# Estrogen withdrawal-induced human breast cancer tumour regression in nude mice is prevented by Bcl-2

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**Abstract We recently showed that estrogen induces expression of the anti-apoptotic protein, Bcl-2 in MCF-7 human breast cancer cells. Since estrogen-dependent breast tumours can regress following estrogen withdrawal, we hypothesized that stable Bcl-2 expression would prevent estrogen-withdrawal induced regression of MCF-7 tumours. We therefore established tumours in ovariectomized female nude mice implanted with an estrogen-release pellet using untransfected MCF-7 cells or MCF-7 cells stably transfected with a Bcl-2 cDNA sense or antisense expression vector. All tumours grew at similar rates indicating that Bcl-2 levels have no effect on tumour formation. After removal of the estrogen pellet, Bcl-2 antisense tumours and untransfected MCF-7 tumours regressed means of 49% and 52%, respectively, after estrogen pellet removal whereas Bcl-2 sense tumours were significantly stabilized. Regressing tumours displayed characteristics of apoptotic cells. These results show that Bcl-2 can prevent hormone-dependent breast tumour regression and are consistent with the notion that decreased Bcl-2 levels following estrogen withdrawal renders hormone-dependent breast tumour cells sensitive to apoptotic regression.**


*Key words:* Bcl-2; Apoptosis; Breast tumour; Estrogen; Regression

## 1. Introduction

Many hormone dependent tumours including estrogen receptor positive human breast cancer tumours respond to hormone deprivation by growth cessation and regression [1]. This observation has led to the widespread use of antiestrogens in the treatment of hormone-dependent breast cancer. An important experimental model for mammary tumour regression has utilized the ability of estrogen receptor positive human breast cancer cells to form hormone-dependent tumours in nude mice. These tumours respond to antiestrogen treatment by growth cessation [2] or in some cases, regression [3,4]. Estrogen ablation by ovariectomy and deprivation by removal of implanted estrogen pellets induces regression of these tumours through the process of programmed cell death or apoptosis [5].

Apoptosis is an active process involving complex molecular signalling events which are yet to be fully understood. Morphologically, apoptosis can be defined by a number of characteristics including chromatin condensation, plasma membrane blebbing and nuclear fragmentation [6]. Often, but not always, apoptosis is accompanied by the activation of endonucleases which cleave genomic DNA into nucleosomal length fragments producing free 3′ hydroxyl ends that can be detected by DNA end-labelling procedures [7]. A critical component of the apoptotic cascade is a family of cysteine proteases (caspases) which consists of over 10 cellular members (reviewed in [8]).

A number of molecules which either positively or negatively regulate apoptosis have been identified. Bcl-2 is among the best studied of these proteins. When expressed at sufficiently high levels, Bcl-2 can suppress apoptosis induced by a wide variety of insults and stimuli [9,10]. Both Bcl-2 family members as well as unrelated molecules regulate apoptosis. For example Bax, Bak and BAD promote apoptosis while Bcl-xL, Mcl-1 and BAG-1 suppress cell death (reviewed in [11,12]). It is not completely understood how Bcl-2 family members regulate apoptosis, however, recent evidence suggests that these proteins can form channels which may participate in the mitochondrial permeability transition which occurs during apoptosis (reviewed in [12]).

We recently showed that estrogen can induce expression of Bcl-2 mRNA and protein in human estrogen receptor positive MCF-7 breast cancer cells [13]. Bcl-2 expression in untreated breast cancer is thus generally associated with a good prognosis based on its association with a differentiated, estrogen receptor positive, EGF-receptor negative phenotype [14]. Estrogen is required to support the growth of hormone-dependent breast cancer cells in vivo. Ablation or treatment with antiestrogen can induce regression of these tumours; however, little is known about the mechanisms involved. In this study we wished to determine if expression of Bcl-2 can prevent estrogen ablation-induced tumour regression in vivo. The results show that reduced Bcl-2 levels in either untransfected MCF-7 cells or in antisense-Bcl-2 transfected cells permits tumour regression following estrogen-pellet removal whereas elevated Bcl-2 significantly prevents regression in this model. Our findings thus support the hypothesis that stable Bcl-2 expression can prevent breast tumour regression and are consistent with the notion that the decrease in Bcl-2 levels following estrogen withdrawal permits tumour regression.

