H.-J. van Slooten, C.J.H. van de Velde and J.H. van Dierendonck

Bcl-2 Family Members and p53 in the Regulation of Apoptosis in Invasive Breast Cancer

Introduction

The administration of chemotherapy to women with metastatic breast cancer (BC) has significantly prolonged disease-free survival, but its impact on overall survival has been disappointing (Early Breast Cancer Trialist's Collaborative Group 1996). Women who at the time of diagnosis have no detectable metastases in axillary lymph nodes and/or at distant sites — generally refered to as node-negative patients — have a high probability of being cured by surgery alone. However, in about one-third of these patients the disease will recur due to the outgrowth of previously undetected micro-metastases. Adjuvant chemotherapy can significantly prolong the mean disease-free survival of node-negative patients, but the dilemma is that treating all these patients with chemotherapeutic drugs results in serious overtreatment.

It is well-established that tumor size and certain histological characteristics (size of the nuclei, differentiation grade and proliferative activity) are sufficiently compelling to make treatment decisions relatively straightforward in approximately half of node-negative BC patients — these patients have either an excellent or a very poor prognosis. But, in the intermediate group, recurrence rate is still 30% and more prognostic information is needed here to identify the high-risk patients (Rosen et al., 1993). With this goal in mind, many studies have attempted to determine which (combination of) tumor characteristics would allow reliable identification of these patients. At present, at least 80 putative prognostic markers have been reported, but most factors are relatively new and from many of these the value has not been fully established. In fact, many factors are somewhat related to the classical histopathological parameters (viz. tumor size, tumor grade and lymph node-status) and to each other and therefore add little new information.

A problem of equal clinical importance is that approximately one-third of BC patients treated with adjuvant chemotherapy does not respond to this treatment. It is obvious that, in order to design optimal treatment strategies for individual patients (whether they have positive nodes or not), it is essential to be able to predict a tumor's response to a specific therapy. Patients likely to be resistant to (a certain combination of) chemotherapeutic drugs might then be selected for treatment with a different and/or more aggressive regimen. But whereas the presence of estrogen and progesteron receptors in BC is widely used to decide whether or not patients should

be given (anti)hormonal therapy, at present no such markers are used to predict response to specific forms of chemotherapy; reliable predictive factors clearly would be of great value for the management of both node-positive and node-negative patients.

Thus, important clinical questions concerning the treatment of BC patients are: (i) which prognostic factors can properly separate the majority of low risk subsets of node-negative patients from those at high risk? (ii) what is the minimal level of risk that justifies systemic adjuvant therapy for node-negative patients? (iii) which factors predict response to chemotherapy and/or hormone therapy or to novel therapeutic approaches in both node-positive and node-negative patients.

Because the development of most clinically used anticancer drugs has been empirical, the molecular mechanisms that determine treatment efficacy remain largely unknown. A more complete understanding of cellular sensitivity c.q. resistance to anti-cancer therapy requires elucidation of mechanisms by which anticancer drugs cause tumor cell death. As outlined by Hickman and co-authors elsewhere in this volume, cellular damage caused by cytotoxic treatments seems insufficient to explain observed anti-tumor effects. Together with the discovery that transformed cells are inherently sensitive to induction of apoptosis by anticancer treatments this has lead to a paradigm shift in our thinking on drug-sensitivity: the majority of drugs used in chemotherapy are now believed to act by inducing a cell death program within tumor cells rather than by disrupting DNA replication or inflicting cellular damage incompatible with cell survival. The sensitivity to induction of apoptotic cell death of certain transformed cell types relative to normal cells may be the main reason for selective killing by radiation or cytotoxic drugs; if this sensitivity is reverted by mutations that disrupt certain apoptotic pathways, tumors may become resistant to a particular type of treatment (Kerr et al., 1994; Reed, 1994). Thus, a detailed understanding of the molecular mechanisms that determine the commitment of a cell to the induction of apoptosis may be crucial for the prediction of treatment responses.

