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Relationship between tamoxifen-induced transforming growth factor β_1 expression, cytostasis and apoptosis in human breast cancer cells

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Summary Previously we have shown that tamoxifen (TAM) induces morphological and biochemical changes typical of apoptosis in oestrogen receptor (ER)-positive MCF-7 or ER-negative MDA-231 human breast cancer cells. In this study the effects of TAM on expression of transforming growth factor β_1 (TGF-β_1) were correlated with the effects on cell cycle kinetics and apoptosis. TAM had similar biphasic effects on both cell lines. Short-term (<6 h) TAM incubation resulted in a slight decrease in TGF-β_1 protein despite an increase in TGF-β_1 mRNA and was associated with an increase in cells in S-phase. No apoptotic effects were noted. Longer (≥ 12 h) TAM incubation induced TGF-β_1 protein (about 3-fold) and mRNA expression (about 2-fold) in both cell lines, and was associated with G_1/G_0 blockade and induction of apoptosis. The accumulation of TAM-induced TGF-β_1 mRNA was increased by cycloheximide, but was not affected by 17 β-oestradiol. Long-term incubation with TAM had no significant effect on TGF-β_1 gene copy number. TAM-induced internucleosomal DNA cleavage was inhibited in both cell lines by the addition of an anti-TGF-β_1 antibody. TAM has dose- and time-dependent effects on TGF-β_1 expression associated with changes in cell cycle kinetics. These effects are independent of ER status and may be the result of a direct regulatory effect of TAM on TGF-β_1 transcription. It also appears that induction of TGF-β_1 plays an important role in TAM-induced apoptosis in breast cancer cells.

Keywords: tamoxifen; apoptosis; transforming growth factor β_1

The mechanism of action of the anti-oestrogen tamoxifen (TAM) is unclear, but it is apparent that TAM has both oestrogen receptor (ER) and non-ER mediated cytostatic activity. Previous observations by our group (Kang and Perry, 1993) and others (Reddel *et al.*, 1984; Dickson *et al.*, 1990) have shown that the time- and dose-dependent growth inhibitory effects of TAM have a biphasic character. TAM may have important biological effects via novel pathways, such as through modulation of transforming growth factor β_1 (TGF-β_1) activity (Butta *et al.*, 1992). TGF-β_1 has either growth inhibitory or growth stimulatory properties, depending on the concentration and cell type (Roberts *et al.*, 1985). TGF-β_1 appears to be an important negative regulator of breast cancer cell growth. ER(+) MCF-7 breast cancer cells (Roberts *et al.*, 1985; Knabbe *et al.*, 1987), and four ER(-) breast cancer cell lines including MDA-231 (Arteaga *et al.*, 1988) secrete and are inhibited by TGF-β_1.

It also appears that TGF-β_1 may be a mediator of TAM-induced cytostasis (Manni *et al.*, 1991). Anti-oestrogens induce TGF-β_1 activity in ER(+) MCF-7 cells *in vitro* (Knabbe *et al.*, 1987), in ER(-) human fetal fibroblast *in vitro* (Colletta *et al.*, 1990), and in both ER(+) and ER(-) human breast cancer *in vivo* (Butta *et al.*, 1992). However, the induction of TGF-β_1 is not an absolute requirement for TAM-induced cytostasis, as demonstrated by other models including long-term passage MCF-7 cells (Zugmaier and Lippman, 1990) and the ER(+) T47-D breast cancer cell line (Murphy and Dotzlaw, 1989). Since TGF-β_1 is rapidly degraded, with the preform having a half-life of 2 h and the active form only 2 min, active synthesis is required for this protein to be expressed (Wakefield *et al.*, 1987).

Recent work in our laboratory has shown that TAM can induce growth stimulation at low dose or growth inhibition and apoptosis at higher doses in either ER(+) MCF-7 or ER(-) MDA-231 human breast cancer cells (Table I; Perry *et al.*, 1995). TAM induced characteristic morphological changes associated with apoptosis, including condensation of chromatin around the nuclear periphery, typical biochemical changes such as internucleosomal DNA fragmentation and the process required protein synthesis. Other investigators have shown that 4-hydroxytamoxifen (Bardon *et al.*, 1987) or toremifene (Warri *et al.*, 1993) can also induce apoptosis in MCF-7 cells. The mechanisms whereby TAM induces apoptosis remain unknown. However, since TAM induces secretion of TGF-β_1, TGF-β_1 may be a mediator of TAM-induced apoptosis. TGF-β_1 induces apoptosis in several model systems including uterine epithelial cells (Rotello *et al.*, 1991), and liver cells *in vitro* and *in vivo* (Oberhamer *et al.*, 1992; Bursch *et al.*, 1993). Increased TGF-β_1 expression and apoptosis are noted in ER-dependent tumours following oestrogen ablation (Kyprianou *et al.*, 1990). TGF-β_1 has been detected early in mammary gland involution (Strange *et al.*, 1992). But there is no direct evidence showing a relationship between TAM-induced TGF-β_1 expression and TAM-induced apoptosis in ER(+) and ER(-) human breast cancer cell lines.

