

## APOPTOSIS IN TUMORIGENESIS AND CANCER THERAPY

Gaël McGill and David E. Fisher

*Division of Pediatric Hematology/Oncology, Dana Farber Cancer Institute and Children's Hospital, Harvard Medical School, 44 Binney St., Boston, MA 02115*

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. The biochemistry of apoptosis
  - 3.1 The executioner and its substrates
  - 3.2 Channeling our attention on the Bcl-2 family
  - 3.3 The Fas way to apoptosis
  - 3.4 The PI3-K/Akt survival pathway
4. Apoptosis and cancer
  - 4.1 Apoptosis and tumorigenesis
  - 4.2 Anoikis and anchorage-independence: implications for tumor metastasis
  - 4.3 Hypoxia and angiogenesis
  - 4.4 Antineoplastic therapies
5. Conclusions
6. Acknowledgments
7. References

### 1. ABSTRACT

Apoptosis is a morphologically and biochemically distinct form of cell death which can be triggered by a variety of extracellular agents during both normal development as well as in adult pathological states. Much progress has recently been made in understanding the molecular pathways which regulate this process as well as new intersections between these. A direct interaction between components of the 'executioner' - the ICE-family of cysteine proteases - and the Bcl-2 family of proteins, which modulate a cell's propensity to undergo apoptosis, has recently been demonstrated. New pathways to cell survival, like the PI3-K/Akt signal transduction pathway, are also providing new clues as to the regulation of cell death by growth factors and extracellular matrix for example. The links which exist between apoptosis and cancer research are several. Genetic alterations in components of the apoptosis pathway occur during tumorigenesis and confer resistance to a variety of physiological (oncogene-induced cell death, loss of adhesion, growth under hypoxia) as well as therapeutic (chemotherapy and radiation) death triggers. Similarly, antineoplastic therapies are thought to induce tumor cell apoptosis, and consequently, common mutations in apoptosis-regulatory genes carry a poor prognosis

Received 6/15/97 Accepted 6/28/97

Send correspondence to: David E. Fisher: Division of Pediatric Hematology/Oncology, Dana Farber Cancer Institute and Children's Hospital, Harvard Medical School, 44 Binney St., Boston, MA 02115, Tel: (617) 632-4916, Fax: (617)632-2085, Email:david\_fisher@dfci.harvard.edu and gmcgill@student.med.harvard.edu

for the patient. A more detailed understanding of the biochemistry of apoptosis and the ways in which it is disabled in tumors will likely reveal new transformation selective death triggers which stimulate cell death in ways independent of components like p53 and increase the therapeutic window of these drugs in the clinics.

### 2. INTRODUCTION

The study of apoptosis is relevant to many aspects of tumor biology which include tumorigenesis, tumor homeostasis, angiogenesis, metastasis, and clinical treatment. Malignant cells often harbor mutations in critical components of the apoptotic pathway which may correlate with poor prognosis. Solid tumors must circumvent the apoptotic pathways which regulate their anchorage-dependent survival in order to metastasize and establish secondary tumor sites. Although chemotherapy was long thought to kill tumor cells by inactivating critical metabolic pathways, it is now recognized that many effective chemotherapeutics trigger the tumor into killing itself by activation of an apoptotic pathway. It is therefore of prime importance for the discovery of improved treatments to better understand the molecular underpinnings of the apoptosis machinery. It is crucial to remember, however, that much of the knowledge we have gained has been derived from the study of 'developmental apoptosis' - or programmed cell death - in primitive organisms amenable to genetic manipulation. Given the highly conserved nature of the apoptosis machinery (at least parts of it), uncovering its molecular details using a variety of *in vitro* and genetic systems will likely provide useful information for cancer therapy.

## Apoptosis in Tumorigenesis

Apoptosis is a morphologically and biochemically distinct form of cell death which can be likened to 'cellular suicide.' The apoptotic cell actively destroys itself in a manner which will neither harm neighboring cells nor induce an inflammatory response. This physiological process is distinct from necrotic cell death, which occurs as a result of severe cell injury and results in swelling and lysis. The genetically programmed death of cells during normal development is sometimes referred to as "programmed cell death," an example of apoptosis in normal physiology. During the development of most, if not all, multicellular organisms, programmed cell death provides an efficient mechanism whereby unwanted cells are eliminated. Its relevance has been documented in a number of instances which include thymocyte maturation, formation of the (wrongly named) interdigital "necrotic zones" during limb morphogenesis, mammary gland involution, spermatogonial and follicular development, and synaptogenesis during neural development among others. Whereas the death triggers specific for each of these systems are as diverse as the systems themselves, common apoptosis "effector pathways" appear to be conserved throughout evolution from nematodes to vertebrates. Pathways leading to and responsible for this widespread phenomenon have been incessantly populated with new trigger, effector, and inhibitor molecules. Accrued interest in deciphering these pathways has, in part, been sparked by the recent understanding that successful antineoplastic therapies may also operate through induction of tumor cell-specific apoptosis.

This review focuses on aspects of apoptosis research which have gained particular attention in recent years. Advances in the 'executioner' machinery, its substrates, the Bcl-2 family, Fas-induced death, and the PI3-K/Akt pathway are described with particular focus on the use of genetic and *in vitro* systems to identify new participants. We subsequently provide an overview of the relevance of these topics in understanding issues specific to tumor biology such as tumorigenesis, anchorage-independent survival and metastasis, tumor-induced angiogenesis, and chemotherapy.

### 3. THE BIOCHEMISTRY OF APOPTOSIS

#### 3.1 The executioner and its substrates

Our molecular understanding of apoptosis has advanced profoundly since its original description by Wyllie and colleagues (1). While the first hallmarks of apoptotic cell death - membrane blebbing, chromatin condensation, and cellular fragmentation into 'apoptotic bodies' - were purely morphological, biochemical hallmarks have superseded these. Apoptosis is now studied as a cascade of proteases and endonucleases, where oligonucleosomal DNA laddering and cleavage of a variety of substrates by cysteine proteases have become the modern "gold-standards." Our knowledge of the

executioner as well as some of its key modulators stems in large part from genetic studies of the nematode *C. elegans*.

Apoptosis is a developmentally programmed process in *C. elegans* whereby the death of individual cells is genetically determined and reproducibly observed. Mutants in which this pattern of programmed death is disturbed were described in the Horvitz laboratory at M.I.T., which led to the discovery of the cell death (*ced*) genes. Nematode mutants in the *ced* genes displayed a variety of phenotypes ranging from defects in engulfment by neighboring cells to excess death or survival (2, 3). In particular, the *ced-3* and *ced-4* genes were found to be required for the death of the normally occurring 131 programmed cell death events during *C. elegans* development. Gain-of-function mutations in the *ced-9* gene suggested it may function to modulate the activity of *ced-3* and *ced-4* (4). Cloning of Interleukin 1-beta Converting Enzyme (ICE) protease as a mammalian homologue for CED-3 provided the first indication that proteases may play a critical role in apoptosis (5, 6). Following this observation, numerous ICE-family proteases have been identified in mammalian cells and are thought to constitute the core of the apoptosis executioner.

The ICE proteases all belong to the cysteine protease subfamily characterized by a cysteine residue at the active site. ICE/*ced-3* homologues contain the conserved QACRG sequence surrounding the catalytic cysteine and show a preference for cleaving substrates after aspartate residues. Phylogenetic relationships among the proteases has led to their subdivision into three families (7). Typically, these enzymes can autocatalytically cleave and activate themselves as well as other ICE-family proteases. This may lead to amplification and diversification of available substrates during the execution phase of apoptosis in cells. The specific amino-acid sequence N-terminal to the target aspartate determines substrate preference among proteases of the ICE family: CPP32-like proteases cleave following DEVD, while ICE-like proteases cleave following YVAD. Specific peptide inhibitors which mimic cellular substrates containing these sequences have proved useful in studying the role and timing of activation of these proteases both in cells and *in vitro*. In the attempt to simplify the nomenclature for this ever-expanding family of enzymes, apoptosis proteases have recently been renamed 'caspases' (or cysteine proteases which cleave after *aspartic acid*), and numbered in function of the chronology of their discovery (8).

The ICE-family proteases known to date (over 10 members in mammals), strongly suggest that, unlike in *C. elegans* or other lower organisms, there may be considerable functional overlap within the death machinery of higher species. Alternatively, the wide variety of target substrates discovered so far in addition to *in vivo* observations afforded by the generation of knock-out mice, suggest that some proteases may have

## Apoptosis in Tumorigenesis

organ/tissue-specific expression patterns and/or cleave specific substrates within the cell. Mice deficient for CPP32, for example, display a brain-restricted apoptosis defect reminiscent of the neuron-specific phenotype of nematodes lacking *ced-3* (9). Mice defective for ICE - the prototype of the apoptosis cysteine proteases - show no significant apoptosis defects during development or in response to apoptosis triggers such as ionizing radiation or dexamethazone, but are resistant to Fas-induced death (10, 11). While both these genetic studies point to the restricted tissue/pathway function of individual proteases in apoptosis, more extensive knock-out studies and crosses should reveal whether this trend holds true for other ICE-homologues.

One of the central questions remains how proteolysis leads to the demise of the cell and its stereotypical changes. It is still unclear which substrates known to date (if any) are instrumental in the death pathway and which are simply biochemical markers of the process. Poly ADP-ribose polymerase (PARP) was one of the first proteins reported to be cleaved during apoptosis (12, 13), and is a target of the Yama/CPP32 protease, caspase-3 (14). PARP's role in recognizing DNA breaks along with the fact that CPP32-mediated cleavage separates its DNA-binding domain from its catalytic domain, has led to the notion that PARP inactivation may be functionally important for efficient DNA fragmentation to occur during late phases of apoptosis (15). However, mice lacking PARP show no obvious defects in apoptosis (16), suggesting that it is not required for apoptosis to occur, although functional homologues could in principle be rescuing PARP.

A wealth of substrates continue to be identified as potentially important signaling intermediates. These include, among others, the nuclear lamins (17-19), the 70kD component of the U1 snRNP (20, 21), DNA-PK (22), Gas2 (23), D4-GDI (24), PKC- $\delta$  (25), SREBPs (26), Huntingtin (27), PAK2 (28) and RB (29,30). Biochemical isolation of other relevant substrates based on their activity to signal apoptosis using *in vitro* systems should provide a clearer picture of the nuclear events downstream of the cysteine proteases. A number of cell-free systems like chicken S/M extracts (13, 31), HeLa cell extracts (32), *Xenopus* extracts (33, 34), as well as extracts of other cells triggered to die in response to a variety of apoptotic triggers (35-37) have already been used to reconstitute portions of the apoptosis pathway *in vitro* and characterize novel apoptosis-inducing activities. One such activity was recently isolated from HeLa cell extracts and termed the DNA fragmentation factor or DFF (38). DFF is cleaved both *in vitro* (following addition of recombinant CPP32 to extracts) and *in vivo* (in U937 cells treated with Staurosporine), and is required for chromatin condensation and DNA laddering to occur. Although the mechanism by which DFF induces DNA fragmentation remains unknown, this new protein is one of the first ICE-

family protease substrate to date with a demonstrated role in signaling downstream events of apoptosis.

### 3.2 Channeling our attention on the Bcl-2 family

Bcl-2 is the mammalian homologue of *ced-9* which, in *C. elegans*, is required to protect cells that normally survive from undergoing programmed cell death (4, 39). It has been the focus of intense study ever since its demonstrated ability to rescue pre-B cells from apoptosis in response to IL-3 withdrawal (40). Originally identified as a result of the t(14;18) translocation in B-cell follicular lymphoma in which its juxtaposition with the IgH enhancer leads to dysregulated overexpression, Bcl-2's causal role in tumorigenesis was directly confirmed in studies where mice expressing a Bcl-2 transgene in lymphoid cells developed B-cell malignancies (41-43). Bcl-2 is now the prototype of a rapidly growing family of interacting proteins which share its ability to modulate apoptosis. A prevalent model has emerged by which a cell's threshold to apoptosis is determined by the levels of pro- and anti-apoptotic members which, through dimerization, act as a survival "rheostat switch" (44). The biochemistry through which Bcl-2 and its 'partners-in-crime' mediate their effects has only recently started to unfold.

Bcl-2 family proteins share several homology regions (Bcl-2-homology/BH domains) crucial for both their dimerization and apoptosis-modulatory functions (44). The BH domains along with the use of the yeast two-hybrid and other methods have been instrumental in the identification of novel interacting proteins such as Bax (45), Bcl-x (46), Bad (47), Bag-1 (48), Bak (49-51), Bik (52), Hrk/harakiri (53) and others. The pro-apoptotic Bax protein, cloned through its ability to co-immunoprecipitate with Bcl-2, was originally found to form homodimers as well as heterodimers with Bcl-2 via the BH1 and BH2 domains (45). Site-directed mutagenesis of these domains in Bcl-2 prevent heterodimer formation with Bax (or Bak) and abrogate its death-repressor activity (54). Interestingly, Bcl-2 mutations permissive for homodimerization, but not heterodimerization with Bax, also abolished Bcl-2 activity suggesting that Bcl-2 requires Bax to exert its death-repressor activity.

It remains unclear, however, who among the pro- and anti-apoptotic members are the key effectors in the apoptosis pathway. Indeed, unlike Bcl-2, not all members appear to require heterodimerization to carry out their protective effect. The Bcl-x transcript is alternatively spliced into a short and long form to yield both pro- and anti-apoptotic factors (Bcl-x<sub>S</sub> & Bcl-x<sub>L</sub> respectively) (46). While the long form resembles Bcl-2 in its dimerization preferences and anti-apoptotic capacity, Bcl-x<sub>L</sub> mutants which prevent its interaction with Bax or Bak can still rescue Sindbis virus-induced apoptosis (55). The short splice form of Bcl-x, although lacking BH1 and BH2 dimerization regions, appears to mediate its death-inducing function by antagonizing Bcl-2 and Bcl-x<sub>L</sub> (46).

## Apoptosis in Tumorigenesis

A simple 'rheostat switch' model whereby both pro- and anti-death molecules require co-interaction to mediate their effects on apoptosis is not sufficient to explain the above observations. Alternatively, death-promoting and death-suppressing Bcl-2 family proteins may operate in mechanistically different ways. The phenotype of Bak knock-out mice which display both an excess and lack of cell death in different tissues supports this possibility (56).

A third region of Bcl-2 homology - BH3 - has been demonstrated to be essential for the activity of the pro-apoptotic proteins. Indeed, although all three BH1, 2, & 3 regions are involved in dimer interface as revealed by structural studies (57), expression of the BH3 domain of Bak alone is sufficient to induce apoptosis (58). A crystal structure of Bcl-x<sub>L</sub> bound to a Bak peptide derived from the BH3 region demonstrates the importance of this domain in mediating protein-protein interaction (59). Bcl-x<sub>L</sub> BH1, BH2, and BH3 domains form an elongated hydrophobic cleft as revealed by NMR and X-ray structures (57). When bound with Bcl-x<sub>L</sub>, the BH3 region of Bak adopts an amphipathic alpha helix and mediates both hydrophobic and electrostatic interactions with the Bcl-x<sub>L</sub> hydrophobic cleft. Mutations in residues observed to form specific contacts within this cleft abolish interaction of the two proteins (59).

Structure determination of Bcl-x<sub>L</sub>, in addition to revealing the overall architecture of the BH dimerization domains, has also revealed a somewhat unexpected structural similarity of this protein to the membrane insertion domain of the diphtheria toxin and related colicins (60). Indeed, comparing Bcl-x<sub>L</sub> (as well as other members like Bcl-2) with the diphtheria toxin reveals that both proteins have two central helices with apolar residues (alpha<sub>5</sub> and alpha<sub>6</sub> in Bcl-x<sub>L</sub>) surrounded by three amphipathic helices (alpha<sub>1</sub>, alpha<sub>3</sub>, and alpha<sub>4</sub> in Bcl-x<sub>L</sub>) (57). The two central hydrophobic helices alpha<sub>5</sub> and alpha<sub>6</sub> of Bcl-x<sub>L</sub> are long enough to span the length of the membrane, and, like the insertional domain of the diphtheria toxin, may require dimerization to form a pH-dependent membrane pore (61). By analogy with the functional role of the insertional domain in toxins, these observations raise the possibility that proteins of the Bcl-2 family may control the passage of ions or other components across the mitochondrial membrane. Alternatively, Bcl-2 family proteins may interact with and regulate the activity of other mitochondrial transport proteins via these helical domains. A recent report that Bcl-x<sub>L</sub> forms pH-sensitive ion-conducting channels in synthetic lipid membranes has reinforced this hypothesis (62).

Until recently, Bcl-2's ability to regulate the apoptosis pathway remained a mechanistic enigma. Based on observations linking Bcl-2-mediated rescue of apoptosis with lower levels of oxygen free radicals as well as Bcl-2's localization to nuclear, ER and mitochondrial membranes, early studies advanced the possibility that Bcl-2 could function in an antioxidant pathway (63, 64).

