Journal of Cellular Biochemistry 88:128–137 (2003)

Paradoxical Role of Apoptosis in Tumor Progression

Katerina V. Gurova and Andrei V. Gudkov*

Department of Molecular Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio

Abstract Tumors frequently acquire resistance to apoptosis that is expected to contribute to malignant phenotype and reduce sensitivity to treatment. In fact, inactivation of *p53* tumor suppressor gene resulting in suppression of apoptosis serves as a negative prognostic marker. Surprisingly, expression of a strong anti-apoptotic protein Bcl-2, another mechanism to avoid apoptosis, was found to be associated with a favorable prognosis. This paradoxical anti-progressor function of Bcl-2 has been explained in literature based on the negative effect of Bcl-2 on cell proliferation. Here, by analyzing accumulated experimental and clinical data, we provide evidence supporting another hypothesis that defines apoptosis as an accelerator of tumor progression. The mechanism of anti-progressor function of Bcl-2 is based on creation of tumors that maintain control of genomic stability by eliminating selective advantages for the cells that acquire resistance to apoptosis through loss of p53. Thus, inhibition of apoptosis does not lead to loss of genomic stability and creates tumor environment that no longer supports further tumor progression and inhibitors of apoptosis can be considered as factors suppressing tumor progression. J. Cell. Biochem. 88: 128–137, 2003. © 2002 Wiley-Liss, Inc.

Key words: apoptosis; tumor progression; genomic stability; p53; Bcl-2

Defects in apoptotic signaling pathways are common in cancer cells [Evan and Vousden, 2001; Zornig et al., 2001 and references therein]. Inhibition of programmed cell death seems to be important for tumor initiation since apoptosis is thought to be involved in the process of eliminating cells with damaged DNA and cells with anomalies in cell cycle regulation (i.e., cells of high risk of malignant transformation). Moreover, impaired apoptosis may enhance tumor progression and promote metastasis by enabling tumor cells survival in circulation and in abnormal cellular microenvironment. Furthermore, inactivation of apoptotic response may increase cancer cell resistance to various forms of therapy [Johnstone et al., 2002, review].

Several oncogenes have been defined among genes encoding negative regulators of apoptosis (prototype is *Bcl-2*). On the other hand, there are multiple examples of tumor suppressor genes among pro-apoptotic genes (prototype is *p53*). This suggests that a loss of apoptosis may be the most important characteristic of malignant cell phenotype presumably associated with an unfavorable prognosis for cancer patients. Surprisingly, the analysis of the results of numerous clinical studies that estimates the prognostic value of various tumor markers indicates that such schematics are frequently oversimplified.

Bcl-2 AS A FAVORABLE PROGNOSTIC MARKER: CLINICAL DATA

The mechanism regulating the escape of tumor cells from apoptosis can be divided into two categories: (i) inactivation of sensors of apoptotic stimuli (such as inactivation of p53, the major mediator of different types of stress) or certain elements of apoptotic machinery (i.e., inactivation of caspases) and (ii) upregulation of anti-apoptotic factors (such as overexpression of Bcl-2, or activation of Akt). Although, both mechanisms are known to contribute to the resistance of apoptosis in many tumors, the analysis of clinical data shows a remarkable difference between them in terms of their association with positive or negative prognosis of the disease. Inactivation of p53 (and elimination of

Grant sponsor: NCI; Grant numbers: CA60730, CA88071, CA75179.

*Correspondence to: Andrei V. Gudkov, Department of Molecular Biology/NC20, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195. E-mail: gudkov@ccf.org

Received 11 September 2002; Accepted 12 September 2002

DOI 10.1002/jcb.10382

p53-dependent apoptosis) correlates with poor clinical outcome and is specific for the tumor at advanced stages of progression [Zhang et al., 1998; Castiglione et al., 1999; Megha et al., 2002]. However, upregulation of *Bcl-2*, another frequent event in tumors that is also associated with inhibition of apoptosis, does not unequivocally correlate with pessimistic prognosis [Joensuu et al., 1994; Binder et al., 1995; Kobayashi et al., 1997; Berardo et al., 1998; Inada et al., 1998; Hamilton and Piccart, 2000 and references therein]. On the contrary, overexpression of Bcl-2 is frequently associated with better clinical outcome and more favorable prognosis confronting the fact that *Bcl-2* as an oncogene should contribute to tumor progression and resistance to treatment [Kobayashi et al., 1997; Berardo et al., 1998; Inada et al., 1998]. This puzzling observation was initially made for breast cancers: overexpression of Bcl-2 was found to positively correlate with higher histological tumor grade, normal ploidy, estrogen receptor positivity, and absence of metastases, all characteristic of a less malignant disease [Joensuu et al., 1994; Binder et al., 1995; Kobayashi et al., 1997]. In colorectal adenoma, Bcl-2 expression is frequently higher than in normal mucosal epithelium, while in carcinoma Bcl-2 levels are decreased compared with adjacent normal tissue [Krajewska et al., 1996]. Similar conclusions can be found in clinical literature describing immunohystochemical analyses of gastric [Liu et al., 1998], prostate [Diaz et al., 2000], cervical [Tjalma et al., 1998], esophageal [Koide et al., 1997], and endometrial carcinomas [Saegusa and Okayasu, 1997].

