Hypersensitivity of Human Testicular Tumors to Etoposide-induced Apoptosis Is Associated with Functional p53 and a High Bax:Bcl-2 Ratio

Christine M. Chresta, John R. W. Masters, and John A. Hickman

Cancer Research Campaign Molecular and Cellular Pharmacology Research Group, School of Biological Sciences, The University of Manchester, G.38, Stopford Building, Oxford Road, Manchester, M13 9PT [C. M. C., J. A. H.], and Institute of Urology and Nephrology, University College London, 67 Riding House Street, London, W1P 7PN [J. R. W. M.], United Kingdom

ABSTRACT

Metastatic testicular cancers are curable, whereas bladder cancers and most other solid tumors are not. Cell lines derived from human testicular (CH, GCT27, and 833K) and bladder (RT4, RT112, and HT1376) tumors retain this differential chemosensitivity in vitro. We have investigated the hypothesis that differential sensitivity to chemotherapy is related to differences in the threshold of susceptibility to undergoing apoptosis. Sensitivity to etoposide was not directly related to the frequency of DNA strand breaks. DNA damage was on average 2-fold greater in the testicular than the bladder tumor cell lines; in contrast, the testicular tumor lines were 15-fold more sensitive to etoposide cytotoxicity than the bladder tumor lines (IC50 values of 19 ± 6 versus 293 ± 180 μM, respectively). Using equidamaging (550 rad equivalents) etoposide treatments, the percentage of cells that underwent drug-induced apoptosis was on average higher in the testicular tumor cell lines than the bladder tumor cell lines. The testicular tumor lines have two characteristics that could confer sensitivity to drug-induced apoptosis. First, they have functional p53: the product of the p53-dependent gene waf-1 was increased after etoposide treatment. Second, the testicular tumor lines expressed relatively high levels of the apoptosis-promoting protein Bax, but there was no expression of the suppressor of apoptosis Bcl-2. In contrast, only one of the three bladder cell lines (RT4) had functional p53, and all of the bladder lines had readily detectable levels of Bcl-2 and low levels of Bax. In the testicular cell lines, increases in p53 and p53-transactivated genes were associated with apoptosis but not arrest in G1. In contrast, in the bladder cell line (RT4), increases in p53 and Waf-1 were associated with both arrest in G1 and apoptosis. The differences in the ratio of Bax:Bcl-2 could contribute to the differential sensitivity of the two tumor types. However, in contrast to earlier reports, the ratio of Bax and Bcl-2 was not perturbed by DNA damage.

INTRODUCTION

Drug resistance is a major problem in tumor chemotherapy, and metastatic cancer is generally incurable. In contrast, testicular tumors rarely develop resistance, and approximately 80% of patients with metastatic disease are cured using combination chemotherapy (1). Thus, testicular tumors may provide clues to the successful treatment of disseminated cancer. Cell lines derived from testicular germ cell tumors maintain their sensitivity to chemo- and radiotherapy in vitro (2, 3). They are sensitive to a wide range of chemotherapeutic agents, especially those that damage DNA (2). Studies in which the sensitive human germ cell tumor lines have been compared to relatively resistant tumors derived from transitional carcinoma of the bladder have shown that drug sensitivity is not directly related to drug target interactions (4, 5). The testicular tumors are 5-fold more sensitive to cis-platin cytotoxicity but on average have lower levels of initial damage (5). Similarly, although the testicular tumor lines on average have higher levels of SSBs3 in response to the topoisomerase poison 4-(9-acridinylamino)-methanesulfon- m-anisidide, when individual lines are analyzed, there is not a direct relationship between the level of damage and cytotoxicity (4).

Recently, the differential sensitivity of cells to chemotherapy has been suggested to be associated with different susceptibilities to undergoing apoptosis (6–10). Sensitivity to DNA-damaging agents and to irradiation has been demonstrated to depend upon the expression of wtp53 (7, 8, 11, 12). After DNA damage, wild-type p53 protein accumulates in the nucleus of the damaged cells by a mechanism that does not require transcription (13, 14). Activated p53 transactivates transcription of p53-dependent genes, waf-1, mdm-2, and gadd45 (15–18). Depending upon the cell type, an elevation of p53 can result in G1 arrest and/or apoptosis (8, 11, 19, 20). These two cellular outcomes are also influenced by growth factors, oncogenes, and activation of cytokine-like signal transduction pathways (21).