In the present study we determined the effects of TAM on TGF-β_1 expression, cell cycle kinetics and DNA cleavage in MCF-7 and MDA-231 cells. We found that TAM had similar biphasic effects on TGF-β_1 expression and cell cycle kinetics in both cell lines. The TAM-induced increase in TGF-β_1 expression strongly correlated with DNA cleavage characteristic of apoptosis, and this DNA cleavage was inhibited by addition of an anti-TGF-β_1 antibody. The data demonstrate that tamoxifen-induced expression of TGF-β_1 is independent of ER status and appears to be directly linked to the observed G_1/G_0 arrest, cytostasis and apoptosis.

Materials and methods

Cell lines

The MCF-7 cell line was originally obtained from the Michigan Cancer Foundation; the MDA-231 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). Cells were maintained in α-minimal essential medium (α-MEM) supplemented with 5% fetal calf serum, glutamine, penicillin and streptomycin. The cells were incubated at 37°C in a 95% air/5% carbon dioxide environment. Forty-eight hours before each experiment the cells were transferred to phenol-red-free media with charcoal dextran stripped-fetal calf serum to remove exogenous oestrogens

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Table 1 Time dependence of 10 μM TAM-induced DNA cleavage and cytostasis[a]

	MCF-7		*MDA-231*	
Time (h)	*DNA cleavage (%)*	*Cell growth (%)*	*DNA cleavage (%)*	*Cell growth (%)*
0	4.8 ± 0.7[b]	100	4.5 ± 0.5	100
6	5.1 ± 0.7	123 ± 6	5.5 ± 0.8	109 ± 8
12	8.6 ± 1.1	63 ± 6	4.8 ± 0.9	81 ± 4
24	21.6 ± 1.6	44 ± 7	18.6 ± 1.8	64 ± 9
48	36.9 ± 2.2	4 ± 3	30.6 ± 2.0	7 ± 2

[a]Adapted from Perry *et al.* (1995). [b]Mean ± s.e.

(Reddel, 1984). No significant spontaneous apoptosis was noted when cells were incubated for up to 7 days in the oestrogen-free medium.

Drugs

Tamoxifen (trans 1-(4-β-dimethylaminoethoxyphenyl)-1, 2-diphenylbut-1-ene; TAM) was kindly provided by Zeneca Pharmaceuticals (Wilmington, DE, USA). A stock solution was prepared in 2% ethanol and stored at −20°C. All of the chemicals used were of reagent grade and were obtained commercially.

Measurement of cell cycle kinetics

Single cell preparations obtained from control cultures and cultures treated with 10 μM TAM for various periods of time were fixed in ice-cold methanol for 15 min. This dose of TAM was chosen based on our previous experiments which demonstrated dose- and time-dependent growth inhibition and apoptosis in MCF-7 and MDA-231 cells (Perry *et al.*, 1995). The cells were removed from the fixative by centrifugation at 900 *g* for 5 min and stained for DNA flow cytometry with 50 μg ml^{-1} propidium iodide. Thirty minutes before analysis, RNase (ribonuclease type A, Sigma, St Louis, MO, USA) was added directly to the stained cell preparations to yield a final concentration of 1.0 mg ml^{-1}, and samples were filtered through 40 μ nominal pore size nylon gauze. Analysis was performed using an Epics V electronically programmable individual cell sorter (Coulter Electronics, Hialeah, FL, USA) equipped with a MDADS graphic display (Coulter), with excitation at 488 nm provided by an argon ion laser operating at 200 mW and fluorescence detected at greater than 550 nm. Single parameter DNA histograms were collected for 10 000 viable cells, and the cell cycle kinetic parameters were calculated from the histograms using the manufacturer's software (Coulter).