Bcl-2 and Bcl-x<sub>L</sub> can prevent hypoxia-induced cell death, however, suggesting that they exert their anti-apoptotic activity by a mechanism other than modulation of oxygen free radicals (65, 66). Based on cellular fractionation studies as well as electron microscopic observation, others hypothesized that Bcl-2 may be involved in nuclear transport (67). The more recent discovery of the extended family of Bcl-2 dimerization partners led to a model whereby ratios of these molecules may determine a cell's susceptibility to apoptosis in response to a variety of apoptotic stimuli (44). While these observations suggest how regulation may occur within the family, they give no hint as to how these proteins are regulated by exterior signals as well as a possible connection with the central apoptosis machinery. In addition to their putative role as channel-forming assemblies, Bcl-2 family proteins have now been found to recruit and interact with several signaling proteins which may regulate their activities.

Although in many cases levels of pro-and anti-apoptotic Bcl-2 family proteins determine a cell's susceptibility to apoptosis, there exist several demonstrated instances where expression levels do not correlate with apoptosis propensity (68, 69). Post-translational modification has been put forth as a possible mechanism for modulating the death activities of these proteins. Recent evidence suggests that trophic factor-induced phosphorylation of both Bcl-2 and Bad may regulate their apoptotic functions. For both Bcl-2 and Bad, phosphorylation has been suggested to have an inhibitory effect. Bcl-2 phosphorylation on serine occurs in response to a variety of stimuli and often correlates with decreased anti-apoptotic potential (70, 71). Bcl-2 has also been proposed to recruit the Raf-1 kinase to outer mitochondrial membranes and induce phosphorylation of Bad (72). This phosphorylation triggers Bad's dissociation from Bcl-x<sub>L</sub> and cytoplasmic association with the 14-3-3 protein (73). While the effect of these modifications on the apoptosis functions of Bcl-x<sub>L</sub> or Bcl-2 are not yet fully understood, they may provide a link between growth factor pathways and regulation of the downstream apoptosis machinery.

An interaction between Bcl-2 family proteins, members of the ICE-family cysteine proteases, and the nematode protein CED-4 has recently been demonstrated by a number of groups (74-76). Considering the epistatic relationship of these genes in *C. elegans* whereby *ced-9* rescues cells from apoptosis by antagonizing the activity of *ced-3* and *ced-4* (4), a direct physical interaction between these proteins was tested. CED-9 appears capable of binding the death-inducing splice form of CED-4 (77) using the yeast two-hybrid system and *in vitro* binding studies (75, 76). Mutations in CED-9 which eliminate its anti-apoptotic activity were found to disrupt its ability to interact with CED-4. These studies point to the importance of CED-4 binding for CED-9 function, and raise the possibility that the CED-4 interaction domain of

## Apoptosis in Tumorigenesis

CED-9 could potentially be used as a bait for identifying mammalian CED-4 homologues.

A key observation which allowed for a CED-4/Bcl-x<sub>L</sub>/ICE interaction to be tested in a mammalian tissue culture system was that the nematode CED-4 protein is sufficient to induce apoptosis when overexpressed in mammalian cells (74). In addition, CED-4-induced apoptosis can be inhibited with Bcl-x<sub>L</sub> or caspase inhibitors suggesting that in mammalian cells, CED-4, under control of the Bcl-2 family, induces death via ICE-family protease activation. Once this was established, Chinnaian *et al.* took advantage of mutant proteins with the binding but not the cytotoxic properties of wild-type molecules in order to overexpress and immunoprecipitate CED-4 without killing cells. They observed that wild-type CED-9 (or Bcl-x<sub>L</sub>) coimmunoprecipitates with CED-4 when cotransfected into 293 cells. In agreement with the yeast two-hybrid studies, immunoprecipitation of CED-4 failed to bring down a CED-9 mutant (lacking BH1 and BH2 domains) which does not block CED-4-induced apoptosis. Similarly, the ability of Bcl-x<sub>L</sub> mutants to inhibit apoptosis correlated with their capacity to interact with CED-4. Coexpression of pro-apoptotic Bcl-2-family members such as BAX, BAK or BIK disrupted the CED-4/Bcl-x<sub>L</sub> interaction.

In addition to its ability to interact with certain anti-apoptotic Bcl-2 family proteins, CED-4 was also observed to immunoprecipitate with ICE family-proteases which contain a large pro-domain (such as ICE or FLICE). Although the functional significance of this binding as well as the mechanism through which CED-4/ICE interaction may activate the protease, these results place CED-4 as a central regulatory switch between the Bcl-2 family and the downstream executioner machinery. The importance of CED-4 as an adaptor between these two families is manifest in the fact that a CED-4 mutant which interacts with CED-3 (but not CED-9) reduces Bcl-x<sub>L</sub>'s ability to coimmunoprecipitate with ICE or FLICE. The fact that CED-4 has no known mammalian homologue explains why the link between these two central apoptosis families has remained elusive until now. The key was to test the activity of the nematode protein in the context of a mammalian cell culture system. Given its central role, CED-4 may present a new therapeutic target for intervention in cancer, for example, where some upstream signaling components of the apoptosis machinery are often disabled.

Finally, an additional signaling activity has recently been attributed to the Bcl-2 family, and involves the release from mitochondria of proteins which may be instrumental in downstream apoptosis events. Several studies have shown that cytochrome c is released from mitochondrial stores upon induction of apoptosis, and that Bcl-2 overexpression is able to prevent this release (78, 79). Interestingly, cytochrome c was previously implicated

in signaling apoptosis through its ability, in combination with dATP, to trigger PARP cleavage in naive HeLa cell extracts (32). Research also indicates that Bcl-2 may exert its anti-apoptotic activity by blocking the release of other death signaling molecules from mitochondria such as an as-of-yet unidentified apoptogenic protease (80). Whatever normally causes the release of either cytochrome c or other signaling entities from mitochondrial stores upon induction of apoptosis, as well as the mechanisms through which these molecules activate the executioner remain to be discovered.

In conclusion, current data point in several directions for a functional role of the Bcl-2 family in apoptosis. Anti-apoptotic members of the bcl-2 family may exert their death-suppressing function through their ability to bind and sequester a CED-4 homologue in mammals. Binding of CED-4 by these proteins presumably interferes with its ability to bind and activate CED-3 homologues like ICE or FLICE. Through dimerization with Bcl-2 or Bcl-x<sub>L</sub>, pro-apoptotic Bcl-2 proteins may, in turn, disrupt their ability to bind CED-4, thereby freeing it for interaction with executioner proteases. Such a model offers an attractive signaling pathway which provides a direct biochemical link between the Bcl-2 family of apoptosis modulators with the cysteine proteases, and fits well with the epistatic relationships described in nematodes. Nevertheless, while the interaction with CED-4, recruitment of Raf-1, formation of ion-channels, and ability to release cytochrome c (and perhaps other apoptosis effectors) from mitochondria are all reported activities of Bcl-2 family proteins, it remains unclear how these diverse activities converge to modulate apoptosis.

### 3.3 The Fas way to apoptosis

Cytokine-induced apoptosis by Fas or tumor necrosis factor (TNF) is now one of the best studied and understood biochemical pathways to cell death which directly links ligand-receptor interactions at the plasma membrane with components of the executioner machinery. During development of the immune system, over 90% of immature thymocytes are deleted as a result of unproductive T cell receptor rearrangements or because they become self-reactive as a result of this rearrangement. Selection can occur in the thymus, in the peripheral circulation, or following the immune response (in the case of mature T cells). Based on the phenotypes of *lpr* (lymphoproliferation) and *gld* (generalized lymphoproliferative disease) mice which harbor defects in the Fas and Fas ligand (FasL) genes respectively (81), Fas-induced apoptosis is thought to be involved in at least two of these processes: peripheral clonal deletion and downregulation following immune reaction (82, 83). In addition, the Fas pathway has been linked to several types of autoimmune disease such as ALPS (autoimmune lymphoproliferative syndrome) and autoimmune diabetes (84-87).

## Apoptosis in Tumorigenesis

FasL is a member of the tumor necrosis factor (TNF) family of membrane and secreted proteins which is displayed on T cells following T cell receptor signaling (88-90). Fas (also referred to as APO-1 or CD95) is a receptor of the TNFR family of cell surface proteins (91, 92). While Fas and TNF receptors are abundantly expressed in a wide variety of tissues (thymus, liver, heart, kidney), FasL and TNF expression patterns (mostly in natural killer cells and activated lymphocytes) appear to be a more relevant indication of where Fas-mediated cell death occurs. Both FasL and TNF undergo metalloprotease-mediated cleavage from the membrane and therefore also exist as soluble forms. It remains unclear whether this cleavage extends their signaling range or whether the shedding of these molecules is a means of downregulating their activity (82). Until recently, the signaling mechanisms by which ligand/receptor interaction resulted in apoptosis remained unknown. The identification of the death domain dimerization motif in the cytoplasmic tails of Fas and TNFR has led to the rapid isolation of a number of crucial intracellular intermediates in the Fas pathway.

The death domain (DD) was originally recognized by homology between Fas and TNFR1. Mutagenesis studies of these receptors revealed this region of approximately 80 residues to be crucial for FasL/TNF-induced apoptosis (93, 94). Several groups identified proteins interacting with Fas and TNFR using the DD as a yeast two-hybrid bait: FADD/MORT1 (Fas-associated protein with death domain), TRADD (TNFR1-associated death domain protein), and RIP (receptor interacting protein) (95-99). The recent NMR structure of the DD reveals the biochemical basis for its ability to oligomerize and mediate signaling (100). In fact, the DD may be somewhat of a misnomer since it is found in a number of receptors not all of which appear to be involved in signaling apoptosis (101). In addition to this domain, the recognition of a death effector domain (DED) in FADD led to the isolation of the FLICE (FADD-like ICE)/MACH (MORT1-associated CED-3 homologue) protein (102, 103). Activation of Fas or TNFR1 is thought to result in the formation of a 'death-inducing signaling complex' (DISC) which leads to interaction between these proteins based on DD and DED interactions. In addition, the recently identified DR3/WSL-1/Apo-3 receptor homologous to TNFR1 and capable of inducing apoptosis via its interaction with TRADD/RIP raises the possibility that other receptors also mediate cytokine-induced cell death (104-106).

A current model for Fas-induced apoptosis involves trimerization of the receptor upon binding a FasL trimer followed by FADD/MORT1 recruitment to the receptor complex via death domain interaction. Binding of the FLICE/MACH protease to FADD/MORT1 via its death effector domain leads to its activation which, in turn, is likely followed by proteolytic activation of other ICE family members. The recent discovery of a new family of viral FLICE-inhibitory proteins (v-FLIPs) found in several

$\gamma$ -herpesvirus and the tumorigenic human molluscipoxvirus points to the importance of FLICE as a central target for inhibiting the Fas/TNFR1 apoptosis pathway (107). By interacting with FADD via their DED, these viral proteins prevent the FADD-dependent recruitment and activation of FLICE, and completely block apoptosis induced via Fas, TRAMP or the as-of-yet unidentified TRAIL receptor (108, 109).

In addition to its role in apoptosis, TNF also induces activation of the NF-kappaB transcription factor. TNFR1 signaling is not as clear and well understood as that of Fas. Following TNF receptor trimerization, both TRADD and FADD/MORT1 can be recruited. It is at this level that the pathways of apoptosis and NF-kappaB activation bifurcate, since TRADD has been shown to have both apoptosis and NF-kappaB signaling properties (97). TRADD interacts with the RIP serine/threonine kinase to induce apoptosis, as well as with members of the TRAF (TNF receptor-associated factor) family to mediate NF-kappaB activation (110). While RIP does not contain a DED, it is its DD which is required to induce apoptosis suggesting an interaction with another signaling molecule. Such a candidate molecule was recently identified as RAIDD (RIP-associated ICH-1/CED-3-homologous protein with a death domain), which in addition to interacting with RIP through its DD, contains a sequence homologous to the 'pro' domain of the ICH-1/CED-3 proteases (111). This region of homology mediates RAIDD's interaction with these proteases and likely leads to their activation. Therefore, at least two potential signaling links have been made between Fas/TNFR1 and the downstream executioner machinery: one via FADD/MORT1 and involving the FLICE/MACH protease, and the other via TRADD, RIP, and RAIDD to activate ICH-1.

Several important questions regarding the Fas/TNFR signaling remain unanswered. How is the decision between FADD/TRADD-mediated death and TRADD-mediated NF-kappaB activation made given that certain TRAFs (TRAF-2 for example) interact with RIP as well as TRADD (99)? Why have cells evolved ways of controlling these two pathways via the same receptor complex? Studies suggest that NF-kappaB activation likely results in the expression of a 'survival gene' since inactivation of this pathway (with IkappaB phosphorylation-defective mutants for example) leads to increased sensitivity to Fas and TNF (112, 113). The potential for modulating apoptosis in this fashion may extend to cancer therapy (114). The ability of NF-kappaB-induced gene expression to antagonize apoptosis induced by other triggers remains to be fully investigated.

Several studies have also demonstrated the requirement for ceramide in TNF-mediated cell killing, suggesting that the signaling pathways to the executioner may not be as direct as anticipated. Ceramide is a lipid second messenger derived from the sphingomyelin cycle, and has been implicated in mediating apoptosis in

## Apoptosis in Tumorigenesis

response to a number of different triggers like radiation (115), chemotherapeutics (116, 117) as well as Fas and TNF (118, 119). Ceramide may nevertheless be a downstream signal in the Fas/TNF pathway since ICE-like activity appears to be necessary for ceramide generation in response to the *Drosophila* REAPER protein which, like Fas and TNFR, contains a death domain (101, 120). Similarly, an ICE-like activity has been suggested to be required for activation of the p38 stress activated kinase pathway by Fas (121).

Another mystery is the mechanism through which FLICE becomes activated as well as its relevant substrates. Considering that other ICE-family proteases are typically activated following cleavage of their 'pro' domain (122), FLICE activation may similarly involve such a modification or cleavage reaction. The ICE protease cascade activated downstream of FLICE also remains poorly understood. The timing of CPP32-like and ICE-like protease activities following Fas ligation has been investigated using specific fluorescent substrates. These studies demonstrate that an ICE-like activity is transiently activated, whereas the CPP32-like activity accumulates during FasL/TNF-induced apoptosis (123). Nevertheless, it is likely that FLICE activation is still an early event in the pathway to irreversible death, given the ability of inhibitors such as Bcl-2 and Bcl-x<sub>L</sub> to block Fas-induced death both *in vitro* and *in vivo* (124-126). In keeping with this notion and the recent emphasis on mitochondrial events in apoptosis signaling, cytochrome c function appears to be impaired in Jurkat cells undergoing Fas-induced death (127). Taken together these observations suggest that Fas/TNFR1-induced apoptosis signals may converge with other classical apoptosis pathways on a conserved executioner machinery.

### 3.4 The PI3-K/Akt survival pathway

During development of the nervous system, excess neurons are produced only to subsequently die by competing for a limited supply of secreted or membrane-bound neurotrophic substances. The 'neurotrophic hypothesis' was validated in the 1950s with the discovery of nerve-growth factor (NGF) - a polypeptide shown to be important for the survival of sensory and sympathetic neurons *in vivo* - and, more recently, with the identification of other neurotrophins such as BDNF, CT-1, NT-3 and CNTF (128). *In vitro* studies showed that PC12 pheochromocytoma cells required NGF for both differentiation and survival. While Ras/Raf/Erk was required for differentiation, PI3-K was found to be crucial for the survival effect of NGF. Indeed, the ability of NGF to prevent apoptosis could be blocked by PI3-K inhibitors wortmannin and LY294002 (129). Treatment of cells ectopically expressing the PDGF-R with PDGF induced PI3-K and protected against apoptosis while PDGF receptor mutants unable to activate PI3-K had no effect. With the recent discovery of the Akt kinase as a downstream PI3-K substrate with anti-apoptotic activity, we

are now one step closer to understanding how PI3-K is linked to the apoptosis machinery.

The protooncogene Akt (also referred to as protein kinase B) is a ubiquitously expressed serine/threonine kinase whose catalytic domain is homologous to that of PKA and PKC family kinases, and which contains an N-terminal Pleckstrin homology (PH) domain. Akt kinase activity is upregulated in the presence of serum in a variety of cell lines in a manner dependent on PI3-K. This was originally shown by Franke *et al.* using a PDGFR-negative cell line into which PDGFR mutants lacking PI3-K binding sites (Y740F & Y751F) were transfected. Alternatively, treatment of cells with the PI3-K inhibitor wortmannin also blocked Akt kinase activity (130). In addition, Akt activation was abrogated by point mutations in the PH domain, suggesting the importance of this domain in regulation of the kinase. These results along with those showing that PI3-K's ability to rescue cells from serum deprivation-induced apoptosis was independent of p70S6K (131) pointed to Akt as a likely candidate. Shortly thereafter it was discovered that IGF-1 or insulin-mediated survival of cerebellar cultures *in vitro* is dependent on Akt (132). Cells transfected with dominant-negative constructs of the kinase - PH domain alone or kinase-dead versions of the protein - induce apoptosis of neurons in the presence of growth factors. Conversely, neurons into which wt-Akt was overexpressed were resistant to serum-deprivation suggesting the role of this kinase in cell survival.