## 2. Materials and methods

### 2.1. Tumour growth in nude mice

Six week-old ovariectomized female nude mice (nu/nu CD-1) were implanted subcutaneously with an estrogen release pellet (60 day release pellet containing 0.72 mg β-estradiol, Innovative Research of America, Sarasota, FL, USA). Two days later three pooled clones

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*Abbreviations:* E2, estrogen; S.D., standard deviation


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of exponentially growing MCF-7 cells expressing either the sense orientation or the antisense orientation of Bcl-2 (MCF-7(Bcl-2S) or MCF-7(Bcl-2AS)) [13] were trypsinized and three million cells were injected subcutaneously into the flanks of the animals. In seven of the animals, MCF-7(Bcl-2AS) cells were injected in one flank while MCF-7(Bcl-2S) cells were injected in the contralateral flank. To rule out the potential influence of factors released by non-regressing tumours on matched tumours in the contralateral flank following estrogen pellet removal, we also injected three animals with MCF-7(Bcl-2AS) cells on both sides and two animals with MCF-7(Bcl-2S) cells on both sides. Four animals were inoculated on one side only with MCF-7(Bcl-2AS) cells and four were inoculated on one side only with MCF-7(Bcl-2S) cells. Five tumours were also generated using untransfected MCF-7 cells. In order to assess the estrogen dependence of tumour growth, four inoculations of MCF-7(Bcl-2S) and four of MCF-7(Bcl-2AS) were performed in sham operated mice which did not receive an estrogen pellet. Tumours were allowed to grow to an average of 300 $mm^3$ before removal of the estrogen pellet. Estrogen pellets were left in four animals inoculated with MCF-7(Bcl-2S) cells for the duration of the experiment.

*2.2. Growth measurements*

Tumour size was calculated every 3 days using calipers to measure length, width and height of the tumour. The tumour volumes were then calculated according to the formula $V = 1/2\ (4\pi/3)(l/2)(w/2)(h)$, where $l$ = length, $w$ = width and $h$ = height [15].

*2.3. In situ end labelling (ISEL)*

ISEL was performed essentially as described by Fliss and Gattinger [16]. Frozen cryostat sections were thawed and fixed in 1% glutaraldehyde. Free 3′ hydroxyl ends were labelled with biotin-16 dUTP (Boehringer Mannheim, Canada) using terminal transferase. Labelled ends were detected by incubation in avidin conjugated-fluoroscein isothiocyanate. Following application of antifade solution, slides were coverslipped and photographed using a fluorescence microscope equipped with a 35-mm camera.

*2.4. Immunohistochemistry*

Tumour specimens derived at various times after removal of the estrogen pellet were fixed in zinc-buffered formalin and embedded in paraffin. After sectioning (5 μm), deparaffinized sections were pretreated by heating in a microwave before incubating with antisera against Bcl-2 family members. Typically, antibodies were diluted 1:500 to 1:1000 (v/v) for polyclonal antibodies Bcl-2 [17], Bax [18], Bcl-x [19], Mcl-1 [20] and Bak [21] and 1:100 of acites for the BAG-1 monoclonal antibody [22]. Proteins were visualized using the avidin-biotin complex diaminobenzidine-based detection method as described in detail elsewhere [17]. Nuclei were counterstained with hematoxylin and closed with De-Pe-X (Fluka, Germany) mounting medium. For all antibodies, the immunostaining procedure was performed in parallel using preimmune serum and in some cases preadsorbed antiserum with 5–10 μg/ml of the synthetic peptide immunogen or recombinant protein to verify antibody specificity. The immunostaining results were arbitrarily scored according to intensity as 0, negative; 1+, weak; 2+, moderate; 3+, strong; 4+, very intense. Results presented for each tumour were based on analysis of serially sectioned slides stained in a minimum of two separate experiments.

*2.5. Immunoblotting*

Unfixed tissues were snap-frozen and pulverized under liquid $N_2$. Following the addition of RIPA buffer, samples were sonicated and incubated for 30 min on ice, then centrifuged at $16\,000 \times g$ for 20 min to remove insoluble protein. Immunoblotting was performed as previously described [13]. Densitometry was performed on immunoblots using the microcomputer imaging densitometry (MCID) software system (Imaging Research, Brock University, St. Catherines, Ont., Canada). Arbitrary density units for each immunoreactive protein band were then normalized to actin band density on the same gel by dividing density units for each band by the density of the actin band in the same lane.