Susceptibility to drug-induced apoptosis is also modulated by a family of proteins that have homology to conserved regions of the bcl-2 gene (22, 23). Apoptosis is promoted by some of the Bcl-2 homologues (Bax, Bak, Bad, and BclXs; Refs. 24–27), whereas other members of the Bcl-2 family (Bcl-2, BclXs, and Bag-1) form dimers with the apoptosis-promoters and inhibit their activity, hence suppressing apoptosis (10, 27–30). The ratio of the various Bcl-2 family members has been suggested to predispose a cell to accelerated or suppressed apoptosis in response to external stimuli (23, 28).

We have used the topoisomerase II poison etoposide to investigate sensitivity of testicular cancer to apoptosis. Etoposide has been used both as a single agent and in combination chemotherapy regimes for the treatment of testicular cancer (31, 32). p53 and Bcl-2 modulate susceptibility to etoposide-induced apoptosis. Thymocytes from p53-null mice are relatively resistant to etoposide-induced apoptosis, especially at low drug concentrations (11). Similarly, mouse bone marrow or B cells in which Bcl-2 protein is overexpressed show resistance to etoposide (33, 34). Testicular germ cell tumors are unusual because they contain high levels of p53 protein, usually indicative of a stabilized mutant form, but have no abnormalities at the gene level (35, 36). Considering the now-established role of wild-type p53 in DNA-damage-induced apoptosis, this could be one of the determinants that explain hypersensitivity of testicular tumors to chemotherapy. In contrast, studies have demonstrated that 30–60% of bladder tumors have mutations in p53 (37, 38). Bcl-2 is not expressed in normal mouse testis, and there are conflicting reports of its presence in the normal bladder (39, 40). As yet, the presence of Bcl-2 has not been described in tumors of these tissues. We have investigated the roles of p53 and Bcl-2 as determinants of chemosensitivity in testicular cancers.

Received 7/26/95; accepted 2/15/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by Project Grant SP2234 from the Cancer Research Campaign (to C. M. C.).

2 To whom requests for reprints should be addressed. Fax: 0161-275-5600.

3 The abbreviations used are: SSB, single-strand break; FBA, filter binding assay; FIGE, field inversion gel electrophoresis; IC, inhibitory concentration (derived from clonogenic assays); cdK, cell cycle-dependent kinase.
MATERIALS AND METHODS

Cell Culture and Cytotoxicity Assays. The origins of the cell lines are detailed in Table I.

All of the cell lines were grown routinely, under identical conditions, in 25-cm² flasks in RPMI 1640 with 10% (v/v) heat-inactivated FCS and 2 mM glutamine at 37°C in a humidified atmosphere of 5% CO₂ in air. Each cell line was used over a maximum of 12 passages to minimize changes that occur during prolonged culture. All cell lines were routinely tested and found to be mycoplasma free.

Drug sensitivity was measured by clonogenic assays. One thousand testicular and 500 bladder tumor cells were plated in 6-well plates (2 cm²) and allowed to adhere overnight. They were then treated for 1 h with either etoposide or drug vehicle (DMSO). The drug was then washed from the cells with three changes of PBS, and the cells were reincubated in fresh medium for 10–14 days. Colonies were stained with methylene blue, and colonies of greater than 50 cells were counted.

DNA Fragmentation FBA. Apoptosis was quantified using the FBA (41). Percentage DNA fragmentation assayed using the FBA corresponded with percentage of apoptosis determined by counting trypan-blue-negative cells detached from the monolayer. The detached cells were demonstrated to be apoptotic by Hoechst 33258 staining and by analysis of DNA integrity using agarose gel electrophoresis (see “Results”).