It should be noted that there are plenty of works that present a different view on the prognostic value of Bcl-2 and show examples of association of Bcl-2 expression with higher tumor grade and less favorable prognosis thus contradicting to the above-described phenomenon. This apparent controversy has been discussed in several reviews [Hamilton and Piccart, 2000 and references therein).

Thus, there are a number of malignancies in which expression of a strong anti-apoptotic factor Bcl-2 is associated with less malignant phenotype and favorable prognosis. On the other hand, frequent loss of powerful pro-apoptotic factor p53 is a characteristic of advanced tumors and is associated with accelerated tumor progression and unfavorable prognosis. Hence, two different genetic events, both frequently acquired by transformed cells and resulting in inhibition of apoptosis, have opposite effects on tumor progression. The reason for this discrepancy may not be necessarily linked to the regulation of apoptosis by Bcl-2 or p53 since both proteins have additional functions. In fact, inactivation of p53 results in numerous changes in cell phenotype reflecting multifunctionality of this protein that includes loss of checkpoint control. Additional functions, such as mild interference with cell cycle progression, have also been assigned to Bcl-2 [Huang et al., 1997]. However, Bcl-2 seems to be much less diverse in its functions than p53, being primarily an anti-apoptotic factor acting on leakage prevention of apoptotic mediators (i.e., cytochrome c) from mitochondria.

In an attempt to elucidate the underlying cause for a "positive" prognostic value of Bcl-2 and why the consequences of Bcl-2 expression creates the effect opposite of p53 suppression, we will review observations concerning the role of Bcl-2 and other anti-apoptotic factors, members of *Bcl*-2 family, in tumor progression and prognosis.

Bcl-2 FAMILY MEMBERS AND TUMOR PROGNOSIS: CLINICAL DATA

Bcl-2 family consists of two groups of structurally related proteins playing opposite roles in apoptosis. They act by regulation of permeability of mitochondrial pores controlling release of cytochrom c and other triggers of caspase activation. Anti-apoptotic members of *Bcl*-2 family that reduce the rate of cell death and contribute to the accumulation of cell mass are often considered as oncogenes, while pro-apoptotic genes causing accelerated cell death are defined as candidate tumor suppressors. However, as in the case with *Bcl*-2 itself, there are reports describing properties of *Bcl*-2 family members that contradict these presumptions. For example, elevated expression of a pro-apoptotic member of *Bcl*-2 family, Bax, was shown to be associated with poor prognosis in esophageal squamous cell carcinoma and Bak in bladder cancer [Haitek et al., 2001; Kurabayashi et al., 2001; Takayama et al., 2001]. Similarly, expression of another pro-apoptotic protein Mcl-1 was defined as unfavorable prognostic marker for lung, head, and neck cancer [Krajewska et al., 1996; Eerola et al., 1999; Hotz et al., 1999].

Importantly, this tendency well fits with another line of observations showing a correlation between tumor aggressiveness and its apoptotic rate. Thus, in breast tumors the significant increase in the proportion of apoptotic cells was observed in recurrent tumors as compared with primary lesions. Consistently, the chance for survival patients carrying primary tumors with higher apoptotic index was significantly shorter [Vakkala et al., 1999]. In colorectal carcinoma, spontaneous apoptosis occurs more frequently in advanced aneuploid tumors [Li et al., 2000]. Numerous examples of a positive correlation between the number of apoptotic cells in the tumors and tumor grade can be found in literature for a large variety of solid tumors [Tanji et al., 1998; Eerola et al., 1999; Hotz et al., 1999; Li et al., 2000; Sjostrom and Bergh, 2001]. Altogether, the accumulated information indicates an increase in spontaneous apoptosis in the course of tumor progression, thus providing additional support for positive prognostic value of anti-apoptotic gene expression. We, therefore, can conclude that there are enough observations contradicting with the conventional view on pro- and anti-apoptotic factors in tumor progression to address this problem experimentally.

Bcl-2 AS AN ANTI-PROGRESSOR: LESSONS FROM EXPERIMENTAL MODELS

One of the first experimental indications of anti-progressor function of Bcl-2 came from the lab of Galina Deichman [Deichman et al., 1998]. Tumor progression in these studies was estimated in a strictly quantitative manner by monitoring acquisition of a malignant phenotypes (such as tumorigenicity and ability to form experimental and spontaneous metastases) during serial of alternate in vitro/in vivo passaging of spontaneously transformed Syrian hamster fibroblasts (originally low tumorigenic and non-metastatic) with gradual selection of increasingly malignant variants that results in isolation of numerous independent strains of extremely tumorigenic and highly metastatic variants. Deichman's group then analyzed how transduction of the original cells with different oncogenes would affect highly reproducible process of tumor progression. Transduction with oncogenic *c-myc* or *v-Ha-ras* did not have any detectable effect on this process, while inactivation of p53 (by a dominant negative mutant) and overexpression of bcl-2 both modified tumor progression in this model. As expected, p53 suppression accelerated selection of metastatic variants. Overexpression of bcl-2, however, caused an opposite effect, resulting in a significant delay in the appearance of metastatic phenotype with no influence on tumorigenicity. This model provides experimental confirmation of the effect described in the above-mentioned works: two anti-apoptotic events, inactivation of p53 and overexpression of bcl-2, resulted in opposite influence on tumor progression. Moreover, this work indicated that bcl-2 and p53 differentially effected specifically the rate of tumor progression measured by speed of accumulation of metastatic variants but did not differ in their effect on tumorigenicity or any other estimated properties of transduced tumor populations, thus questioning the reputation of bcl-2 as an oncogene by ruling out its direct anti-tumor effect.

