Effects of Estrogen on Apoptotic Pathways in Human Breast Cancer Cell Line MCF-7

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Abstract

We utilized a reverse transcription-PCR method to examine the effect of estrogen on the expression of mRNA for Bcl-2 and Bax, two modulatory proteins in the apoptotic pathway, in human breast cancer cell line MCF-7. We found that the bcl-2 mRNA levels in the cells exposed to 17ß-estradiol were higher than those of control cells. Although the relative bax mRNA levels remained unchanged, the changes in bcl-2 mRNA level occurred in a time- and concentration-dependent fashion. In addition, pretreatment with 17ß-estradiol protected MCF-7 cells from apoptosis. Our study provides evidence that responses of breast epithelial cells toward a steroid sex hormone involve regulation of the apoptotic pathway.

Introduction

Apoptosis (programmed cell death) is a normal physiological phenomenon that can be observed in various tissues. Cells undergoing apoptosis are characterized by distinct biochemical and morphological changes (1). It is known that apoptosis plays a role in differentiation processes, such as formation of digits during embryogenesis and selection of lymphocyte populations. In addition, perturbation of the apoptotic pathway has been found to be associated with tumorigenesis (2, 3). The protein Bcl-2 has been shown to be a component of the apoptotic pathway. Overproduction of Bcl-2 protein resulted in blockage of apoptosis and increased survival upon external “death” stimuli (4). The antiapoptotic activity of Bcl-2 is thought to be modulated by the protein Bax, and it has been proposed that the Bcl-2:Bax protein ratio may determine whether a cell would undergo apoptosis (5, 6).

Materials and Methods

Cell Culture and Total RNA Isolation. MCF-7 cells were maintained in medium A [RPMI 1640 with 2 mm L-glutamine, 3.5 ng/ml hydrocortisone, 1.5 ng/ml insulin, 100 units/ml penicillin, and 100 μg/ml streptomycin] with 5% fetal bovine serum in 75-cm² flasks (Falcon, Lincoln Park, NJ) and grown in 24-well plates (5 X 10⁵ cells/well in 3 ml of medium B [phenol red-free RPMI 1640 containing 2 mm glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 5% charcoal dextran-treated fetal bovine serum]). Total RNA was isolated from MCF-7 cells grown in 6-well Costar plates (5 X 10⁵ cells/well in 3 ml of medium B) by a method described previously (10).

Quantitation of bcl-2 and bax mRNA Levels. We utilized a competitive reverse transcription-PCR method to quantitate bcl-2 and bax mRNA levels (11). The procedure used one set of primers to amplify both target cDNA and an externally added MIMIC of known concentration. MIMICs for both bcl-2 and bax quantitation were constructed with the use of the PCR-MIMIC construction kit (Clontech, Palo Alto, CA) following the manufacturer’s directions. The primer pairs used for amplification of bcl-2 and bax mRNA and quantitation of MIMIC fragments are listed in Table 1. We routinely used 1 μg of RNA for cDNA synthesis (Clontech). After first-strand cDNA synthesis reaction, the cDNA was made up to 100 μl final volume, and 4 μl were used for PCR. A typical PCR consisted of 0.2 μM dNTP, 2 μM MIMIC, 4 μl cDNA, 0.2 μM of each primer, PCR buffer, and Taq polymerase.

Results

Effects of Estrogen on bcl-2 and bax mRNA Level. To examine the effects of estrogen on the apoptotic pathway, we used the human breast cancer cell line MCF-7 and monitored mRNA expression of apoptosis-related proteins Bcl-2 and Bax. The MCF-7 cells are a good model because they expressed estrogen receptor and, as in our initial study, also expressed both bcl-2 and bax mRNA (data not shown). A competitive reverse transcription-PCR protocol was used to determine bcl-2 and bax mRNA level. The primer for synthesis of MIMIC and specific message bands. All results were normalized against G3PDH mRNA quantitated with the use of identical PCR conditions. Results are expressed as level of expression relative to G3PDH. The primer pairs and competitor for G3PDH were purchased from Clontech.

Apoptotic Death Assay. We used DNA fragmentation as the criteria for apoptotic cell death. DNA fragmentation was measured with the use of the cell death ELISA (Boehringer Mannheim, Indianapolis, IN). MCF-7 cells (1 X 10⁴ cell) were plated in each well of 24-well plates. After appropriate treatment, the cells were washed once with PBS, and 0.5 ml lysis buffer was added. After a 30-min incubation, the supernatant was recovered and assayed for DNA fragments according to manufacturer’s protocol. Each treatment was performed in triplicate. Additional plates identical treated as above were analyzed for cell number with the use of the sulforhodamine assay (12).