Conventional Electrophoresis of DNA. After drug treatment, apoptotic cells detached from the monolayer. These cells were collected by centrifugation and combined with the monolayer cells that had been detached by trypsinisation. Cells (1 X 10⁶) were washed in PBS then lysed in 250 µl sarkosyl lysis solution (0.2% sarkosyl, 2 mM NaCl, and 0.04 mM EDTA; pH 10) containing protease K at 0.5 mg/ml. The lysate was incubated overnight at 45°C then diluted with one volume of TE (10 mM Tris HCl pH 7.4; 1 mM EDTA) buffer, and DNA was precipitated by addition of two volumes of 95% ethanol (−20°C). Precipitates were allowed to form at −20°C for 30 min and then collected by centrifugation at 4°C. The pellets were resuspended in TE and incubated with 20 µg/ml RNase for 1 h at 37°C before electrophoresis on 1% agarose gels in Tris-borate buffer at 40 V overnight.

Field Inversion Electrophoresis of DNA. Supernatant and monolayer cells (4 X 10⁶) were collected as described for conventional electrophoresis. They were then prepared for FIEGE as described (42). Under the conditions used, DNA fragment sizes of 10–750-kbp are resolved and all DNA fragments greater than 750–800 kb that enter the gel migrated at the same rate. Molecular weight standards of 50–1000 kb and 0.1–200 kb (Sigma Chemical Co.) were used to determine the size of DNA fragments.

Western Blotting. After drug treatment, supernatant and adherent cells were combined as described for conventional electrophoresis. Cells (3 X 10⁶) were labeled for 30 h with 0.015 µCi [³⁵S]thymidine/ml (specific activity, 56 mCi/mmol; Amersham, England). Proteins were transferred to Immobilon-P membrane in 10 mM CAPS buffer at 70 V for 3 h. Gels were stained with Coomassie blue to confirm even loading.

Antibodies. The antibodies used for this study were p53, Ab-6 (DO1), from Oncogene science (Cambridge, MA); Waf-1, EA10 Ab, a kind gift from Dr. W. S. El-Deiry (Johns Hopkins, Baltimore, MD); Mdm2, Ab 2A10, a kind gift from Prof. A. Levine (Princeton, NJ); Bax, N-20, from Santa Cruz Biotech (Santa Cruz, CA); and Bel-2, Ab-124, from Dako (High Wycombe, England). Goat anti-rabbit or mouse horseradish peroxidase conjugated secondary antibodies (Promega, Madison, WI) were used as described by the manufacturers, and bands were visualized using enhanced chemiluminescence reagents from Amersham (Amersham, England).

Alkaline Elution. DNA SSBs were measured by alkaline elution (pH 12.1) as described by Kohn et al. (43). Cells in early logarithmic phase growth (1–2 X 10⁶/ml) were labeled for 30 h with 0.015 µCi [³¹P]thymidine/ml (specific activity, 56 mCi/mmol; Amersham, England), washed, and reincubated in fresh medium for 2 h. The cells were then exposed to etoposide for 1 h. After drug treatment, cells were rinsed with ice-cold PBS and then scraped into vesse at 0°C to minimize the possibility of reversal of damage. Cells were collected by centrifugation and resuspended in ice-cold PBS. Cells (8 X 10⁵) were loaded onto each filter and rinsed with 10 ml ice-cold PBS before lysis. For measurement of DNA, SSBs cells were lysed using a sarkosyl lysis solution (0.2% sodium dodecyl sarcosine, 2 mM NaCl, and 0.04 mM EDTA; pH 10) onto 2-µm polycarbonate filters (Millipore). DNA was eluted for 15 h at 0.04 ml/min with tetraethylammonium hydroxide (pH 12.1) containing 0.1% SDS. DNA remaining on the filter and in the filter funnels was released as described (43). The frequency of SSBs induced by etoposide was converted to rad equivalents using a calibration graph derived from the number of SSBs produced by a known X-ray dose.

