mutations sequenced so far correspond to mutations that have been reported previously with high frequency in human tumors (reviewed in [64]). Thus, immortalizing primary cells with E1A selects for rare clones that have suppressed apoptosis and have sustained mutations in p53. These results are in agreement with another study in which immortalization of murine fibroblasts is accompanied by p53 mutations (65).

E1B 19-kDa Protein Blocks Apoptosis Induced by Wild-type p53

To assess the ability of E1B 19-kDa protein to prevent p53-mediated apoptosis, the E1B 19-kDa gene was introduced into a E1A plus p53(val135) transformant at the restrictive temperature. Two independent 19-kDa-expressing lines were obtained. Upon shift down to the permissive temperature, wild-type p53-dependent viability loss is prevented in E1B 19-kDa-expressing lines, but not in control lines (62). Therefore, expression of the E1B 19-kDa protein is sufficient to block apoptosis upon the return of p53 to the wild-type conformation in transformed cell lines. Thus, the E1B 19-kDa protein has been identified as a modifier of p53 function (62). Interestingly, wild-type p53 still had some capacity to suppress growth in the 19-kDa-expressing transformants, suggesting that growth suppression and apoptosis functions of p53 may be separable (62).

Apoptosis May Be the Normal Cellular Response to Inappropriate Deregulation of Growth Control

Mutations of p53 are among the most common genetic alterations found in human cancer (64). Mice engineered through homologous recombination to have lost both p53 alleles develop normally, indicating that p53 is not essential for normal growth and development in the mouse (66). The p53 null mice and humans heterozygous for germline mutations in p53 possess a greatly elevated incidence of tumor formation (66, 67). Thus, the most conspicuous function of p53 is that of a tumor suppressor. This function is important, if not the most important, defense against cancer and is the subject of intense investigation by many laboratories.

One approach to elucidating p53 function has been to reintroduce the wild-type p53 gene into transformed cells that have lost it. When this is done, p53 will either suppress growth, causing a cell cycle block at G_1/S (59–61), or induce apoptosis (58, 62, 68, 69). It is not yet clear why p53 acts differently in these situations. One explanation is that the growth arrest and apoptotic functions of p53 are separable and/or depends on the cellular factors. Some transformed cell lines may even have sustained mutations, merely by virtue of being selected for growth in culture, making them resistant to the induction of apoptosis by p53. Nonetheless, these studies suggest that p53 can act as a tumor suppressor in two possible ways, by inducing growth arrest or death, depending on the circumstances.

p53 is normally expressed at low levels due to a short half-life in most, if not all, normal cells (reviewed in [70]). Low level p53 expression must not be detrimental under most circumstances. The p53 protein levels in cells are dramatically increased by agents that damage DNA (71, 72). Normally, cells respond to moderate amounts of DNA damage by cell cycle arrest concomitant with transient p53 accumulation, followed by repair and reentry into the cell cycle (72, 73). Transformed cells either lacking p53 or expressing mutant p53 do not arrest in response to DNA damage and continue cycling. Therefore, p53 may act as cell cycle checkpoint to sense DNA damage and prevent entry into S phase until DNA repair is complete (72, 73). One prediction from these observations is that cells lacking wild-type p53 function would accumulate genetic alterations at an accelerated rate. In support of this prediction, loss of p53 function is associated with gene amplification events, substantiating a role for p53 in regulating genomic stability (74, 75).

p53 may function differently by triggering apoptosis under conditions of irreparable DNA damage, as would exist in many transformed cells and perhaps in virus-infected cells. Introduction of DNA damage will cause apoptosis (reviewed in [30]). One prediction is that cells lacking normal p53 function would be resistant to induction of apoptosis in response to DNA damage, whereas those retaining p53 function would die. This alternate pathway to growth arrest and repair for induction of cell suicide would permit selective elimination of abnormal, damaged, or infected cells, and would enhance the survival of the host (62).