*2.6. DNA fragmentation assay*

Tumours were homogenized in a buffer containing 10 mM Tris-HCl, 25 mM EDTA and 100 mM NaCl then lysed and centrifuged after making the slurry 1% with sodium dodecyl sulfate. DNA fragments were precipitated from the supernatant, phenol/chloroform extracted, reprecipitated then treated with RNase prior to agarose gel electrophoresis.

## 3. Results

*3.1. Bcl-2 prevents estrogen ablation-induced tumour regression*

Since our previous findings have shown that estrogen induces Bcl-2 production in MCF-7 cells, we wished to test the hypothesis that constitutive expression of Bcl-2 might prevent estrogen ablation-induced tumour regression. Since Bcl-2 antisense expression does not alter the growth of MCF-7 cells in the presence of E2, we used Bcl-2(AS) cells as a control for the Bcl-2(S) clones.

Since most mice had bilateral tumours, estrogen pellets were removed when the largest tumour reached approximately 300 $mm^3$. In most cases, one tumour (either sense or antisense) reached this volume before the other regardless of what combination of tumours were present in the mouse. The time to reach maximum tumour volume was also variable between animals and was independent of tumour type. Furthermore, regression of tumours, indicated in Fig. 1, was not dependent on the tumour volume at the time of estrogen pellet removal or the combination of tumour types present in the mouse. Animals were sacrificed after a mean of $17 \pm 3$ days following estrogen pellet removal. Tumours allowed to regress significantly longer than this became too small to perform immunohistochemical analysis. There was no significant difference between average tumour volumes for matched sets of MCF-7(Bcl-2S and AS) tumours grown in the same mouse ($273 \pm 72$ $mm^3$ for Bcl-2(S) and $264 \pm 42$ $mm^3$ for Bcl-2(AS)). Together these data show that there is no difference between the growth of Bcl-2 sense and antisense tumours in the presence of estrogen.

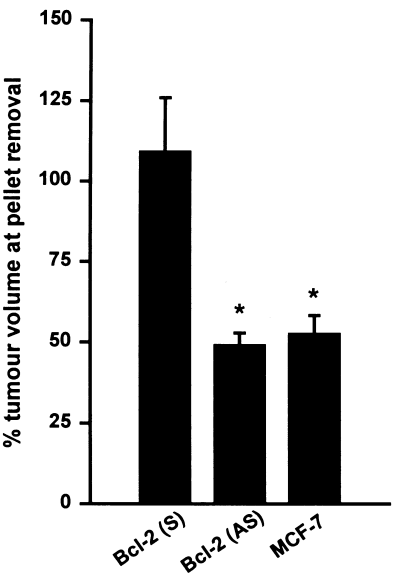


Fig. 1. Tumour regression following estrogen pellet removal. MCF-7(Bcl-2S): $n = 14$, and MCF-7(Bcl-2AS): $n = 15$ and untransfected MCF-7: $n = 5$. Final tumour volumes at tumour removal were divided by tumour volumes at removal of the estrogen pellet. Bars represent standard error. Asterisks indicate significant differences in final tumour volumes between both MCF-7(Bcl-2AS) ($P < 0.001$) and MCF-7 untransfected tumours ($P < 0.04$) and MCF-7(Bcl-2S) tumours (one-tailed $t$-test).

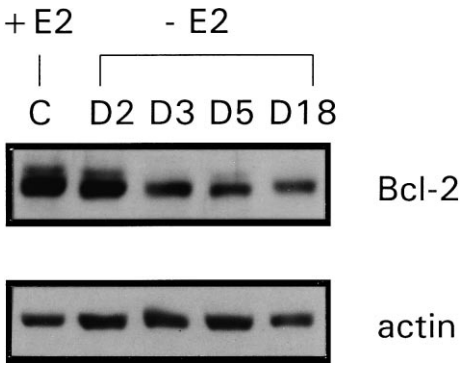


Fig. 2. Estrogen withdrawal decreases Bcl-2 expression in MCF-7 tumours. Extracts from control (C) MCF-7 tumours at the time of E2 pellet removal (+E2), and at days 2, 3, 5 and 18 following pellet removal (−E2) were subjected to immunoblot analysis with Bcl-2 antibody. Reactivity with actin antibody was used as a loading control.