The ability of PI3-K and Akt to promote survival is not limited to cells of neuronal origin, however, as it was found that either activated PI3-K or Akt protects Myc-overexpressing Rat1 cells from undergoing apoptosis upon growth factor withdrawal. Indeed, as serum deprivation is not only an apoptotic trigger for neurons but also for transformation-selective apoptosis in fibroblasts (133), the ability of PI3-K/Akt pathway to rescue factor-dependent survival in these cells was tested by several groups (134, 135). Transfection of a constitutively active mutant of the p110 catalytic subunit of PI3K was found to block apoptosis induced by serum starvation in Myc-Rat-1 cells. V12 Ras's ability to rescue cells was also tested as the p110 point-mutant used in these experiments (p110 K227E) is thought to mimic the conformational change induced by binding of PI3-K to activated Ras. V12 Ras, however, enhanced rather than suppressed serum-deprivation induced death suggesting that Ras could mediate both pro- and anti-apoptotic signals. Using Ras mutants which differ in their ability to activate downstream effectors like Raf versus PI-3K, Kauffmann-Zeh *et al.* demonstrated that interaction with Raf promotes apoptosis while interaction with PI3-K is necessary for survival (135). In addition, Akt was necessary to mediate this effect as shown by the ability of dominant-negative (kinase-dead) Akt constructs to promote apoptosis.

## Apoptosis in Tumorigenesis

These results have been confirmed in careful studies by Kennedy *et al.* demonstrating the ability of dominant active forms of Akt to protect cells from serum withdrawal-induced death (134). In the attempt to understand how Akt promotes survival, levels of Bcl-2 and Bcl-x<sub>L</sub> proteins were tested and found to remain unchanged in Akt-overexpressing cells. Interestingly, however, while Bcl-2 can overcome apoptosis induced by Wortmannin, dominant negative (kinase-dead) Akt antagonizes the ability of Bcl-2 to rescue cells in low serum, suggesting an interplay between these proteins in preventing apoptosis. Perhaps not surprisingly, inhibition of PARP cleavage activity was shown to correlate with Akt-induced survival of these cells, establishing a role for Akt kinase upstream of apoptosis proteases with CPP32-like activity.

Additional pathways which could activate Akt in PI3-K-independent ways as well as Akt substrates important for preventing cell death remain to be discovered. So far, Akt is thought to be regulated via the action of at least two pathways - one involving PI3-K and the other dependent on an Akt-kinase (136). PI3-K regulates Akt by controlling phosphatidylinositol-3,4,5-P3 (PtdIns-3,4,5-P3) and phosphatidylinositol-3,4-P2 (PtdIns-3,4-P2) levels. Akt homo-oligomerizes upon binding PtdIns-3,4-P2 via its PH domain. This interaction, however, is not sufficient for full activation which requires additional phosphorylation in its catalytic loop (threonine 308) and on its C-terminal regulatory domain (serine 473). Since Akt is recruited to the plasma membrane through binding of its PH domain to PtdIns-3,4-P2, it may thus be brought into proximity of such a kinase. Although both MAPKAP-2 and the recently purified PDK1 kinase (activated by either PtdIns-3,4,5-P3 or PtdIns-3,4-P2) have been shown to phosphorylate Akt on its catalytic loop *in vitro*, it remains to be seen whether phosphorylation by these kinases plays a significant role *in vivo* (137). The role of the downstream GSK-3 in mediating Akt's apoptosis protective effects also remains unclear at the time. It will be interesting to see what links exist between the central apoptosis machinery and the Akt pathway, and whether Akt can protect cells from apoptosis induced by a variety of other triggers such as UV or ionizing radiation, although it has already been shown to be important in apoptosis upon loss of matrix adhesion (138).

## 4. APOPTOSIS AND CANCER

In the recent past, molecular oncologists focused their studies primarily on the cellular pathways controlling proliferation. Neoplastic disease was typically envisaged as resulting from defects in these pathways leading to excess cell division. By extension, cancer therapies, when successful, were thought to act by selectively targeting rapidly cycling cells. Therapeutic index could, for practical purposes, be conceptualized as a ratio of mitotic rates between normal and malignant cells. During the last five years, however, overwhelming evidence has accumulated which suggests that

the other side of the balance - the rate of cell death - is just as important. A central theme in much of cancer research today is the ability of tumor cells to resist apoptosis in response to triggers which typically induce cell cycle arrest or death in their untransformed counterparts. We review the importance of apoptosis in modulating central processes in cancer biology such as tumorigenesis, anchorage-independent survival, tumor-induced angiogenesis, and antineoplastic therapies.

### 4.1 Apoptosis and tumorigenesis

Apoptosis is now widely accepted to play a role in tumorigenesis. In the same way that programmed cell death by apoptosis may have evolved as a mechanism for regulating cell numbers and their interactions during normal development, apoptosis may also serve to eliminate cells gone "awry" in the adult organism. In fact, many proteins which are typically known as regulators of cellular proliferation also are potent inducers of apoptosis. The *c-myc* protooncogene, an early growth response gene required for G1/S transition upon mitogenic stimulation, has been shown to play apparently divergent roles in modulating both proliferation and apoptosis.

Deregulated Myc expression is observed in a variety of tumors, and its enforced expression in animals leads to cellular transformation and tumorigenesis (139). In culture, fibroblasts transformed with Myc display increased growth kinetics when cultured in the presence of adequate serum growth factors. Upon serum withdrawal, however, they undergo rapid apoptosis, the kinetics of which correlate with cellular levels of Myc protein (133). In contrast, serum starvation in untransformed fibroblasts typically results in growth arrest. This transformation-selective response is also observed following a number of apoptotic triggers such as radiation and chemotherapy (see below). Myc activates apoptosis in other cell types as well (140, 141), and can cooperate with Bcl-2 to transform haematopoietic cells (142, 143). This scenario is observed *in vivo* during follicular lymphagenesis, whereby Myc overexpression leads to malignant growth instead of apoptosis when placed in the context of Bcl-2's anti-apoptotic effect (144).

While a number of other cellular as well as viral oncoproteins have dual roles in regulating both proliferation and apoptosis (145-147), many of these require p53 to promote cell death. The p53 tumor suppressor was recognized as a regulator of apoptosis following the observation that transfection or activation of wild-type p53 in tumor cells can result in rapid cell death (148, 149). Following these initial observations, a large number of studies demonstrated that most cytotoxic triggers like radiation or chemotherapeutics require functional p53 to induce apoptosis (150-153). Similarly, serum withdrawal-induced death in Myc-overexpressing cells or IL-3-dependent thymocytes is p53-dependent (154-156). Several studies in transgenic mice have confirmed the notion that p53's tumor suppressive role *in vivo* is closely linked to its ability to induce apoptosis (157, 158).



## Apoptosis in Tumorigenesis

The adenovirus E1A gene, like Myc, sensitizes cells to apoptosis induced by serum withdrawal, ionizing radiation, and a variety of chemotherapy agents (159, 160). E1A is thought to mediate its effect on cell death at least in part by stabilizing p53 protein levels (160). Similar to the cooperation between Myc and Bcl-2 oncoproteins in follicular lymphoma, transformation of primary cells by adenovirus 5 requires both E1A and E1B (161). E1A's ability to stimulate proliferation through binding and inactivation of pRB is 'balanced' by its apoptosis-inducing activity in the absence of E1B. The E1B gene encodes two transforming proteins - 19K and 55K - both of which disable the p53 pathway to apoptosis (162). Therefore, in the absence of p53 mutations or the E1B proteins, cells overexpressing E1A eventually die by apoptosis (160). The human papilloma virus (HPV), responsible for the majority of cervical carcinomas, triggers an analogous molecular scenario upon infection (163). The E7 protein, like E1A, hijacks the cell cycle machinery through binding pRB, and will trigger apoptosis when expressed alone (164, 165). Coexpression of the E6 protein, which stimulates ubiquitin-mediated degradation of p53, cancels E7's cell death response and leads to cellular transformation (166).

Other proteins which directly regulate cell growth or growth response genes stimulate apoptosis when overexpressed, and point to a role for cell cycle regulation in the life/death decisions of a cell. Deregulated expression of proteins like pRB, cyclin D, or E2F-1 have been shown to affect apoptosis. In fact, characterization of E2F-1 knock-out mice by several groups reveals the paradox whereby this transcription factor can induce tumors as well as promote apoptosis and suppress proliferation *in vivo* (167, 168)! During the cell cycle the E2F-1 protein is released upon pRB phosphorylation in G1 to transactivate genes involved in S phase entry (169). When expressed alone in the absence of serum or in combination with a growth suppressor gene like p53, E2F-1 induces S phase entry followed by apoptosis (170, 171). Deregulated cyclin D1 expression has been shown to have the same consequences (172). As might be expected, however, the opposite effect on apoptosis has been observed when pRB is overexpressed in cells (173, 174). Therefore, in the context of a growth arrest signal like serum starvation, high p53 levels, or DNA damage (see below), deregulated expression of critical cell cycle progression effectors can trigger the cell into apoptosis. While these observations clearly indicate that signaling pathways to growth and apoptosis are tightly linked, they do not reveal the mechanism by which these proteins induce apoptosis. A key question therefore is whether genes like Myc, E2F, pRB and p53 mediate apoptosis through the same pathways by which they regulate the cell cycle.

Conflicting reports in the literature make it unclear as to whether apoptosis is triggered as a result of conflicting growth signals or whether the regulatory

proteins we have discussed so far have distinct apoptosis-inducing activities of their own (175). Myc-induced apoptosis, for example, often correlates with elevated cyclin A expression and activation of cyclin A-dependent kinases cdk2 and cdc2 (176, 177). Inappropriate cell cycle activation of cdc2 leads to cell death (178), which, in other systems, can be suppressed by dominant negative cdks or cdk-inhibitors (179, 180). Similarly, overexpression of either cdc25 or ornithine decarboxylase - two of Myc's transcriptional targets - is sufficient to induce apoptosis (181, 182), and antisense inhibition of cdc25 can block Myc-induced cell death (147). While these studies indicate a role for these proteins in regulating apoptosis, other reports suggest that Myc-induced cyclin A and cell cycle progression does not mediate apoptosis (183, 184). The ability of Myc-overexpressing cells to be triggered into apoptosis throughout the cell cycle also supports this model (133, 185, 186). Studies on the transcription factor p53 and its mechanism of apoptosis-induction have also yielded contradictory results.

p53 regulates the expression of a number of genes in response to extracellular cues such as hypoxia or DNA damage (187). Two alternative cellular responses occur as a result of p53 induction: growth arrest in the G1 phase of the cell cycle or apoptosis. p53's transcriptional targets include Gadd45, a gene thought to be involved in DNA repair (188), p21<sup>*cip1/waf1*</sup>, a G1-specific cdk-inhibitor (189), and Mdm-2, a negative feedback regulator of p53 (190, 191). While p53-mediated transactivation of these target genes is thought to be crucial for DNA damage-induced growth arrest (187), it remains unclear whether p53-mediated transcription of these genes is needed to signal apoptosis.

Myc-mediated apoptosis has been shown to require p53 in a manner independent of p21 induction (154), and p21-deficient mice - while defective in G1 arrest - undergo normal development and apoptosis (192). These data support the idea that p53's cell cycle regulatory function may be separate from its role in apoptosis. The *bax* gene was recently found to be a transcriptional target of p53 and is upregulated in response to a variety of p53-dependent apoptosis triggers (193). However, *bax* induction does not always correlate with p53-dependent apoptosis (145), and thymocytes from *bax* knock-out mice undergo normal p53-mediated apoptosis ((56). In some systems, p53-dependent apoptosis can occur without the apparent need of any new transcription or translation (154, 194). Some reports demonstrate that transactivation-defective p53 mutants lose their ability to mediate apoptosis induced by E1A (195), while others find that p53's transactivation activity does not correlate with apoptotic activity (196, 197). A human-tumor derived p53 mutant which retains cell-cycle arrest activity but is defective for apoptosis has also been described (198). p53-interacting proteins which may modulate its transactivation-independent activities have been identified and include WT1, the Wilms tumor suppressor gene

## Apoptosis in Tumorigenesis

product (199), and the XPB and XPD helicases (197). Therefore, p53 may mediate both transactivation-dependent and independent signaling pathways to apoptosis.

In summary, many oncogenes which stimulate cellular proliferation also potentially induce apoptosis. Although it still remains unclear whether these genes kill cells by mechanisms related to the manner in which they promote proliferation, tumors which exhibit deregulated expression of these oncogenes typically select genetic aberrations which inactivate apoptosis. p53 loss or high Bcl-2 levels are common examples of such aberrations, and often correlate with poor prognosis in the clinics (see below). These observations have provided insights on the *in vivo* process of 'multi-step' tumorigenesis, whereby loss of growth control as well as inactivation of the apoptosis pathway are central for the survival and proliferation of tumors.

### 4.2 Anoikis and anchorage-independence: implications for tumor metastasis

The extracellular matrix (ECM) mediates a number of pivotal processes from cellular migration during gastrulation to tissue homeostasis in the adult (200, 201). Only recently, however, has the ECM's importance as a suppressor of apoptosis been recognized (202). 'Anchorage-dependence' describes the requirement of certain cell types for ECM adhesion in order to proliferate. In the same way that trophic factors prevent apoptosis of "factor-dependent" cells, adhesion to the ECM - a process largely mediated by the integrin family of cell surface receptors (203) - has also been shown to be crucial for the survival of certain anchorage-dependent cells. The ability to grow imbedded within a semi-solid matrix has been extensively studied as a property cells can acquire during tumorigenesis, and changes in integrin expression patterns and levels during progression of tumors from benign to more malignant phenotypes have been described (204, 205). The realization that ECM adhesion is important for cell *survival* as well as proliferation has broadened our understanding of anchorage-dependence and motivated researchers to decipher the adhesion-mediated signaling pathways which inhibit apoptosis. Triggering adhesion-specific death pathways in transformation-selective ways may allow us to prevent anchorage-independent proliferation, a likely first step in the progression of tumors to metastasis.

Similar to the cellular responses to DNA damage or serum starvation, loss of matrix adhesion in primary cells can lead to one of two fates: growth arrest or apoptosis. Primary fibroblasts which are detached from ECM slow their rate of protein synthesis and eventually growth arrest in the G<sub>1</sub> phase of the cell cycle (206, 207). Indeed, integrin-mediated signals are known to regulate the cell cycle machinery at the level of the transcription and translation of various cyclins and cdk inhibitors (reviewed in 208). The behavior of primary fibroblasts

contrasts with that of epithelial and endothelial cells which undergo rapid apoptosis when denied ECM adhesion - a process termed 'anoikis' (209).

Human umbilical vein endothelial cells plated on agarose or MDCK cells incubated in saturating amounts of either soluble ECM components or RGD inhibitory peptides are prevented from adhering and rapidly undergo anoikis (209-211). Several instances of developmentally-regulated programmed cell death triggered as a result of ECM degradation have also been reported. Mammary epithelial cells which require beta<sub>1</sub> integrin-mediated adhesion to survive are typically eliminated by apoptosis upon matrix degradation during mammary gland involution (212, 213). Similarly, during frog metamorphosis, the transcriptional activation of the matrix metalloproteinase stromelysin-3 by thyroid hormone results in apoptosis of primary intestinal epithelial cells due to remodeling of the ECM (214). During cavitation of the vertebrate embryo, inner endodermal cells which fail to contact the basement membrane are triggered into apoptosis, while those in direct contact with it are rescued (215). The mechanism by which integrin-mediated adhesion suppresses anoikis in these cell-types, however, remains unclear.

Given the central role of integrins in mediating adhesion to the ECM, their putative function in transducing survival signals has come under scrutiny. Transfection of certain integrins, for example, is sufficient to rescue melanoma cells from anoikis in a three-dimensional dermal collagen matrix (216). Focal adhesion kinase (FAK), which is known to bind and transmit signals from the integrins (217), has recently been linked to anoikis in primary cells (218, 219). The ECM has also been suggested to regulate the activity of common apoptosis signaling intermediates like p53 (220, 221), Bcl-2 (222), the Jun-N-terminal kinase (223), and ICE (212). Alternatively, integrins, like oncogenes, may signal through their effect on anchorage-dependent cell-cycle progression (208). It also remains to be seen which specific integrin heterodimer combinations mediate protective effects in distinct cell-types. Whatever the precise molecular signals may be, oncogenic transformation in epithelial and endothelial cells has been suggested as a mechanism for escaping this regulation, and achieving anchorage-independent survival and growth characteristics.