Flow Cytometry. Cells were treated with an IC₅₀ concentration of etoposide for 2 h; they were then washed and reincubated in the presence of nocodazole (0.4 µg/ml) for 22 h. Two controls were used, nocodazole alone and drug vehicle (DMSO). At 24 h, cells were detached from the plastic by trypsinisation then washed in saline buffer. Cells (1 X 10⁶/per sample) were fixed in 70% ethanol and stored at 4°C until FACS analysis. Cells were rehydrated in buffered saline, treated with RNase (500 units/ml) at 37°C for 15 min, and finally stained with propidium iodide (50 µg/ml) for 20 min. Cell cycle analysis was performed using a Becton Dickinson fluorescent activated cell analyzer, and data was analyzed using cell lysis software.

RESULTS

Differential Sensitivity to Etoposide

Clonogenic assays were performed to establish the sensitivity of the cell lines to a 1-h exposure to etoposide. Fig. 1 shows that the testicular tumor cell lines were significantly more sensitive to etoposide than the bladder cell lines, with no overlap in the IC₅₀ values between the two cancer cell types. On average, loss of clonogenicity was 16-fold greater in the testicular than in the bladder cell lines, with a
2–3-fold variation within each cell type (Table 2). The bladder cell line HT1376 was exceptionally resistant, showing 40% viability after treatment with 360 μM etoposide.

Comparison of Cytotoxicity Produced by Equidamaging Doses of Etoposide

Etoposide-induced DNA damage was measured by alkaline elution in the two tumor cell types. DNA damage was higher in the testicular tumors; on average, the frequency of SSBs induced by 2 μM etoposide was 3.8-fold greater in the testicular cell lines than in the bladder cell lines (Fig. 2A). When DNA damage was correlated with cytotoxicity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>DNA Damage (RE)</th>
<th>Loss of Clonogenicity (%)</th>
<th>Apoptosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>532 ± 28.2</td>
<td>87.22 ± 6.31</td>
<td>39.78 ± 6.52</td>
</tr>
<tr>
<td>Bladder</td>
<td>270 ± 54.4</td>
<td>5.43 ± 7.68</td>
<td>8.6 ± 2.25</td>
</tr>
</tbody>
</table>

DNA damage was determined after a 1 h treatment with etoposide. Colonies were counted to assess loss of clonogenicity 12–14 days after a 1-h pulse treatment with etoposide. Apoptosis was measured following 4 h continuous treatment.

Table 2 Differential sensitivity of testicular and bladder tumor cell lines to 20 μM etoposide-induced DNA damage, cytotoxicity, and apoptosis

The testicular lines 833K and GH were more sensitive than OCT27; the percentage of DNA cleavage following 550RE SSBs was 75% for 833K and 70% for GH, whereas OCT27 had only 25% DNA cleavage. Therefore, we determined whether the susceptibility to undergoing apoptosis was different between the two cell types.

Induction of Apoptosis with Etoposide

Apoptosis after 4 h Treatment with Etoposide (IC₅₀). Apoptosis is characterized by cell shrinkage, detachment from monolayers, chromatin condensation, and the nonrandom fragmentation of DNA. To determine whether apoptosis could be detected in both cell types, they were treated for 4 h with a concentration of etoposide that reduced clonogenicity by 90% (IC₅₀). After a 4-h drug treatment, a significant percentage of the cells detached from the monolayer. Detached and monolayer cells were analyzed by staining with Hoechst 33258 for condensed chromatin, a characteristic morphological feature of apoptosis, and by trypan blue uptake for necrosis. Trypan blue uptake was only evident in HT1376, the exceptionally resistant bladder tumor cell line; in this cell line, only 11% of cells detached, and 63% of these were trypan blue positive. In the remaining two bladder cell lines, RT4 and RT112, and in the testicular cell lines, detached cells excluded trypan blue and had condensed chromatin. Analysis of DNA integrity by conventional electrophoresis and FAGE revealed nonrandom cleavage of DNA in the cell lines that exhibited morphological features of apoptosis. Cleavage to 50-kb fragments and inter nucleosomal cleavage were detected in all of the testicular lines and in the bladder cell lines RT4 and RT112 (Fig. 3). There was no evidence of DNA cleavage in HT1376. The >750-kb band of DNA on the FlOE gels represents unresolved fragments and is a product of the etoposide-induced DNA damage (42). The 750 Kb band was present in all cell types, confirming that HT1376 received the etoposide damage but did not couple it to apoptosis.