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2 The abbreviations used are: MIMIC, competitor DNA fragment; G3PDH, glyceraldehyde 3-phosphate dehydrogenase.
ESTROGEN AFFECTS APOPTOSIS

A
Control  +Estradiol

1 2 3 1 2 3

Fig. 1. Effects of 17ß-estradiol on bcl-2 and bax mRNA level. A, representative gel showing reverse transcription-PCR for bcl-2 and bax. Lanes 1–3: 1:3 serial dilution of MIMIC. B, relative bcl-2 and bax mRNA levels in control and 17ß-estradiol (10^{-9} M)-treated MCF-7 cells. Cells were treated with or without estradiol for 48 h.

Table 1 Primer pairs for construction of MIMIC and amplification of bcl-2 and bax mRNA

<table>
<thead>
<tr>
<th>MIMIC</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>bcl-2 mRNA</td>
<td>5'-ACTTGGCAGCTGGGAGGAGGCTTT</td>
<td>5'-GCCCATCTTCCAGATGGTGAGCTGGAGCTTT</td>
</tr>
<tr>
<td>bax RNA</td>
<td>5'-CGACTTGGCAGCTGGGAGGAGGCTTT</td>
<td>5'-GCCCATCTTCCAGATGGTGAGCTGGAGCTTT</td>
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</tbody>
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17ß-estradiol, and the relative level of bcl-2 and bax mRNA was quantitated. Shown in Fig. 1A is a representative gel for analysis of PCR products. We found that treatment with 17ß-estradiol resulted in an increase in bcl-2 mRNA level, but produced no change in bax mRNA level (Fig. 1B). Observing a change in relative bcl-2 mRNA level upon treatment with 17ß-estradiol, we then further characterized this effect. We found that the increase in bcl-2 mRNA level with 17ß-estradiol was time dependent (Fig. 2A) for up to 48 h. In addition, the increase in bcl-2 mRNA level accompanied the increase in estradiol concentration (Fig. 2B). The effect of 17ß-estradiol on bcl-2 mRNA levels was seen at estradiol concentrations as low as 10^{-11} M and plateaued at 10^{-10} M.

Effect of Tamoxifen on Estrogen-stimulated bcl-2 mRNA Expression. To determine whether the increase in bcl-2 mRNA was due to specific interaction between estradiol and estrogen receptor, we examined the effect of tamoxifen, an antiestrogen, on estradiol-stimulated bcl-2 expression. We found that addition of tamoxifen (10^{-6} M) completely inhibited the stimulatory effects of estradiol, thus supporting the possibility that increased in bcl-2 mRNA levels involve the interaction of estradiol with the estrogen receptor.

Effects of Estrogen on Apoptosis. The observation that estradiol increases bcl-2 mRNA level would suggest that estradiol may inhibit...
apo-otic pathways. We then examined the effect of pretreatment of cells with estradiol on apoptosis induced by tamoxifen. Cells were incubated with and without 17β-estradiol (10^{-10} M) for 24 h, by which time media were replaced with media containing tamoxifen. Apoptotic cell death was determined by monitoring DNA fragmentation. We found that cells pretreated with 17β-estradiol (10^{-10} M) appeared to be more resistant to tamoxifen-induced apoptosis (Fig. 3).

Discussion

In summary, we have demonstrated that treatment of MCF-7 cells with 17β-estradiol resulted in increased bcl-2 mRNA levels but did not affect bax mRNA levels. Estradiol stimulated a time- and concentration-dependent increase in bcl-2 mRNA. The estradiol-stimulated increase could be blocked by tamoxifen, suggesting that it was mediated by an interaction between estradiol and estrogen receptor. Moreover, pretreatment of MCF-7 cells with estradiol appeared to protect cells from apoptosis.

Our in vitro study using MCF-7 cells as a model provides direct evidence that steroid sex hormones such as estrogen can inhibit apoptosis by increasing the production of Bcl-2, an antiapoptotic protein. Our finding is consistent with clinical studies that correlated detection of Bcl-2 protein with expression of estrogen receptor (7, 8). These findings also support the hypothesis (7) that modulation of apoptosis may be a mechanism by which estrogen influences breast cancer risk. In addition, additional characterization of the role of estrogen in apoptotic pathways could lead to design of antitumor drug(s) that target this pathway.

The differential regulation of bcl-2 and bax mRNA by estrogen is interesting. One may hypothesize that, at least in the case of breast epithelial cells, cell survival depends on external mitogenic signal. The survival of cell is enhanced by an increase in the production of antiapoptotic protein Bcl-2 to counteract death signal by Bax.

References

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