Another piece of evidence supporting anti-progressor role of *bcl-2* came from the analysis of transgenic mouse models describing paradoxical inhibition of carcinogenesis by bcl-2 [De La Coste et al., 1999; Vail et al., 2001]. L-PK-*c-myc* transgenic animals develop hepatic tumors that resulted from targeted expression of *c-myc* oncogene in the liver [De La Coste et al., 1999]. Two phases of abnormal hyperproliferation of hepatocytes are observed in these animals. The first one occurs at 1 month of age and is accompanied by the high rate of apoptosis among new hepatocytes, resulting in the clearance of the majority of dysplastic cells by 2 months of age. During the next relatively "quiescent" period, heterologous dysplastic changes accumulated in the liver varying from slight to severe dysplasia. The second phase of hyperproliferation occurred at the 5th month after birth resulting in the appearance of neoplastic foci and liver tumors developed in 80% of mice between 6 and 8 months. These animals were crossed with L-PK-*bcl-2* transgenic mice expressing high levels of bcl-2 protein in the liver without any detectable phenotypic changes. This experiment was likely planned with the expectation to observe rapid tumor formation accelerated by anti-apoptotic function of bcl-2. To authors' surprise, the effect of *bcl-2* transgene was quite the opposite: authors observed a strong and highly significant inhibition of carcinogenesis in double transgenic animals, as compared with the single transgenic *c-myc*

littermates. Remarkably, the first wave of hyperproliferation and the appearance of dysplastic foci were not affected, even though apoptosis was considerably inhibited. In this study, negative influence of bcl-2 on cell proliferation ("slow re-entry of quiescent cells into cell cycle") was suggested as a potential mechanism of "tumor suppressor" function of bcl-2. The decrease of mitotic index in hepatocytes during the second hyperproliferative phase seems to support this idea. However, the lack of effect of bcl-2 on the first proliferation wave points to another explanation of the phenomenon: bcl-2 does not inhibit myc-induced over-proliferation per se but rather slows down the progression of primary hyperplastic pre-malignant lesions by interfering with additional accumulation of genetic events that cooperate with *c-myc*. Importantly, substitution of *bcl-2* transgene with dominant negative *p53* mutant was accompanied with acceleration of tumorigenic effect of *c-myc* transgene [De la Coste et al., 1999]. At the same time, the use of bcl_{XL} transgene, another anti-apoptotic bcl-2 family member, failed to cause the effect of bcl-2. However, due to the insufficient information on the effect of bcl_{XL} transgene on apoptosis and proliferation rate in liver, it is impossible to rate the significance of this result.

Similar results were obtained using another transgenic model of hepatocarcinogenesis: mice carrying *TGF*-α transgene under the control of metallothionein (MT1) promoter that is highly expressed in the liver [Vail et al., 2001]. These mice are characterized by a highly elevated proliferation of hepatocytes especially profound in young animals and remaining high up to the point of tumor development. Introduction of *bcl-2* under the same promoter into germ line of MT1-*TGF*-α mice caused a decrease in the incidence of HCC, delayed appearance of tumors that were characterized by a slow growth rate. As in the previous example, *bcl-2* transgene did not affect the initial stages of carcinogenesis, including hyperproliferation and appearance of dysplastic adenomas, but suppressed progression of pre-malignant lesions towards invasive cancer.

Thus, in both cases the "tumor suppressor" function of bcl-2 is likely to be a reflection of its effect on tumor progression rather than on tumor initiation. This anti-progressor function of bcl-2 is hard to explain by its negative influence on the cell cycle documented in several in vitro and in vivo experiments [Huang et al., 1997; Furth et al., 1999], since no differences in proliferation rates were observed at early stages of carcinogenesis. One could argue that slower growth of tumors in *bcl-2* transgenic mice might be the result of anti-proliferative effect of bcl-2. However, this difference is likely to be the reflection of slower progression of these tumors rather than a direct effect of bcl-2 overexpression: introduction of *bcl-2* into tumorigenic cell lines usually results in development of faster growing tumors [Kajiwara et al., 1999; Gurova et al., 2002].

ANTI-PROGRESSOR FUNCTION OF APOPTOSIS: A HYPOTHETICAL MODEL

Thus, the analysis of clinical and experimental data brings us to a conclusion that Bcl-2 is capable of suppressing tumor progression. This function does not seem to be limited to Bcl-2-mediated effect on cell proliferation. The hypotheses explaining these results can be summed in two groups. According to one view, Bcl-2 has no active causative role in reducing tumor progression rates but rather it is a neutral marker of certain types of slow-progressing tumors, the properties of which are determined by various Bcl-2-unrelated factors. For example, it was demonstrated that expression of Bcl-2 is positively regulated by estrogen [Teixeira et al., 1995; Kandouz et al., 1996], and therefore Bcl-2 serves as a favorable prognostic marker simply as a marker of relatively benign hormone responsive breast tumors. This obviously cannot be a general explanation, since Bcl-2 has been reported a positive prognostic marker in colorectal and gastric tumors that are not estrogen-dependent [Krajewska et al., 1996; Inada et al., 1998] Another example of the same type is negative regulation of Bcl-2 expression by some p53 mutants [Haldar et al., 1994] that can result in a decrease in expression of Bcl-2 in advanced tumors with mutated p53. Again, this is not likely to be a universal mechanism, since *Bcl-2* regulation by p53 mutants is more an exception rather than a general rule. However, this phenomenon (lower level of Bcl-2 in advanced tumors) can be also explained by a switch to another anti-apoptotic member of Bcl-2 family, Bcl_{XL} accompanied by parallel decrease in Bak expression, observed in some tumors [Krajewska et al., 1996; Eguchi et al., 2000].

