Glucocorticoids Inhibit Apoptosis during Fibrosarcoma Development by Transcriptionally Activating Bcl-xL

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Glucocorticoids influence many physiological processes, and in particular apoptosis, often with opposite effects depending on the cell type examined. We found that during fibrosarcoma development there is a strong increase in apoptosis at the tumor stage, which is repressed by dexamethasone to levels observed in normal fibroblasts. The anti-apoptotic Bcl-2 family protein Bcl-xL is induced by dexamethasone at the transcriptional level at all stages of fibrosarcoma development. The ligand-activated glucocorticoid receptor (GR) activates the Bcl-x promoter in transient transfection experiments, and GR binds to specific Bcl-x promoter sequences in vitro and in vivo. Furthermore, a GR antagonist abolishes this effect, indicating that Bcl-xL induction is mediated by GR. Importantly, exogenous Bcl-xL inhibits apoptosis and caspase-3 activity in fibrosarcoma cells to levels found in dexamethasone-treated fibroblasts. We conclude that Bcl-xL is a key target mediating the anti-apoptotic effects of glucocorticoids during fibrosarcoma development. These observations provide further understanding of the molecular basis of glucocorticoid regulation of cell death during tumorigenesis.

Glucocorticoids exert different effects on apoptosis and cell growth depending on the tissues examined. In some cell types, for example thymocytes and some leukemia cell lines, treatment with glucocorticoids induces apoptosis (1). This has led to their common use as chemotherapeutic agents in lymphomas and leukemias (2). In contrast, glucocorticoids have been reported to inhibit apoptosis in a number of other cell types, including glial and astrocytoma cell lines (3), fibroblasts (4), hepatoma cells (5), gastric cancer cell lines (6), and mammary epithelial cells (7, 8).

The glucocorticoid receptor (GR)† belongs to a superfamil of transcription factors that includes receptors for steroid and thyroid hormones, retinoic acid, and vitamin D₃ (9). GR is normally localized in the cytoplasm in a non-active state in a complex that includes Hsp90. Upon hormone binding, GR changes conformation and migrates to the nucleus, where it induces or represses transcription by binding to specific DNA sequences on target genes (10).

Apoptosis, triggered by a variety of intra- and extracellular signals, is important for normal development, to maintain tissue homeostasis, and as a defense strategy against the emergence of cancer (11, 12). The apoptotic program is executed by a family of cysteine proteases called caspases, which are activated by proteolytic cleavage (13, 14). Once activated, effector caspases cleave a variety of cellular substrates including structural components, regulatory proteins, and other caspases, resulting in the orchestrated collapse of the cell characteristic to apoptosis.

Bcl-2 family proteins play critical roles in the control of apoptosis. Two major groups of Bcl-2 family proteins exist; the pro-survival members, including Bcl-2, Bcl-xL, Bcl-w, Mcl-1, etc., and the pro-apoptotic members, including Bax, Bak, Bok, etc. (see Ref. 15 for review). The ratio between these two groups of family members determines whether a cell will live or die. Downstream of this checkpoint lie the caspase pathway and mitochondria dysfunction, major execution events that lead to irreversible cell death (16). Alterations in the expression of anti-apoptotic members such as Bcl-2 and Bcl-xL have been implicated in tumorigenesis in both clinical cases and transgenic models (17). In addition, Bcl-2 members are also important determinants of anticancer drug sensitivity (18).

The conversion of a normal cell to a neoplastic one occurs in multiple steps (19), and one approach to studying this process has been to employ transgenic mice (20). Mice carrying the bovine papillomavirus type I genome develop dermal fibrosarcomas in a process that involves distinct proliferative stages. These are the normal fibroblasts (NF), and then two histological grades of hyperplasia, mild fibromatosis (MF) and aggressive fibromatosis (AF). Finally, at lower frequency, dermal fibrosarcomas (FS) develop (21). The first molecular distinction between the AF and the FS cells to be identified was a dramatic increase in ligand-dependent GR transcriptional activity in FS cells (22). This increase does not result from changes in the intracellular levels of GR, hormone-dependent nuclear translocation, or specific DNA binding activity, all of which are unaltered throughout the progression. Moreover, analysis of the tumors formed in mice upon inoculation of AF or FS cells indicates a direct correlation between GR transcriptional activity and tumorigenic potential (22).