Western analysis of TGF-β_1 expression

Breast cancer cells were treated with 10 μM TAM for up to 48 h, washed twice with cold phosphate-buffered saline (PBS) and sonicated for 30 s at 0–4°C. Cytosolic protein (50 μg per lane) was resolved using 12.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by electrophoretic transfer to polyvinylidene difluoride membranes according to the manufacturer's directions (BioRad, Richmond, CA, USA). Western immunoblotting was performed using rabbit anti-human TGF-β_1 polyclonal antibody (Oncogene Science, Uniondale, NY, USA). After soaking in TTBS (100 mM Tris-HCl, pH 7.5, 0.9% sodium chloride, and 0.1% Tween-20), the membranes were incubated with anti-TGF-β_1 antibody (1:500) for 6 h at room temperature in TTBS (containing 5% goat serum). The filters were then washed three times with TTBS. Biotinylated goat anti-rabbit IgG was used as the secondary antibody (Vector Laboratories, Burlingame, CA, USA) and colour developed using a commercially obtained avidin–biotin–peroxidase staining kit (Vector).

Hybridisation probes

TGF-β_1 cDNA was originally from Dr R Dernyck (Genentech, San Francisco, CA, USA). Glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA was obtained from American Type Culture Collection. The above DNAs were subcloned in SP6 vectors (Promega Biotec, Madison, WI, USA) and used as templates for synthesis of high-specific activity (10^9 c.p.m. μg^{-1}) ^{32}P-labelled cDNA probes.

Isolation of RNA and Northern analysis

Total RNA from breast cancer cells treated with 10 μM TAM for up to 48 h was isolated by the guanidinium isothiocyanate method (Maniatis *et al.*, 1982). Poly (A +)-RNA was purified using an oligo(dt +)-cellulose column (× 2). An aliquot of 5 μg of each mRNA preparation was size fractionated using a 1% formaldehyde–agarose gel. The RNA gel was stained with ethidium bromide before transfer to a nitrocellulose membrane to monitor the integrity of 18 S and 28 S rRNA bands. Filters were prehybridised in 1 M sodium chloride, 10% dextran sulphate, 50% formamide and 0.1% SDS at 42°C for 12 h and then hybridised in 5 × standard saline citrate (SSC, 1.5 M sodium chloride, 0.15 M sodium citrate), 1 × Denhardt's solution, 0.1 mg ml^{-1} salmon sperm DNA, 10% dextran sulphate, 45% formamide and 0.05% SDS with 10^6 c.p.m. ml^{-1} ^{32}P-labelled TGF-β_1 cDNA or GADPH cDNA (as the control) at 42°C for 10 h. Filters were then treated with four 5 min washes in 2 × SSC, 0.1% SDS at room temperature and then three 15 min washes in 0.2 × SSC, 0.1% SDS at 60°C. Autoradiography was then performed at −70°C for 72 h. Relative abundance of TGF-β_1 transcripts was assessed by densitometry, normalised to the density of the GADPH transcripts.

Other experiments were performed to examine the effects of certain inhibitors on TAM-induced TGF-β_1 mRNA expression. Cells were treated for 24 h with 10 μM TAM alone, 10 μM TAM plus 10 μM 17β-oestradiol, or with 10 μM plus 0.35 μM cycloheximide. This concentration of cycloheximide inhibited protein synthesis by greater than 90% and when given alone did not induce TGF-β_1 expression or induce apoptosis (data not shown).

Isolation of DNA and Southern analysis

To measure TGF-β_1 gene copy number, Southern analysis was performed as described by others (Ueda *et al.*, 1987). Genomic DNA was extracted from breast cancer cells treated with 10 μM TAM for up to 120 h and then digested overnight with a 2-fold excess of the *Eco*RI restriction endonuclease. An aliquot of 10 μg of each sample was then resolved by electrophoresis on a 0.8% agarose gel and transferred to Nytran membranes (Schleicher & Schuell, Keene, NH, USA) using 10 × SSC. The blotted DNAs were then hybridised under high-stringency conditions (68°C) with 10^6 c.p.m. ml^{-1} of ^{32}P-labelled TGF-β_1 cDNA. Filters were treated with four 5 min washes in 1 × SSC, 0.1% SDS at room temperature, followed by three 15 min washes in 0.1 × SSC, 0.1% SDS at 55°C. Autoradiography was performed using Kodak X-OMAT AR film with Cronex intensifying screens at −75°C for 72 h. Equal loading of DNA was confirmed by ethidium bromide staining of the gels and by rehybridisation of the filters with a GADPH probe.