An important reason for the relative paucity of clinical data regarding tumor responsiveness to chemotherapy is that most clinical trials do not contain a control group of untreated patients. When all treatment-arms in a trial include some form of adjuvant therapy, the association of a given marker with poor survival can be an indication of aggressive tumor growth or of increased chemoresistance or both. Our studies on material from EORTC Trial 10854 gave us the opportunity to circumvent this problem by selecting a subset of premenopausal node-negative patients randomized to receive either one course of peri-operative chemotherapy with a combination of 5-fluorouracil, epirubicin and cyclophosphamide (1xFEC) or no such adjuvant treatment (Van Slooten et al., 1996; Clahsen et al., in press). In a second study we used material from EORTC Trial 10902 in which patients with operable BC were randomized to receive either pre- or post-operative chemotherapy. This enabled us to directly assess primary tumor response (defined by clinical measurements as well as a mammographical assessment of response) and correlate this response with the expression of a number of molecular markers, including two factors involved in regulation of apoptotic cell death, Bcl-2 and p53.

The Bcl-2 Gene Family in the Genesis of Breast Cancer

The Bcl-2 proto-oncogene, which was initially identified on the basis of the t(14:18) chromosomal translocation found in human B-cell follicular lymphomas (Tsujimoto et al., 1984), is the founding member of a family of proteins comprising proteins that can either repress (e.g. Bcl-2, Bcl-Xl, CED-9, Mcl-1, Bcl-W, and A1) or promote apoptosis (e.g. Bax, Bcl-Xs, Bad, and Bak) (Korsmeyer, 1995). The members of this family possess one or more of four homologous domains – invariant residues within these regions are essential for their respective functions (Yin et al., 1994).To date, most research has been focussed on Bcl-2 demonstrating that overexpression protects cells against induction of apoptosis by a variety of stimuli, including irradiation and most clinically used chemotherapeutic drugs (Campos et al., 1993).

Many Bcl-2 family proteins can physically interact with each other, forming a complex network of homo- and heterodimers. Recent data have shown that homodimerization of Bax, and not heterodimerization with Bcl-2, seems essential for its apoptosis-inducing capacity and that Bcl-2 can rescue cells from the lethal effects of Bax without heterodimerizing with it (Zha and Reed, 1997). While an *in vivo* competition exists between Bcl-2 and Bax — as will be discussed in more detail in chapter 15, the ratio of these proteins ultimately determines the cellular sensitivity to the induction of cell death — each seems to be able to regulate apoptosis independently (Knudson & Korsmeyer, 1997) and also Bak can accelerate chemotherapy-induced cell death independently of its heterodimerization with Bcl-Xl and Bcl-2 (Simonian et al., 1997).

A number of Bcl-2 family members may function as pore-forming proteins, reminiscent of bacterial toxins like diphteria toxin (Schendel et al., 1997). One concept is that Bcl-2 and Bcl-Xl directly or indirectly block the mitochondrial release of cytochrome c (Kharbanda et al., 1997; Yang et al., 1997; Kluck et al., 1997, Kim et al., 1997), which is involved in the activation of proteases (caspases) required for the execution/degradation phase of the apoptotic process (Zou et al., 1997). But although inhibition of the cytochrome c-induced cascade of caspase activities could be an important function of anti-apoptotic Bcl-2 family members, Li et al. recently provided evidence that in some cells cytochrome c release is not required to induce cell death and that in these cells Bcl-Xl is still able to inhibit cell death induced by Fas or tumor necrosis factor (Li et al., 1997b).

Nevertheless, the relative expression levels of pro- and anti-apoptotic Bcl-2 family members may regulate the level of caspase activity and by doing so determine the cells' sensitivity to apoptotic stimuli. However, Bcl-2 may not only act upstream of caspases, but can also be a target of these enzymes: a remarkable recent finding is that caspase-3 can convert Bcl-2 into a Bax-like death effector (Cheng et al., 1997). Moreover, Bcl-2 can also bind to a variety of cytoplasmic proteins (Reed, 1994), including the calcium-binding protein calcineurin. As a result of latter interaction, calcineurin is unable to promote the nuclear translocation of transcription factor NF-AT, which is required for expression of interleukin-2 and other cytokines involved in T-cell proliferation (Shibasaki et al., 1997).