After removal of the estrogen pellet clear differences in the behaviour of Bcl-2 sense and antisense tumours were noted. Fig. 1 shows the mean ratios of tumour volumes at the time of estrogen pellet removal to the volume at the time of tumour removal. Mean volumes for Bcl-2(AS) tumours at the termination of the experiment were less than 50% of the volume of the tumour at estrogen pellet removal and all MCF-7 untransfected control tumours regressed to a similar extent (mean 52%). Strikingly, the Bcl-2(S) tumour volumes at the time of tumour harvest remained significantly stabilized compared with their volumes at E2 pellet removal.

No regression was observed unless the estrogen release pellet was removed indicating that regression was dependent on estrogen ablation (data not shown). Four MCF-7(Bcl-2S) tumours were also grown with an estrogen pellet present throughout the entire experiment. These tumours also grew continuously and were used for studies of Mcl-1 expression (see below).

If decreased Bcl-2 expression is critical for regression then wild-type MCF-7 cells which consistently regress following E2 withdrawal should contain reduced levels of Bcl-2 following pellet removal. To determine this we removed MCF-7 tumours at the time of removal of the E2 pellet and on several days thereafter. Immunoblot analysis of extracts from these tumours in Fig. 2 shows that, as expected, Bcl-2 levels were highest in the tumour exposed to E2. Levels declined rapidly between days 2 and 3 and remained low after pellet removal.

### 3.2. *Regressing tumours but not stable tumours undergo programmed cell death*

To determine if Bcl-2 expression prevented apoptosis in stable tumours, we performed several assays to detect dying and apoptotic cells. First, sections from regressing Bcl-2 antisense and stable Bcl-2 sense tumours were assayed for the presence of oligonucleosomal length DNA, a hallmark of apoptosis. This was determined by the labelling of free 3′ DNA ends using terminal deoxynucleotidyl transferase. Typical results presented in Fig. 3A show that antisense tumours contain a large number of nuclei which can be labelled in the transferase reaction whereas Bcl-2(S) tumours display virtually no in situ end labelling (Fig. 3b). When the percentage of labelled nuclei in sections from six Bcl-2(AS) and five Bcl-2(S) was calculated, regressing tumours contained significantly