To understand cancer progression it is important to determine the mechanisms by which signaling proteins influence proliferation and apoptosis at different stages of the tumorigenic process. Here we have examined the effects of dexamethasone on apoptosis in the multistep tumorigenic pathway.
of key GR target mediating the inhibition of apoptosis during fibrosarcoma progression.

EXPERIMENTAL PROCEDURES

DNA Plasmid Constructions—The plasmid containing 3.2 kb (−2392 to −94) of the 5′-region of the murine Bcl-x promoter linked to the luciferase reporter gene (23) was a kind gift of Gabriel Nunez (Ann Arbor, MI), and was designated Bcl-x3.2. Serial promoter deletion fragments were cloned into pGL2-basic (Promega) as follows: Bcl-x:2.8 (−2829 to −94) as a BgII and HindIII fragment; Bcl-x:0.6 (−679 to −94) by SmaI and partial HindIII digestion; Bcl-x:0.2 (−299 to −94) as an XhoI/HindIII fragment, and Bcl-x:0.1 (−199 to 0) as a KpnI and HindIII 110-bp fragment.

Frames containing the putative GREs from the Bcl-x promoter were cloned into a TK<sub> reporter</sub> luciferase reporter construct: RE1 contains sequences of the murine Bcl-x promoter from −2963 to −2268. This fragment was amplified by PCR using Bcl-x:3.2 as template and the oligonucleotides A (5′-GTTCCTCCAAAGATGCCAATG-3′) and C (5′-AAATGCGGATCCTGACTGACTG-3′). The product was digested with BamHI and inserted into TK<sub>109</sub> luciferase. RE2 sequences from −2963 to −2826, which were amplified by PCR using the oligonucleotides A and D (5′-CTCTGGTATAGCTGTGGGCTGCC-3′). The PCR product was digested with BamHI and BgII. RE3 contains sequences −2829 to −2826 of the Bcl-x promoter, which was amplified by PCR using the oligonucleotides B (5′-AAGGTTGATCTCCTATGGTAC-3′) and C. The product was digested with BamHI.

The putative GREs from the Bcl-x promoter were mutated using the following oligonucleotides (nucleotides that were changed are underlined) and their reverse complements: P1 (5′-CTCTGGTGGCCCAACACTGC-3′), P2 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P3 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P4 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P5 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P6 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′).

The QuikChange XL site-directed mutagenesis kit (Stratagene) was used following oligonucleotides (nucleotides that were changed are underlined) and their reverse complements: P1 (5′-CTCTGGTGGCCCAACACTGC-3′), P2 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P3 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P4 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P5 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′), P6 (5′-CTCTGGTCCAGAAGACAAAGAAGGTTGC-3′).

Several cultures were tested from each stage of the tumorogenic process: normal fibroblasts (23784, 40950); mild fibromatosis (14249, 39614, 27877); aggressive fibromatosis (BPV3, BPV7, BPV21); verrucous fibrosarcoma (BPV1, BPV2, BPV11). For experiments involving the ability of cellular AP-1 components JunB and c-Jun is apparent in AF and FS cells (22, 50), but dramatically increased GR activity is unique to the tumor cells (22).

TABLE I

|        | NF | MF | AF | FS |
|--------|----|----|----|----|
| BPV-1 RNA | −  | +  | +  | +  |
| E5, E6 | −  | −  | +  | +  |
| JunB, c-Jun | +  | +  | +  | +  |
| GR activity | +  | +  | +  | +  |

(Sigma) were used as primary antibodies, followed by incubation with the corresponding secondary antibody (horseradish peroxidase-conjugated; Bio-Rad). Protein-antibody complexes were visualized by an enhanced chemiluminescence immunoblotting detection system (Amer sham Biosciences).