Comparison of Susceptibility to Undergoing Apoptosis Using Equimolar and Equidamaging Concentrations of Etoposide. Apoptosis was quantified for a range of etoposide concentrations using the FBA (41). As shown in Fig. 4, the testicular cell lines are more sensitive than the bladder cell lines to etoposide-induced apoptosis at all concentrations studied. However, there was variation in susceptibility to undergoing apoptosis within each tumor cell type. The testicular lines 833K and GH were more sensitive than GCT27; the percentage of DNA fragmentation of these cell lines after a 4-h treatment with 20 μM etoposide was significantly greater than in the bladder cell lines. The testicular cell lines also showed a significantly greater loss of viability for a given frequency of DNA damage (Fig. 2B). This suggests that chemosensitivity is related to post-DNA damage events. Therefore, we determined whether the susceptibility to undergoing apoptosis was different between the two cell types.
etoposide was 46, 40, and 32%, respectively (Fig. 4). The bladder cell line RT4, which has functional p53, is also included, the difference is less significant (0.1 > P > 0.05).

Expression of p53 in Testicular and Bladder Cell Lines

The absence of mutations in the p53 gene of testicular germ cell tumors (35, 36) suggests a potential mechanism for hypersensitivity to drug-induced apoptosis in which wild-type p53 would promote apoptosis. p53 protein levels, in the presence and absence of etoposide, were measured by immunoblotting of whole cell extracts (see Fig. 5). All of the testicular tumor cell lines had, as expected, high basal levels of p53 protein that were unchanged after drug treatment. High basal p53 protein levels in bladder cancer usually indicate the presence of a p53 form stabilized by mutation (44). This is consistent with the absence of apoptosis and the high level of drug resistance seen in this cell line.

Etoposide-induced Expression of Waf-1 Protein

To determine whether p53 was able to transactivate transcription, levels of the cdk inhibitor Waf-1 protein were determined. Waf-1 has previously been demonstrated to show a p53-dependent increase in response to DNA damage (15). Waf-1 levels were determined by immunoblotting of whole cell lysates after treatment with IC10 (90% of cells survive) or IC90 (10% of cells survive) concentrations of etoposide for 4 h (Fig. 6A). Levels of p53 were also determined for comparison. Waf-1 was found to increase to low levels in the testicular lines GCT27 < GH < 833K and to high levels in RT4, the most sensitive bladder cell line. A study of the kinetics of p53 and Waf-1 expression was performed using 833K (testicular) and RT4 (bladder), to determine when p53 and Waf-1 levels were maximally induced (Fig. 6B). p53 protein was maximal after a 2 h treatment, and Waf-1 levels were maximal by 4 h. Thus, the absence of detectable increases of Waf-1 in RT112 and HT1376 (Fig. 6A) is unlikely to be a result of an inappropriate sampling time. Treatment with low concentrations of etoposide also resulted in increases in the p53 transactivated gene Mdm-2 in the testicular lines and RT4 (data not shown).

Expression of Bax and Bcl-2: Basal Levels and Effect of DNA Damage

p53 has been reported to differentially regulate the expression of two of the Bcl-2 family members; it promotes the transactivation of bax, an accelerator of apoptosis, and has been reported to repress the expression of bcl-2, a suppressor of apoptosis (46, 47). We therefore determined whether Bax and Bcl-2 were regulated in the testicular and bladder cell lines after etoposide treatment. Two concentrations of etoposide were used, IC10 and IC90, and samples were taken at 4 h, the time at which Waf-1 levels were maximal (see Fig. 6B). Surprisingly,
FUNCTIONAL p53 IN CHEMOSENSITIVE TUMORS

testicular cancer. The response of solid tumors to chemotherapy is influenced by several factors, including tumor size and vascularization. However, cell lines derived from testicular and bladder tumors, when cultured in vitro, retain their relative chemosensitivities and reflect the sensitivities observed in the clinic. This suggests that differential drug sensitivity is an inherent property of the testicular and bladder tumor cell types (2, 48, 49). We have investigated the threshold of susceptibility to undergoing apoptosis in testicular and bladder tumor cell lines and have determined whether this is related to p53 function.