Under certain conditions, induction of apoptosis seems to depend on cell cycle progression and the activity of proteins involved in cell cycle regulation: e.g., cyclins

and cyclin-dependent kinases (Kranenburg et al., 1996) or their inhibitors (Wang et al., 1997). In that context it is intriguing that overexpression of Bcl-2 has been found to promote the departure of cells from the cell cycle, and to inhibit the transition from a resting to a cycling state (Pietenpol et al., 1994; Mazel et al., 1996). This property has also described for Bcl-X_L and E1B19kD and seems to be molecularly separable from the anti-apoptotic function of these proteins (Huang et al., 1997).

Recent data suggest a possible link between Bcl-2 and Bax and $p27^{KIP}$, an inhibitor of cyclin dependent kinases that functions as a 'brake' in the cell cycle. In thymocytes, which are normally quiescent, $p27^{KIP}$ needs to be degraded in order to get apoptosis — this degradation seems to be enhanced by Bax, whereas Bcl-2 does the opposite (Gil Gómez, personal communication).

Because of its importance as an inhibitor of apoptosis, we hypothesized that Bcl-2 might be useful as a molecular marker for both prognosis and treatment response in BC. In normal human breasts, high Bcl-2 expression is observed in the lobular ducts, whereas intralobular ducts show remarkable variability of Bcl-2 expression, possibly reflecting the hormonal regulation of Bcl-2 (Van Slooten et al., 1996). Bcl-2 is preferentially expressed in well-differentiated, estrogen receptor-positive breast tumors and associated with good prognosis (Bhargava et al., 1994; Gee et al., 1994; Joensuu et al., 1994; Leek et al., 1994; Gasparini et al., 1995; Hellemans et al., 1995; Silvestrini et al., 1996). In invasive BC, we and others observed a strong inverse correlation between Bcl-2 and proliferative activity (Joensuu et al., 1994; Silvestrini et al., 1994; Van Slooten et al., 1996; Van Slooten et al., in press). In line with these findings our data show that, both in invasive BC and ductal carcinoma *in situ*, lack of Bcl-2 expression is strongly associated with increased levels of apoptosis, high proliferative activity and high tumor grade, suggesting the existence of high levels of cell turnover in tumors of patients with poor prognosis.

Also in other tumor types a correlation seems to exist between high proliferative activity and high apoptotic activity and high tumor grade (Lipponen and Aaltomaa, 1994; Aihara et al., 1995; Du et al., 1996; Isacson et al., 1996; Koshida et al., 1996; Shoji et al., 1996). It is tempting then to hypothesize that these data reflect the connection between Bcl-2 expression, decreased growth fraction and tumor cell differentiation. In theory, a direct link between protection against apoptosis and a reduction of proliferation could function as a hardwired defense protecting cells against oncogenic transformation, complementary to the induction of apoptosis/senescence by oncogenic mutations. The existence of such a dual mechanism could explain the finding that in many cancer types Bcl-2 is preferentially expressed in slowly proliferating, well-differentiated tumors associated with good prognosis. It would also provide an explanation for the counter-intuitive finding that in many cancer types, loss of Bcl-2 expression — in spite of the resulting increase in tumor apoptosis — is associated with tumor progression. Long-lived cells with increased resistance to apoptosis pose a potential hazard to a multicellular organism — they are much more likely than short-lived apoptosis-sensitive cells to sustain and survive the multiple mutations needed for transformation into a cancer cell.

The importance of reducing the proliferative potential of long-lived cells may be demonstrated by the high rate at which tumors develop from breast epithelial

cells. These cells are present in the body over a long period of time, but also have to retain significant proliferative potential and may therefore be more susceptible to transformation than other long-lived cells.

