Carcinogenesis and apoptosis: paradigms and paradoxes

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Apoptosis is a physiological process of cell elimination, which is important for both maintenance of cellular homeostasis, and cell proliferation and differentiation. Disturbances in the cell death process might lead to uncontrolled cell growth and to tumor formation. In addition, proper function of the apoptotic machinery is critical for tumor susceptibility to treatment. Many pro-apoptotic and anti-apoptotic genes have been cloned and their significance for the proper function of the apoptotic pathways carefully investigated. However, the precise role of these genes and their products in cancer development is less clear. Here, we will discuss some of the current paradigms and paradoxes concerning the involvement of apoptotic genes in carcinogenesis.

Introduction

In the early 1970s the term apoptosis was coined and described as a basic biological phenomenon with wide-ranging implications in tissue kinetics (1). The authors suggested that this form of cell death is important not only for the spontaneous elimination of potentially malignant cells and for therapeutically induced tumor regression but also for tumor progression. Kerr et al. (1) speculated further that hyperplasia might result from decreased apoptosis rather than increased mitosis. Presently, it is generally agreed that cell populations are tightly regulated by their rates of proliferation, differentiation and death. Dysfunction of any one of these processes can result in either uncontrolled cell growth or uncontrolled cell death. Yet, even 30 years after the above-mentioned seminal paper was published, it remains ambiguous as to what degree cell proliferation and/or cell death must occur to initiate tumor formation. According to Hanahan and Weinberg (2), at least six essential alterations in cell physiology dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. These characteristics all have molecular bases and, importantly, combinations of these features can lead to uncontrolled growth. Searching for a balance between cell proliferation, differentiation and death remains a challenge for many cell biologists.

From the time when apoptosis was first suggested to serve as a barrier against cancer, it has been assumed that genes involved in positive regulation of cell death might act as anti-tumorigenic genes, whereas, in contrast, genes involved in negative regulation of cell death might act as oncogenes. Accordingly, the importance of some oncogenes for the cell death process was investigated in detail. Moreover, during the past several decades dozens of pro-apoptotic and anti-apoptotic genes were cloned, and their significance for proper function of the apoptotic pathways was carefully investigated. However, the importance of these genes and their products for cancer development is less clear. Although recently, the role of the cell death machinery in the treatment of cancer has been discussed in detail in many review articles (3–8), analysis of the role of cell death in tumorigenesis is still very rare. Here, we will discuss current knowledge about the involvement of apoptotic genes in carcinogenesis.

Bcl-2 family proteins in carcinogenesis

Early evidence supporting the hypothesis that genes and proteins, which play a role in tumorigenesis, might be involved in the negative regulation of cell death came from the observation that Bcl-2, which was originally cloned from the t(14;18) translocation breakpoint found in follicular B-cell lymphomas, was able to rescue lymphoid and myeloid cells from an otherwise inevitable death caused by withdrawal of the cytokine, interleukin-3 (9,10) Moreover, it was shown that mutations in bcl-2 can, in turn, promote lymphomagenesis and influence the sensitivity of tumor cells to chemotherapy and radiotherapy. At present, the Bcl-2 family of proteins together with related members is known to include more than 30 proteins with either pro-apoptotic or anti-apoptotic functions, suggesting that they might also play different roles in carcinogenesis (11).

Do pro-survival Bcl-2 family members act as oncogenes? Indeed, expression of Bcl-2 in transgenic mice confirmed the hypothesis that inhibition of apoptosis can lead to cancer, as these mice develop B cell lymphomas and leukemias [for review, see (11)]. However, enforced expression of Bcl-2 alone is only weakly tumorigenic, and tumor incidence in these mice was relatively low (5–10%) within the first year of life and was associated with long latency periods. Hence, the fact that most bcl-2 transgenic mice develop tumors only when they are old suggests that Bcl-2 overexpression by itself is not sufficient for malignant transformation. The lifespan of B lymphoid tumors is significantly prolonged by bcl-2 transgene expression, suggesting that Bcl-2 overexpression provides a predisposition for the development of B-cell lymphomas (12). Importantly, plasmacytomas from bcl-2 transgenic mice carried rearrangements of the c-myc proto-oncogene, which promotes abnormal cell proliferation and tumorigenesis. It is likely that Bcl-2 promotes neoplastic transformation by prolonging the lifespan of normally short-lived cells, allowing them to accumulate additional oncogenic mutations. The observation that, as a result of combination of a
bcl-2 and a c-myc transgene, leukemias develop extremely rapidly, much more so than in mice bearing either transgene alone, supports this assumption. In addition, double transgenic (bcl-2/c-myc) mice show dramatically accelerated appearance of breast cancer (13). However, in pancreatic β-cell tumors c-Myc, rather than Bcl-2, co-operates with Bcl-X<sub>L</sub> (14). Bcl-2 co-operates not only with c-Myc in tumor formation but can also do so with the pim-1 and v-abl oncogenes in lymphogenesis and plasmacytoma development, respectively (15,16). Importantly, overexpression of Bcl-2 together with downregulation of some pro-apoptotic proteins might lead to tumor formation. For example, loss of Fas expression (lpr mutation) and Bcl-2 overexpression acted synergistically in the development of AML (17). Although comparative analysis of tumor rates in different Bcl-2 transgenic strains revealed that the VavP-Bcl-2 mice older than 10 months developed follicular lymphomas (18), it seems that in a majority of cases Bcl-2 has an oncogenic potential, but acts as an oncogene only in cooperation with other proteins, presumably, proto-oncogenes (Figure 1).