Analysis of DNA fragmentation

Approximately 4 × 10^6 breast cancer cells were seeded into 75 cm^2 flasks (Falcon, Becton Dickson). After 24 h the cells

Table II Effects of TAM on cell cycle kinetics

	G_1/G_0 (%)		S (%)		G_2/M (%)	
Time (h)	*MCF-7*	*MDA-231*	*MCF-7*	*MDA-231*	*MCF-7*	*MDA-231*
0	45 ± 3[a]	51 ± 2	31 ± 5	29 ± 5	24 ± 4	20 ± 5
6	44 ± 4	42 ± 3	36 ± 5	38 ± 6	20 ± 3	20 ± 3
12	53 ± 3	50 ± 3	28 ± 4	29 ± 6	19 ± 3	21 ± 4
24	66 ± 4	58 ± 4	24 ± 6	24 ± 2	10 ± 5	18 ± 3
48	73 ± 4	70 ± 5	20 ± 3	18 ± 5	7 ± 3	12 ± 2

[a]Mean ± s.e.

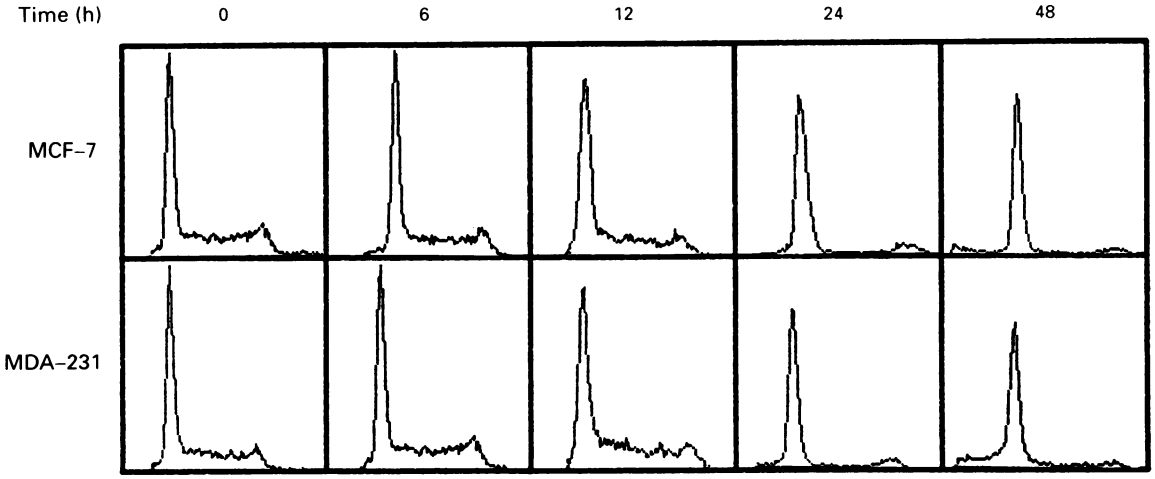

Figure 1 DNA histograms of MCF-7 and MDA-231 cells treated with 10 μM TAM for various periods of time. The results shown are typical of the five assays performed.

were treated at 37°C for 24 h with 10 μM TAM alone or with addition of anti-TGF-β_1 antibody (Oncogene Science). Cellular DNA from each treatment group was isolated from 5×10^6 cells. The cells were washed in PBS and the cell pellet was resuspended in 1.0 ml of 0.15 M sodium chloride, 0.015 M sodium citrate, 10 mM EDTA (pH 7.0), containing 1% (w/v) sodium lauryl sarkosinate and 0.5 mg ml^{-1} proteinase K. Proteolytic digestion was allowed to proceed at 50°C for 2 h. The DNA was precipitated with two volumes of absolute ethanol, resuspended in 10 mM Tris-HCl, 1 mM EDTA buffer (pH 8.0), heated to 70°C and loaded onto a 1.6% agarose gel. Pulse-field electrophoresis was carried out in 40 mM Tris-acetate, 1 mM EDTA (pH 8.0), until the marker dye had migrated 4–5 cm. The gels were stained with ethidium bromide and DNA was visualized under UV light.