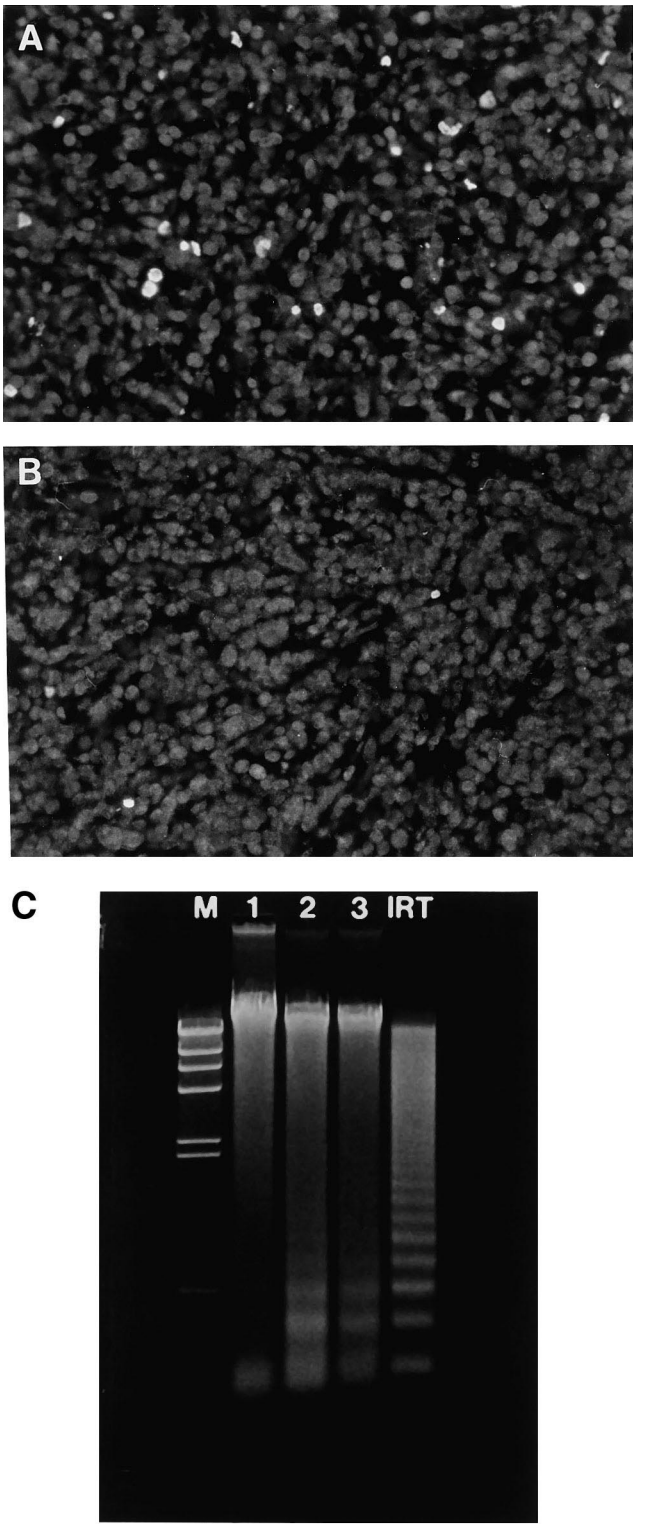


Fig. 3. In situ end labelling and DNA fragmentation assay in MCF-7 tumour sections. In situ end labelling was performed as described in Section 2 to detect free 3′ hydroxyl groups indicative of apoptosis. Sections are from: (A) a representative regressing MCF-7(Bcl-2AS) and (B) a stable MCF-7(Bcl-2S) tumour. C: DNA from a stable MCF-7(Bcl-2S) tumour (lane 1) and two regressing MCF-7(Bcl-2AS) tumours (lanes 2 and 3) was assayed for the presence of oligonucleosomal length DNA ladders. M: lambda *Hin*dIII digest and IRT: irradiated rat thymocytes.

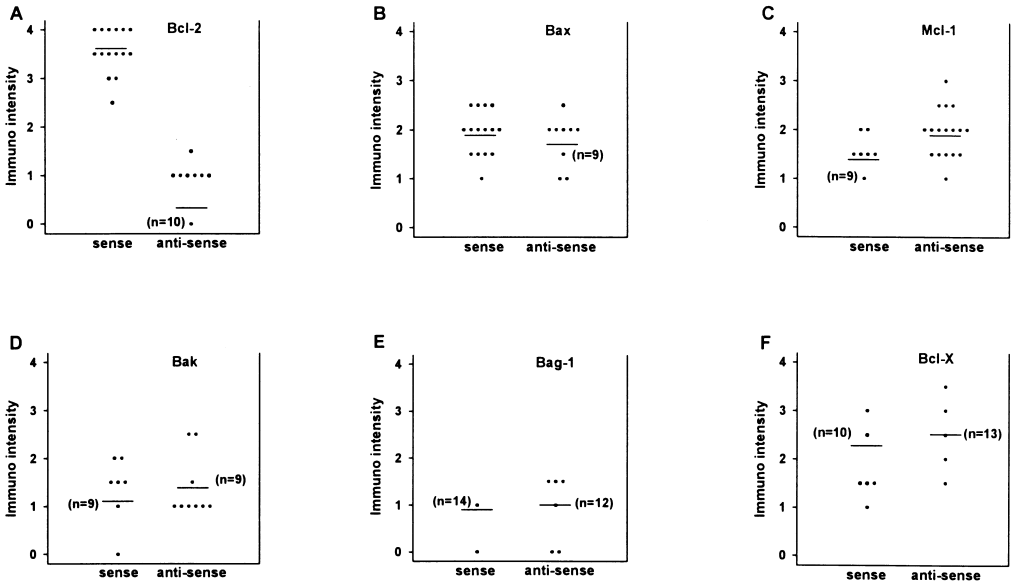


Fig. 4. Immunostaining of MCF-7(Bcl-2S and AS) tumours. Zinc-buffered formalin-fixed, paraffin-embedded tumours were sectioned and immunostained with antisera specific for apoptosis regulatory proteins. A summary of immunohistochemical analysis of apoptotic proteins in MCF-7(Bcl-2S) and MCF-7(Bcl-2AS) tumours scored as described in Section 2. Serial sections of all tumours were stained with antibodies against human (A) Bcl-2; (B) Bax; (C) Mcl-1; (D) Bak; (E) Bag-1; and (F) Bcl-x. Individual tumours are represented by each point. When more than seven tumours scored the same intensity the point is preceded by the number of tumours in parentheses.

more labelled nuclei than the stable Bcl-2(S) tumours (mean ± S.E., 3.0 ± 0.6% vs. 1.1 ± 0.3%, respectively, $P < 0.05$, *t*-test). Secondly, we assessed these tumours for the presence of oligonucleosomal length DNA fragments. Fig. 3C shows that the DNA 'laddering' pattern can be seen in two regressing antisense tumours following estrogen pellet removal but not in a non-regressing MCF-7(Bcl-2S) tumour. Together these results show that tumour stability is associated with an inhibition of apoptosis.