E1A expression will mimic DNA damage by inducing p53 accumulation (76, 77; L. Rao and E. White, in preparation). It is possible that by forcing abnormal proliferation, E1A may inadvertently cause the accumulation of DNA damage (78, 62). p53 may respond in turn by accumulating and inducing apoptosis. Alternatively, p53 may respond directly to perturbation of growth control by E1A by inducing apoptosis. That is, induction of growth arrest by p53 may be incompatible with simultaneous growth stimulation by E1A, thereby resulting in apoptosis (62). Interestingly, amplification of c-*myc* proto-oncogene expression, which is associated with similar growth-promoting activities as E1A, has been shown to induce apoptosis (79). *bcl-2* will suppress c-*myc*-induced apoptosis and collaborate with c-*myc* in a transformation assay (52, 80, 81), analogous to our observations with E1A and *bcl-2*. (19). Additional functional similarities between E1A and c-*myc*

(82, 83) suggest that some common aspects of growth deregulation may be associated with induction of apoptosis.

Regardless of whether apoptosis results from deregulation of cell growth or some downstream consequence thereof, transformation may require the induction of proliferation to be coupled to suppression of apoptosis (62). Until recently, oncogenic transformation was thought to be the process by which controls on cell growth were perturbed in such a way that proliferation was sustained inappropriately. However, the products of some oncogenes (E1A, and c-*myc*) that induce proliferation also induce death and are insufficient for transformation. If cell death is inhibited, a function supplied by another category of oncoproteins (*bcl-2*, E1B 19 kDa, and E1B 55 kDa), transformation can take place efficiently. The multistep process of transformation may, therefore, result from deregulation of cell growth coupled to suppression of cell death (19, 47, 84). Finally, evidence is accumulating for a role of the product of the p53 tumor suppressor gene in regulation of apoptosis. The observations that wild-type p53 can induce death when expressed in infected or transformed cells and that the loss of the p53 function can lead to cancer have suggested that cell death may be a defense at the cellular level against cancer and viral infection. Thus, regulation of apoptosis may be an important aspect in the development of cancer and in an integral aspect of the function of oncogenes and tumor suppressor genes.

The mechanism by which the E1B 19-kDa protein interferes with the ability of p53 to induce apoptosis has not yet been established, nor is it known whether the transcriptional and growth arrest functions of p53 are similarly altered. The 19-kDa protein could interfere with the p53 protein by direct by physical association, analogous to other DNA tumor virus-transforming proteins that produce inactive complex formation with p53 contributing to its sequestration (reviewed in [85]) or degradation (86). An alternate possibility is that the 19-kDa protein acts upstream of p53, preventing the induction of p53 levels in response to E1A, and thereby apoptosis. On the other hand, the 19-kDa protein may act downstream of p53 by altering chromatin structure, inhibiting the endonuclease, or providing a "second signal" analogous to the mechanism of apoptosis inhibition proposed for growth factors (reviewed in [31]). The 19-kDa protein has been localized to the nuclear envelope, where it could potentially influence nuclear events and signal transduction (87). Identification of the cellular proteins that associate with the 19-kDa protein *in vivo* and mediate its biologic activity may resolve these issues.

Apoptosis involves multiple pathways and is a multistep process. For example, the adenovirus E3 gene products will block apoptosis by TNF-α but not E1A (reviewed in [44]), nor will Bcl-2 block all forms of apoptosis (56, 89). The availability of numerous inducers and inhibitors of apoptosis will permit the identification of the different pathways and the points at which regulation occurs. The E1B 19-kDa protein possesses the capacity to override the induction of apoptosis by p53 (62), and since it can also prevent apoptosis by TNF-α and Fas antigen, one prediction is that p53 mediates these pathways also. The function of p53 in apoptosis may, however, be restricted to those pathways involved in preventing cancer and minimizing the consequences of viral infection, since p53 expression is, for the most part, not overtly required for growth and differentiation (66). Whether induction of apoptosis by p53 requires new gene expression, as in some apoptotic pathways (glucocorticoids), or is independent of transcription, as in others (TNF-α), remains to be determined. p53 does modulate transcription, both positively and negatively (89–94), and may also regulate progression through S phase of the cell cycle (72, 73, 95, 96). The relationship between these activities of p53 and induction of apoptosis is under investigation.