Results

Effects of TAM on cell cycle kinetics

MCF-7 or MDA-231 cells were treated with 10 μM TAM for various periods of time and subjected to flow cytometric analysis. The results revealed that TAM treatment of 6 h resulted in an increase in cells entering S-phase (Table II). TAM treatment for ⩾ 24 h induced a time-dependent blockade in G_1/G_0 (Figure I, Table II). There was no significant difference between the cell cycle kinetic changes induced in the ER(+) MCF-7 and the ER(−) MDA-231 cell lines. Breast cancer cells were also treated with 1–50 μM TAM for 24 h. TAM also induced a dose-dependent blockade of MCF-7 and MDA-231 cells in G_1/G_0, and the dose-dependence and degree of the blockade were similar in both cell lines (data not shown).

Effects of TAM on TGF-β_1 protein determined by Western blot

Cytosolic protein was isolated from breast cancer cells treated with 10 μM TAM for up to 48 h, and the protein

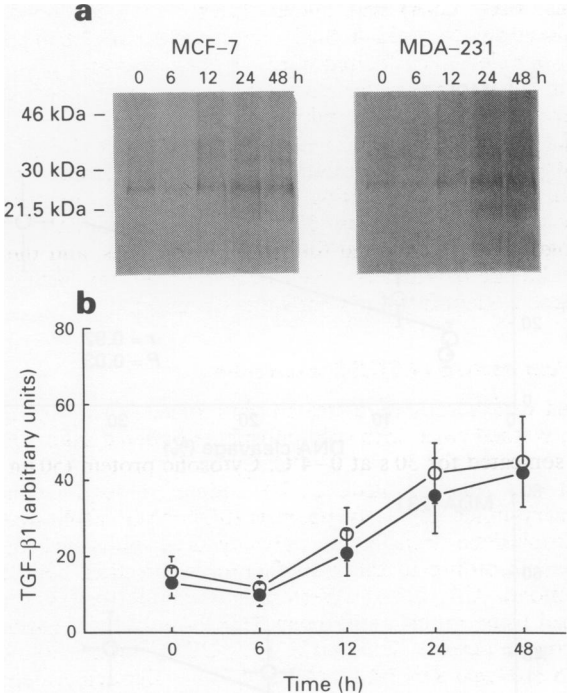

Figure 2 Effects of TAM on TGF-β_1 protein in MCF-7 and MDA-231 cells. (**a**) Cells were treated with 10 μM TAM for up to 48 h and subjected to Western blotting. (**b**) TGF-β_1 bands from four separate experiments quantified using laser densitometry, mean ± s.e. (○, MCF-7; ●, MDA-231).

subjected to electrophoretic separation and immunoblotting. The results revealed that TAM had a time-dependent effect on TGF-β_1 protein (Figure 2). There was no significant difference in the degree or time course of the TGF-β_1 induction between MCF-7 and MDA-231 cells. TAM treatment

for 6 h resulted in a slight but non-significant decrease of TGF-β_1 protein, accompanied by a slight increase in cell growth (Table I) and an increase in the number of cells entering S-phase (Table II). TAM treatment for 12–48 h resulted in a progressive increase of TGF-β_1 protein. This increase in TGF-β_1 protein preceded the G_1/G_0 block (Figure 1) and correlated with DNA cleavage (Figure 3). TAM also caused a dose-dependent induction of TGF-β_1 protein, which was similar in both cell lines (data not shown).

Effects of TAM on TGF-β_1 mRNA expression determined by Northern blot

Total cellular RNA from breast cancer cells treated with 10 μM TAM for up to 48 h was isolated and subjected to Northern blot hybridisation (Figure 4). There was no significant difference in relative abundance of TGF-β_1 transcripts between MCF-7 and MDA-231 cells under control conditions. TAM treatment increased mRNA expression in both cell lines, but the induction was faster in ER(+) MCF-7 cells, with maximal levels of TGF-β_1 mRNA reached by 6 h. Maximal levels of TGF-β_1 mRNA in ER(−) MDA-231 cells were reached by 12 h.

A variety of inhibition experiments were then performed to determine the effects on TAM-induced TGF-β_1 mRNA expression. Cells were incubated for 24 h with 10 μM TAM alone or with TAM and various inhibitors. Again, TAM significantly increased TGF-β_1 mRNA expression in MCF-7 (□) and MDA-231 (■) cells compared with saline-treated control cells (Figure 5). The addition of 10 μM 17β-oestradiol failed to block TAM induction of TGF-β_1 mRNA in either cell line, suggesting that TAM induction of TGF-β_1 is independent of ER. Inhibition of protein synthesis by cycloheximide significantly increased the accumulation of TAM-induced TGF-β_1 mRNA in both cell lines, suggesting that TAM may also induce protein(s) which negatively regulate