Oncogenic transformation of endothelial or epithelial cells as well as treatments which reduce cell-cell contacts between these cells rescues them from anoikis (202). Transfection of MDCK or human endothelial cells with v-Ha-*ras* or v-*src* oncogenes abrogates anoikis (209, 211). Similarly, inducible c-H-*ras* expression rescues rat intestinal epithelial cells cultured in three-dimensional growth conditions (224). Although the protective mechanism of transformation in these cell types is not yet fully understood, Ras-induced PI3-K leading to Akt

## Apoptosis in Tumorigenesis

activation may be a crucial ECM-mediated survival signal (138). Ras has been suggested to harbor both pro- and anti-apoptotic potentials depending on the downstream cellular pathways it activates (135), and therefore differences in the effects of Ras-transformation on anoikis propensity may be more complex than anticipated. In certain contexts such as overexpression of nuclear oncogenes like Myc or E1A combined with serum starvation, Ras' pro-apoptotic signaling capacity may be 'dominant' over its ability to activate Akt. In fibroblasts which lose matrix adhesion, Ras-transformation in combination with either Myc or E1A does not rescue from anoikis, but instead profoundly activates it (221). Despite differences between cell types, matrix contact in primary cells may promote cell survival via integrin-mediated Ras activation of PI3-K/Akt (138).

Treatment of MDCK cells prior to matrix detachment with either scatter factor or the phorbol ester TPA - both of which lead to disassembly of epithelial intercellular junctions (225, 226) - also confers resistance to anoikis (209). Other developmental signals, in addition to scatter factor, which induce epithelial-mesenchymal transitions might similarly affect a cell's propensity to anoikis. Thus regulation of the survival signals from matrix attachment may themselves be subject to regulation during development. In contrast, a colon carcinoma cell line required intercellular contact for survival in suspension (227); integrins instead of intercellular junctions appeared to mediate this rescue. These observations suggest that a cell's response to the loss of ECM may depend on the status of cell-cell junctions and the signaling pathways they trigger. The loosening of intercellular contact is a likely prerequisite for migration as well as survival in suspension - two characteristics typically shared by primary fibroblasts. The effect of cell-cell contact on sensitivity to anoikis is likely to be cell-type specific. Unlike epithelial/endothelial cells which typically form tight cellular sheets *in vivo* as a result of intercellular connections, fibroblasts are highly motile and migratory and do not typically display extensive cell-cell contacts. Therefore, the apparent disparities in anoikis sensitivity observed between different cell-types in culture may reflect the *in vivo* behaviors and intracellular pathways of the cells.

In summary, detachment from the ECM triggers apoptosis in cell types such as epithelial and endothelial cells, while inducing cell cycle arrest in primary fibroblasts. Cell-type differences may also extend to transformed epithelial/endothelial cells, which may resist anoikis, and transformed fibroblasts, which are sensitized to it. The existence of ECM-mediated survival pathways suggests that tumor cells may acquire anchorage-independent growth and survival characteristics by circumventing an otherwise 'default' apoptotic pathway (228). This may be the case for the  $\alpha_5\beta_1$  integrin, for example, which has been suggested to send growth arrest/apoptosis signals in fibroblasts or colon carcinoma

cells when not ligated to fibronectin (221, 229). Interestingly, there is evidence to suggest that this same receptor induces apoptosis in hematopoietic cells when ligated to fibronectin (230). Therefore tumors arising from a variety of cell types may employ different strategies for circumventing anoikis: selection of mutations which result in constitutive activation of integrin-mediated survival pathways, or loss-of-function mutations in genes required to complete apoptosis in response to the loss of matrix adhesion. It will be interesting to see which signal transduction pathways underlie the basic behavioral difference between different cell types, and which genetic alterations are required to alleviate anoikis and allow for metastatic growth of tumors.

### 4.3 Hypoxia and angiogenesis

The influence of microenvironment on the carcinogenesis and progression of solid tumors is now well recognized. Transformed cells may not only accumulate genetic mutations which allow for unchecked cell cycle progression and anchorage-independent survival, but also undergo selective pressure to adapt to low nutrient/oxygen conditions. Hypoxia, which commonly occurs as a result of increasing tumor mass and lack of vasculature, is sensed by cells and leads to activation of the hypoxia inducible factor 1 (HIF-1) (231). This basic-helix-loop-helix transcription factor has been shown to have several transcriptional targets including the erythropoietin (EPO) gene and vascular endothelial growth factor (VEGF) (232). The p53 tumor suppressor is also induced by hypoxia, although it remains unclear whether this occurs via HIF-1 (233).

Hypoxia is also a trigger for apoptosis (as well as necrosis) in transformed cells *in vitro* (234, 235), and correlates well spatially with areas of apoptosis *in vivo* (236). Likely as a result of its ability to modulate p53 protein levels and trigger apoptosis, hypoxia has been suggested to provide a selective pressure for expansion of apoptosis-resistant/p53-deficient cells at the core of solid tumors *in vivo* (236). Interestingly, solid tumors which have hypoxic regions typically carry a poor prognosis suggesting a possible correlation between hypoxia's ability to select for apoptosis-deficient cells and subsequent tumor resistance to chemotherapy or radiation.

In addition to conferring a survival advantage to p53-deficient tumor cells, hypoxia also induces a number of other cellular changes in order for cells to adapt to low nutrient/oxygen conditions (237). This adaptive response is carried out in a number of ways including upregulation of metabolic pathways (238, 239) as well as stimulation of angiogenic signaling pathways to increase tumor perfusion (240). The 'angiogenic switch' during tumorigenesis has been suggested to result from a shift in the local balance of angiogenic activators and inhibitors. Hypoxia has been shown to induce a number of genes whose expression may 'trip' the angiogenic switch (241). The VEGF promoter, for example, contains HIF-1 binding sites and is activated

## Apoptosis in Tumorigenesis

by low oxygen as well as glucose starvation (242-244). Furthermore, oncogenic transformation by c-Ha-ras acts synergistically with the effects of hypoxia to stimulate VEGF reporter expression *in vitro* (232). Hypoxia's role in selecting for p53-deficient cells may also indirectly influence the local angiogenesis balance since mutation of p53 is typically associated with the loss of the angiogenic inhibitor thrombospondin-1 (245).

The effects of angiogenesis on the growth and metastatic potential of tumors have been extensively studied. Light microscopic and immunohistochemical studies have revealed a direct correlation between the invasive and metastatic potential of a tumor and the number and density of microvessels surrounding it (246, 247). The discovery of molecules such as angiostatin (248) and endostatin (249) have demonstrated how depletion of antiangiogenic factors following removal of a primary tumor can directly affect the angiogenic balance and stimulate the growth of local and distant metastases (250). Since preventing angiogenesis can block the growth of both the primary tumor and its metastatic outposts, it has been the focus of much attention in the development of new cancer therapies. Recent studies aiming to understand the mechanisms of angiogenesis and potential ways of interfering with tumor-induced vascularization have revealed that matrix adhesion of proliferating vascular endothelial cells is required for their survival (251).

Endothelial cell proliferation is stimulated by a number of soluble factors like the acidic or basic FGFs, VEGF, and other factors, most of which can be released by hypoxic tumor cells (240). Neovascularization, however, requires that endothelial cells accomplish a number of other tasks such as matrix degradation and migration to appropriate sites (252). These processes involve extensive changes in cell-matrix recognition pathways. For example, two cytokine-dependent angiogenic pathways in endothelial cells have been shown to induce expression of distinct integrin receptors. Basic FGF- or TNF $\alpha$ -induced angiogenesis was found to be dependent on the  $\alpha_v\beta_3$  integrin, while VEGF- or TGF $\alpha$ -induced angiogenesis depended on  $\alpha_v\beta_5$  (253).

$\alpha_v\beta_3$  and  $\alpha_v\beta_5$  integrins may affect not only the migratory capabilities of cells but also their requirement for matrix in order to block apoptosis (251). This anchorage-dependent requirement appears to be specific to proliferating vascular endothelial cells implying that agents which target these integrin-ECM interactions would be quite specific and not affect normal vasculature. Short cyclic RGD-containing peptides which mimic the integrin's target recognition sequence on ECM proteins have been used in several mouse models and shown to be effective in blocking neovascularization (254, 255). Specifically, their mechanism of action in inhibiting angiogenesis has been linked to their ability to induce apoptosis of the newly forming vasculature. Therefore, the

discovery of "anchorage-dependent" survival signals suggest new targets not only for blocking processes such as anchorage-independent tumor cell growth, but also for interfering with tumor-induced angiogenesis by initiating anoikis of proliferating vascular endothelial cells.

### 4.4 Antineoplastic Therapies

Accrued interest in deciphering apoptotic signaling pathways has, in part, been sparked by the recent understanding that successful antineoplastic therapies induce tumor cell apoptosis rather than killing them as a result of direct insult to DNA. Traditional radiation biology has hypothesized that ionizing radiation kills cancer cells by overwhelming their mitotic and/or metabolic needs, resulting in disarray and death. The same reasoning applied to chemotherapeutics which cause DNA damage or inhibit DNA modifying enzymes. These explanations, however, do not account for the fact that certain slow-growing tumors respond well to therapies while rapidly growing ones can be completely immune to the same treatments (175). Instead, examination of tumor samples using immunohistochemical assays specific for apoptotic cell death has revealed that death by apoptosis, not necrosis, often follows radiation or chemotherapy (256, 257). In addition, clinical evidence has suggested prognostic links between treatment outcome and distinct molecular genetic alterations which are known to disable the cellular apoptosis pathway (258). As noted above, the foremost example of such a mutated gene is the tumor suppressor p53.

Mutations in p53 are found in over 50% of all malignancies making it one of the most frequently aberrant genes in human cancer (259, 260). Although a number of different mutants of p53 are observed in human cancers, they cluster as missense mutations throughout the DNA binding region and in four 'hotspots' (261). Most of these mutations impair p53's ability to bind DNA and result in a loss of transactivation potential. Mice deficient in p53 show minimal developmental defects, but have a higher incidence of spontaneous tumors (262, 263), similar to humans with Li-Fraumeni syndrome (with p53 germline heterozygosity (264)). In addition to its undisputed role in tumorigenesis, p53 also has prognostic value in terms of response to therapy since most triggers of apoptosis induce and may require p53. From a clinical standpoint, mutations in p53 are usually a poor prognostic indication in a variety of tumor types including gastrointestinal, hematopoietic, breast, and genito-urinary cancers (152, 265-277). Conversely, highly curable tumors like pediatric ALL, testicular, and Wilms tumors often correlate with wild-type p53 status (175). In other tumors such as cervical cancer or sarcomas, although p53 status is wild-type, the protein may be sequestered in an inactive or degraded state due to the effects of viral oncoproteins such as HPV E6 and Mdm-2.

Cell culture studies using E1A/ras-transformed mouse embryo fibroblasts derived from either wild-type or p53 deficient mice have demonstrated that classical

## Apoptosis in Tumorigenesis

therapeutics including etoposide and adriamycin as well as ionizing radiation employ p53 to induce apoptosis (150). These observations were subsequently confirmed *in vivo* in a nude mice solid tumor model (278). Similarly, transgenic mice carrying a p53-responsive lacZ construct confirm the *in vitro* data and demonstrate that p53 modulates radiation and drug sensitivity *in vivo* (279, 280). A number of genes in addition to p53 have been demonstrated to affect the cytotoxicity of drugs and radiation *in vitro*, and are suspected to have the same effects *in vivo*. Bcl-2 and Bcl-x<sub>L</sub>, for example, inhibit chemotherapy-induced apoptosis in neuroblastoma cells (281, 282). The relevance of this observation is confirmed by the *in vivo* data that a significant number of primary neuroblastomas harbor elevated Bcl-2 levels which correlate with aggressive tumor behavior and poor prognosis (283, 284). Bcl-2 has been shown to modulate drug-induced apoptosis in other systems as well (285, 286) although, surprisingly, high Bcl-2 expression has been correlated with improved prognosis in breast cancer (287-291).

Since tumors accumulate mutations which increase their resistance to certain environmental apoptosis triggers like hypoxia as well as to classical antineoplastic therapies, it is crucial to learn how to manipulate the downstream apoptosis machinery in new, perhaps more direct, ways. Finding new therapeutic triggers which induce tumor cell apoptosis in a manner independent of p53 has important clinical implications and remains a central focus in this area of apoptosis research. Similarly, shifting the balance of Bcl-2 family proteins in tumor cells may also trigger cell death. Paclitaxel (also called taxol or taxotere), a drug which acts p53-independently in patients, may exert its cytotoxic effects through its ability to phosphorylate and inhibit Bcl-2 downstream of p53 (71). Interestingly, Bax has been shown to enhance the cytotoxicity of chemotherapeutics like paclitaxel, vincristine and doxorubicin (but not etoposide or hydroxyurea) in a p53-independent fashion (292). In addition, new drugs like Apoptin are being discovered which, at least *in vitro*, appear to kill transformed cells which lack p53 and overexpress Bcl-2 (293). Understanding how a tumor cell's environment makes it more or less susceptible to certain drugs may also add therapeutic strategies. For example, both hypoxia and the ECM have been shown to affect the cell's response to cytotoxic drugs or radiation.

How might ECM affect a cell's capacity to respond to classical antineoplastic therapies? An early study shows that intercellular contact can modify the cell's ability to repair DNA following radiation-induced damage (294). In some instances, blocking intercellular contact using integrin-targeted monoclonal antibodies is sufficient to induce apoptosis (227). Similarly, cells in contact with basement membrane ECM are more resistant to conventional therapies than ones lacking adhesion (295-298). The survival pathways activated upon ECM

adhesion which have recently been the focus of anoikis research may therefore provide a broad resistance to cytotoxic treatments. Topotecan - a topoisomerase I inhibitor - is significantly more effective in inducing apoptosis in a variety of tumor lines when cells are treated in suspension (299). Therefore, therapeutic agents which specifically target tumor cell adhesion mechanisms similar to the cyclic RGD peptides used as angiogenesis inhibitors could potentially synergize with other apoptotic triggers. In combination with 'classical' therapies such as radiation or chemotherapeutics, these may potentiate their cytotoxic effects *in vivo* by downregulating survival signals like Akt (138) or Bcl-2 (222).

Hypoxia is another clear example of a microenvironment which directly determines the efficacy of certain treatments like radiation *in vivo*. Radiation is known to require oxygen in order to fix the DNA damage, a process referred to as the "Oxygen Effect" (300). Free radicals produced on the DNA can either decay or, in the presence of oxygen, form DNA-O<sub>2</sub> intermediates which typically result in double-strand breaks. Therefore, areas of low oxygen tension in solid tumors (caused by chronic or acute hypoxia) have been observed to be more resistant to the cytotoxic effects of radiation (301). Radiosensitizers - compounds which act as oxygen mimetics and potentiate the effects of radiation in such hypoxic environments - have been used in order to circumvent this problem (302, 303), and are under active clinical investigation.

Another approach whereby therapies may halt tumor cell proliferation and induce apoptosis has been to target the activity of dominant oncogenes. Farnesyltransferase inhibitors block the activity of the enzyme farnesyltransferase which modifies a number of small G proteins like Ras and trigger localization to the plasma membrane (304). Since this localization is required for Ras' signaling activities, inhibiting this process was hoped to abrogate its signaling potential and inhibit Ras-induced transformation. These inhibitors may also block Rho, another small G-protein involved in actin cytoskeletal organization (305). Other studies have confirmed a role for Rho in Ras-induced transformation (306) and shown that Rho can induce apoptosis via the production of ceramides (307). These studies indicate that other downstream effectors of Ras may also provide good therapeutic targets. The fact that fibroblasts forced into suspension appear more sensitive to farnesyltransferase inhibitors than adherent ones (308) again points to the importance of combining drugs with different targets in order to more potently induce apoptosis in a transformation-selective way.

## 5. CONCLUSIONS

Apoptosis research remains a field in full expansion with critical discoveries being reported almost weekly. However, despite the enormous progress which

## Apoptosis in Tumorigenesis

has been made so far, we still know relatively little about the regulation and intersection of the central pro- and anti-apoptotic pathways inside the cell. Although recent biochemical data suggest ways in which Bcl-2 family proteins interact with and modulate cysteine protease activity, the precise biochemical mechanisms for this modulation as well as the role of putative signaling intermediates like cytochrome C remain to be discovered. Continued study of these cell death signaling pathways will hopefully provide a wealth of new targets for apoptosis-inducing therapies in the clinics. Finding cell death triggers which work independently of p53 may enhance the development of antineoplastic therapies. In addition, the study of p53 positive tumors which are resistant to apoptosis may assist in identifying additional death modulators downstream of p53. Understanding which oncogenically-induced apoptotic activities remain in tumor cells could further advance our ability to trigger apoptosis in a transformation-selective manner.