Do other anti-apoptotic members of the Bcl-2 family act as oncogenes? There is accumulating evidence supporting a positive answer to this question. Thus, transgenic overexpression of Bcl-X<sub>L</sub> induced lymphogenesis, or development of pancreatic β-cell tumors, and overexpression of Mcl-1 resulted in B-cell lymphomas. Although, mutations in bcl-x, mcl-1, bcl-w or al genes have not been identified as a cause of tumors, high level of expression of the corresponding proteins can contribute to carcinogenesis due to oncogenic activation of their upstream regulators (e.g. NF-kB) [for review see (11)].

Bcl-w, which is expressed in almost all murine myeloid cell lines analyzed and in a wide range of tissues (19), is frequently overexpressed in colorectal adenocarcinomas and appears to play a role in the progression from adenoma to adenocarcinoma in the colorectal epithelium. Bcl-w is also expressed in a majority of infiltrative gastric adenocarcinomas, and it may suppress tumor cell death by blocking JNK activation (20). Upregulation of A1/Bfl-1 and the serine/threonine kinase, Pim-1, is essential for in vitro transformation and in vivo leukemogenesis mediated by BCR/ABL (21). Overexpression of this protein was also observed in human gastric cancer. Together with the E1A oncogene, A1 plays a role in transformation of primary epithelial cells. Another anti-apoptotic protein from the same family, Mcl-1, promotes survival in a spectrum of hematopoietic cell types in transgenic mice (22). Mcl-1 expression is also required for the survival of multiple myeloma cells. Further, an elevated level of Mcl-1 was detected in prostate cancers, B-cell chronic lymphocytic leukemia (B-CLL) and leukemic relapse in AML and ALL. In B-CLL patients, higher levels of Mcl-1 were strongly correlated with failure to achieve complete remission after single-agent therapy (23). Taken together these findings indicate that anti-apoptotic members of the Bcl-2 family might be involved in tumor progression either directly or in conjunction with some oncogenes or pro-apoptotic genes (Figure 1).

Do pro-apoptotic members of the Bcl-2 family act as anti-oncogenes? According to the concept that genes involved in positive regulation of cell death might act as antitumorigenic genes, it follows that pro-apoptotic Bcl-2 family members should act as tumor suppressors. Indeed, bax-null mice acquire few spontaneous tumors (24). However, evidence for Bax functioning as a tumor suppressor came from the observation that loss of Bax accelerates tumorigenesis in transgenic mice expressing a truncated SV40 large T antigen that blocks Rb but not p53 (25). Moreover, loss of Bax reduces apoptosis occurring in pre-neoplastic mammary lesions. Bax deficiency also decreases p53-induced apoptosis and, consistently, does not accelerate tumorigenesis in p53<sup>−/−</sup> mice (26,27). Interestingly, p53-deficiency is much more oncogenic than overexpression of Bcl-2 or loss of pro-apoptotic Bcl-2 proteins, such as Bax. There are accumulating evidence that bax or bak are mutated in some human gastric and colorectal cancers (28,29), as well as in leukemias (30), although it is unclear whether these mutations contribute to tumor formation.

Recently, it was shown that suppression of Bif-1 expression, a protein regulating Bax/Bak activity, was associated with an enhanced ability of HeLa cells to form colonies in soft agar and tumors in nude mice, suggesting that the loss of this protein may contribute to tumorigenesis via downregulation of the pro-apoptotic function of Bax/Bak (31) (Figure 1).