Cell Retardation Assays—Analysis of DNA-protein interactions was performed as described (22). The following oligonucleotides were used: consensus GRE-TAT (22), P1 (5′-TGCCCAACAGTACCTTGTGAAAA-GAC-3′), P2 (5′-TGTCGACAGAACAGCGTCTTTCGAGGCAATGT-GTGCCACAG-3′), and P3 (5′-ATATTGACAGAGCTTGCTGAGGGCTC-3′), and P4 (5′-TTGACCAGAAGCGTCACTGACG-3′). Northern blot analysis was performed as described previously (26) except for at least 16 h and treated with or without 100 nM dexamethasone for 6 h. The change in absorbance of each sample was used for PCR amplification (30 cycles) with the r

RESULTS

Cell Death Is Reduced by Dexamethasone Treatment—Cell proliferation increases during fibrosarcoma development in vivo (27) and in cultured cells (21), and it is inhibited by dexamethasone, a GR agonist (53). We examined the effects of glucocorticoids on cell death during fibrosarcoma progression. We used low-passage primary cell lines representative of the four stages in this multistep pathway: NF, MF, AF, and FS.
cells (see Table I). Cells from each stage were cultured in the absence and presence of dexamethasone, and cell growth was observed for 7 days at 24-h intervals. In the absence of hormone, cells from the earlier stages of the progression proliferated at a lower rate, as reported previously, whereas FS cells increased rapidly in number (21). Cells cultured in the presence of dexamethasone appeared flattened (data not shown) and were fewer in number. Trypan blue exclusion was used as a marker for viability of the cells. The number of dead cells, particularly among FS cells. Fig. 1 shows that Bcl-2 migrated as a doublet and remained constant in all cell types throughout tumor progression. In contrast, Bcl-xL expression clearly decreased during fibrosarcoma progression. However, in the presence of dexamethasone its expression was strongly induced, correlating with the much reduced level of apoptosis in dexamethasone-treated FS cells (Fig. 1).

Analysis of the expression of anti-apoptotic proteins (Fig. 2B) showed that Bcl-2 migrated as a doublet and remained constant in all cell types throughout tumor progression. In contrast, Bcl-xL expression clearly decreased during fibrosarcoma progression. However, in the presence of dexamethasone its expression was strongly induced, correlating with the much reduced level of apoptosis in dexamethasone-treated FS cells (Fig. 1).

The combination of changes in the expression levels of apoptotic proteins in FS cells, together with elevated caspase-3 activity, correlates with the increase in apoptosis at the tumor stage, suggesting that Bcl-2 proteins play a role in regulating apoptosis during fibrosarcoma development. Furthermore, Bcl-xL induction in response to hormone in FS cells makes Bcl-xL a candidate for mediating the reduction in apoptosis at the tumor stage.

**Bcl-xL Expression Is Induced by Dexamethasone**—A more detailed analysis of expression of Bcl-xL protein during fibrosarcoma progression showed a clear increase in the fold induction of the protein by dexamethasone as fibrosarcoma progresses (Fig. 3A). This pattern of hormone-dependent induction of expression correlates with the transition in GR transactivation activity observed during fibrosarcoma progression (22) and suggests that GR might be involved directly in Bcl-xL regulation. The analysis presented involved representative cell cultures from the four stages of the pathway (NF 40950, MF...
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14249, AF BPV3, FS BPV1). However, we assayed the expression of Bcl-x<sub>L</sub> in two or more independent cell lines from each stage (see “Experimental Procedures”) and found that all lines derived from a particular stage displayed consistent results. To determine the time course of Bcl-x<sub>L</sub> induction in FS cells, we examined protein levels at various times after dexamethasone treatment (Fig. 3B). Induction of Bcl-x<sub>L</sub> protein could be detected as early as 2–4 h following exposure to the hormone and remained elevated for 24 h.

**Bcl-x Transcription Is Induced by Dexamethasone**—The rapid increase in the level of Bcl-x<sub>L</sub> protein by dexamethasone in FS cells suggested that this induction occurred at the transcriptional level. Therefore, we examined Bcl-x mRNA expression after exposure to dexamethasone in all stages of fibrosarcoma development. Northern blot analysis revealed a major band of ~3-kb mRNA in all cell stages (Fig. 4A), as reported previously (32). In the absence of hormone, the expression level of the Bcl-x transcript was reduced during tumor development, similar to the observed decrease in protein levels. Bcl-x mRNA was increased by the presence of dexamethasone.