## 6. ACKNOWLEDGMENTS

We wish to acknowledge and apologize to all those whose work in the field was not directly referenced here. G.M. is a predoctoral fellow of the Howard Hughes Medical Institute and a Sandoz Fellow. D.E.F. is supported by grants from the NIH and is Fellow of the Pew Foundation and the James S. McDonnell Foundation.

## 7. REFERENCES

1. Kerr, J. F., Wyllie, A. H., and Currie, A. R.: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257 (1972)
2. Ellis, H. M. and Horvitz, H. R.: Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 44, 817-829 (1986)
3. Hedgecock, E., Sulston, J. E., and Thomson, N.: Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science* 220, 1277-1280 (1983)
4. Hengartner, M. O., Ellis, R. E., and Horvitz, H. R.: *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* 356, 494-499 (1992)
5. Miura, M., Zhu, H., Rotello, R., Hartweg, E. A., and Yuan, J.: Induction of apoptosis in fibroblasts by IL-1 beta-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* 75, 653-660 (1993)
6. Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M., and Horvitz, H. R.: The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1beta-converting enzyme. *Cell* 75, 641-652 (1993)
7. Chinnaiyan, A. M. and Dixit, V. M.: The cell-death machine. *Curr Biol* 6, 555-562 (1996)
8. Alnemri, E. S., Livingston, D. J., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wong, W. W., and Yuan, J.: Human ICE/CED-3 protease nomenclature. *Cell* 87, 171 (1996)
9. Kuida, K., Zheng, T. S., Na, S., Kuan, C.-Y., Yang, D., Karasuyama, H., Rakic, P., and Flavell, R. A.: Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* 384, 368-372 (1996)
10. Kuida, K., Lippke, J. A., Ku, G., Harding, M. W., Livingston, D. J., Su, M. S.-S., and Flavell, R. A.: Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. *Science* 267, 2000-2003 (1995)
11. Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., Salfeld, J., Towne, E., Tracey, D., Wardwell, S., Wei, F.-Y., Wong, W., Kamen, R. and Seshadri, T.: Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. *Cell* 80, 401-411 (1995)
12. Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E., and Poirier, G. G.: Specific proteolytic cleavage of poly(ADP-ribose) polymerase: An early marker of chemotherapy-induced apoptosis. *Cancer Res* 53, 3976-3985 (1993)
13. Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G., and Earnshaw, W. C.: Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature* 371, 346-347 (1994)
14. Tewari, M., Quan, L. T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M.: Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* 81, 801-9 (1995)
15. Earnshaw, W. C.: Apoptosis: lessons from *in vitro* systems. *TiCB* 5, 217-219 (1995)
16. Wang, Z.-Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M., and Wagner, E. F.: Mice lacking ADPRT and poly(ADP-ribosylation) develop normally but are susceptible to skin disease. *Genes Dev* 9, 509-520 (1995)
17. Rao, L., Perez, D., and White, E.: Lamin proteolysis facilitates nuclear events during apoptosis. *J Cell Biol* 135, 1441-1455 (1996)

## Apoptosis in Tumorigenesis

18. Takahashi, A., Alnemri, E. S., Lazebnik, Y. A., Fernandes-Alnemri, T., Litwack, G., Moir, R. D., Goldman, R. D., Poirier, G. G., Kaufmann, S. H., and Earnshaw, W. C.: Cleavage of lamin A by Mch2alpha but not CPP32: Multiple interleukin 1beta converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. *Proc Natl Acad Sci USA* 93, 8395-8400 (1996)
19. Neamati, A., Fernandez, A., Wright, S., Kiefer, J., and McConkey, D. J.: Degradation of lamin B1 precedes oligonucleosomal DNA fragmentation in apoptotic thymocytes and isolated thymocyte nuclei. *J Immunol* 154, 1693-1700 (1994)
20. Casciola-Rosen, L. A., Miller, D. K., Anhalt, G. J., and Rosen, A.: Specific cleavage of the 70-kDa protein component of small nuclear ribonucleoprotein is a characteristic biochemical feature of apoptotic cell death. *J Biol Chem* 269, 30757-30760 (1994)
21. Tewari, M., Beidler, D. R., and Dixit, V. M.: CrmA-inhibitable cleavage of the 70 kDa protein component of the U1 small nuclear ribonucleoprotein during Fas-and tumor necrosis factor-induced apoptosis. *J Biol Chem* 270, 18738-18741 (1995)
22. Song, Q., Less-Miller, S. P., Kumar, S., Zhang, N., Chan, D. W., Smith, G. C. M., Jackson, S. P., Alnemri, E. S., Litwack, G., Khanna, K. K., and Lavin, M. F.: DNA-dependent protein kinase catalytic subunit: a target for an ICE-like protease in apoptosis. *EMBO J* 15, 3238-3246 (1996)
23. Brancholini, C., Benedetti, M., and Schneider, C.: Microfilament reorganization during apoptosis: the role of Gas2, a possible substrate for ICE-like proteases. *EMBO J* 14, 5179-5190 (1995)
24. Na, S., Chuang, R. H., Tiru, T. G., Hanke, J. H., Bokoch, G. M., and Danley, D. E.: D4-GDI, a substrate for CPP32, is proteolyzed during Fas-induced apoptosis. *J Biol Chem* 271, 11209-11213 (1996)
25. Emoto, Y., Manome, Y., Meinhardt, G., Kasaki, H., Kharbanda, S., Robertson, M., Ghayur, T., Wong, W. W., Kamen, R., Weichselbaum, R., and Kufe, D.: Proteolytic activation of protein kinase C  $\delta$  by an ICE-like protease in apoptotic cells. *EMBO J* 14, 6148-6156 (1995)
26. Wang, X., Zelenski, N. G., Yang, J., Sakai, J., Brown, M. S., and Goldstein, J. L.: Cleavage of sterol regulatory element binding proteins (SREBPs) by CPP32 during apoptosis. *EMBO J* 15, 1012-1020 (1996)
27. Goldberg, Y. P., Nicholson, D. W., Rasper, D. M., Kalchman, M. A., Koide, H. B., Graham, R. K., Bromm, M., Kazemi-Esfajani, P., Thornberry, N. A., Vaillancourt, J. P., and Hayden, M. R.: Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nature Genetics* 13, 442-449 (1996)
28. Rudel, T. and Bokoch, G. M.: Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. *Science* 276, 1571-1574 (1997)
29. An, B. and Dou, Q. P.: Cleavage of the retinoblastoma protein during apoptosis: An interleukin 1beta- converting enzyme-like protease as candidate. *Cancer Res* 56, 438-442 (1996)
30. Jänicke, R. U., Walker, P. A., Lin, S. Y., and Porter, G.: Specific cleavage of the retinoblastoma protein by an ICE-like protease in apoptosis. *EMBO J* 15, 6969-6978 (1996)
31. Lazebnik, Y. A., Cole, S., Cooke, C. A., Nelson, W. G., and Earnshaw, W. C.: Nuclear events of apoptosis in vitro in cell-free mitotic extracts: A model system for analysis of the active phase of apoptosis. *J Cell Biol* 123, 7-22 (1993)
32. Liu, X. S., Kim, C. N., Yang, J., Jemmerson, R., and Wang, X. D.: Induction of apoptotic program in cell-free extracts - requirement for dATP and cytochrome c. *Cell* 86, 147-157 (1996)
33. Newmeyer, D. D., Farschon, D. M., and Reed, J. C.: Cell-free apoptosis in *Xenopus* egg extracts: inhibition by Bcl-2 and requirement for an organelle fraction enriched in mitochondria. *Cell* 79, 353-64 (1994)
34. Evans, E. K., Lu, W., Strum, S. L., Mayer, B. J., and Kornbluth, S.: Crk is required for apoptosis in *xenopus* egg extracts. *EMBO J* 16, 230-241 (1997)
35. Enari, M., Hase, A., and Nagata, S.: Apoptosis by a cytosolic extract from Fas-activated cells. *EMBO J* 14, 5201-5208 (1995)
36. Martin, S. J., Newmeyer, D. D., Mathias, S., Farschon, D. M., Wang, H. G., Reed, J. C., Kolesnick, R. N., and Green, D. R.: Cell-free reconstitution of fas-, uv radiation- and ceramide-induced apoptosis. *EMBO J* 14, 5191-5200 (1995)
37. Fearnhead, H. O., McCurrach, M. E., O'Neill, J., Zhang, K., Lowe, S. W., and Lazebnik, Y.A.: Oncogene-dependent apoptosis in extracts from drug-resistant cells. *Genes Dev* 11, 1266-1276 (1997)
38. Liu, X., Zou, H., Slaughter, C., and Wang, X.: DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* 89, 175-184 (1997)

## Apoptosis in Tumorigenesis

39. Hengartner, M. O. and Horvitz, H. R.: *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell* 76, 665-676 (1994)
40. Vaux, D. L., Cory, S., and Adams, J. M.: *Bcl-2* gene promotes a haemopoietic cell survival and cooperates with *c-myc* to immortalize pre-B cells. *Nature* 335, 440-442 (1988)
41. Veis, D. J., Sorenson, C. M., Shutter, J. R., and Korsmeyer, S. J.: *Bcl-2*-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 75, 229-40 (1993)
42. Nakayama, K., Nakayama, K., Negishi, I., Kuida, K., Sawa, H., and Loh, D. Y.: Targeted disruption of *Bcl-2* alpha beta in mice-occurrence of grey hair, polycystic kidney disease, and lymphocytopenia. *Proc Natl Acad Sci USA* 91, 3700-3704 (1994)
43. Kamada, S. A. A., Shinto, Y., Tsujimura, T., Takahashi, T., Noda, T., Kitamura, Y., Kondoh, H., and Tsujimoto, Y.: *Bcl-2* deficiency in mice leads to pleiotropic abnormalities: Accelerated lymphoid cell death in the thymus and spleen, polycystic kidney, hair hypopigmentation, and distorted small intestine. *Cancer Res* 55, 354-359 (1995)
44. Oltvai, Z. N. and Korsmeyer, S. J.: Checkpoints of dueling dimers foil death wishes. *Cell* 79, 189-192 (1994)
45. Oltvai, Z. N., Millman, C. L., and Korsmeyer, S. J.: *Bcl-2* heterodimerizes *in vivo* with a conserved homolog, *Bax*, that accelerates programmed cell death. *Cell* 74, 609-19 (1993)
46. Boise, L. H., Gonzalez, G. M., Postema, C. E., Ding, L., Lindsten, T., Turka, L. A., Mao, X., Nunez, G., and Thompson, C. B.: *bcl-x*, a *bcl-2*-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* 74, 597-608 (1993)
47. Yang, E., Zha, J., Jockel, J., Boise, L. H., Thompson, C. B., and Korsmeyer, S. J.: *Bad*, a heterodimeric partner for *Bcl-x<sub>L</sub>* and *Bcl-2*, displaces *Bax* and promotes cell death. *Cell* 80, 285-291 (1995)
48. Takayama, S., Sato, T., Krajewski, S., Kochel, K., Irie, S., Millan, J. A., and Reed, J. C.: Cloning and functional analysis of *BAG-1*: a novel *Bcl-2*-binding protein with anti-cell death activity. *Cell* 80, 279-284 (1995)
49. Chittenden, T., Harrington, E. A., O'Connor, R., Flemington, C., Lutz, R. J., Evan, G. I., and Guild, B. C.: Induction of apoptosis by the *Bcl-2* homologue *Bak*. *Nature* 374, 733-736 (1995)
50. Farrow, S. N., White, J. H. M., Martinou, I., and Brown, R.: Cloning of a *bcl-2* homologue by ioninteraction with adenovirus E1B 19K. *Nature* 374, 731-733 (1995)
51. Kiefer, M. C., Brauer, M. J., Powers, V. C., Wu, J. J., Umansky, S. R., Tomei, L. D., and Barr, P. J.: Modulation of apoptosis by the widely distributed *Bcl-2* homologue *Bak*. *Nature* 374, 736-739 (1995)
52. Boyd, J. M., Gallo, G. J., Elangovan, B., Houghton, A. B., Malstrom, S., Avery, B. J., Ebb, R. G., Subramanian, T., Chittenden, T., Lutz, R. J., and Chinnadurai, G.: *Bik1*, a novel death-inducing protein shares a distinct sequence motif with *Bcl-2* family proteins and interacts with viral and cellular survival-promoting proteins. *Oncogene* 11, 1921-1927 (1995)
53. Inohara, N., Ding, L., Chen, S., and Nunez, G.: *Harakiri*, a novel regulator of cell death, encodes a protein that activates apoptosis and interacts selectively with survival-promoting proteins *Bcl-2* and *Bcl-x<sub>L</sub>*. *EMBO J* 16, 1686-1694 (1997)
54. Yin, X. M., Oltvai, Z. N., and Korsmeyer, S. J.: *BH1* and *BH2* domains of *Bcl-2* are required for inhibition of apoptosis and heterodimerization with *Bax*. *Nature* 369, 321-323 (1994)
55. Cheng, E. H.-Y., Levine, B., Boise, L. H., Thompson, C. B., and Hardwick, J. M.: *Bax*-independent inhibition of apoptosis by *bcl-x(l)*. *Nature* 379, 554-556 (1996)
56. Knudson, C. M., Tung, K. S., Tourtellotte, W. G., Brown, G. A., and Korsmeyer, S. J.: *Bax*-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* 270, 96-99 (1995)
57. Muchmore, S. W., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H. S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S.-L., Ng, S.-C., and Fesik, S. W.: X-ray and NMR structure of human *Bcl-x<sub>L</sub>*, an inhibitor of programmed cell death. *Nature* 381, 335-341 (1996)
58. Chittenden, T., Flemington, C., Houghton, A. B., Ebb, R. G., Gallo, G. J., Elangovan, B., Chinnadurai, G., and Lutz, R. J.: A conserved domain in *Bak*, distinct from *BH1* and *BH2*, mediates cell death and protein binding functions. *EMBO J* 14, 5589-5596 (1995)
59. Sattler, M., Liang, H., Nettesheim, D., Meadows, R. P., Harlan, J. E., Eberstadt, M., Yoon, H. S., Shuker, S. B., Chang, B. S., Minn, A. J., Thompson, C. B., and Fesik, S. W.: Structure of *Bcl-x<sub>L</sub>*-*Bak* peptide complex: recognition between regulators of apoptosis. *Science* 275, 983-986 (1997)