A group of pro-apoptotic proteins in the Bcl-2 family, the so-called BH3-only proteins, also seem to play a role in suppression of tumorigenesis (Figure 1). For example, Bim-deficient mice develop chronic myelomonocytic leukemia. However, this occurs only at 2 years of age, again suggesting that Bcl-2 family proteins by themselves are very poor inducers of tumorigenesis (32). Supporting this assumption, it was found that some BH3-only proteins, i.e. Noxa and Puma, are regulated by p53 and may act as tumor suppressors (33,34). Recently, it was further shown that transcription factor Slug, which is a target for p53, antagonizes p53 by repressing Puma (35). Since Slug is aberrantly expressed in various tumors, it might contribute to tumorigenesis by repressing the expression of Puma or perhaps other BH3-only proteins. Bik/Bik/Nbk, also belonging to this subfamily, is induced in response to overexpression of the adenoviral transforming protein E1A in a manner dependent on p53 (36). Loss of Bik/Bik/Nbk is a common feature of clear-cell renal carcinoma (RCC). Whereas strong expression of this protein was found in the renal tubuli and in epithelial lining of the glomerula, a consistent loss of expression was observed in primary RCC tissue and RCC cell lines. Mutation of bik/bik/nbk is rare; however,
deletion of this gene at 22q13.2 is frequent. In addition to loss of heterozygocity, DNA methylation mediates transcriptional silencing of this gene. Importantly, loss of Bik/Blk/Nbk coincides with failure to express Bim, whereas Puma, Bid and BNIP3 are readily detectable and, in case of Puma, inducible by p53 (37).

Recently, it was also shown that although loss of Bim does not in itself elevate tumor incidence, a loss of a single allele of Bim dramatically accelerates leukemogenesis in mice overexpressing a myc transgene during B lymphopoiesis, suggesting a tumor suppressor role for Bim in B cells (38). This protein can act as a tumor suppressor also in the formation of epithelial solid tumors (39).

Human bmf gene is located at 15q14 within the region that harbors a putative tumor suppressor that is lost in metastatic, but not primary, tumors of breast, lung and certain other tissues (40). Consistently, Bmf is activated by loss of cell attachment (anoikis), a process that is considered to be a safeguard against metastatic tumor growth (40). Thus, one might conclude that blocking apoptosis with Bcl-2 family proteins, or loss of function of pro-apoptotic proteins from the same family, together with either loss of other components of the apoptotic pathways, such as p53, or overexpression of certain oncogenes, would greatly facilitate tumorigenesis.

Caspases and tumorigenesis

Since the caspase family of proteases plays an important role in both the signaling and execution phases of apoptosis, it is conceivable that low expression or dysregulation of caspase function might influence the apoptotic process and result in inappropriate cell proliferation. Expression of execution caspases in tumor cells has been investigated in detail, but the results are conflicting. Thus, as compared with normal cells, expression of caspase-3 in some tumor cells was found to be upregulated, but in some tumors it was downregulated (41,42). Either a positive or a negative correlation between expression of this protein and malignant grade/stage of the tumor has also been documented (41,43). In pancreatic tumors and in neuroblastomas nuclear localization of caspase-3 was found to correlate with low malignancy. Several reports describe that high level of caspase-3, measured by immunohistochemistry, might be an indicator of a good prognosis for treatment of lung carcinomas (44,45). However, in vitro investigation of caspase-3 activity in colorectal carcinomas revealed that high activity of caspase-3 correlated with poor prognosis (46).

It seems that more work is required to draw any final conclusions about the relationship between caspase-3 status, apoptotic rate and prognosis in human tumors. The association between the rate of apoptosis and expression of several other relevant molecules (Bcl-2, pro- and active caspase-3, and caspase-7) was studied in 61 primary breast carcinomas (47). Interestingly, high level of the anti-apoptotic protein Bcl-2 frequently coincided with increased expression of pro-apoptotic molecules, such as caspase-3 and caspase-7. However, expression of caspase-3 or -7 did not correlate with the extent of apoptosis or any clinical pathological features, except overrepresentation of ER+ status in tumors expressing caspase-3. These findings indicate a general dysregulation of spontaneous apoptosis in primary breast tumors; however, it is unclear whether this dysregulation is a primary or secondary event in cancer.