Another group of hypotheses makes a functional link between Bcl-2 and reduced tumor progression. Any attempts to connect anti-apoptotic and anti-progressor functions of Bcl-2 seems to contradict common sense, which tells that inhibition of apoptosis should facilitate tumor cell survival and resistance to treatment and, therefore, should be the property of more aggressive tumors. In fact, inactivation of p53 fits this expectation resulting in rapidly progressing tumors with no p53-mediated apoptosis. Hence, it would be logical to look for the mechanisms of anti-progressor function of Bcl-2 among those properties of this factor that are unrelated to apoptosis (exactly as it has been proposed in above-cited studies), such as anti-proliferative effects of Bcl-2. There is, however, another simple model that can (i) explain anti-progressor function of Bcl-2 independently of any additional properties of this protein besides anti-apoptotic ones and (ii) resolve the problem of opposite effects of Bcl-2 expression and p53 inactivation on tumor progression.

Tumor progression can be viewed as Darwin's selection of cell variants under conditions of a strong competition for rapid accumulation. Two factors can greatly accelerate this process: (i) high degree of genetic variability, generating many phenotypic variants for testing, and (ii) effective elimination of the less adapted ones, providing space for expansion of the winners. Overexpression of Bcl-2 and loss of p53 both act by blocking apoptosis and therefore should presumably slow down the selection rate by reducing cell turnover in the population (factor (ii)). But do they cause similar effects on generation of genetic variability?

From the first glance, suppression of apoptosis (regardless of the mechanism) is expected to increase in degree of genetic variability. The role of apoptosis as a genomic stability keeper was suggested based on the p53 studies in which repression of p53-mediated apoptosis was accompanied with dramatic increase in mutation rate. This putative link, however, is not more than a correlation, since it has never been accurately checked in functional assays, which among the multiple p53 functions is responsible for the control of genomic stability. Hence p53 function as a "guardian of the genome" may not necessarily be attributed to apoptosis.

To clarify this problem, we directly compared the effects of Bcl-2 overexpression and p53 inactivation, both resulting in suppression of apoptosis, on the genetic stability determined by a standard assay based on a frequency of *CAD* gene amplification in cell population maintained in constant presence of CAD protein inhibitor PALA [Gurova et al., 2002]. The results of these experiments came as a surprise: while p53 suppression had an expected effect, causing several orders of magnitude-fold increase in the frequency of gene amplification, ectopic expression of bcl-2 (that was enough to suppress apoptosis) did not result in a detectable change in genomic stability. This result showed that (i) apoptosis is not an essential factor of control over genomic stability and (ii) although inactivation of p53 and expression of bcl-2 both suppress apoptosis, they have different effect on genomic stability: in contrast to p53 inactivation, bcl-2 overexpression does not make cell permissive to gene amplification and genetically unstable. This finding pointed to a potential cause of dramatic differences between the effects of p53 inactivation and Bcl-2 expression on tumor progression and provided an important support for our hypothesis on "anti-progressor" function of bcl-2.

CRUCIAL EXPERIMENT: Bcl-2 PREVENTS SELECTION OF GENETICALLY UNSTABLE p53-DEFICIENT CELLS IN EXPERIMENTAL TUMORS

To directly check how tumor progression depends on apoptosis sensitivity of the original tumor, we created tumor cell population differing in their apoptotic potential and monitored the expansion of "more malignant" rare variants inside such tumors in vivo [Gurova et al., 2002]. As "a more malignant variants, we used cells either with inactivated p53 or with overexpressed bcl-2, both resistant to DNA damaging agents and hypoxia due to suppression of apoptosis. Instead of waiting for a infrequent spontaneous appearance of such variants in growing tumor, we mixed small number of GFP-labeled p53-deficient or bcl-2 overexpressing cells (prepared in vitro by transduction of a dominant negative mutant of *p53* or *bcl-2*) with an excess of parental cells, possessing wild type p53 and expressing low levels of bcl-2, and monitored their proportion at different stages of growth of subcutaneous tumors in mice. The first important observation made was that both cell variants were accumulated with the same rates inside growing

tumors: by the 12th day of growth, the parental cells in the tumors were almost completely substituted by green fluorescent cells. We concluded that p53 deficiency and bcl-2 expression provided tumor cells with similar selective advantages in a primary tumor. However, as it was mentioned above, the outcome of overgrowth of selected cell variants is very different: in one case (p53 inhibition), we have a fast progressing tumor, while in the other case (bcl-2 expression) the tumor is characterized by a reduced progression rate [Deichman et al., 1998].