### *3.3. Expression of Bcl-2 is insufficient for tumorigenesis*

Inoculation of ovariectomized mice which had been sham operated but did not receive an estrogen pellet with MCF-7(Bcl-2(AS)) cells (four inoculations) or MCF-7(Bcl-2(S)) cells (four inoculations) did not produce tumours indicating that Bcl-2 is insufficient for permitting tumour growth in the absence of estrogen.

### *3.4. Expression of apoptotic proteins in Bcl-2 sense and antisense tumours*

We wished to confirm the constitutive expression of Bcl-2 in sense tumours in vivo and to determine whether changes in the expression of other apoptosis-regulating proteins occur in tumours which might contribute to their regression or stabilization. We therefore evaluated the levels of Bcl-2, Bax, Mcl-1, BAG-1, Bak and Bcl-x by immunohistochemistry on sections from each tumour (Fig. 4). As expected, all sense Bcl-2 tumours exhibited intense Bcl-2 immunostaining (mean ± S.D. of 3.57 ± 0.46 on the relative scale) while antisense tumours contained little or small amounts of Bcl-2 immunoreactivity (mean 0.44 ± 0.56) (Fig. 4A).

No significant differences between the immunointensity scores of all of the other apoptosis regulatory proteins were detected in sense vs. antisense tumours except for Mcl-1 (Fig. 4C). Mcl-1 levels varied in a reciprocal manner with respect to Bcl-2 expression. While Bcl-2 antisense tumour cells contained Mcl-1 immunostaining at moderate to high levels (mean 1.94 ± 0.50), Bcl-2 sense tumours expressed this protein at significantly lower levels (mean 1.27 ± 0.37, $P < 0.001$, unpaired *t*-test). Importantly, while Bcl-2 antisense tumours exhibited homogeneous immunostaining for Mcl-1, Bcl-2 sense tumours were generally comprised of fewer than 50% Mcl-1 immunopositive cells (not shown).

Both Bax and Bcl-x expression were easily detectable by immunohistochemical analysis in Bcl-2 sense and antisense tumours. No significant difference between Bcl-2(S) and (AS) immunointensity for either of these proteins was observed (Fig. 4B and F, (Bax) and (Bcl-x), respectively), although Bax immunointensity was slightly higher ($P = 0.06$) in Bcl-2(S) expressing tumours compared with (AS) tumours (mean scores of 1.93 ± .46 and 1.65 ± 0.39, respectively). Bcl-x was expressed at a mean immunointensity level of 2.23 ± 0.56 in Bcl-2(S) tumours compared with 2.50 ± 0.40 in Bcl-2(AS) tumours. Bak immunostaining also was not significantly different in the two types of tumour (means of 1.17 ± 0.49 (Bcl-2(S) and 1.44 ± 0.46 (Bcl-2(AS)). We observed high levels of Bak immunostaining in fibroblasts and macrophages which had infiltrated and surrounded sections of the tumour confirming that the low levels of Bak observed in tumours did not reflect an artifact of the staining procedure (Fig. 4D). BAG-1 immunostaining was essentially the same in Bcl-2(S) and Bcl-2(AS) tumours (means of 0.93 ± 0.26 and 0.97 ± 0.41, respectively) (Fig. 4E).

Sections of tumours were also stained with preimmune serum corresponding to each of the indicated antibodies. In all instances sections were negative for immunoreactivity with preimmune serum (not shown), thus confirming the specificity of these results.