Several observations suggest that downregulation of pro-caspase-3 expression level correlates with progression of gastric carcinoma and gastric malignant lymphoma (48). Moreover, concomitant downregulation of pro-caspase-3 and PTEN was suggested to be an important determinant in the progression of primary malignant gastric lymphoma (48,49). Interestingly, high expression of pro-caspase-3 was negatively correlated with lymph node metastasis of gastric lymphoma, as well as of non-small-cell lung cancer (45,48). Progression of melanoma was shown to be associated with loss of the transcription factor AP-2-alpha, which is preferentially cleaved by caspase-6. Immuno-histochemical analysis of AP-2-alpha and active caspase-6 revealed high levels of these proteins in primary melanomas and metastasis thereof as compared with their levels in naevi (50). This study supports the notion that the activation of caspases does not inevitably result in apoptosis but may also contribute to tumor progression; in this particular case to melanoma progression.

A correlation between caspase expression and tumor development does not seem to be restricted to execution caspases (Figure 1). Thus, loss of caspase-1 mRNA and protein was observed in gastric cancer, both tissue and cell lines (51). This loss was associated with pTNM stage, lymph node metastasis and poor prognosis. In metastatic malignant melanomas expression of caspase-1 was related to both tumor burden and the clinical response to treatment (52).

A deficiency of caspase-8, an apical caspase in receptor-mediated killing, has recently been described in small-cell lung cancer (SCLC) and in neuroblastomas (53–55). Moreover, the loss of caspase-8 protein corresponded to a loss of caspase-8 gene expression, which was found to be due to methylation of the promotor in the majority of the cell lines examined. With rare exceptions, cell lines harboring a methylated allele lacked the corresponding unmethylated allele, indicating either biallelic methylation or loss of the unmethylated allele. Treatment of SCLC cell lines lacking caspase-8 expression with the demethylating agent 5-aza-2’-deoxycytidine (5-dAzaC) restored gene expression in the majority of SCLC cell lines tested. This restoration resulted in expression of the protein as well as its ability to participate in the formation of a functional DISC complex, thereby sensitizing tumor cells to drug-induced apoptosis. In addition to the DNA methylation, found in different tumors, three mutation sites in caspase-8 have recently been reported. A mutation that modifies the stop codon of caspase-8 and adds an Alu repeat to the coding region was found in head and neck cancer cell lines (56). Another missense mutation (alanine to valine) at the caspase-8 codon 96 was found in a neuroblastoma cell line lacking caspase-8 expression (57). Deletion of the leucine 62 in caspase-8, which was observed in human vulval squamous carcinoma cells, dramatically altered the pro-apoptotic function of caspase-8 (58). This mutation prevents interaction of pro-caspase-8 with FADD and, hence, the activation of the caspase cascade. The reported observations of caspase-8 changes in cancer cells, including gene deletion, methylation and point mutation, identify mechanisms by which some tumors, including lung carcinoma, Ewing tumors, neuroblastoma and melanomas, may escape caspase-8-mediated cell death. Modulation of caspase-8 and FLICE-inhibitory protein expression was also suggested to be a potential mechanism of EBV tumorigenesis in Burkitt’s lymphoma (59) and of increased malignant potential of endometrial carcinomas (60). Since lack of expression of caspase-8 was found in a
subset of both high-grade SCLC and low-grade (carcinoid) neuroendocrine lung tumors, but not in NCSLC, which usually lack neuroendocrine characteristics, it was postulated that caspase-8 might function as a tumor suppressor in neuroendocrine lung tumors, similarly to neuroblastomas (also of neuroendocrine origin) (61). Like in the case of caspase-8, silencing mutations in caspase-9 were also found to be associated with the development of neuroblastomas, suggesting a potential tumor suppressor function also for this apical caspase (62). However, a more recent detailed investigation of the status of caspase-8 in neuroblastoma cells showed that suppression of caspase-8 expression occurs during the establishment of neuroblastoma metastases in vivo and that reconstitution of caspase-8 expression in deficient neuroblastoma cells suppressed their metastasis (63). Importantly, caspase-8 status did not predict primary tumor growth; rather, caspase-8 selectively potentiated cell death in neuroblastoma cells invading the collagenous stroma at the tumor margin. In this situation cell death was initiated by unligated integrins. Loss of either caspase-8 or integrin rendered these cells refractory to integrin-mediated death, allowed cell survival in the stromal microenvironment and promoted metastasis. Altogether, these findings define caspase-8 as a metastasis suppressor gene that regulates the survival and invasive capacity of neuroblastoma cells. However, future investigations are required to understand how general this observation is (Figure 1).