## Apoptosis in Tumorigenesis

60. Parker, M. W. and Pattus, F.: Rendering a membrane protein soluble in water: a common packing motif in bacterial protein toxins. *TiBS* 18, 391-395 (1993)
61. London, E.: Diphtheria toxin: membrane interaction and membrane translocation. *Biochim Biophys Acta* 1113, 25-51 (1992)
62. Minn, A. J., Velez, P., Schendel, S. L., Liang, H., Muchmore, S. W., Fesik, S. W., Fill, M., and Thompson, C. B.: Bcl-x<sub>L</sub> forms an ion channel in synthetic lipid membranes. *Nature* 385, 353-357 (1997)
63. Hockenbery, D. M., Oltvai, Z. N., Yin, X. M., Millman, C. L., and Korsmeyer, S. J.: Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75, 241-251 (1993)
64. Kane, D. J., Sarafin, T. A., Auton, S., Hahn, H., Gralla, F. B., Valentine, J. C., Ord, T., and Bredesen, D. E.: Bcl-2 inhibition of neural cell death: decreased generation of reactive oxygen species. *Science* 262, 1274-1276 (1993)
65. Shimizu, S., Eguchi, Y., Kosaka, H., Kamiike, W., Matsuda, H., and Tsujimoto, Y.: Prevention of hypoxia-induced cell death by Bcl-2 and Bcl-x<sub>L</sub>. *Nature* 374, 811-813 (1995)
66. Jacobson, M. D. and Raff, M. C.: Programmed cell death and Bcl-2 protection in very low oxygen. *Nature* 374, 814-816 (1995)
67. Krajewski, S., Tanaka, S., Takayama, S., Schibler, M. J., Fenton, W., and Reed, J. C.: Investigations of the subcellular distribution of the BCL-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum and other mitochondrial membranes. *Cancer Res* 53, 4701-4714 (1993)
68. Strasser, A., Harris, A. W., Huang, D. C., Krammer, P. H., and Cory, S.: Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. *EMBO J* 14, 6136-6147 (1995)
69. Gajewski, T. F. and Thompson, C. B.: Apoptosis meets signal transduction: Elimination of a BAD influence. *Cell* 87, 589-592 (1996)
70. May, W. S., Tyler, P. G., Ito, T., Armstrong, D. K., Qatsha, K. A., and Davidson, N. E.: Interleukin-3 and bryostatin-1 mediate hyperphosphorylation of BCL2 in association with suppression of apoptosis. *J Biol Chem* 269, 26865-26870 (1994)
71. Haldar, S., Jena, N., and Croce, C. M.: Inactivation of Bcl-2 by phosphorylation. *Proc Natl Acad Sci USA* 92, 4507-4511 (1995)
72. Wang, H.-G., Rapp, U. R., and Reed, J. C.: Bcl-2 targets the protein kinase Raf-1 to mitochondria. *Cell* 87, 629-638 (1996)
73. Zha, J., Harada, H., Yang, E., Jockel, J., and Korsmeyer, S. J.: Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X<sub>L</sub>. *Cell* 87, 619-628 (1996)
74. Chinnaiyan, A. M., O'Rourke, K., Lane, B. R., and Dixit, V. M.: Interaction of CED-4 with CED-3 and CED-9: A molecular framework for cell death. *Science* 275, 1122-1126 (1997)
75. Wu, D., Wallen, H. D., and Nunez, G.: Interaction and regulation of subcellular localization of CED-4 and CED-9. *Science* 275, 1126-1129 (1997)
76. Spector, M. S., Desnoyers, S., Hoepfner, D. J., and Hengartner, M. O.: Interaction between the C. elegans cell-death regulators CED-9 and CED-4. *Nature* 385, 653-656 (1997)
77. Shaham, S. and Horvitz, H. R.: An alternatively spliced c-elegans CED-4 RNA encodes a novel cell death inhibitor. *Cell* 86, 201-208 (1996)
78. Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T.-I., Jones, D. P., and Wang, X.: Prevention of Apoptosis by Bcl-2: Release of cytochrome c from mitochondria blocked. *Science* 275, 1129-1132 (1997)
79. Kluck, R. M., Bossy-Wetzel, E., Green, D. R., and Newmeyer, D. D.: The release of cytochrome c from mitochondria: A primary site for Bcl-2 regulation of apoptosis. *Science* 275, 1132-1136 (1997)
80. Susin, S. A., Zamzami, N., Castedo, M., Hirsch, T., Marchetti, P., Macho, A., Daugas, E., Geuskens, M., and Kroemer, G.: Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. *J Exp Med* 184, 1331-1341 (1996)
81. Nagata, S. and Suda, T.: Fas and Fas ligand: *lpr* and *gld* mutations. *Immun Today* 16, 39 (1995)
82. Nagata, S.: Apoptosis by death factor. *Cell* 88, 355-365 (1997)
83. Rozzo, S. J., Eisenberg, R. A., Cohen, P. L., and B.L., K.: Development of the T cell receptor repertoire in *lpr* mice. *Semin Immunol* 6, 19-26 (1994)
84. Fisher, G. H., Rosenberg, F. J., Straus, S. E., Dale, J. K., Middleton, L. A., Lin, A. Y., Strober, W., Lenardo, M. J., and Puck, J. M.: Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 81, 935-946 (1995)

## Apoptosis in Tumorigenesis

85. Rieux, L. F., Le, D. F., Hivroz, C., Roberts, I. A., Debatin, K. M., Fischer, A., and de Villartay, J.P.: Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* 268, 1347-1349 (1995)
86. Benoist, C. and Mathis, D.: Cell death mediators in autoimmune diabetes-no shortage of suspects. *Cell* 89, 1-3 (1997)
87. Chernovsky, A. V., Wang, Y., Wong, F. S., Visintin, I., Flavell, R. A., Janeway, C. A., and Matis, L. A.: The role of Fas in autoimmune diabetes. *Cell* 89, 17-24 (1997)
88. Brunner, T., Mogil, R., LaFace, D., Yoo, N., Mahboubi, A., Echeverri, F., Martin, S., Force, W., Lynch, D., Ware, C., and Green, D.: Cell-autonomous Fas (CD95)/Fas-ligand interactions mediates activation-induced apoptosis in T-cell hybridomas. *Nature* 373, 441-444 (1995)
89. Dhein, J., Walczak, H., Bäuml, C., Debatin, K., and Krammer, P.: Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* 373, 438-441 (1995)
90. Ju, S., Panka, D., Cui, H., Ettinger, R., El-Khatib, M., Sherr, D., and Stanger, B.: Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 373, 444-448 (1995)
91. Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S., Sameshima, M., Hase, A., Seto, Y., and Nagata, S.: The polypeptide encoded by the cDNA for the human cell surface antigen Fas can mediate apoptosis. *Cell* 66, 233-243 (1991)
92. Oehm, A., Behrmann, I., Falk, W., Pawlita, M., Maier, G., Klas, C., Li-Weber, M., Richards, S., Dhein, J., Trauth, B. C., Ponsing, H., and Krammer, P.H.: Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily: sequence identity with the Fas antigen. *J Biol Chem* 267, 10709-10715 (1992)
93. Itoh, N. and Nagata, S.: A novel protein domain required for apoptosis: mutational analysis of human Fas antigen. *J Biol Chem* 268, 10932-10937 (1993)
94. Tartaglia, L. A., Ayres, T. M., Wong, G. H. W., and Goeddel, D. V.: A novel domain within the 55kd TNF receptor signals cell death. *Cell* 74, 845-853 (1993)
95. Boldin, M., Varfolomeev, E., Panczer, Z., Mett, I., Camonis, J., and Wallach, D.: A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J Biol Chem* 270, 7795-7798 (1995)
96. Chinnaiyan, A., O'Rourke, K., Tewari, M., and Dixit, V.: FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81, 505-512 (1995)
97. Hsu, H., Xiong, J., and Goeddel, D. V.: The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell* 81, 495-504 (1995)
98. Stanger, B. Z., Leder, P., Lee, T. H., Kim, E., and Seed, B.: RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. *Cell* 81, 513-523 (1995)
99. Hsu, H. L., Shu, H. B., Pan, M. G., and Goeddel, D. V.: TRADD-TRAF2 and tradd-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 84, 299-308 (1996)
100. Huang, B., Eberstadt, M., Olejniczak, E. T., Meadows, R. P., and Fesik, S. W.: NMR structure and mutagenesis of the Fas (Apo-1/CD95) death domain. *Nature* 384, 638-641 (1996)
101. Feinstein, E. and Kimchi, A.: The death domain: a module shared by proteins with diverse cellular functions. *TiBS* 20, 342-344 (1995)
102. Boldin, M. P., Goncharov, T. M., Goltsev, Y. V., and Wallach, D.: Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/Apo-1- and TNF receptor-induced cell death. *Cell* 85, 803-815 (1996)
103. Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J. D., Zhang, M., Gentz, R., Mann, M., Krammer, P. H., Peter, M. E., and Dixit, V. M.: Flice, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/Apo-1) death-inducing signaling complex. *Cell* 85, 817-827 (1996)
104. Chinnaiyan, A. M., O'Rourke, K., Yu, G.-L., Lyons, R. H., Garg, M., Duan, D. R., Zing, L., Gentz, R., Ni, J., and Dixit, V. M.: Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science* 274, 990-992 (1996)
105. Kitson, J., Raven, T., Jaing, Y.-P., Goeddel, D. V., Giles, K. M., Pun, K.-T., Grinham, C. J., Brown, R., and Farrow, S. N.: A death-domain-containing receptor that mediates apoptosis. *Nature* 384, 372-375 (1996)
106. Marsters, S. A., Sheridan, J.P., Donahue, C.J., Pitti, R.M., Gray, C.L., Goddard, A.D., Bauer, K.D., and Ashkenazi, A.: Apo-3, a new member of the tumor necrosis factor receptor family, contains a death domain and activates apoptosis and NF-kappaB. *Curr Biol* 6, 1669-1676 (1996)

## Apoptosis in Tumorigenesis

107. Thome, M., Schneider, P., Hofmann, K., Fickenscher, H., Meinel, E., Neipel, F., Mattmann, C., Burns, K., Bodmer, J. L., Schroter, M., Scaffidi, C., Kramer, P. H., Peter, M. E., and Tschopp, J.: Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. *Nature* 386, 517-521 (1997)
108. Wiley, S. R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C.P., Nicholl, J.K., Sutherland, G.R., Smith, T.D., Rauch, C., and Smith, C.A.: Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3, 673-682 (1995)
109. Bodmer, J.-L., Burns, K., Schneider, P., Hofmann, K., Steiner, V., Thome, M., Bornand, T., Hahne, M., Schroter, M., Becker, K., Wilson, A., French, L.E., Browning, J.L., MacDonald, H.R. and Tschopp J.: TRAMP, a novel apoptosis-mediating receptor with sequence homology to tumor necrosis factor receptor 1 and Fas(Apo-1/CD95). *Immunity* 6, 79-88 (1997)
110. Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V.: TRAF2-mediated activation of NK-kappa B by TNF receptor 2 and CD40. *Science* 269, 1424-1427 (1995)
111. Duan, H. and Dixit, V. M.: RAIDD is a new 'death' adaptor molecule. *Nature* 385, 86-89 (1997)
112. Beg, A. A. and Baltimore, D.: An essential role for NF-kappaB in preventing TNF-alpha induced cell death. *Science* 274, 782-784 (1996)
113. Liu, Z.-G., Hsu, H., Goeddel, D. V., and Karin, M.: Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. *Cell* 87, 565-576 (1996)
114. Wang, C. Y., Mayo, M. W., and Baldwin, A. S.: TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. *Science* 274, 724 (1996)
115. Santana, P., Pena, L. A., Haimovitzfriedman, A., Martin, S., Green, D., McLoughlin, M., Cordoncardo, C., Schuchman, E. H., Fuks, Z., and Kolesnick, R.: Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis. *Cell* 86, 189-199 (1996)
116. Bose, R., Verheij, M., Haimovitz, F. A., Scotto, K., Fuks, Z., and Kolesnick, R.: Ceramide synthase mediates daunorubicin-induced apoptosis: an alternative mechanism for generating death signals. *Cell* 82, 405-414 (1995)
117. Jaffrezou, J. P., Levade, T., Bettaieb, A., Andrieu, N., Bezombes, C., Maestre, N., Vermeersch, S., Rousse, A., and Laurent, G.: Daunorubicin-induced apoptosis: triggering of ceramide generation through sphingomyelin hydrolysis. *EMBO J* 15, 2417-2424 (1996)
118. Tepper, C. G., Jayadev, S., Liu, B., Bielawska, A., Wolff, R., Yonehara, S., Hannun, Y. A., and Seldin, M. F.: Role for ceramide as an endogenous mediator of Fas-induced cytotoxicity. *Proc Natl Acad Sci USA* 92, 8443-8447 (1995)
119. Obeid, L. M., Linardic, C. M., Karolak, L. A., and Hannun, Y. A.: Programmed cell death induced by ceramide. *Science* 259, 1769-1771 (1993)
120. Pronk, G. J., Ramer, K., Amiri, P., and Williams, L. T.: Requirement of an ice-like protease for induction of apoptosis and ceramide generation by REAPER. *Science* 271, 808-810 (1996)
121. Juo, P., Kuo, C. J., Reynolds, S. E., Konz, R. F., Raingeaud, J., Davis, R. J., Biemann, H. P., and Blenis, J.: Fas activation of the p38 mitogen-activated protein kinase signaling pathway requires ICE/CED-3 family proteases. *Mol Cell Biol* 17, 24-35 (1997)
122. Martin, S. J. and Green, D. R.: Protease activation during apoptosis: death by a thousand cuts? *Cell* 82, 349-52 (1995)
123. Enari, M., Talianian, R. V., Wong, W. W., and Nagata, S.: Sequential activation of ice-like and CPP32-like proteases during fas-mediated apoptosis. *Nature* 380, 723-726 (1996).
124. Itoh, N., Tsujimoto, Y., and Nagata, S.: Effect of bcl-2 on Fas antigen-mediated cell death. *J Immunol* 151, 621-627 (1993)
125. Boise, L. H., Minn, A. J., Noel, P. J., June, C. H., Accavitti, M. A., Lindsten, T., and Thompson, C. B.: CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-x<sub>L</sub>. *Immunity* 3, 87-98 (1995)
126. Rodriguez, I., Matsuura, K., Khatib, K., Reed, J. C., Nagata, S., and Vassalli, P.: A bcl-2 transgene expressed in hepatocytes protects mice from fulminant liver destruction but not from rapid death induced by anti-Fas antibody injection. *J Exp Med* 183, 1031-1036 (1996)
127. Krippner, A., Matsuno-Yagi, A., Gottlieb, R., and Babior, B.: Loss of function of cytochrome c in Jurkat cells undergoing Fas-mediated apoptosis. *J Biol Chem* 271, 21629-21636 (1996)
128. Oppenheim, R. W.: Neurotrophic survival molecules for motoneurons: An embarrassment of riches. *Neuron* 17, 195-197 (1996)
129. Yao, R. and Cooper, G. M.: Requirement for phosphatidylinositol-3 kinase in the prevention of

## Apoptosis in Tumorigenesis

- apoptosis by nerve growth factor. *Science* 267, 2003-2006 (1995)
130. Franke, T. F., Yang, S.-I., Chan, T. O., Datta, K., Kaslaukas, A., Morrison, P. K., Kaplan, D. R., and Tschlis, P. N.: The protein kinase encoded by the Akt proto-*Oncogene* is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* 81, 727-736 (1995)
131. Yao, R. and Cooper, G. M.: Growth factor-dependent survival of rodent fibroblasts requires phosphatidylinositol 3-kinase but is independent of pp70(S6K) activity. *Oncogene* 13, 343-351 (1996)
132. Dudek, H., Datta, S. R., Frank, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E.: Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 275, 661-664 (1997)
133. Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock, D. C.: Induction of apoptosis in fibroblasts by c-myc protein. *Cell* 69, 119-128 (1992)
134. Kennedy, S. G., Wagner, A. J., Conzen, S. D., Jordan, J., Bellacosa, A., Tschlis, P. N., and Hay, N.: The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev* 11, 701-713 (1997)
135. Kauffmann-Zeh, A., Rodriguez-Viciana, P., Ulrich, E., Gilbert, C., Coffey, P., Downward, J., and Evan, G. I.: Suppression of c-Myc-induced apoptosis by Ras signaling through PI(3)K and PKB. *Nature* 385, 544-548 (1997)
136. Franke, T. F., Kaplan, D. R., and Cantley, L. C.: P13K: Downstream AKTion blocks apoptosis. *Cell* 88, 435-437 (1997)
137. Alessi, D. r., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R. J., Reese, C. B., and Cohen, P.: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B. *Curr Biol* 7, 261-269 (1997)
138. Kwaja, A., Rodriguez-Viciana, P., Wennström, S., Warne, P. H., and Downward, J.: Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. *EMBO J* 16, 2783-2793 (1997)
139. Marcu, K. B., Bossone, S. A., and Patel, A. J.: myc function and regulation. *Annu Rev Biochem* 61, 809-860 (1992)
140. Askew, D. S., Ashmun, R. A., Simmons, B. C., and Cleveland, J. L.: Constitutive c-myc expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene* 6, 1915-1922 (1991)
141. Shi, Y., Glynn, J. M., Guilbert, L. J., Cotter, T. G., Bissonnette, R. P., and Green, D. R.: Role for c-myc in activation-induced apoptotic cell death in T cell hybridomas. *Science* 257, 212-214 (1992)
142. Reed, J. C., Cuddy, M., Haldar, S., Croce, C., and Nowell, P.: BCL2-mediated tumorigenicity of a human T-lymphoid cell line: Synergy with Myc and inhibition by BCL2 antisense. *Proc Natl Acad Sci USA* 87, 3660-3664 (1990)
143. Strasser, A., Harris, A. W., Bath, M. L., and Cory, S.: Novel primitive lymphoid tumors induced in transgenic mice by cooperation between myc and bcl-2. *Nature* 348, 331-333 (1990)
144. McDonnell, T. J. and Korsmeyer, S. J.: Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). *Nature* 349, 254-256 (1991)
145. Canman, C. E. and Kastan, M. B.: Induction of apoptosis by tumor suppressor genes and oncogenes. *Sem Cancer Biol* 6, 17-25 (1995)
146. Shen, Y. and Shenk, T. E.: Viruses and apoptosis. *Curr Opin Genetics Dev* 5, 105-111 (1995)
147. Hunter, T.: Oncoprotein Networks. *Cell* 88, 333-346 (1997)
148. Yonish-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A., and Oren, M.: Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* 352, 345-347 (1991)
149. Levy, N., Yonish-Rouach, E., Oren, M., and Kimchi, A.: Complementation by wild-type p53 of interleukin-6 effects on M1 cells-induction of cell cycle exit and cooperativity with c-myc suppression. *Mol Cell Biol* 13, 7942-7952 (1993)
150. Lowe, S. W., Ruley, H. E., Jacks, T., and Housman, D. E.: p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 74, 957-967 (1993)
151. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T.: p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 362, 847-849 (1993)
152. Lotem, J. and Sachs, L.: Hematopoietic cells from mice deficient in wild-type p53 are more resistant to induction of apoptosis by some agents. *Blood* 82, 1092-1096 (1993)
153. Lee, J. M. and Bernstein, A.: p53 mutations increase resistance to ionizing radiation. *Proc Natl Acad Sci USA* 90, 5742-5746 (1993)