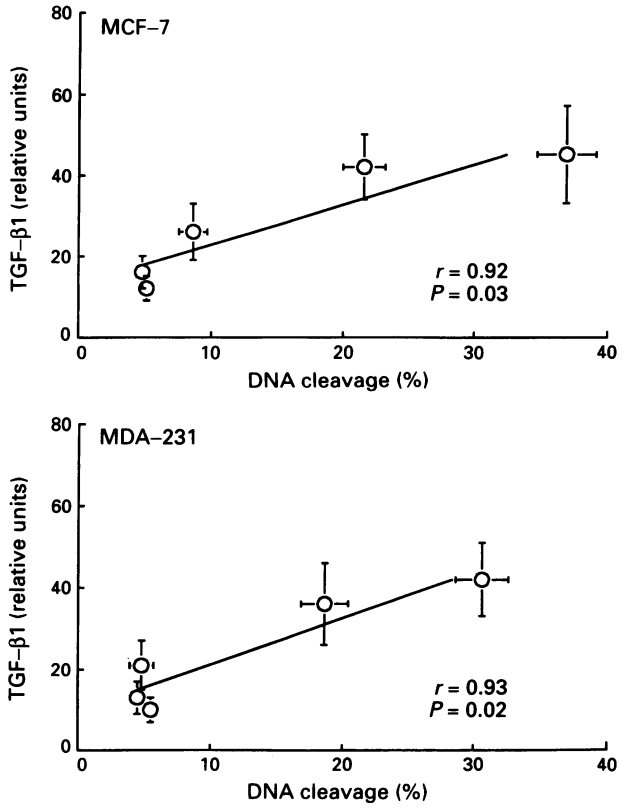

Figure 3 Correlation between TAM-induced TGF-β_1 protein expression and internucleosomal DNA cleavage. MCF-7 and MDA-231 cells were treated with 10 μM for up to 48 h. TGF-β_1 protein was determined by Western blotting as shown in Figure 2. DNA cleavage data is from Perry *et al.* (1995). Results shown are mean ± s.e. of four separate experiments.

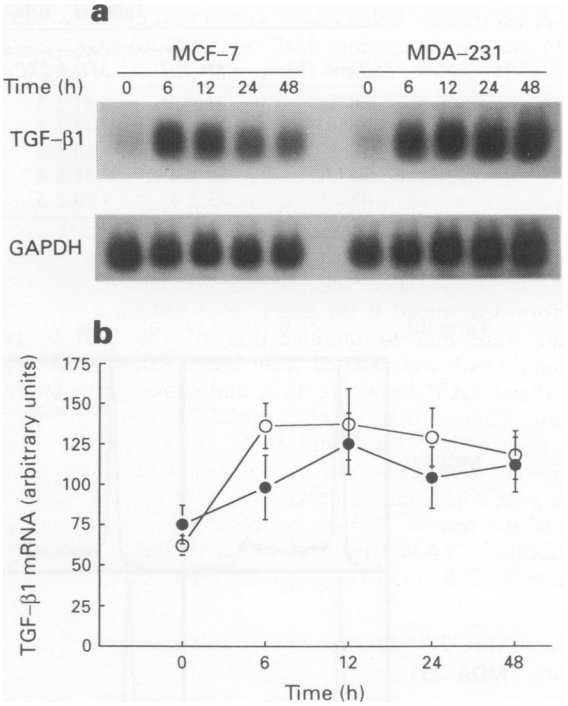

Figure 4 Effects of TAM on TGF-β_1 mRNA expression. (**a**) MCF-7 cells and MDA-231 cells were treated with 10 μM TAM for up to 48 h and subjected to Northern blotting. (**b**) Relative TGF-β_1 mRNA levels of four separate experiments quantified by laser densitometry and normalised to the GAPDH transcript, mean ± s.e. (○, MCF-7; ●, MDA-231).