P53 and Clonal Evolution in Breast Cancer

The p53 tumor suppressor gene is among the most frequently mutated genes in human cancer (Hainaut et al., 1997). In BC, mutations have been reported to occur in 15-50 % of cases, depending on the stage of the disease and the method of detection (Pietilainen et al., 1995). The p53 gene encodes a phosphoprotein which has been shown to play a critical role in controlling cell proliferation, cellular differentiation and apoptosis (Ko and Prives, 1996). The levels and activities of wildtype (wt) p53 have been shown to increase in response to irradiation and DNA damaging agents — high levels of p53 may induce apoptosis, whereas relatively low levels cause cell cycle arrest (Chen et al., 1996).

As was discussed in a previous chapter, loss of p53 function is an important factor in tumor development and progression, presumably through reduced tumor apoptosis and/or reduced induction of senescence (Howes et al., 1994; Symonds et al., 1994; Serrano et al., 1997). In response to DNA damage, p53 is involved in activation of DNA repair mechanisms and may prevent expansion of cells with damaged DNA by inducing apoptosis or senescence. The latter has been found to be dependent on elevated expression of the p53-inducible gene p21/WAF1 (Brown et al., 1997).

Animal work clearly demonstrated that inactivation of p53 is associated with treatment resistance (Lowe et al., 1993a and 1993b; Merrit et al., 1994), but at present no unequivocal evidence has been obtained of the existence of a similar association in invasive BC.

The mechanisms by which wt-p53 protein induces apoptosis are not completely understood, but p53-mediated upregulation of Bax, downregulation of Bcl-2 and activation of caspase-3 are likely to be involved (Selvakumaran et al., 1994; McCurrach et al., 1997). Bax seems to be an important downstream-effector of p53 (Yin et al., 1997) and its importance is highlighted by the discovery of frameshift mutations within the *bax* gene in subsets of colorectal and gastric cancers (Rampino et al., 1997; Yamamoto et al., 1997). In invasive BC, mutation of p53 is negatively correlated with immunodetected Bcl-2, but no significant correlation exists between Bax and p53 status (Van Slooten et al., in press).

Increased genetic instability leading to accumulation of genetic alterations may be one of the most important factors driving tumor progression in BC. In colon cancer, inactivation of p53 seems to take place prior to the occurrence of major DNA rearrangements and aneuploid clonal divergence, which is in line with the concept that inactivation of p53 allows proliferation of cells with a damaged genome, and enables the genesis of aneuploid tumor subclones. Also in BC, loss of p53 function is associated with rapid tumor growth, aneuploidy, high tumor grade and with poor prognosis (Thor et al., 1992; Allred et al., 1993; Silvestrini et al., 1996). Moreover, mutations affecting the zinc-binding domains of the gene, critical for DNA binding

of p53 (L2-L3), where reported to be associated with a particularly poor prognosis (Børresen et al,. 1996) and possibly with increased chemoresistance (Aas et al., 1996). We hypothesized that such an aggressive tumor phenotype would be characterised by increased levels of proliferation and/or reduced levels of apoptosis. However, no such phenotypical differences existed between p53 mutations inside or outside the zinc-binding domains (Van Slooten et al., submitted). Unexpectedly and in contradiction with its proposed function as 'guardian of the genome', in BC loss of p53 function was associated with increased rather then decreased levels of apoptosis. The presence of a simultaneous increase in the level of mitosis suggests the existence of increased cell turnover in p53-mutated tumors.

In line with these results it has been found that after crossing p53-deficient mice with mammary tumor-susceptible Wnt-1 transgenic mice, the off-spring all developed mammary tumors, and did so much sooner than their $p53^{+/+}$ counterparts. p53-deficient tumors had high average growth rates, i.e., showed much higher percentages of mitotic figures, but, remarkably, also no decrease in percentages of apoptotic cells compared to $p53^{+/+}$ tumors (Jones et al. 1997). Together with our own data this suggests that p53 mutations are associated with the development of a phenotype with accelerated cell turnover. The possibility that p53-deficient tumors are the end-result of many more cell divisions (thereby enhancing the mutation rate), combined with the supposed role of p53 in preventing chromosomal abberations, may contribute to rapid clonal evolution and thereby the generation of aggressive and/or treatment-resistant variants. Our finding of similar associations between p53, proliferation and apoptosis in ductal carcinoma *in situ* suggests that the development of such a dangerous phenotype may be an early event in BC. Whereas the observed increase in p53-independent tumor apoptosis may result from a loss of cell cycle control, these tumors may in fact be quite resistant to induction of p53-dependent apoptosis, e.g. as induced by genotoxic treatments.