Effective tumor progression requires high degree of genetic variability that is achieved by inactivation of p53 genomic stability control mechanism. As we had shown, such cells can be quickly accumulated in tumor presumably due to their resistance to apoptosis. However, what would happen if the original cell population was already resistant to apoptosis? Would then p53-deficient cells have a chance for accumulation? We experimentally addressed these questions by creating tumors that formed from excess of bcl-2-overexpressing cells mixed with a small number of GFP-labeled p53-deficient variants and monitored the proportion of green fluorescent cells in the course of tumor growth. The results were remarkably different from what we saw in the previous experiments: p53-deficient cells failed to accumulate in tumor population if it consists of the cells expressing bcl-2, thus preventing tumor from the expansion of genetically unstable cells and subsequent fast progression.

Similar conclusion has been made in a recent work from Scott Lowe's group [Schmitt et al., 2002a], which used a mouse model of *myc*-driven lymphomas to compare biological effects of inactivation of p53, suppression of caspase 9 (acting downstream of p53) and expression of Bcl-2, all resulting in suppression of apoptosis. All three variants showed similar selective advantages before apoptosis-sensitive variants. However, apoptosis-defective lymphomas that retain intact p53 genes do not display the checkpoint defects and gross aneuploidy that are characteristic of p53 mutant tumors. At the same time, they completely alleviate pressure to inactivate p53 during lymphomagenesis. Authors concluded that apoptosis is the only p53 function selected against during lymphoma development, whereas defective cell-cycle checkpoints and genomic instability are mere byproducts of p53 loss. Thus, similar conclusions were made after independent analyses of different experimental tumor models.

Bcl-2 AS AN "ANTI-PROGRESSOR": PUTATIVE MECHANISM AND PRACTICAL OUTCOMES

Based on all the information discussed above, we propose the following model which explains why *Bcl-2*can be a positive prognostic marker (Fig. 1). With an increase in size of a primary tumor that has wild type *p53* and low Bcl-2 expression, hypoxic conditions inside the tumor create conditions that favor Darwin's selection of cells with reduced sensitivity to hypoxia-induced apoptosis. Escape from death can be achieved equally well by either activation of anti-apoptotic gene expression (i.e., *Bcl-2*) or from the loss of pro-apoptotic factors (i.e., *p53*) [Graeber et al., 1996; Shimizu et al., 1996].

Since the appearance of either mutation is a rare event and the expansion of mutated cell clone progresses rapidly, the event that came first had a strong chance dominate in the tumor and prevent the expansion of mutations resulting in the same phenotypic alteration through an alternative mechanism. This means that in real tumors p53 inactivation or Bcl-2-overexpression are likely to be alternative genotypes that are rarely combined together. If *p53* mutation occurs first, it will result in the formation of a tumor with a high level of genetic instability, leading to higher rates of progression, metastases, invasion, and poor prognosis. If the *Bcl-2*-overexpressing clone appears first, this will lead to formation of a tumor in which the control of genomic stability is unaffected and which no longer favors selection of *p53*-deficient cells. Such a tumor would be characterized by low rates of progression and favorable prognosis.

In fact, there are some clinical observation confirming that the two tumor markers, *p53*-deficiency and Bcl-2 overexpression, are rarely expressed together in one tumor. Bcl-2 protein was abundant in the cell lines obtained from breast, head, and neck carcinomas that maintain wild type *p53* [Joensuu et al., 1994; Berardo et al., 1998; Castiglione et al., 1999, Tete, 1999; Lazaris et al., 2000]. On the contrary, p53 accumulation (indicative of *p53* mutations) was associated with low Bcl-2 expression in breast cancer [Berardo et al., 1998; van Slooten et al., 1998].