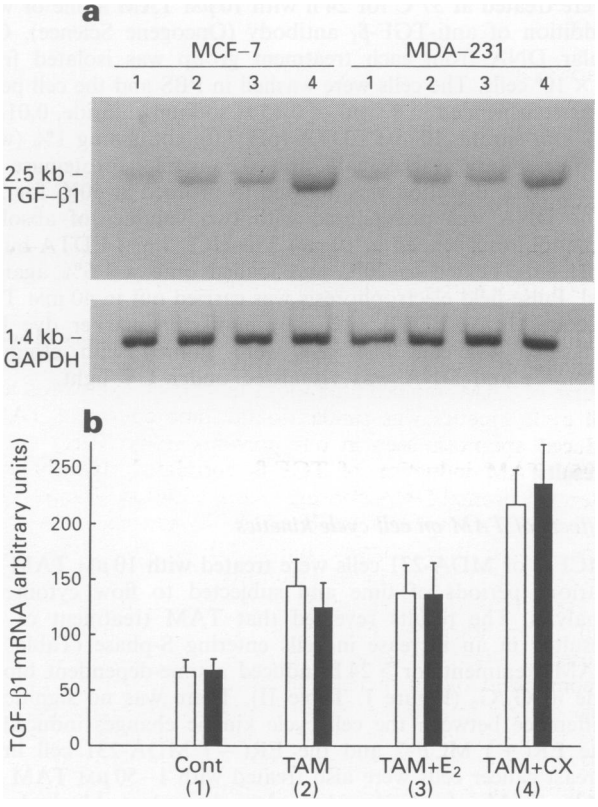

Figure 5 Effects of 17β-oestradiol and cycloheximide on TAM induction of TGF-β_1 mRNA expression. MCF-7 and MDA-231 cells were treated with TAM and various inhibitors for 24 h. (**a**) A representative Northern blot. Cells were incubated for 24 h with saline (lane 1), 10 μM TAM (lane 2), 10 μM TAM + 10 μM 17β-oestradiol (lane 3) or 10 μM TAM + 0.35 μM cycloheximide (lane 4). (**b**) Relative TGF-β_1 mRNA levels of four separate experiments quantified by laser densitometry and normalised to the GADPH transcript, mean ± s.e. (□, MCF-7; ■, MDA-231).

TGF-β_1 expression. Others have shown that cycloheximide in certain models can increase death-associated mRNAs (Owens *et al.*, 1991). Cycloheximide alone had no effect on TGF-β_1 expression (data not shown).

Effects of TAM on TGF-β_1 gene copy number

Because the TAM-induced increase in TGF-β_1 expression occurred over a relatively short time scale, it seemed unlikely that this increase was due to gene amplification. Nonetheless, TAM can rapidly effect cellular genes (Lau *et al.*, 1991). We therefore determined if the effects of TAM on TGF-β_1 expression were due to amplification of the TGF-β_1 gene. Genomic DNA was isolated from breast cancer cells treated with 10 μM TAM for up to 48 h, and subjected to Southern blotting. There was no significant difference in TGF-β_1 gene copy number in MCF-7 and MDA-231 cells under control conditions, and TAM had no significant effect on TGF-β_1 DNA gene copy number (data not shown). Thus, the TAM-induced increase in TGF-β_1 expression was not due to gene amplification. TAM treatment for up to 120 h also did not increase TGF-β_1 gene copy number (data not shown).

Effects of Anti-TGF-β_1 antibody on TAM-induced DNA cleavage

TAM induction of TGF-β_1 highly correlated with internucleosomal DNA cleavage (Figure 3). Therefore, the ability of TAM to induce internucleosomal DNA cleavage was determined in the presence or absence of anti-TGF-β_1 antibody. TAM alone induced DNA cleavage typical of apoptosis in MCF-7 and MDA-231 cells (Figure 6). The addition of an irrelevant isotype control antibody failed to inhibit TAM-induced DNA cleavage. However, the addition of anti-TGF-β_1 antibody inhibited TAM-induced DNA cleavage in both cell lines.

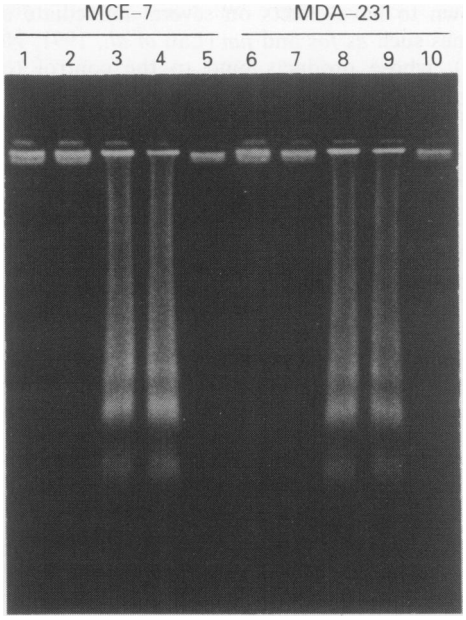

Figure 6 Effects of anti-TGF-β_1 antibody on TAM-induced DNA cleavage. MCF-7 and MDA-231 cells were treated with 10 μM TAM alone or along with antibody for 24 h. DNA was isolated and then subjected to agarose gel electrophoresis to measure DNA cleavage. Cells were treated with saline (lanes 1 and 6), 50 ng ml^{-1} anti-TGF-β_1 (lanes 2 and 7), 10 μM TAM (lanes 3 and 8), 10 μM TAM + 50 ng ml^{-1} IgG_{2a} (lanes 4 and 9), and 10 μM TAM + 50 ng ml^{-1} anti-TGF-β_1 (lanes 5 and 10). The results shown are typical of the four experiments performed.