Interestingly, this situation could be very similar for colon cancers; Fazeli and coworkers recently reported that the appearance of highly dysplastic cells in human late-stage colon adenomas, which usually coincides with loss of heterozygozity at the p53 locus, did not correlate with any reduction in the incidence of apoptosis and it was proposed that wt-p53 retards the progression from benign adenoma to malignancy by mechanism(s) other than the promotion of apoptosis (Fazeli et al. 1997). It seems relevant in this context that very low levels of wt-p53 protein have been shown to protect cells from apoptosis rather than facilitate this process (Lassus et al. 1996). It has been found that a link may exist between loss of estrogen receptor, genomic lesions in the p53 gene as well as in genes involved in hereditary BC (BRCA1 and BRCA2 respectively), and high fractional allelic loss (a measure of genetic instability), being all associated with poor prognosis (Sato et al., 1991; Schmutzler et al., 1997). Also, molecular changes in the signaling pathway from tyrosine kinase receptors (like epidermal growth factor receptor and c-erbB-2), which involves oncogene products such as Ras and the Raf-1 protein kinase, can induce estrogen-independent growth and, in the case of Raf-1 activation, apoptosis (El-Ashry et al., 1997). In a mouse model of mammary tumorigenesis, c-*erb*B-2/neu has been demonstrated to cooperate with mutant p53, resulting in the development of high grade, aneuploid

Table I. Four-year Disease-Free Survival and Hazard Ratios for Treatment Effect by p53 and Bcl-2 scores.

Parameter	PeCT		Control		HR (95% CI)	P value
	N/O	DFS (%)	N/O	DFS(%)		
p53						
Negative	191/28	88.0	168/43	77.0	0.51 (0.31-0.82)	<0.01
Positive	39/10	76.0	42/10	80.0	1.12 (0.47-2.70)	0.80
Bcl-2						
Low score (0-2)	60/14	79.5	52/18	67.6	0.55 (0.27-1.12)	0.09
High score (3-6)	161/21	87.0	150/31	81.0	0.61 (0.35-1.06)	0.07

N = total number of patients; O = number of events; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; PeCT, peri-operative (poly)chemotherapy.
(P53 data: Clahsen et al., 1998; Bcl-2 data: Van Slooten et al., 1996).

tumors, exhibiting increased proliferation and apoptosis, in contrast to tumors arising in p53-null mice, which show reduced apoptosis (Li et al., 1997). In addition, in Non-Hodgkin lymphomas increased levels of apoptosis and proliferation were reported to exist in the subset of Bcl-2 negative lymphomas with mutated p53, which are associated with poor prognosis (Takano et al., 1997).

Therefore, in BC, loss of p53 function and loss of expression of estrogen receptors and Bcl-2 may be associated with an aggressive phenotype, already present in the pre-invasive stage and characterized by increased cell turnover, increased genetic instability and rapid clonal evolution.

Predicting Response to Pre-Operative Chemotherapy in Breast Cancer

Unless triggered by an appropiate stimulus (e.g. DNA damage), wt-p53 protein is rapidly degraded, has a short half-life and low intracellular levels. Stabilization of p53 protein in the absence of a stimulus is always a hallmark of loss of function secondary to a mutation or interaction with viral or cellular oncoproteins (Blagosklonny, 1997).

In the premenopausal node-negative patients entered into EORTC Trial 10854, p53 protein accumulation, in contrast to Bcl-2, predicted a lack of response to 1xFEC as defined by disease-free survival time (see table I). Surprisingly, p53 seemed to predict treatment resistance in patients who developed distant metastases, but not in patients who developed local recurrence (van Slooten et al., unpublished data).