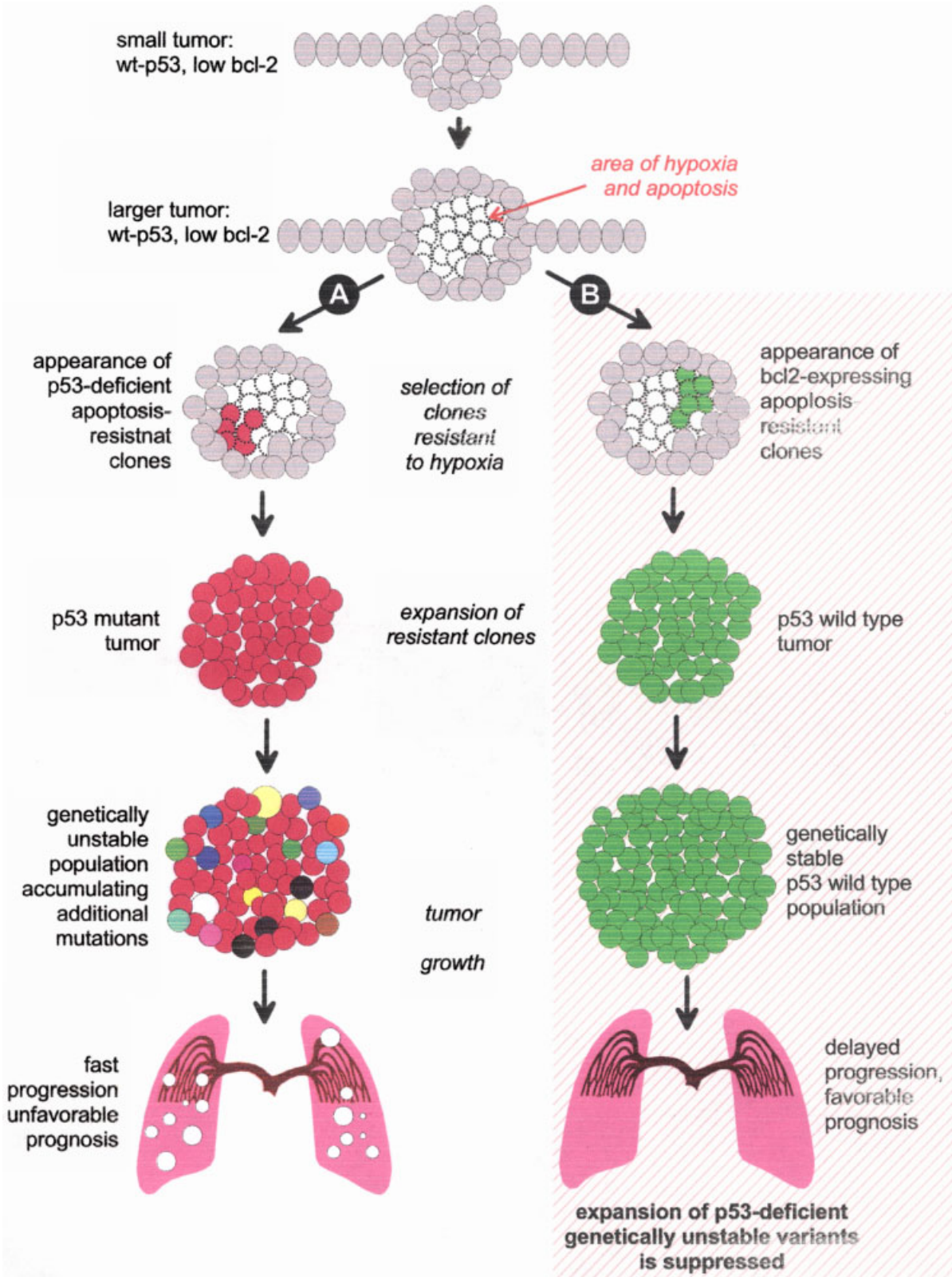

Fig. 1. Alternative scenarios of progression in the tumors that acquired resistance to apoptosis through two different genetic mechanisms (see explanation in the text). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Moreover, in some experimental system, we can find examples of the mutual exclusion of these two anti-apoptotic factors: erythroleukemia cell lines, induced by F-MuLV frequently overexpress bcl-2 and this event was shown to precede the emergence of p53 mutations, suggesting that bcl-2 expression may delay *p53* mutation in leukemic cells [Howard et al., 2001]. Interestingly, the majority of F-MuLV-induced erythroleukemia cell lines established from primary tumors induced in p53-deficient mice express low to negligible levels of bcl-2 [Howard et al., 2001].

Data from the transgenic mouse model confirmed our prediction from the other side: enforced acceleration of apoptosis by overexpression of *bax*, potential tumor suppressor, induced tumorigenesis on a tumor-predisposed *p53* deficient background, even though apoptosis rate of thymocytes (to which transgene expression was restricted in this model) in lck^{pr}-*bax* mice was increased, comparing with $p53^{-/-}$ animals [Knudson et al., 2001]. Although, authors explained this unusual fact by the putative positive influence of *bax* on cell growth rate (what we certainly can not exclude), it well fits our model postulating faster progression of apoptosis-prone tumors.

In summary, there is a series of puzzling experimental and clinical observations indicating that Bcl-2 expression, associated with apoptosis suppression, can act to slow down tumor progression, while p53 inactivation, though also resulting in apoptosis suppression, has stimulating effect on tumor progression. We have suggested a plausible explanation for this apparent discrepancy by showing that Bcl-2 expression has no effect on control of genomic stability but provide cells in vivo with strong selective advantages, thus devaluating and preventing expansion of cells with mutant p53 that are capable of rapid progression due to genomic instability. Thus apoptosis could be viewed as a factor promoting tumor progression by stimulating rapid turnover and provoking selection of malignant cells with mutant p53. These considerations demand a new outlook on the role of apoptosis in cancer origin and stimulate an experimental exploration of a new cancer prevention and treatment strategy that is based on the use of pharmacological inhibitors of programmed cell death.

It is important to stress that lack of selective advantageous for genetically unstable p53-deficient variants in the tumors formed by apoptosis resistant cells is not absolute and may be devaluated under conditions of cancer treatment. Schmitt et al. [2002b] has recently shown that treatment of Bcl-2-expressing mouse lymphomas with chemotherapeutic drugs results in senescent-like growth arrest in the tumor, creating conditions that favor selection of p53-deficient variants resuming growth and further tumor progression.

ACKNOWLEDGMENTS

Authors' research on the role of cell–cell interactions and p53-mediated apoptosis in tumor progression is supported by grants from NCI CA60730, CA88071 and CA75179.

REFERENCES

Berardo MD, Elledge RM, de Moor C, Clark GM, Osborn CK, Allred DC. 1998. Bcl-2 and apoptosis in lymph node positive breast carcinoma. Cancer 82:1296–1302.

Binder C, Marx D, Overhoff R, Binder L, Schauer A, Hiddemann W. 1995. Bcl-2 protein expression in breast cancer in relation to established prognostic factors and other clinicopathological variables. Ann Oncol 6:1005–1010.