## Apoptosis in Tumorigenesis

154. Wagner, A. J., Kokontis, J. M., and Hay, N.: Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce  $p21^{waf1/cip1}$ . *Genes Dev* 8, 2817-2830 (1994)
155. Hermeking, H. and Eick, D.: Mediation of c-Myc-induced apoptosis by p53. *Science* 265, 2091-2093 (1994)
156. Canman, C. E., Gilmer, T. M., Coutts, S. B., and Kastan, M. B.: Growth factor modulation of p53-mediated growth arrest versus apoptosis. *Genes Dev* 9, 600-611 (1995)
157. Symonds, H., Krall, L., Remington, L., Saenz, R. M., Lowe, S., Jacks, T., and Van Dyke, T.: p53-dependent apoptosis suppresses tumor growth and progression in vivo. *Cell* 78, 703-711 (1994)
158. Donehower, L. A., Godley, L. A., Aldaz, M., Pyle, R., Shi, Y.-P., Pinkel, D., Gray, J., Bradley, A., Medina, D., and Varmus, H. E.: Deficiency of p53 accelerates mammary tumorigenesis in Wnt-1 transgenic mice and promotes chromosomal instability. *Genes Dev* 9, 882-895 (1995)
159. White, E., Cipriani, R., Sabbatini, P., and Denton, A.: Adenovirus E1B 19-kilodalton protein overcomes the cytotoxicity of E1A proteins. *J Virol* 65, 2968-2978 (1991)
160. Lowe, S. W. and Ruley, H. E.: Stabilization of the p53 tumor suppressor is induced by adenovirus 5 E1A and accompanies apoptosis. *Genes Dev* 7, 535-545 (1993)
161. Rao, L., Debbas, P., Sabbatini, P., Hockenberry, D., Korsmeyer, S., and White, E.: The adenovirus E1A proteins induces apoptosis which is inhibited by the E1B 19K and Bcl-2 proteins. *Proc Natl Acad Sci USA* 89, 7742-7746 (1992)
162. Debbas, M. and White, E.: Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B. *Genes Dev* 7, 546-554 (1993)
163. zur Hausen, H.: Human papillomaviruses in the pathogenesis of anogenital cancer. *Virology* 184, 9-13 (1991)
164. Dyson, N., Howley, P. M., Munger, K., and Harlow, E.: The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 243, 934-937 (1989)
165. Whyte, P., Buchkovich, K. J., Horowitz, J. M., Friend, S. H., Rayback, M., Weinberg, R. A., and Harlow, E.: Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. *Nature* 334, 124-129 (1988)
166. White, A. E., Livanos, E. M., and Tlsty, T. D.: Differential disruption of genomic integrity and cell cycle regulation of normal fibroblasts by the HPV oncoproteins. *Genes Dev* 8, 666-677 (1994)
167. Field, S. J., Tsai, F. Y., Kuo, F., Zubiaga, A. M., Kaelin, W. G., Livingston, D. M., Orkin, S. H., and Greenberg, M. E.: E2F-1 functions in mice to promote apoptosis and suppress proliferation. *Cell* 85, 549-561 (1996)
168. Yamasaki, L., Jacks, T., Bronson, R., Goillot, E., Harlow, E., and Dyson, N. J.: Tumor induction and tissue atrophy in mice lacking E2F-1. *Cell* 85, 537-548 (1996)
169. Weinberg, R. A.: The retinoblastoma protein and cell cycle control. *Cell* 81, 323-330 (1995)
170. Wu, X. and Levine, A. J.: p53 and E2F-1 cooperate to mediate apoptosis. *Proc Natl Acad Sci USA* 91, 3602-3606 (1994)
171. Shan, B. and Lee, W.-H.: Deregulated expression of E2F-1 induces S-phase entry and leads to apoptosis. *Mol Cell Biol* 14, 8166-8173 (1994)
172. Kranenburg, O., Vandereb, A., and Zantema, A.: Cyclin D1 is an essential mediator of apoptotic neuronal cell death. *EMBO J* 15, 46-54 (1996)
173. Qin, X. Q., Livingston, D. M., Kaelin, W. J., and Adams, P. D.: Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. *Proc Natl Acad Sci USA* 91, 10918-22 (1994)
174. Haas-Kogan, D., Kogan, S. C., Levi, D., Dazin, P., T'Ang, A., Fung, Y. K., and Israel, M. A.: Inhibition of apoptosis by the retinoblastoma gene product. *EMBO J* 14, 461-472 (1995)
175. Fisher, D. E.: Apoptosis in cancer therapy: crossing the threshold. *Cell* 78, 539-542 (1994)
176. Hoang, A. T., Cohen, K. J., Barrett, J. F., Bergstrom, D. A., and Dang, C. V.: Participation of cyclin A in Myc-induced apoptosis. *Proc Natl Acad Sci USA* 91, 6875-6879 (1994)
177. Meikrantz, W., Gisselbrecht, S., Tam, S. W., and Schlegel, R.: Activation of cyclin A-dependent protein kinase during apoptosis. *Proc Natl Acad Sci USA* 91, 3754-3758 (1994)
178. Shi, L., Nishioka, W. K., Th'ng, J., Bradbury, E. M., Litchfield, D. W., and Greenberg, A. H.: Premature p34<sup>cdc2</sup> activation required for apoptosis. *Science* 263, 1143-1145 (1994)
179. Wang, J. and Walsh, K.: Resistance to apoptosis conferred by cdk inhibitors during myocyte differentiation. *Science* 273, 359-361 (1996)

## Apoptosis in Tumorigenesis

180. Meikrantz, W. and Schlegel, R.: Suppression of apoptosis by dominant negative mutants of cyclin-dependent protein kinases. *J Biol Chem* 271, 10205-10209 (1996)
181. Bello-Fernandez, C., Packham, G., and Cleveland, J.: The ornithine decarboxylase gene is a transcriptional target of c-Myc. *Proc Natl Acad Sci USA* 90, 7804-7808 (1993)
182. Galaktionov, K., Chen, X. C., and Beach, D.: Cdc25 cell-cycle phosphatase as a target of c-myc. *Nature* 382, 511-517 (1996)
183. Rudolph, B., Saffrich, R., Zwicker, J., Henglein, B., Muller, R., Ansorge, W., and Eilers, M.: Activation of cyclin-dependent kinases by myc mediates induction of cyclin a, but not apoptosis. *EMBO J* 15, 3065-3076 (1996)
184. Packham, G., Porter, C. W., and Cleveland, J. L.: C-myc induces apoptosis and cell cycle progression by separable, yet overlapping, pathways. *Oncogene* 13, 461-469 (1996)
185. Evan, G. I., Brown, L., Whyte, M., and Harrington, E.: Apoptosis and the cell cycle. *Curr Opin Cell Biol* 7, 825-834 (1995)
186. Harrington, E. A., Bennett, M. R., Fanidi, A., and Evan, G. I.: c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J* 13, 3286-3295 (1994)
187. Levine, A. J.: p53, the cellular gatekeeper for growth and division. *Cell* 88, 323-331 (1997)
188. Smith, M. L., Chen, I.-T., Zhan, Q., Bae, I., Chen, C.-Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M., and Fornace, A. J.: Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science* 266, 1376-1379 (1994)
189. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B.: WAF1, a potential mediator of p53 tumor suppression. *Cell* 75, 817-825 (1993)
190. Barak, Y., Juven, T., Haffner, R., and Oren, M.: Mdm2 expression is induced by wild-type p53 activity. *EMBO J* 12, 461-468 (1993)
191. Wu, X., Bayle, H., Olson, D., and Levine, A. J.: The p53-mdm2 autoregulatory feedback loop. *Genes Dev* 7, 1126-1132 (1993)
192. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J., and Leder, P.: Mice lacking *p21<sup>CIP1</sup>/WAF1* undergo normal development, but are defective in G1 checkpoint control. *Cell* 82, 675-684 (1995)
193. Miyashita, T. and Reed, J. C.: Tumor suppressor p53 is a direct transcriptional activator of the human *bax* gene. *Cell* 80, 293-299 (1995)
194. Caelles, C., Helmberg, A., and Karin, M.: p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature* 370, 220-223 (1994)
195. Sabbatini, P., Lin, J., Levine, A. J., and White, E.: Essential role for p53-mediated transcription in E1A-induced apoptosis. *Genes Dev* 9, 2184-2192 (1995)
196. Haupt, Y., Rowan, S., Shaulian, E., Vousden, K. H., and Oren, M.: Induction of apoptosis in HeLa cells by trans-activation-deficient p53. *Genes Dev* 9, 2170-2183 (1995)
197. Wang, X. W., Vermeulen, W., Coursen, J. D., Gibson, M., Lupold, S. E., Forrester, K., Xu, G. W., Elmore, L., Yeh, H., Hoeijmakers, J., and Harris, C. C.: The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. *Genes Dev* 10, 1219-1232 (1996)
198. Rowan, S., Ludwig, R. L., Haupt, Y., Bates, S., Lu, X., Oren, M., and Vousden, K. H.: Specific loss of apoptotic but not cell-cycle arrest function in a human tumor derived p53 mutant. *EMBO J* 15, 827-838 (1996)
199. Maheswaran, S., Engelbert, C., Bennett, P., Heinrich, G., and Haber, D. A.: The WT1 gene product stabilizes p53 and inhibits p53-mediated apoptosis. *Genes Dev* 9, 2143-2156 (1995)
200. Lin, C. Q. and Bissell, M. J.: Multi-faceted regulation of cell differentiation by extracellular matrix. *FASEB J* 7, 737-744 (1993)
201. Gumbiner, B. M.: Cell adhesion: The molecular basis of tissue architecture and morphogenesis. *Cell* 84, 345-357 (1996)
202. Ruoslahti, E. and Reed, J. C.: Anchorage dependence, integrins, and apoptosis. *Cell* 77, 477-478 (1994)
203. Hynes, R. O.: Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* 69, 11-25 (1992)
204. Schwartz, M. A.: Signaling by integrins: implications for tumorigenesis. *Cancer Res* 53, 1503-1506 (1993)
205. Plantefaber, L. C. and Hynes, R. O.: Changes in integrin receptors on oncogenically transformed cells. *Cell* 56, 281-290 (1989)

## Apoptosis in Tumorigenesis

206. Ben-Ze'ev, A., Farmer, S. R., and Penman, S.: Protein synthesis requires cell-surface contact while nuclear events respond to cell shape in anchorage-dependent fibroblasts. *Cell* 21, 365-372 (1980)
207. Campisi, J. and Medrano, E. E.: Cell cycle perturbations in normal and transformed fibroblasts. *J Cell Physiol* 114, 53-60 (1983)
208. Assoian, R. K.: Anchorage-dependent cell cycle progression. *J Cell Biol* 136, 1-4 (1997)
209. Frisch, S. M. and Francis, H.: Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol* 124, 619-626 (1994)
210. Meredith, J. E., Fazeli, B., and Schwartz, M. A.: The extracellular matrix as a cell survival factor. *Mol Biol Cell* 4, 953-961 (1993)
211. Re, F., Zanetti, A., Sironi, M., Polentarutti, N., Lanfrancone, L., Dejana, E., and Colotta, F.: Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J Cell Biol* 127, 537-546 (1994)
212. Boudreau, N., Sympton, C. J., Werb, Z., and Bissell, M. J.: Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. *Science* 267, 891-893 (1995)
213. Alexander, C. M., Howard, E. W., Bissell, M. J., and Werb, Z.: Rescue of mammary epithelial cell apoptosis and entactin degradation by a tissue inhibitor of metalloproteinase-1 transgene. *J Cell Biol* 135, 1669-1677 (1996)
214. Patterson, D., Hayes, W. P., and Shi, Y.-B.: Transcriptional activation of the metalloproteinase gene *stromelysin-3* coincides with thyroid hormone-induced cell death during frog metamorphosis. *Dev Biol* 167, 252-262 (1995)
215. Coucouvanis, E. and Martin, G. R.: Signals for death and survival: a two-step mechanism for cavitation in the vertebrate embryo. *Cell* 83, 279-287 (1995)
216. Montgomery, A. M. P., Reisfeld, R. A., and Cheresch, D. A.: Integrin  $\alpha_5\beta_3$  rescues melanoma cells from apoptosis in three-dimensional dermal collagen. *Proc Natl Acad Sci USA* 91, 8856-8860 (1994)
217. Schaller, M. D., Borgman, C. A., Cobb, B. C., Reynolds, A. B., and Parsons, J. T.: pp125<sup>FAK</sup> a structurally distinctive protein-tyrosine kinase associated with focal adhesion. *Proc Natl Acad Sci USA* 89, 5192-5196 (1992)
218. Hungerford, J. E., Compton, M. T., Matter, M. L., Hoffstrom, B. J., and Otey, C. A.: Inhibition of pp125<sup>FAK</sup> in cultured fibroblasts results in apoptosis. *J Cell Biol* 135, 1383-1390 (1996)
219. Frisch, S. M., Vuori, K., Ruoslahti, E., and Chan-Hui, P.-Y.: Control of adhesion-dependent cell survival by focal adhesion kinase. *J Cell Biol* 134, 793-799 (1996)
220. Nikiforov, M. A., Hagen, K., Ossovskaya, V. S., Connor, T. M., Lowe, S. W., Deichman, G. I., and Gudkov, A. V.: p53 modulation of anchorage independent growth and experimental metastasis. *Oncogene* 13, 1709-1719 (1996)
221. McGill, G., Shimamura, A., Bates, R., Savage, R. E., and Fisher, D. E.: Loss of matrix adhesion leads to rapid transformation-selective apoptosis in fibroblasts. *J Cell Biol*, 1997 (In Press)
222. Zhang, Z., Vuori, K., Reed, J. C., and Ruoslahti, E.: The  $\alpha_5\beta_1$  integrin supports survival of cells on fibronectin and up-regulates bcl-2 expression. *Proc Natl Acad Sci USA* 92, 6161-6165 (1995)
223. Frisch, S. M., Vuori, K., Kelaita, D., and Sicks, S.: A role for Jun-N-terminal kinase in anoikis; suppression by Bcl-2 and Crm-A. *J Cell Biol* 135, 1377-1382 (1996)
224. Rak, J., Mitsuhashi, Y., Erdos, V., Huang, S.-n., Filmus, J., and Kerbel, R. S.: Massive programmed cell death in intestinal epithelial cells induced by three-dimensional growth conditions: suppression by mutant c-H-ras oncogene expression. *J Cell Biol* 131, 1587-1598 (1995)
225. Ojakian, G. K.: Tumor promoter-induced changes in the permeability of epithelial cell tight junctions. *Cell* 23, 95-103 (1981)
226. Stoker, M., O'Neill, C., Berryman, S., and Waxman, V.: Anchorage and growth regulation in normal and virus-transformed cells. *Intl J Cancer* 3, 683-693 (1968)
227. Bates, R. C., Buret, A., van helden, D. F., Norton, M. A., and Burns, G. F.: Apoptosis induced by inhibition of intercellular contact. *J Cell Biol* 125, 403-415 (1994)
228. Raff, M. C.: Social controls on cell survival and cell death. *Nature* 356, 397-400 (1992)
229. Varner, J. A., Emerson, D. A., and Juliano, R. L.: Integrin  $\alpha_5\beta_1$  expression negatively regulates cell growth: Reversal by attachment to fibronectin. *Mol Biol Cell* 6, 725-740 (1995)
230. Sugahara, H., Kanakura, Y., Furitsu, T., Ishihara, K., Oritani, K., Ikeda, H., Kitayama, H., Ishikawa, J., Hashimoto, K., Kanayama, Y., and Matsuzawa, Y.:

## Apoptosis in Tumorigenesis

Induction of programmed cell death in human hematopoietic cell lines by fibronectin with very late antigen 5. *J Exp Med* 179, 1757-1766 (1995)

231. Guillemin, K. and Krasnow, M. A.: The hypoxic response: Huffing and HIFing. *Cell* 89, 9-12 (1997)

232. Mazure, N. M., Chen, E. Y., Yeh, P., Laderoute, K. R., and Giaccia, A. J.: Transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. *Cancer Res* 56, 3436-3440 (1996)

233. Graeber, T. G., Peterson, J. F., Tsai, M., Monica, K., Fornace, A. J., and Giaccia, A. J.: Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol Cell Biol* 14, 6264-6277 (1994)

234. Spiro, I., Rice, G. C., Durand, R. E., Stickler, R., and Ling, C. C.: Cell killing, radiosensitization and cell cycle redistribution induced by chronic hypoxia. *Int J Radiol Oncol Biol Phys* 10, 1275-1280 (1984)

235. Yao, K.-S., Clayton, M., and O'Dwyer, P. J.: Apoptosis in human adenocarcinoma HT29 cells induced by exposure to hypoxia. *J Natl Cancer Inst* 87, 117-122 (1995)

236. Graeber, T. G., Osmanian, C., Jacks, T., Housman, D. E., Koch, C. J., Lowe, S. W., and Giaccia, A. J.: Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 379, 88-91 (1996)