Material from patients treated with preoperative polychemotherapy offers the unique possibility to directly monitor the response of the primary tumor. For that reason, a

project was started to assess in breast tumors from patients, who had recieved four courses of FEC preoperatively (EORTC trial 10902), the relationship between a number of molecular markers and treatment response. Both biopsies taken prior to chemotherapy and surgically removed tumor material were collected, and mammographies were taken before and after chemotherapy and scored according to WHO criteria, as well as to a system that takes into account changes in the mammographical density of the lesion. It was found though that neither p53 accumulation nor expression of Bcl-2 was significantly associated with mammographical response of the primary tumor to chemotherapy.

The failure of Bcl-2 expression to predict response to chemotherapy, although in conflict with *in vitro* data, seems to be in line with its failure to predict response to disease-free survival after 1xFEC (see table I). Moreover, the complex interactions between the various anti- and pro-apoptotic members of the *bcl-2* gene family may require assessment of the ratio between various family members for an adequate prediction of treatment response.

However, because of the large body of experimental evidence highlighting the important role of p53 in treatment resistance (see also the contribution of Lowe et al. and Hickman et al to this volume), and the clinical finding of an association of p53 accumulation with lack of response to 1xFEC in BC patients, the absence of an association between p53 accumulation and mammographical response to pre-operative chemotherapy is difficult to explain. Sjögren and colleagues reported that although in their series of 316 BC patients confirmed p53 mutations were associated with treatment resistance, p53 accumulation as detected by immunohistochemistry was not Sjörgen et al., 1996). But the strong correlation we observed between p53 mutations detected by DNA analysis and immunohistochemistry respectively makes it unlikely that the lack of any significant association between tumor response and p53 mutations was solely due to 'false positive' and/or 'false negative' staining results.

As mentioned above, in BC loss of p53 function seems to be associated with reduced efficacy of polychemotherapy in preventing the outgrowth of distant metastases, but not in reducing the outgrowth of tumor cells at the site of the primary tumor. One can thus not exclude the possibility that p53 is a poor predictor of the response of a primary tumor to pre-operative chemotherapy. In fact, the magnitude of the response of the primary tumor may have little value in predicting the effect of chemotherapy on the overall survival of BC patients. Supporting this view is the finding of Linn and coworkers, that whereas coexpression of p53 and P-glycoprotein did not predict response to pre-operative chemotherapy, it strongly predicted overall survival (Linn et al., 1996).

It should be mentioned that evaluation of primary tumor response (e.g. decrease in tumor size assessed by mammography or by using calipers is difficult. In fact, several investigators found that this type of evaluation did not correspond to pathological response (e.g. as assessed by microscopical evaluation) or local recurrence-free survival. In contrast, a complete pathological response was significantly associated with improved local recurrence-free survival and overall survival (Vinnicombe et al., 1996; Brain et al.. 1997). Thus, evaluation of tumor response by an experienced pathologist seems to be the most reliable predictor of response in patients treated

with pre-operative chemotherapy. For these reasons, an important question to answer in the POCOB study is which associations exist between p53 status, initial tumor response assessed either by mammographical, clinical or pathological parameters and overall survival.

The finding that p53 activation can induce apoptosis as well as senescence may result in different types of tumor response in chemotherapy. Of note, in this respect, is the finding in colon cancer cells growing *in vitro* that loss of p21-mediated cell cycle arrest in response to irradiation resulted in an increase in apoptotic cells and a decrease in senescent cells. Moreover, in immune-deficient mice p21-/- cells were found to be more sensitive to irradiation then their p21+/+ counterparts (Waldman et al., 1997). Because p21 expression is mediated by p53, it is not unlikely that p53 mutated tumors initially respond to genotoxic treatment, but with time, due to the failure to undergo permanent cell cycle arrest, resume growth. In a wt-p53 tumor on the other hand, many cells may respond to therapy by undergoing senescence, resulting in a (temporary) decrease in tumor growth rate rather than a measurable decrease in tumor volume.