The frequency of DNA damage produced by etoposide was determined and compared to the percentage apoptosis produced by equivalent etoposide treatments. As can be seen in Figs. 2B and 4B, for a

Fig. 7. Etoposide-induced G1 arrest. Cells were treated with an IC50 concentration of etoposide for 1 h; they were then washed and reincubated in the presence of nocodazole (0.4 μg/ml) for 22 h. Control cells received either nocodazole alone or drug vehicle alone (DMSO). Addition of nocodazole prevented cells that were released from an etoposide-induced G2 arrest from moving through the cycle synchronously and appearing as an apparent G1 checkpoint. The percentage of cells in G1 in the presence of nocodazole alone is presented relative to the percentage in G1 after etoposide (IC50) and nocodazole. Cell cycle analysis was performed using a Becton Dickinson fluorescent activated cell analyzer, and data were analyzed using cell lysis software.

Bax protein was not increased in any of the six cell lines under any treatment conditions (Fig. 8). However, the endogenous levels of Bax were higher in the testicular tumor lines than in the bladder. To detect Bax in the bladder lines, the gel had to be exposed to film for an increased length of time, resulting in a dark background, as can be seen in Fig. 8. The basal levels of Bc1-2 protein differed dramatically between testis and bladder. Immunoblotting demonstrated that Bc1-2 was present in all of the bladder cell lines but undetectable in the testicular cell lines (see Fig. 9). There was no detectable Bc1-2 protein in the testicular lines, even when gels were loaded with high concentrations of protein (150 μg) and films overexposed. The levels of Bc1-2 protein in the bladder tumor cell lines were unchanged after a 4-h treatment with etoposide (IC50).

DISCUSSION

In the present study we determined whether susceptibility to apoptosis could be the mechanism that confers chemosensitivity to testicular cancer. The response of solid tumors to chemotherapy is influenced by several factors, including tumor size and vascularization. However, cell lines derived from testicular and bladder tumors, when cultured in vitro, retain their relative chemosensitivities and reflect the sensitivities observed in the clinic. This suggests that differential drug sensitivity is an inherent property of the testicular and bladder tumor cell types (2, 48, 49). We have investigated the threshold of susceptibility to undergoing apoptosis in testicular and bladder tumor cell lines and have determined whether this is related to p53 function.

The frequency of DNA damage produced by etoposide was determined and compared to the percentage apoptosis produced by equivalent etoposide treatments. As can be seen in Figs. 2B and 4B, for a
FUNCTIONAL p53 IN CHEMOSENSITIVE TUMORS

Thus, the cell lines that had functional p53 (i.e., all of the testicular tumor cell lines and the bladder cell line RT4) were sensitive to etoposide-induced apoptosis. Mdm-2 expression was also studied as a marker of p53 function and similar results to those observed for Waf-1 induction were obtained in the six cell lines (results not shown).

Increases in p53 after DNA damage have been associated with two cellular outcomes, arrest in G1 and apoptosis (8, 11, 19–21). These two outcomes are cell type dependent and are probably related to expression of the p53-dependent gene waf-1, a potent inhibitor of cdks (15, 21). Immunoblotting was used to measure Waf-1 protein levels in response to etoposide-induced DNA damage. In the testicular cell lines, there was a transitory increase of Waf-1 to low levels, but no G1 checkpoint. However, in one of the bladder cell lines, RT4, there was a prolonged increase of Waf-1 to high levels, and a G1 checkpoint was observed (Figs. 6 and 7). The checkpoint/apoptosis outcome is affected by the level of the Waf-1 protein (21, 56). Zhang et al. (56) have shown that greater than one Waf-1 molecule is required per ternary complex (Waf-1, PCNA, cdk, cyclin) for inhibition of the cdk to occur. Therefore the differences in absolute Waf-1 protein levels between the testicular and bladder tumor cells could be responsible for the observed cellular outcomes. The basal levels of Waf-1 are modulated by growth factors and can be regulated by constitutive activation of protein kinases (raf and src) involved in cytokine pathways (21, 57). Further studies are required to determine whether there is differential activation of growth-related kinases between the testicular and bladder tumor cell lines. However, src and ras have been described to be activated in bladder carcinomas (58–60). The p53-dependent apoptosis/G1-arrest decision is also modulated by transcription factors involved in cell cycle progression (myc, myb, and E2F). Overexpression of either myc, myb, or E2F results in p53-dependent apoptosis rather than arrest (61–63). N-myc and c-myc have been detected in germ cell tumors (55, 64, 65). Again, further studies are required to determine whether activation of oncogenes contributes to the observed differences in the basal levels of expression of Waf-1 and growth arrest/apoptosis in the testicular and bladder tumor cell lines.