237. Hochachka, P. W., Buck, L. T., Doll, C. J., and Land, S. C.: Unifying theory of hypoxia tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proc Natl Acad Sci USA* 93, 9493-9498 (1996)

238. Warburg, O.: On respiratory impairment in cancer cells. *Science* 124, 269-270 (1956)

239. Semenza, G. L., Roth, P. H., Fang, H., and Wang, G. L.: Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* 269, 23757-23763 (1994)

240. Hanahan, D. and Folkman, J.: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86, 353-364 (1996)

241. Fandrey, J.: Hypoxia-inducible gene expression. *Respiration Physiology* 101, 1010 (1995)

242. Shweiki, D., Neeman, M., Itin, A., and Keshet, E.: Induction of vascular endothelial growth factor expression by hypoxia and glucose deficiency in multicell spheroids:

implications for tumor angiogenesis. *Proc Natl Acad Sci USA* 92, 768-772 (1995)

243. Shweiki, D., Itin, A., Soffer, D., and Keshet, E.: Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 359, 843-845 (1992)

244. Hlatky, L., Tsionou, C., Hahnfeldt, P., and Coleman, C. N.: Mammary fibroblasts may influence breast tumor angiogenesis via hypoxia-induced vascular endothelial growth factor upregulation and protein expression. *Cancer Res* 54, 6083-6086 (1994)

245. Dameron, K., Volpert, O., Tainsky, M., and Bouck, N.: Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 265, 1582-1585 (1994)

246. Weidner, N., Carroll, P. R., Flax, J., Blumenfeld, W., and Folkman, J.: Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* 143, 401-409 (1993)

247. Weidner, N., Semple, J. P., Welch, W. R., and Folkman, J.: Tumor angiogenesis and metastasis correlation in invasive breast carcinoma. *N Engl J Med* 324, 1-8 (1991)

248. O'Reilly, M. S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R. A., Moses, M., Lane, W. S., Cao, Y., Sage, E. H., and Folkman, J.: Angiostatin: A novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis Lung Carcinoma. *Cell* 79, 315-328 (1994)

249. O'Reilly, M. S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W. S., Flynn, E., Brikhead, J. R., Olsen, B. R., and Folkman, J.: Endostatin: An endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88, 277-285 (1997)

250. Fidler, I. J. and Ellis, L. M.: The implications of angiogenesis for the biology and therapy of cancer metastasis. *Cell* 79, 185-188 (1994)

251. Varner, J. A., Brooks, P. C., and Cheresch, D. A.: The integrin  $\alpha_v\beta_3$ : Angiogenesis and apoptosis. *Cell Adhesion and Comm* 3, 367-374 (1995)

252. Bischoff, J.: Cell adhesion and angiogenesis. *J Clin Invest* 99, 373-376 (1997)

253. Friedlander, M., Brooks, P. C., Shaffer, R. W., Kincaid, C. M., Varner, J. A., and Varner, D. A.: Definition of two angiogenic pathways by distinct  $\alpha_v$  integrins. *Science* 270, 1500-1502 (1995)

254. Brooks, P. C., Montgomery, A. M. P., Rosenfeld, M., Reisfeld, R. A., Hu, T., Klier, G., and Cheresch, D. A.:



## Apoptosis in Tumorigenesis

- Integrin  $\alpha_v\beta_3$  antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 79, 1157-1164 (1994)
255. Hammes, H.-P., Brownlee, M., Jonczyk, A., Sutter, A., and Preissner, K. T.: Subcutaneous injection of a cyclic peptide antagonist of vitronectin receptor-type integrins inhibits retinal neovascularization. *Nature Med* 2, 529-533 (1996)
256. Eastman, A.: Activation of programmed cell death by anticancer agents: cisplatin as a model system. *Cancer Cells* 2, 275-280 (1990)
257. Hickman, J. A.: Apoptosis induced by anticancer drugs. *Cancer Met Rev* 11, 121-139 (1992)
258. Fung, C. Y. and Fisher, D. E.: p53: from molecular mechanisms to prognosis in cancer. *J Clin Oncol* 13, 808-811 (1995)
259. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C.: p53 mutations in human cancers. *Science* 253, 49-53 (1991)
260. Hollstein, M., Rice, K., Greenblatt, M. S., Soussi, T., Fuchs, R., Sorlie, T., Hovig, E., Smith-Sorenson, B., Montesano, R., and Harris, C. C.: Database of p53 gene somatic mutations in human tumors and cell lines. *Nucl Acids Res* 22, 3551-3555 (1994)
261. Vogelstein, B. and Kinzler, K. W.: p53 function and dysfunction. *Cell* 70, 523-526 (1992)
262. Donehower, L., Harvey, M., Slagle, B., McArthur, M., Montgomery, C., Butel, J., and Bradley, A.: Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. *Nature* 356, 215-221 (1992)
263. Harvey, M., McArthur, M. J., Montgomery, C. A., Butel, J. S., Bradley, A., and Donehower, L. A.: Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. *Nature Genet* 5, 225-229 (1993)
264. Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A., and Friend, S. H.: Germline p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250, 1233-1238 (1990)
265. el Rouby, S., Thomas, A., Costin, D., Rosenberg, C. R., Potmesil, M., Silber, R., and Newcomb, E. W.: p53 gene mutation in B-cell chronic lymphocytic leukemia is associated with drug resistance and is independent of MDR1/MDR3 gene expression. *Blood* 82, 3452-3459 (1993)
266. Sander, C. A., Yano, T., Clark, H. M., Harris, C., Longo, D. L., Jaffe, E. S., and Raffeld, M.: p53 mutation is associated with progression in follicular lymphomas. *Blood* 82, 1994-2004 (1993)
267. Fossa, S. D., Berner, A. A., Jacobsen, A. B., Waehre, H., Kvarstein, B., Urnes, T., Ogreid, P., Johansen, T. E., Silde, J., and Nesland, J. M.: Clinical significance of DNA ploidy and S-phase fraction and their relation to p53 protein, c-erbB-2 protein and HCG in operable muscle-invasive bladder cancer. *Br J Cancer* 68, 572-578 (1993)
268. Allred, D. C., Clark, G. M., Elledge, r., Fuqua, S. A., Brown, R. W., Chamness, G. C., Osborne, C. K., and McGuire, W. L.: Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J Natl Cancer Inst* 85, 200-206 (1993)
269. Neri, A., Baldini, L., Trecca, D., Cro, L., Polli, E., and Maiolo, A. T.: p53 gene mutations in multiple myeloma are associated with advanced forms of malignancy. *Blood* 81, 128-135 (1993)
270. Isola, J., Visakorpi, T., Holli, K., and Kallioniemi, O. P.: Association of overexpression of tumor suppressor protein p53 with rapid cell proliferation and poor prognosis in node-negative breast cancer patients. *J Natl Cancer Inst* 84, 1109-1114 (1992)
271. Florenes, V. A., Oyjord, T., Holm, R., Skrede, M., Borresen, A. L., Nesland, J. M., and Fodstad, O.: TP53 allele loss, mutations and expression in malignant melanoma. *Br J Cancer* 69, 253-259 (1994)
272. Wada, M., Bartram, C. R., Nakamura, H., Hachiya, M., Chen, D., Borenstein, J., Miller, C. W., Ludwig, L., Hansen-Hagge, T. E., Ludwig, W., Reiter, A., Mizoguchii, H., and Koeffler, H. P.: Analysis of p53 mutations in a large series of lymphoid hematologic malignancies of childhood. *Blood* 82, 3163-3169 (1993)
273. Silvestrini, R., Bennini, E., Daidone, M. G., Veneroni, S., Boracchi, P., Cappelletti, V., Di Fronzo, G., and Veronesi, U.: p53 as independent prognostic marker in lymph node-negative breast cancer patients. *J Natl Cancer Inst* 85, 965-970 (1993)
274. YeARGIN, J., Cheng, J., and Haas, M.: Role of the p53 tumor suppressor gene in the pathogenesis and in the suppression of acute lymphoblastic T-cell leukemia. *Leukemia* 6, 85S-91S (1992)
275. YeARGIN, J., Cheng, J., Yu, A. L., Gjerset, R., Bogart, M., and Haas, M.: P53 mutation in acute T cell lymphoblastic leukemia is of somatic origin and is stable during establishment of T cell acute lymphoblastic leukemia cell lines. *J Clin Invest* 91, 2111-2117 (1993)

## Apoptosis in Tumorigenesis

276. Sawan, A., Randall, B., Angus, B., Wright, C., Henry, J. A., Ostrowski, J., Hennessy, C., Lennard, T. W., Corbett, I., and Home, C. H.: Retinoblastoma and p53 gene expression related to relapse and survival in human breast cancer: an immunohistochemical study. *J Pathol* 168, 23-28 (1992)
277. Starzynska, T., Bromley, M., Ghosh, A., and Stern, P. L.: Prognostic significance of p53 overexpression in gastric and colorectal carcinoma. *Br J Cancer* 66, 558-562 (1992)
278. Lowe, S. W., Bodis, S., McClatchey, A., Remington, L., Ruley, H. E., Fisher, D. E., Housman, D. E., and Jacks, T.: p53 status and the efficacy of cancer therapy in vivo. *Science* 266, 807-810 (1994)
279. Komarova, E. A., Chernov, M. V., Franks, R., Wang, K., Armin, G., Zelnick, C. R., Chin, D. M., Bacus, S. S., Stark, G. R., and Gudkov, A. V.: Transgenic mice with a p53-responsive *lacZ*: p53 activity varies dramatically during normal development and determines radiation and drug sensitivity *in vivo*. *EMBO J* 16, 1391-1400 (1997)
280. Gottlieb, E., Haffner, R., King, A., Asher, G., Gruss, P., Lonai, P., and Oren, M.: Transgenic mouse model for studying the transcriptional activity of the p53 protein: age- and tissue-dependent changes in radiation-induced activation during embryogenesis. *EMBO J* 16, 1381-1390 (1997)
281. Dole, M., Nunez, G., Merchant, A. K., Maybaum, J., Rode, C. K., Bloch, C. A., and Castle, V. P.: Bcl-2 inhibits chemotherapy-induced apoptosis in neuroblastoma. *Cancer Res* 54, 3253-3259 (1994)
282. Dole, M. G., Jasty, R., Cooper, M. J., Thompson, C. B., Nunez, G., and Castle, V. P.: Bcl-x<sub>L</sub> is expressed in neuroblastoma cells and modulates chemotherapy-induced apoptosis. *Cancer Res* 55, 2576-2582 (1995)
283. Reed, J. C., Meister, L. M., Tanaka, S., Cuddy, M., Yum, S., Geyer, C., and Pleasure, D.: Differential expression of bcl-2 protooncogene in neuroblastoma and other tumors of neural origin. *Cancer Res* 51, 6529-6538 (1991)
284. Castle, V. P., Heidelberger, K. P., Bromberg, J., Ou, X., Dole, M., and Nunez, G.: Expression of the apoptosis-suppressing protein Bcl-2 in neuroblastoma is associated with poor stage disease, unfavorable histology and N-myc amplification. *Am J Pathol* 143, 1542-1550 (1993)
285. Miyashita, T. and Reed, J. C.: Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* 81, 151-157 (1993)
286. Kamesaki, S., Kamesaki, H., Jorgensen, T. J., Tanizawa, A., Pommier, Y., and Cossman, J.: Bcl-2 protein inhibits etoposide-induced apoptosis through its effects on events subsequent to topoisomerase-II induced DNA strand breaks and their repair. *Cancer Res* 53, 4251-4256 (1993)
287. Hellemans, P., van Dam, P. A., Weyler, J., van Oosterom, A. T., Buytaert, P., and Van Marck, E.: Prognostic value of bcl-2 expression in invasive breast cancer. *Br J Cancer* 72, 354-360 (1995)
288. Lipponen, P., Pietilainen, T., Kosma, V. M., Aaltomaa, S., Eskelinen, M., and Syrjanen, K.: Apoptosis suppressing protein bcl-2 is expressed in well-differentiated breast carcinomas with favourable prognosis. *J Pathol* 177, 49-55 (1995)
289. Hurlimann, J., Larrinaga, B., and Vala, D. L.: Bcl-2 protein in invasive ductal breast carcinomas. *Virchows Archiv* 426, 163-168 (1995)
290. Joensuu, H., Pytkkanen, L., and Toikkanen, S.: Bcl-2 protein expression and long-term survival in breast cancer. *Am J Pathol* 145, 1191-1198 (1994)
291. Gee, J. M., Robertson, J. F., Ellis, I. O., Willsher, P., McClelland, R. A., Hoyle, H. B., Kyme, S. R., Finlay, P., Blamey, R. W., and Nicholson, R. I.: Immunocytochemical localization of BCL-2 protein in human breast cancers and its relationship to a series of prognostic markers and response to endocrine therapy. *Int J Cancer* 59, 619-628 (1994)
292. Strobel, T., Swanson, L., Korsmeyer, S., and Cannistra, S. A.: BAX enhances paclitaxel-induced apoptosis through a p53-independent pathway. *Proc Natl Acad Sci USA* 93, 14094-14099 (1996)
293. Danen-Van Oorschot, A. A. M., Fischer, D. F., Grimbergen, J. M., Klein, B., Zhuang, S.-M., Falkenburg, J. H. F., Blackendorf, C., Quax, P. H. A., Van der Eb, A. J., and Noteborn, M. H. M.: Apoptin induces apoptosis in human transformed and malignant cells but not in transformed cells. *Proc Natl Acad Sci USA* 94, 5843-5847 (1997)
294. Durand, R. E. and Sutherland, R. M.: Effects of intercellular contact on repair of radiation damage. *Exp Cell Res* 71, 75-80 (1972)
295. Fridman, R., Giaccone, G., Kanemoto, T., Martin, G. R., Gazdar, A. F., and Mulshine, J. L.: Reconstituted basement membrane (matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer lines. *Proc Natl Acad Sci USA* 87, 6698-6702 (1990)
296. Fuks, Z., Vlodavsky, I., Andreeff, M., McLoughlin, M., and Haimovitz-Friedman, A.: Effects of extracellular matrix on the response of endothelial cells to radiation *in vitro*. *Eur J Cancer* 28A, 725-731 (1992)

## Apoptosis in Tumorigenesis

297. Kobayashi, H., Man, S., Kapitan, S. J., Teicher, B. A., and Kerbel, R. S.: Acquired multicellular-mediated resistance to alkylating agents in cancer. *Proc Natl Acad Sci USA* 90, 3294-3298 (1993)
298. Kerbel, R. S., Brauer, M. J., Powers, V. C., Wu, J. J., Umansky, S. R., Tomei, L. D., and Bar, P. J.: Multicellular resistance: a new paradigm to explain aspects of acquired drug resistance of solid tumors. *Cold Spring Harbor Symp. Quant. Biol Mol Gen Cancer* 59, 661-672 (1995)
299. Whitacre, C. M. and Berger, N. A.: Factors affecting topotecan-induced programmed cell death: adhesion protects cell from apoptosis and impairs cleavage of poly(ADP-Ribose) polymerase. *Cancer Res* 57, 2157-2163 (1997)
300. Hall, E.: Radiobiology for the radiobiologist, Fourth Ed., Lippincott Company, Philadelphia, PA (1994)
301. Moulder, J. E. and Rockwell, S.: Tumour hypoxia: its impact on cancer therapy. *Cancer Metastasis Rev* 5, 313-341 (1987)
302. Fowler, J. F., Adams, G. E., and Denekamp, J.: Radiosensitizers of hypoxic cells in solid tumors. *Cancer Treat Rev* 3, 227-256 (1976)
303. Dische, S.: Chemical sensitizers for hypoxic cells - a decade of experience in clinical radiotherapy. *Radiother Oncol* 3, 97-115 (1985)
304. Gibbs, J., Oliff, A., and Kohl, N. E.: Farnesyltransferase inhibitors: Ras research yields a potential cancer therapeutic. *Cell* 77, 175-178 (1994)
305. Lebowitz, P. F., Davide, J. P., and Prendergast, G. C.: Evidence that farnesyltransferase inhibitors suppress transformation by interfering with Rho activity. *Mol Cell Biol* 15, 6613-6622 (1995)
306. Qiu, R.-G., Chen, J., McCormick, F., and Symons, M.: A role for Rho in Ras transformation. *Proc Natl Acad Sci USA* 92, 11781-11785 (1995)
307. Esteve, P., del Peso, L., and Lacal, J. C.: Induction of apoptosis by rho in NIH3T3 cells requires two complementary signals. Ceramides function as a progression factor for apoptosis. *Oncogene* 11, 2657-2665 (1995)
308. Lebowitz, P. F., Sakamuro, D., and Prendergast, G. C.: Farnesyl transferase inhibitors induce apoptosis of Ras-transformed cells denied substratum attachment. *Cancer Res* 57, 708-713 (1997)