The finding that p53 mutated tumors may be associated with increased clonal evolution may have implications for the use of pre-operative CT in the treatment of BC. Experimental evidence indicates that following irradiation, in line with the function of p53 as 'guardian of the genome', the rate at which mutations accumulate in cell populations lacking functional p53 is dramatically increased compared to cell populations with wildtype p53 (Phillips et al., 1997). Thus, in theory, treatment of a large population of p53-mutated tumor cells with DNA-damaging drugs (as is the case with pre-operative (poly)chemotherapy) may increase the likelihood that unfavourable mutations accumulate, eventually resulting in a very poor treatment outcome. Future investigations will have to determine whether in BC patients with p53-mutated tumors the pre- or postoperative timing of chemotherapy affects clinical outcome.

Summary

Whereas in lymph-node negative, premenopausal BC patients high levels of Bcl-2 expression are clearly associated with reduced levels of apoptosis, reduced proliferation, and good prognosis, they do not predict response to chemotherapy (1xFEC). p53 mutations (both in- and outside the zinc-binding domains) are associated with increased levels of both apoptosis and proliferation, indicating increased cell turnover. p53 accumulation seems to predict response to chemotherapy in premenopausal, lymph-node negative patients, but only in patients who ultimately develop distant metastasis. Neither Bcl-2 expression nor p53 accumulation predicted response to pre-operative CT.

Our data suggest that p53 has little impact on the apoptosis rate in breast cancers. One possibility is that wildtype p53 induces senescence in cells with mutations in growth regulatory genes and that p53 inactivation permit cells to proceed crisis and immortalization. Mutations leading to increased cell divisions also increase the rate of (p53-independent) apoptosis and Bcl-2 counteracts both apoptosis and cell division activities. As outlined in figure 1, loss of p53 function and loss of Bcl-2 may be

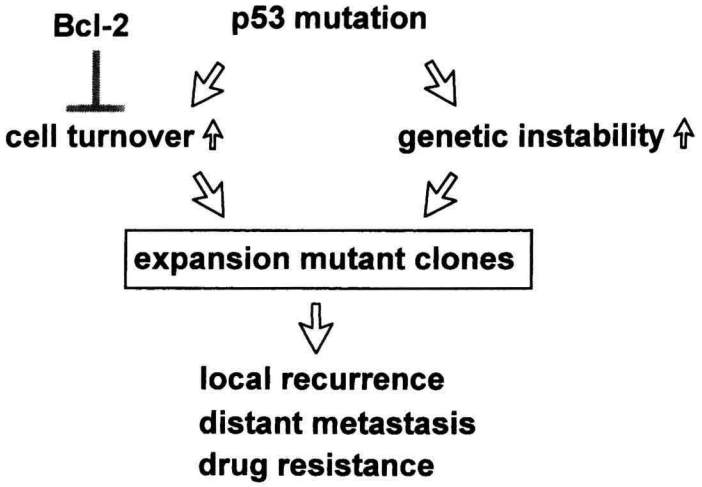

Fig. 1. P53 as an important mediator of the rate at which mutant cell clones expand within a tumor cell population, thereby affecting the rate of tumor progression. Inactivation of p53, by removing cell cycle blocks, increases proliferation rate and the rate of p53-independent (proliferation associated) apoptosis, leading to an increase in cell turnover. On the other hand, failure to delete cells with damaged DNA from the proliferating population through p53-dependent apoptosis/senescence as well as aberrations in mitotic checkpoints result in increased genetic instability. These two mechanisms may synergistically enhance the generation and expansion of mutant clones within a tumor cell population. Finally, the increased clonal evolution in tumors with inactivated p53 leads to increased rate of local recurrence, distant metastasis and drug resistance.

associated with an aggressive phenotype, already present in the pre-invasive stage and characterised by an increase in both cell turnover and genetic instability. This may result in rapid clonal evolution, which in turn may enhance tumor progression and treatment resistance.

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