To investigate the regulation of Bcl-x promoter activity by dexamethasone, we transiently transfected a luciferase reporter construct containing a 3.2-kb genomic fragment of the murine Bcl-x promoter, Bcl-x(3.2), together with a -3-kb mRNA in all stages of fibrosarcoma progression. Northern blot analysis of Bcl-x mRNA levels using a 725-bp fragment of the Bcl-x<sub>L</sub> CDNA (IMAGE 1395857) as probe. The membrane was also probed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A promoter linkage of the Bcl-x(3.2) luciferase reporter construct in NF, MF, AF, and FS cells. The cells were either untreated (black bars) or treated for 36 h with 100 nm dexamethasone (shaded bars). Transcriptional activity of endogenous GR was analyzed. In all transfection experiments, luciferase activity was normalized to the β-galactosidase activity of a co-transfected reporter. The values presented are the mean of at least five different experiments. Normalized luciferase activity units are ×10<sup>3</sup> in all experiments. C and D, expression of the Bcl-x(3.2) reporter in FS cells in response to increasing concentrations of dexamethasone (C) or to various hormone treatments, 100 nm dexamethasone, and/or the GR antagonist RU 40555 (RU) (D).

A series of luciferase vectors were constructed containing 5′ fragments of the 3.2-kb region of the Bcl-x promoter (Fig. 5A) and were transiently transfected into FS cells. Analysis of these fragments showed a clear reduction in the ability of the promoter to respond to dexamethasone, from around 8–10-fold induction using the 3.2-kb fragment to a small but significant (p < 0.005) induction using the most proximal region (Fig. 5B). The highest induction from the latter was obtained using Bcl-x(0.1), suggesting that this region (−199 to −94) contains a proximal weak glucocorticoid response element (GRE). Although this fragment does not contain classical consensus GRE sequences, it does contain putative binding sites for factors known to interact with GR, such as C/EBP (33) and Oct-1 (34). However, it appears that the major hormone-inducible promoter sequences are located within −3292 to −679.

To investigate further the glucocorticoid-responsive sequences within the Bcl-x promoter, the murine genomic Bcl-x sequence (GenBank™ accession number AF 088904) was screened for potential GR binding sites by computer analysis (MatInspector program, San Diego Workbench). Three candidate sequences, P1–P3 (with P2 containing two adjacent sites), were identified with some homology to the consensus GRE sequence GGTCANNTGTGCT (35) in the sequence between −2944 and −2316 (Fig. 5C). To test whether these putative GREs (or GRE-like elements) are sufficient to mediate hormone induction, three fragments containing these sequences (RE1, −2963 to −2268, RE2, −2963 to −2826 and RE3, −2349 to −2268, Fig. 5C) were cloned into a luciferase reporter plasmid driven by a TK<sub>109</sub> promoter (TK<sub>109</sub>-Luciferase). All three reporter plasmids displayed a clear induction of transcription in response to dexamethasone treatment (Fig. 5D). The largest
Fig. 5. Bcl-x promoter analysis. A, Bcl-x promoter fragments. Dex, dexamethasone. B, activity of the Bcl-x luciferase constructs shown in A in FS cells. The values presented are the mean of four different transfection experiments done in duplicates. Cells were untreated (black bars) or treated for 36 h with 100 nM dexamethasone (shaded bars). C, putative dexamethasone-responsive sequences within the Bcl-x promoter. D and E, activity of the Bcl-x constructs shown in C in FS cells (D) and in COS-7 cells (E) with conditions as described in B. F, triple mutant (mut) reporter resulting from the mutation of 2 bp in each of the three GREs (P1, P2, and P3) in the Bcl-x(3.2) reporter construct. G, transcriptional activity of the wild type (wt) and triple mutant Bcl-x(3.2) reporters (mut) with conditions as described in B.

fragment, RE1, which encompasses all four putative GREs, displayed the strongest transcriptional activity, followed by RE2 and RE3. Importantly, these sequences also conferred hormone responsiveness when co-transfected with a GR expression vector into COS-7 cells (Fig. 5E) although to a lesser extent than a reporter construct driven by a consensus GRE (TAT1, one copy of the GRE from the tyrosine aminotransferase gene promoter in front of the TK109 promoter). A construct containing an unrelated sequence (consensus thyroid response element, TK) upstream of the TK109-luciferase did not respond to dexamethasone. These results confirm that dexamethasone-responsive sequences of the Bcl-x promoter lie within (−2944 to −2826) and (−2330 to −2268).