Castiglione F, Sarotto I, Fontana V, Destifanis M, Venturino A, Ferro S, Cardaropoli S, Orengo MA, Porcile G. 1999. Bcl-2, p53 and clinical outcome in series of 138 operable breast cancer patients. Anticancer Res 19: 4555–4563.

De la Coste A, Mignon A, Fabre M, Gilbert E, Porteu A, Van Dyke T, Kahn A, Christine P. 1999. Paradoxical inhibition of c-myc-induced carcinogenesis by Bcl-2 in transgenic mice. Cancer Res 59:5017–5022.

Deichman GI, Matveeva VA, Kashkina LM, Dyakova N, Uvarova E, Nikiforov M, Gudkov A. 1998. Cell transforming genes and tumor progression: In vivo-unified secondary phenotypic cell changes. Int J Cancer 75:277–283.

Diaz JI, Pow-Sang JM, Mora LB, Seign JD, Cantor AB, Dalton WS. 2000. Cytometric analysis of Fas and Bcl-2 expression in normal prostatic epithelium and prostate cancer. Urol Oncol 5:149–154.

Eerola AK, Ruokolainen H, Soini Y, Raunio H, Paakko P. 1999. Accelerated apoptosis and low bcl-2 expression associated with neuroendocrine differentiation predict shortened survival in operated large cell carcinoma of the lung. Pathol Oncol Res 5:179–186.

Eguchi H, Suga K, Saji H, Toi M, Nakachi K, Hayashi SI. 2000. Different expression patterns of Bcl-2 family genes in breast cancer by estrogen receptor status with special reference to pro-apoptotic Bak gene. Cell Death Differ 7:439–446.

Evan GI, Vousden KH. 2001. Proliferation, cell cycle and apoptosis in cancer. Nature 411:342–348.

Furth PA, Bar-Peled U, Li M, Lewis A, Laucirica A, Jager R, Weiher H, Russel RG. 1999. Loss of anti-mitotic effects of Bcl-2 with retention of anti-apoptotic activity during tumor progression in a mouse model. Oncogene 18:6589–6596.

Graeber TG, Osmanian S, Jacks T, Housman DE, Koch CJ, Lowe S, Giaccia AJ. 1996. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors. Nature 379:88–91.

Gurova KV, Kwek SS, Koman IE, Kamarov AP, Kandel E, Nikiforov MA, Gudkov AV. 2002. Apoptosis inhibitor as a suppressor of tumor progression: expression of Bcl-2 eliminates selective advantages for p53-deficient cells in the tumor. Cancer Biol Ther 1:39–44.

Haitek A, Posch B, El-Baz M, Mokhtar AA, Susani M, Ghoneim MA, Marberger M. 2001. Bilharzial related, organ confined, muscle invasive bladder cancer: Prognostic value of apoptotic markers, p53, E-cadherin, epidermal growth factor receptor and c-rebB-2. J Urol 165:1481–1487.

Haldar S, Negrini M, Monne M, Sabbioni S, Croce CM. 1994. Down-regulation of bcl-2 by p53 in breast cancer cells. Cancer Res 54:2095–2097.

Hamilton A, Piccart M. 2000. The contribution of molecular markers to the prediction of response in the treatment of breast cancer: A review of the literature on HER-2, p53, and BCL-2. Ann Onco 11:647–663.

Hotz MA, Bosq J, Zbaeren P, Reed J, Schwab G, Krajewski S, Brousset P, Borner MM. 1999. Spontaneous apoptosis and the expression of p53 and Bcl-2 family proteins in locally advanced head and neck cancer. Arch Otolaryngol Head Neck Surg 125:417–422.

Howard JC, Li Q, Chu W, Zochodne B, Kapoor M, Ung Y, Rosen K, Ben-David Y. 2001. Bcl-2 expression in F-MuLV-induced erythroleukemias: A role for the anti-iapoptotic action of Bcl-2 during tumor progression. Oncogene 20:2291–2300.

Huang DCS, O'Reilly LA, Strasser A, Cory S. 1997. The anti-apoptosis function of Bcl-2 can be genetically separated from its inhibitory effect on cell cycle entry. EMBO J 16:4628–4638.

Inada T, Kikuyama S, Ichikawa A, Igarashi S, Ogata Y. 1998. Bcl-2 expression as a prognostic factor of survival of gastric carcinoma. Anticancer Res 18:2003–2010.

Joensuu H, Pylkkanen L, Toikkanen S. 1994. Bcl-2 protein expression and long term survival in breast cancer. Am J Pathol 145:1191–1198.

Johnstone RW, Ruefli AA, Lowe SW. 2002. Apoptosis: A link between cancer genetics and chemotherapy. Cell 108:153–164.

Kajiwara T, Tatkeuchi T, Ueki T, Moriyama N, Ueki K, Kakizoe T, Kawabe K. 1999. Effect of Bcl-2 overexpression in human prostate cancer cells in vitro and in vivo. Int J Urol 6:520–525.

Kandouz M, Siromachkova M, Jacob D, Chretien Market B, Therwath A, Gompel A. 1996. Antagonism between estradiol and progestin on Bcl-2 expression in breast cancer cell line. Int J Cancer 68:120–125.

Knudson CM, Johnson GM, Lin Y, Korsmeyer SJ. 2001. Bax accelerates tumorigenesis in p53-deficient mice. Cancer Res 61:659–655.