Using cDNA array-based expression profiling, reduced expression of the caspase-5 gene was shown in highly metastatic subpopulations of lung cancer (64). Combination of this method with polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis suggests that caspase-5 might be a suppressor gene of highly metastatic potential in lung cancer. Comparing differential gene expression profiles established by cDNA microarray analysis between normal cells, primary carcinoma cells and metastatic carcinoma cells revealed downregulation of caspase-3, as well as specific methylation of this gene, only in metastatic cells, suggesting that caspases, indeed, might act as tumor suppressor genes. Whether this phenomenon is specific for caspase-3,-5 and -8 is still unclear and requires additional investigation.

It has been shown previously that 10 out of 19 metastatic melanoma cell lines, and 10 out of 24 melanoma specimens, were characterized by a low level of Apaf-1, a protein essential for mitochondrial-mediated caspase activation (65). The authors proposed that Apaf-1 downregulation contributes to chemoresistance in malignant melanoma. However, this may be a late event during tumor progression, since a majority of in situ melanomas were positive for Apaf-1. Several recent publications, in which more than 400 pigmented lesions were analyzed, support that downregulation of Apaf-1 occurs during melanoma progression (66,67). Deletions in the locus containing Apaf-1 and an undetectable level of this protein were found in in situ melanomas, and lymph node and visceral metastases (68,69). However, more recently two groups reported that only very few metastatic melanomas were negative for Apaf-1, questioning the role of this protein in tumor formation and susceptibility to treatment (70,71). Hence, it seems that mere work is required to clarify the role of this protein in both processes.

**Inhibitor of apoptosis proteins and carcinogenesis**

The inhibitor of apoptosis proteins (IAPs) are a family of proteins that are able to regulate apoptosis when ectopically expressed in cells. It was suggested that the inhibitory effect of IAPs is mediated by the presence in their molecules of one or more baculoviral IAP repeat (BIR) motifs. Although, it was recently found that X-linked IAP (XIAP) indeed binds and inhibits caspase activity, cIAP1 and 2 are able to bind caspases but do not directly inhibit their activity (72). Many IAPs also have a caspase activation recruitment domain (CARD) and another zinc-binding motif, the RING domain, which can recruit E2-ubiquitin-conjugating enzymes and catalyze the transfer of ubiquitin onto target proteins [for review, see (73)]. Given the importance of IAPs as downstream inhibitors of apoptosis, it was suggested that their expression might link to both tumor formation and chemoresistance.

It was recently shown that in non-differentiated pheochromocytoma PC-12 cells the level of Apaf-1 is high, but that it drops dramatically during differentiation, which renders the mature neurons resistant to cell death (74). The reduction in the level of Apaf-1 is accompanied by increased protection by the IAPs. Like in melanoma, where the level of Apaf-1 was also deceased during tumor progression, it appears that the level of Apaf-1 and the functional state of the IAPs play important roles in the regulation of differentiation and proliferation of tumor cells.

The prognostic importance of IAP members has also been examined; however, with inconsistent results. Elevated levels of IAPs were found in a wide variety of cancer cell lines and primary tumor biopsies. It was also suggested that IAPs could function as oncogenes. Thus, chromosomal amplification of the 11q21–q23 region, which encompasses both c-IAP1 and c-IAP2, was observed in a variety of malignancies, and esophageal squamous cell carcinomas frequently display amplification of IAPs. In addition, it was recently reported that a translocation involving genes for cIAP2 and MALT1/paracaspase commonly occurs in mucosa-associated lymphoid tissue (MALT) lymphomas (75). This translocation results in the formation of a fusion protein with the N-terminal half containing the BIR domains of cIAP2 and the C-terminal half containing the protease-like parts of MALT1/paracaspase (Figure 1). Primary pulmonary MALT lymphomas were characterized by frequent and heterogeneous cytogenetic abnormalities, including not only the translocations described above, but also aneuploidy and mutations in the pro-apoptotic molecule, Bcl-10 (76). It was also shown that self-oligomerization of the cIAP2/MALT1 fusion protein causes deregulated ubiquitin ligase activity of MALT1/paracaspase (77). The chimeric protein targets NEMO for ubiquitination and, thereby, activates the NFkB pathway. Consistent with this finding, NEMO ubiquitination is increased in t(11;18)(q21; q21)-positive MALT lymphoma samples, suggesting a link between translocation, MALT1/paracaspase-mediated deregulation of ubiquitin ligase activity, constitutive NFkB activation and promotion of tumorigenesis. Interestingly, Bcl-10 acts upstream of MALT1/paracaspase in a pathway required for activation of NFkB. Thus, although abnormal expression, activity and/or subcellular localization of Bcl-10 or MALT1/paracaspase resulted from chromosomal translocations and lead to constitutive NFkB activation, protection from apoptosis and uncontrolled cellular proliferation, the role of IAP as an inhibitor of caspases in this process is unclear.