Finally, two point mutations in positions known to be important for GR-dependent transcription in the context of a consensus GRE (35) were introduced in each of the three putative GREs, and their effect on dexamethasone-dependent induction of the Bcl-x promoter was determined in transient transfection assays. Single and double combinations of these mutations decreased Bcl-x inducibility by dexamethasone in an additive fashion (data not shown), whereas introduction of two point mutations in each of the putative GREs (Fig. 5F) resulted in strong reduction of dexamethasone-dependent transcriptional activity in FS cells (Fig. 5G). In NF cells the response to dexamethasone is much weaker, but the inhibitory effect of mutating the GREs was still detectable. Although mutation of these binding sites does not abolish dexamethasone-dependent transcriptional activation of the Bcl-x promoter in FS cells totally, this was not surprising, because we know that the Bcl-x promoter contains other weak proximal putative GREs (Fig. 5B), and it is still conceivable that there are further weak putative GREs that remain unidentified. However, our results suggest that the integrity of the three GREs we have identified is required for GR-dependent activation of transcription.

**GR Binds to the Bcl-x Promoter in Vitro and in Vivo**—The ability of GR to bind to the identified GRE sequences was examined by gel mobility shift assays. We incubated nuclear extracts from hormone-treated FS cells in the presence of labeled oligonucleotides containing either one of the putative GREs (Fig. 5C, P1–3). A retarded complex was observed using P1 (Fig. 6A, lane b), P2 (lane g), and P3 (lane j) that migrated at the same position as that originated by binding of FS cell extracts to the consensus GRE-TAT (lane m). This complex has been shown to represent GR protein specifically bound to the GRE sequences (22). Incubation with 50-fold excess of the corresponding unlabeled oligonucleotides abolished binding to these sequences (Fig. 6A, lanes c, f, h, and i), whereas 200-fold excess of nonspecific unlabeled oligonucleotide had no effect (lanes d, h, l, and o), confirming the specificity of the binding reactions. The same experiment was performed with extracts from all four stages of the tumorigenesis pathway, and as expected from previous findings (22), GR binds to these sequences at all cell stages with similar efficiency (data not shown). Furthermore, binding of GR to the labeled consensus GRE (Fig. 6B, lane b) is disrupted by 50-fold excess of the unlabelled putative P1 (lane c), P2 (lane d), and P3 (lane e), which is as efficient as using the same molar excess of unlabelled consensus GRE (S) (lane f). In the reverse experiment, binding to all labeled putative GREs, P1, P2, and P3 (Fig. 6C, lanes b, e, and h), could be abolished by competition with unlabelled consensus GRE (S) (lanes c, f, and i). In summary, these experiments demonstrate that GR can specifically bind in vitro to non-consensus GRE sequences in the Bcl-x promoter.

To determine whether GR interacts directly with this region of the Bcl-x promoter in vivo, we used the chromatin immunoprecipitation assay. Using an antibody raised against GR to immunoprecipitate sequences bound by GR, we observed increased occupancy by GR at the Bcl-x promoter after addition of dexamethasone (Fig. 7, compare lanes 7 and 8), whereas no signal could be amplified from a fragment corresponding to a control element from the Bak promoter, which lacks functional GREs. These observations were dependent on the use of an antibody raised against GR to immunoprecipitate efficiently the sequences of the Bcl-x promoter containing putative GREs, whereas a control antibody (also rabbit serum, against cyclin A) failed to recruit either of the promoter sequences in a ligand-dependent manner (lanes 5 and 6). Thus, GR binds to the functional GREs sequences identified in the Bcl-x promoter in vivo.