To ensure that our visual assessment by immunohistochemistry of the apoptosis regulatory protein expression in tumours was accurate, we performed immunoblot analysis of these proteins using lysates derived from each tumour followed by

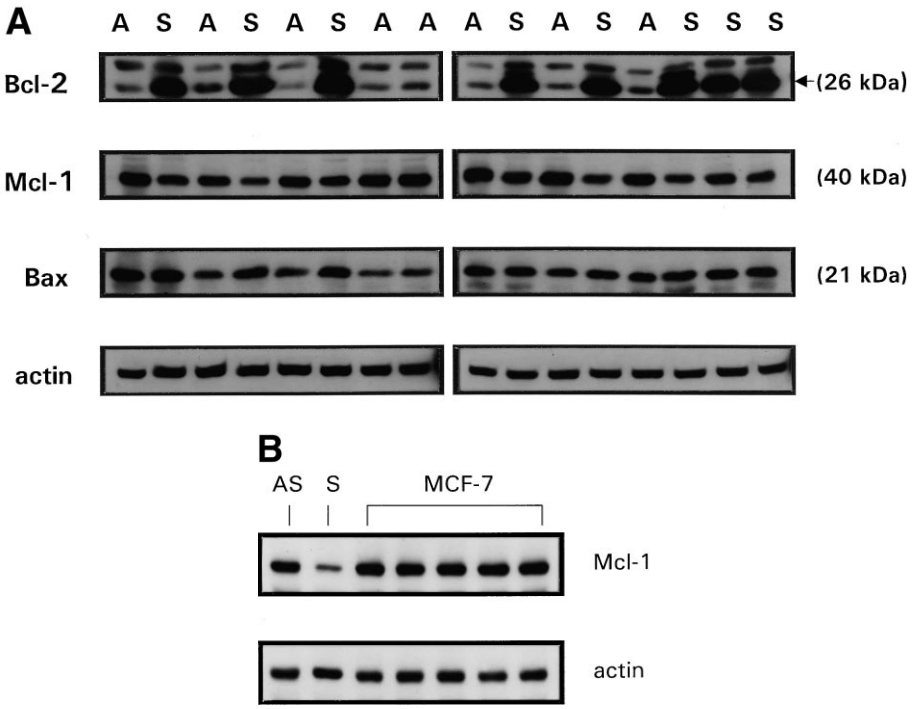


Fig. 5. A: Analysis of apoptotic regulatory proteins from MCF-7 tumours. Extracts from sets of contralateral tumours (pairs from left to right) were analyzed by immunoblot for expression of Bcl-2, Mcl-1 and Bax. Samples are marked A, antisense and S, sense. B: Analysis of Mcl-1 in untransfected MCF-7 tumours. Extracts from five regressing MCF-7 tumours, a Bcl-2(AS) and a Bcl-2(S) tumour were subjected to immunoblot analysis for Mcl-1 expression.

densitometric quantification. Consistent with the immunohistochemistry, immunoblot analysis did not detect significant differences between sense and antisense tumours for Bak or Bcl-x or BAG-1 expression (not shown). Also in agreement with immunointensity results, the immunoblot analysis in Fig. 5A shows that Mcl-1 protein levels in Bcl-2(S) tumours appeared reduced. Densitometric analysis of Mcl-2 corrected for actin resulted in mean values ± S.D. of 1.02 ± 0.19 for Bcl-2(AS) and 0.70 ± 0.13 for Bcl-2(S), indicating a significant difference in Mcl-1 expression between Bcl-2(S) and Bcl-2(AS) ($P < 0.05$; Student's $t$-test). In the absence of E2, Bcl-2(AS) and untransfected MCF-7 cells contain similar levels of Bcl-2 (not shown). Accordingly, Fig. 5B shows that Mcl-1 levels were also elevated in regressing untransfected MCF-7 tumours compared with Bcl-2(S) tumours.

Since the results of immunohistochemistry suggested a small but not statistically significant increase in Bax levels in Bcl-2(S) tumours, the blot in Fig. 5A was also incubated with anti-Bax antibody. While omission of the first lane containing a Bcl-2(AS) sample yielded a mean density value of 0.60 ± 0.16 which is significantly elevated ($P < 0.05$, Student's $t$-test) compared with Bcl-2(S) tumours (mean 0.83 ± 0.10), inclusion of the first Bcl-2(AS) value rendered the differences insignificant. These results suggest that a subset of Bcl-2(S) tumours do contain elevated Bax levels.