Although it is not known how Bcl-2 blocks apoptosis, the functional interchangeability with the E1B 19-kDa protein suggests that both may act by the same mechanism. Similar functional substitution of the *ced-9* gene of *Caenorhabditis elegans*, which inhibits programmed cell death instigated by the products of *ced-3* and *ced-4* (34), with *bcl-2* (97), illustrate the conserved nature of the apoptosis program. The E1B 19K and Bcl-2 proteins both localize to nuclear envelope and ER membranes (87, 98) and some limited amino acid sequence similarity between the two is apparent (Verwaerde and White, unpublished). It is likely since E1A and E1B modulate the p53-dependent apoptotic pathway, that Bcl-2 will also block apoptosis by wild-type p53. Conversely, it will be interesting to test the ability of the E1B 19-kDa protein and mutant p53 to functionally replace Bcl-2 in glucocorticoid-induced apoptosis (99) and hematopoietic cell differentiation (56, 88).

Regulation of apoptosis by viruses is not restricted to our observations with adenovirus because effects on apoptosis have been reported in other viral systems. Examples of both induction and suppression of apoptosis upon infection have been reported, and in some cases, the specific viral gene products involved have been identified (Table I). Induction of apoptosis upon infection contributes to the pathology of human immunodeficiency virus (100–102), chicken anemia virus (103), bovine herpesvirus type 1 (104), and perhaps others. Moreover, viral gene products have been identified as inhibitors of apoptosis. These counterparts to the E1B 19-kDa protein include the Epstein-Barr virus latent membrane protein-1 (105), the p35 protein of Baculovirus (106), and the γ34.5 protein of human

Table I. Viral and Cellular Regulators of Apoptosis or Programmed Cell Death

	Function
Inducers of apoptosis	
Adenovirus E1A	Proliferation, transformation, infection, modulation of transcription, cell cycle control
c-myc	Proliferation, transformation, transcription
TNF-α	Anticancer, antiviral activities
Fas antigen	Negative selection
Wild-type p53	Tumor suppressor gene product, transcription, cell cycle control
ced-3, ced-4	*Caenorhabditis elegans* development
Hormones	Homeostasis, differentiation
Other tumor suppressor genes?	Anticancer activity
Viral infection	Growth deregulation? DNA damage?
Inhibitors of apoptosis	
bcl-2	Oncogenic when overexpressed, blocks apoptosis in hematopoietic cells in response to growth factor withdrawal, CD-3, irradiation, glucocorticoids, maintains B cell memory, role in T cell maturation
Adenovirus E1B 19 kDa	Transformation, infection, blocks apoptosis by TNF-α, Fas, wild-type p53, function can be replaced by Bcl-2
Adenovirus E1B 55 kDa	Infection, transformation, inactivates p53
Epstein-Barr virus LMP-1	Latency, transformation, upregulates Bcl-2
ced-9	*C. elegans* development, function can be replaced by Bcl-2
Mutant p53	Inhibitor of wild-type p53
Adenovirus E3	Infection, blocks apoptosis by TNF-α
Baculovirus p35	Infection
Herpes simplex virus γ34.5	Infection
Growth factors	Second signal?
Other oncogenes?	?

herpes simplex virus type 1 (107). As with the adenovirus E1B 19-kDa protein, these viral inhibitors of apoptosis function to enhance virulence or virus persistence during infection. Both the E1B 19-kDa protein and LMP-1 have transforming activity, suggesting that by inhibiting apoptosis, these viral proteins may promote oncogenicity (108). Of these viral inhibitors of apoptosis, only the adenovirus E1B 19-kDa, which affects p53 (62), and Epstein-Barr virus LMP-1, which upregulates Bcl-2 levels (105), have so far yielded insights into the mechanism of apoptosis suppression.

Ultimately, apoptosis may turn out to be not only an important developmental process, but a common cellular response to viral infection and transformation. Induction of cell death upon infection or transformation may represent a general defense mechanism at the cellular level. As a consequence, many viruses and oncogenes may encode functions to suppress apoptosis. Other tumor suppressor genes may function, not only to block cell growth, but to induce cell death, providing an altruistic and irreversible means to retain the viability of a multicellular organism. Moreover, the discovery that apoptosis is an important aspect of oncogenic transformation provides an opportunity to design new strategies for intervention in the treatment of cancer.

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