**Exogenous Expression of Bcl-xL Reduces Apoptosis in FS Cells**—Apoptosis is reduced by dexamethasone in FS cells, and this correlates with transcriptional activation of the Bcl-x promoter and increased expression of the anti-apoptotic protein, Bcl-xL. To determine whether enhanced Bcl-xL expression is
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**DISCUSSION**

Apoptosis has been found to be widespread in many tumors (36, 37) and yet limited in others (38). GR can display either pro-apoptotic or anti-apoptotic activity depending on cell context (39, 40). We show here that in the absence of glucocorticoids apoptosis increases during fibrosarcoma development, whereas in the presence of dexamethasone it is reduced to those levels found in the earlier stages of the tumorigenic pathway.

Few studies of caspase activity during tumor development have been reported. Caspase-3-like activity has been observed to increase during the development of colorectal carcinoma (41), thus representing a situation similar to that found in the absence of glucocorticoids during fibrosarcoma development. Although involved in some forms of apoptosis, caspase-1 also promotes inflammatory responses by regulating cytokine signaling (42). Increasing caspase-1 activity during fibrosarcoma development may therefore have implications not only for apoptosis but also for inflammation.

Our results demonstrate that decreasing Bcl-x<sub>L</sub> expression results primarily from decreasing Bcl-x transcription. This correlates inversely with increasing transcription of the BPV-1 genome and E5, E6, and E7 oncogene expression in MF, AF, and FS cells (21, 53). Both BPV-1 E6 and E7 oncoproteins sensitize cells to apoptosis induced by tumor necrosis factor-α (43); thus it can be speculated that these proteins may inhibit Bcl-x transcription. Notably, the angiogenic growth factor bFGF increases Bcl-x expression in epithelial cells (44). Be-

**Fig. 6. Gel retardation assays.** In vitro DNA binding of GR from nuclear extracts of dexamethasone-treated FS cells. A, the various ³²P end-labeled oligonucleotides used as probes (P1–P3) are indicated at the top. Competition reactions were performed using 50-fold excess of the corresponding unlabeled oligonucleotide (specific same as labeled) probe (S) (lanes c, f, k, and n) or 200-fold excess of a nonspecific oligonucleotide (N) (lanes d, h, l, and o). B, a ³²P end-labeled consensus GRE-TAT oligonucleotide used as probe. Competition reactions with 50-fold excess of unlabeled oligonucleotides (P1–3, lanes c–e, respectively, as well as GRE, S, lane f) or nonspecific oligonucleotides (N, lanes g–i). C, labeled probes are indicated at the top. Competition reactions were performed with unlabeled GRE (S, lanes c, f, and i) or nonspecific oligonucleotide (N, lanes d, g, and j). The asterisk indicates the position of a nonspecific complex. Lane a (in A–C), and lanes e and i (in A) represent incubation in the absence of extract.

**Fig. 7. ChIP assay.** Cells were untreated or were incubated for 1 h with dexamethasone (Dex). Binding of GR to the Bcl-x promoter in FS cells was determined in vivo with the ChIP assay (lanes 7 and 8). As controls, sample lysates were also incubated with an antibody against cyclin A (lanes 5 and 6) or without antibody (lane 2), and PCR amplifications included primers designed to detect a control segment from the endogenous Bak promoter that lacks functional GREs. Lane 1 is the molecular weight marker; lanes 3 and 4 represent input signals obtained from 0.5% input chromatin, whereas ~3% of the immunoprecipitated material was amplified. IP Ab, immunoprecipitation antibody.