The function of p53 in apoptosis is unclear. p53 is both a transactivator and a repressor of transcription, but it is not clear whether either of these activities of p53 are required for apoptosis (45, 66–68). p53 has been described to differentially regulate two of the Bcl-2 family members that control apoptosis: Bax, an accelerator of apoptosis, is up-regulated, and Bcl-2, a suppressor of apoptosis, is down-regulated (46, 47, 69). This forms an attractive potential mechanism for p53-induced apoptosis that was supported by studies of Zhan et al. (70), who demonstrated up-regulation of Bax mRNA levels in cells undergoing apoptosis after genotoxic stress. We determined whether the Bax/Bcl-2 ratio was perturbed during etoposide-induced apoptosis. Immunoblotting failed to detect any changes in expression of either Bax or Bcl-2 after drug treatment (Figs. 8 and 9). There are several potential explanations for the disparity between our results and those of Miyashita et al. (46) and Zhan et al. (70). First, Bcl-2 and Bax proteins have a long half-life; therefore, a modest alteration in protein synthesis may not be detected at 4 h. However, there was no increase in Bax RNA at 4 h (data not shown). In M1 murine leukemia cells containing a temperature-sensitive p53, incubation at the permissive temperature for 3 h results in increases in both Bax RNA and protein (47). An alternative explanation is that the increases in Bax expression are cell type specific; in support of this, Bax protein was not up-regulated in Baf-3 cells after activation of p53 (21). Although we did not observe regulation of these proteins during apoptosis, the endog-
functional p53 in chemosensitive tumors

enous expression of the two proteins differed significantly between the testicular and bladder tumor cell types. The testicular tumor lines expressed relatively high levels of the apoptosis-promoting protein Bax, but not the suppressor of apoptosis Bcl-2. In contrast, the bladder lines had low levels of Bax and readily detectable levels of Bcl-2. The differences in the ratio of these two proteins could contribute to the differential sensitivity of the two tumor types to etoposide (23). There are several other Bcl-2 family members that also contribute to drug sensitivity [e.g., Bcl-X (27) and Bak (25)]. Preliminary results have shown that Bcl-X is expressed in both cell types, but to higher levels in the bladder. Bax is also expressed in both cell types at the RNA level.4

In summary, the sensitivity of testicular tumors to chemotherapy in the clinic may, at least in part, be related to the susceptibility of this tumor type to DNA-damage-induced apoptosis. Characterization of the testicular tumor cell lines at the molecular level revealed three characteristics that would contribute to the chemosensitive phenotype. First, the testicular tumors have high levels of p53, presumably in a latent state; p53 further increases in levels after DNA damage and functions as a transactivator of transcription. Second, in the testicular tumor cell lines, increases in p53 resulted in apoptosis rather than G1 arrest, an outcome presumably dictated by the cell background (as yet not described for this cell type). Finally, the relative ratio of Bax to Bcl-2 would also favor accelerated apoptosis in the testicular cell lines, in which, presumably, Bax-Bax homodimers promote cell death. The absence of Bcl-2, a pleiotropic suppressor of apoptosis, in the testicular tumors is a striking difference between the two tumor types.

REFERENCES


Hypersensitivity of Human Testicular Tumors to Etoposide-induced Apoptosis Is Associated with Functional p53 and a High Bax:Bcl-2 Ratio

Christine M. Chresta, John R. W. Masters and John A. Hickman


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/8/1834

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.