Previous studies have suggested that Mcl-1 may be a weak 'short term' anti-death protein, allowing the cell a brief window of survival following cell differentiation or responses to death signals [23]. Since a variety of cell death stimuli have been reported to induce transient increases in Mcl-1 [24], we wondered whether estrogen withdrawal itself or the expression of Bcl-2 influences Mcl-1 expression. We therefore compared Mcl-1 levels in four Bcl-2(S) tumours grown continuously in the presence of the estrogen pellet with those of four Bcl-2(S) tumours which had been allowed to remain in the animal for 15 days following estrogen pellet removal. The results indicated that the level of Mcl-1 did not vary in Bcl-2(S) tumours whether or not they were grown continuously in the presence of estrogen (not shown). Thus, Bcl-2 expression, not estrogen, appears to somehow repress Mcl-1 expression in these breast tumour cells.

## 4. Discussion

It has been known for many years that estrogen ablation results in breast tumour regression; however, the underlying mechanism is not known. We previously demonstrated that Bcl-2 expression is under the control of estrogen in MCF-7 breast cancer cells. We thus postulated that estrogen withdrawal might result in apoptosis, in part, by lowering Bcl-2 levels. In the present study we show that MCF-7 and MCF-7(Bcl-2AS) tumours expressing little or no Bcl-2 undergo estrogen-ablation induced cell death. In contrast most tumours which constitutively express high levels of Bcl-2 are resistant to the regression inducing effects of estrogen withdrawal. It should be pointed out that ectopically expressed Bcl-2 levels were higher than physiological levels in the presence of E2 (estimated by comparison between control MCF-7 in Fig. 2 to Bcl-2(S) samples in Fig. 4), and it is a formal possibility that these levels were a factor in prevention of regression. However, insofar as Bcl-2(S) clones behave similarly to control MCF-7 cells with respect to tumour formation and stabilization of tumours after E2 withdrawal was associated with reduced apoptosis, Bcl-2 appears to function primarily in an

anti-apoptotic capacity in these cells. Thus, estrogen deprivation in vivo results in one or more death signals which can be inhibited by Bcl-2 in breast cancer cells.

Bax immunoreactivity displayed a range of intensities in a high proportion of cells. In some Bcl-2 expressing tumours, Bax expression was higher than in antisense tumours, perhaps reflecting the ability of Bcl-2 to stabilize the Bax protein [25] or the ability of cells with higher Bax levels to survive and accumulate in tumours where Bcl-2 is provided. In addition, the expression of both proapoptotic Bax and Bak in MCF-7 cells might render them exquisitely susceptible to estrogen deprivation induced cell death in the absence of Bcl-2. Overall, the absence of changes in any of the other pro- or anti-apoptotic proteins in regressing or stable tumours, respectively, suggests that alterations in Bcl-2 expression are sufficient to regulate estrogen-withdrawal induced breast tumour regression.

A striking inverse correlation between Bcl-2 and Mcl-1 expression was observed in the tumours. Mcl-1 was first described as a transiently induced Bcl-2 related protein in differentiating human myeloid leukemia cells [26]. In complex epithelial tissues, Bcl-2 and Mcl-1 are expressed in opposing gradients. In the latter, Bcl-2 is expressed in less differentiated cells while Mcl-1 is expressed in the more differentiated cells [27]. More recently Mcl-1 has been shown to be an early response gene, induced rapidly by signals for either cell differentiation or death. Consequently, it has been speculated that Mcl-1 may play a role as a 'short term effector of viability', allowing the cell a brief period of time to make decisions regarding the ultimate response to a death inducing signal [28]. In this context, Mcl-1 may partially substitute for Bcl-2 in those tumours which express little or no Bcl-2 although it is clearly unable to prevent regression-associated apoptosis.

In summary, low Bcl-2 levels mediated by antisense transcript expression and/or estrogen withdrawal are associated with regression of MCF-7 tumours following estrogen withdrawal. Stable Bcl-2 expression can prevent tumour regression following estrogen withdrawal consistent with the notion that estrogen promotes the survival of hormone-dependent breast cancer cells at least in part by increasing Bcl-2 expression. Thus, the reduction of Bcl-2 levels by antiestrogens or ovariectomy likely plays a role in the process of regression of hormone-dependent adenocarcinomas of the breast by rendering the cells sensitive to apoptotic stimuli that occur as a result of E2 deprivation. It will be important to extend these observations to additional estrogen-dependent human breast lines and primary tumours in animal models to further define the role of Bcl-2 in tumour regression.

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