**Fig. 8. Over-expression of Bcl-x<sub>L</sub> in FS cells.** A, FS cells were transfected with a Bcl-x<sub>L</sub> expression vector or control vector and treated with 100 nM dexamethasone (Dex) for 48 h. Whole cell extracts were analyzed for Bcl-x<sub>L</sub> protein expression. B, number of dead cells in Bcl-x<sub>L</sub>-transfected FS cell cultures. Values represent the mean of 12 independent counts (×10⁴). C, caspase-3 enzyme activity in Bcl-x<sub>L</sub>-transfected FS cells. Caspase activity is given in absorbance units (×10⁻³).
Increased expression of Bcl-xL and reduced apoptosis correlate in a glucocorticoid-dependent manner during fibrosarcoma development. This phenomenon results in the repression of Bcl-xL expression in fibrosarcoma cells (53) and suggests that the presence of ligand-activated GR is sufficient to induce Bcl-xL expression. Further, specific activation of the Bcl-x promoter by dexamethasone requires the cooperation of the various GREs identified, because deletion or mutation of these elements results in a reduction in responsiveness. This observation supports the prediction made by Nordeen and colleagues (35) that natural response elements are suboptimal and that cooperation among these individually weak sites contributes to the inducibility of the promoter. Because Bcl-x expression is higher in untreated NF than in untreated FS cells, one possibility is that the Bcl-x promoter is repressed in FS cells and that dexamethasone treatment results in de-repression. Although this may be the case, the reduced expression of Bcl-x in FS cells is unlikely to involve GR binding to GREs in the Bcl-x promoter, because their mutation does not affect transcription in the absence of hormone. Importantly, Bcl-xL is responsible for the ability of dexamethasone to inhibit apoptosis in fibrosarcoma cells, as shown by ectopic expression of Bcl-xL. As expression of Bcl-xL decreases during fibrosarcoma development in the absence of glucocorticoids, increasing up-regulation of Bcl-xL expression by dexamethasone serves to maintain Bcl-xL levels during tumor development, and thus apoptosis remains approximately constant in effect, Bcl-xL expression becomes increasingly dependent on glucocorticoids during fibrosarcoma development. Increased Bcl-xL expression and reduced apoptosis correlate in FS cells with decreased caspase-3, but not caspase-1, activity in response to glucocorticoids. Such a correlation has also been reported by Messmer et al. (49). Furthermore, levels of apoptosis in hormone-treated FS cells are comparable to those in earlier cell types, despite the presence of higher caspase-1 activity. These data indicate that caspase-3 is likely to play a more important role than caspase-1 in determining apoptosis in FS cells.

It may be noteworthy that we also observed dexamethasone regulation of Bcl-x expression in both normal and transformed human mammary epithelial cells. Apoptosis of immortalized mouse mammary epithelial cells can be decreased by dexamethasone treatment, associated with increased Bcl-x mRNA expression, within 2 h of hormone treatment (8). Intriguingly, an increase in Bcl-xL protein expression following dexamethasone treatment was readily detectable in transformed SK-BR-3 breast cancer cells but was lower in normal epithelial cells (data not shown). This represents a parallel situation to that observed during fibrosarcoma progression, where the ability of hormone to induce Bcl-xL expression increases during tumor development; it may reflect the ability of normal cells to limit, or of tumor cells to enhance, regulation of gene expression by specific steroid hormone receptors as has been suggested previously (22). Such a phenomenon may be a result of increased expression of steroid hormone receptor co-factors during tumor development, and indeed co-activators such as E6-AP and AIB1 are overexpressed in certain tumor types (50, 51).

It is interesting to consider that the discovery of regulation of Bcl-xL expression by glucocorticoids during tumorigenesis may have clinical relevance. Glucocorticoids such as dexamethasone are used as anti-emetics in the treatment of several cancers. One study has identified a strong negative correlation between Bcl-xL expression and sensitivity to a wide variety of cytotoxic agents in 60 cancer cell lines (18). Our results suggest that Bcl-xL expression (and thus cell survival) may be promoted by dexamethasone. Therefore, glucocorticoid treatment of tumors may reduce the cytotoxic effects of chemotherapy on tumor cells and may be contraindicated in those tumors with highly inducible Bcl-xL expression. A similar situation may exist in malignant gliomas, where glucocorticoids have been reported to interfere with chemotherapy response (52). Importantly, indications that expression of Bcl-xL may be more glucocorticoid-dependent in tumor cells than in normal cells suggest that glucocorticoid therapy could have a certain degree of tumor specificity.

The enhanced expression of Bcl-xL by GR during fibrosarcoma development reflects the unexpected transition in the transcriptional activity of GR and reveals an alternative mode of regulation for steroid hormone receptors. In summary, increased GR transcriptional activity is likely to exert a protective effect by enabling the reduction of apoptosis at the tumor stage of fibrosarcoma development and, therefore, prolonging tumor cell survival.

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