Survivin is also a BIR-domain containing protein, which shows significant differential expression between malignant and non-malignant cells. It is absent, or present at very low levels, in normal adult tissue but appears at increased levels
in a wide variety of solid tumors and hematological malignancies (78). Data from several groups suggest that survivin expression can identify the lesions at highest risk for malignant transformation and invasion. Hence, its presence in body fluids might serve as a diagnostic marker and predictive sign of treatment outcome (79). It seems that the deregulated survivin gene expression observed in different tumors and its role in tumorigenesis are related to its function during mitosis, rather than to its inhibition of apoptosis (80) (Figure 1).

Polymorphic variations in apoptotic genes in cancer patients

Until now, the Arg/Pro codon 72 polymorphism of the p53 gene is the only apoptosis-associated single nucleotide polymorphism (SNP) that was subjected to a systematic analysis. However, there are some other SNPs in apoptotic genes, which have already been examined with respect to cancer risk, although their functional significance has not been strictly proven (Figure 1). The most extensive studies have been devoted to the TNF-alpha gene polymorphisms, and associations with several major cancer types have been reported (81). A role in cancer susceptibility has also been suggested for the SNPs located in the FAS promoter region (82,83). There is also a series of publications assessing a tumor-associated role of the non-coding G4C14-to-A4T14 allelism (84). Protective effects have been observed for the DR4 polymorphism and bladder cancer risk, and for the CASP8 variant and breast cancer proneness (85,86). It is important to emphasize that these initial reports remain to be confirmed in independent studies.

Furthermore, most of the apoptosis-associated SNPs have not yet been subjected to studies of gene–disease interactions. The number of validated coding SNPs in cell death genes is relatively modest [for review, see (87)], although this estimate would increase by an order of magnitude if one would also consider non-coding and/or non-validated gene polymorphisms. Assessment of the functional significance of SNPs residing in apoptotic genes seems to be especially interesting. Ideally, data on the functional relevance of particular SNPs should guide the selection of polymorphic candidates for subsequent large-scale case–control studies.

Conclusions

Carcinogenesis is a complex process that is driven by tight interactions between oncogene activation, tumor suppressor inactivation and the cell death machinery. A major problem with the stream of observations suggesting differences in expression of a variety of genes and proteins between normal and tumor cells is the fact that in most cases it is not known whether these changes are required for initiation of the tumor or its progression. Since reduced apoptosis is generally associated with tumorigenesis, it was suggested that genes involved in the negative regulation of this process might have an oncogenic potential, whereas genes involved in the positive regulation of apoptosis might act as tumor suppressor genes. There is now accumulating evidence that some pro-apoptotic genes, in combination with other genes, indeed might fulfill tumor suppressor functions. In addition, it appears that in many cases negative regulators of apoptosis might act as oncogenes, but only in cooperation with other proteins, presumably, proto-oncogenes. Moreover, in many tumors these anti-apoptotic proteins co-exist with elevated levels of pro-apoptotic molecules, such as active caspase-3 and caspase-7. At first sight, these results seem paradoxical. However, considering that, for example, the Bcl-2 family includes more than 30 proteins, each of which fulfills either anti-apoptotic or pro-apoptotic functions, it seems reasonable that the ratio between these proteins, rather than overexpression of one particular member of this family, might influence tumor formation and/or the susceptibility of the tumor cells to undergo apoptosis. Accumulating evidence further suggests that downregulation of pro-apoptotic proteins, in combination with the expression of other structural and regulatory proteins, are essential for metastatic progression. It seems that the overall status of the cell death machinery, rather than just the expression levels of the individual proteins, might explain how it affects tumorigenesis. Hence, although it is obvious that impairment of the cell suicide process is an important factor in tumor development, it is equally clear that additional work is required to understand the exact route by which all known, or yet unknown, apoptotic genes and proteins operate in this complex process.

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