Kobayashi S, Iwase H, Ito Y, Yamashita H, Iwata H, Yamashita T, Ito K, Toyama T, Nakamura T, Masaoka A. 1997. Clinical significance of bcl-2 gene expression in human breast cancer tissues. Breast Cancer Res Treat 42:173–181.

Koide N, Koike S, Adachi W, Amano J, Usuda N, Nagata T. 1997. Immunohystochemical expression of bcl-2 protein in squamous cell carcinoma of the esophagus. Surg Today 27:685–691.

Krajewska M, Moss SF, Krajevski S, Song K, Holt PR, Reed JC. 1996. Elevated expression of Bcl-X and reduced Bak in primary colorectal adenocarcinoma. Cancer Res 56:2422–2427.

Kurabayashi A, Furihata M, Matsumoto M, Ohtsuki Y, Sasaguri S, Ogoshi S. 2001. Expression of Bax and apoptosis-related proteins in human esophageal squamous cell carcinoma including dysplasia. Mod Pathol 14:741–747.

Lazaris AC, Lenardi I, Kavantzas N, Kandiloros D, Adamapoulos G, Davaris P. 2000. Correlation of tumor markers p53, bcl-2 and cathepsin-D with clinicopathological features and desease-free survival in laringeal squamous cell carcinoma. Pathol Int 50:717–724.

Li L, Yan L, Wang Z, Liu Z, Wei Y, Huang G. 2000. Change of apoptotic status in human colorectal adenoma-carcinoma stages sequences and its correlation with carcinogenesis and prognosis. Clin Med J 113:886–888.

Liu HF, Liu WW, Fang DC, Men RP. 1998. Expression of bcl-2 protein in gastric carcinoma and its significance. World J Gastroenterol 4:228–230.

Megha T, Ferrari F, Benvenuto A, Bellan C, Lalinga AV, Lazzi S, Bartolommei S, Cevenini G, Leoncini L, Tosi P. 2002. p53 mutation in breast cancer. Correlation with cell kinetics and cell of origin. J Clin Pathol 55:461–466.

Saegusa M, Okayasu I. 1997. Bcl-2 is closely correlated with favorable prognosric factors and inversely associated with p53 protein accumulation in endometrial carcinomas: Immunohistochemical and poymerase chain reaction/loss of heterozygosity findings. J Cancer Res Clin Oncol 123:429–434.

Schmitt CA, Fridman JS, Yang M, Baranov E, Hoffman RM, Lowe SW. 2002a. Dissecting p53 tumor suppressor functions in vivo. Cancer Cell 1:289–298.

Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, Lowe SW. 2002b. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 109:335–346.

Shimizu S, Eguchi Y, Kamiike W, Itoh Y, Hasegawa J, Yamabe K, Otsuki Y, Matsuda H, Tsujimoto Y. 1996. Induction of apoptosis as well as necrosis by hypoxia and predominant prevention of apoptosis by bcl-2 and bcl-X. Cancer Res 56:2161–2166.

Sjostrom J, Bergh J. 2001. How apoptosis is regulated, and what goes wrong in cancer. BMJ 322:1538–1539.

Takayama T, Nagao M, Sawada H, Yamada Y, Emoto K, Fujimoto H, Ueno M, Hirao S, Nakajima Y. 2001. Bcl-X expression in esophageal squamous cell carcinoma: Association with tumor progression and prognosis. J Surg Oncol 78:116–123.

Tanji N, Yokoyama M, Sugamoto T, Takeuchi M, Terada N. 1998. Apoptosis in prostatic adenocarcinomas: A study of relationship to Ki-67 and Bcl-2 protein expression. Anticancer Res 18:1111–1116.

Teixeira C, Reed JC, Pratt MA. 1995. Estrogen promotes chemoterapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. Cancer Res 55:3902–3907.

Tete S, Pappalardo S, Fioroni M, Salini L, Imperatrice AM, Perfetti G. 1999. Bcl-2, p53, Ki67 and apoptotic index in cancerous and precancerous lesions of the oral mucosa. Minerva Stomatol 48:419–425.

Tjalma W, De Cuyper E, Weyler J, Van Marck E, De Pooter C, Albertyn G, van Dam P. 1998. Expression of bcl-2 in

invasive and in situ carcinoma of the uterine cervix. Am J Obstet Gynecol 178(1 Pt 1):113–117.

Vail M, Pierce RH, Fausto N. 2001. Bcl-2 delays and alters hepatic carcinogenesis induced by transforming growth factor? Cancer Res 61:594–601.

Vakkala M, Lahteenmaki K, Raunio H, Paakko P, Soini Y. 1999. Apoptosis during breast carcinoma progression. Clin Cancer Res 5:319–324.

Van Slooten HJ, van de Vijver MJ, van de Velde CJ, van Dierendonck JH. 1998. Loss of bcl-2 in invasive breast cancer is associated with high rates of cell death, but also with increased proliferative activity. Br J Cancer 77:789–796.

Zhang GJ, Kimijima I, Abe R, Watanabe T, Kanno M, Hara K, Tsuchiya A. 1998. Apoptotic index correleates to bcl-2 and p53 protein expression, histological grade and prognosis in invasive breast cancers. Anticancer Res 18:1989–1998.

Zornig M, Hueber A, Baum W, Evan G. 2001. Apoptosis regulators and their role in tumorigenesis. Biochim Biophys Acta 1551:F1–F37.