Discussion

TAM has time- and dose-dependent effects on TGF-β_1 expression and cell cycle kinetics in MCF-7 and MDA-231 cells. The effects of TAM on cell cycle kinetics have been well described by our laboratory (Kang and Perry, 1993) and others (Osborne *et al.*, 1983). TAM blocks both MCF-7 and MDA-231 cells in G_1/G_0, indicating that the effect of TAM on cell cycle kinetics is independent of ER. The results of this study show a good correlation between TAM induction of TGF-β_1 and cell cycle blockade in G_1/G_0, which may at least partially explain the cytostatic effects of TAM. The time course of TAM-induced alterations in TGF-β_1 expression and cell cycle kinetics was similar to the time course of TAM-induced apoptosis seen in our previous study (Perry *et al.*, 1995). TAM induction of TGF-β_1 correlated strongly with internucleosomal DNA cleavage. Also, addition of anti-TGF-β_1 antibody completely inhibited TAM-induced DNA cleavage. These data suggest that TGF-β_1 is an important mediator of TAM-induced apoptosis.

The effects of TAM on TGF-β_1 expression are complex and independent of ER status. TAM treatment for 6 h caused a slight decrease in TGF-β_1 expression which was accompanied by an increase in cell growth and the percentage of cells in S-phase. This decrease in TGF-β_1 expression was probably caused at the translational level since TGF-β_1 mRNA levels were increased during this time interval. TAM treatment of $\geqslant$ 12 h caused an increase in TGF-β_1 expression which correlated highly with and preceded the observed G_1/G_0 arrest. This induction of TGF-β_1 was accompanied by a parallel increase in TGF-β_1 mRNA and occurred in the absence of TGF-β_1 gene amplification, suggesting TAM has regulatory effects at the transcriptional or post-transcriptional levels.

TAM induction of TGF-β_1 precedes apoptosis, since TGF-β_1 mRNA was induced by 6–12 h and DNA cleavage and apoptosis in our previous study was not observed until 12–24 h (Table I) (Perry *et al.*, 1995). In the present study addition of anti-TGF-β_1 antibody inhibited TAM-induced internucleosomal DNA cleavage (Figure 6), a strong indication that induction of TGF-β_1 by TAM triggers apoptosis. Cycloheximide treatment significantly increased accumulation of TAM-induced TGF-β_1 mRNA, an increase typically seen with death-associated mRNAs (Owens *et al.*, 1991). TGF-β_1 may trigger apoptosis by activation of signal transduction pathways (Rotello *et al.*, 1991), by effects on c-*myc* expression (Moses *et al.*, 1990; Taetle *et al.*, 1993), or by effects of G_1 phase cyclins (Mercer, 1993).

Although MCF-7 cells are slightly more sensitive to TAM-induced apoptosis (Perry *et al.*, 1995), in this study we found no significant difference between the two cells lines in the degree or time course of TAM induction of TGF-β_1. Oestrogen inhibits TAM-induced cytostasis and apoptosis in MCF-7 cells but not in MDA-231 cells, suggesting that TAM induces apoptosis via both ER-dependent and ER-independent pathways (Perry *et al.*, 1995). However, oestrogen did not inhibit TAM-induced TGF-β_1 expression in the present study, indicating that TAM may induce TGF-β_1 through an ER-independent mechanism.

TAM has previously been shown to elicit TGF-β_1 production in MCF-7 cells (Knabbe *et al.*, 1987), as well as from fibroblasts lacking ER (Colletta *et al.*, 1990). Low doses of TAM ($\leqslant$ 1 μM) induce TGF-β_1 expression even though there is no significant effect on TGF-β_1 mRNA expression (Knabbe *et al.*, 1987). Similar results have been shown by Arrick *et al.* (1990) in a panel of ER(+) and ER(−) cell lines, indicating that TAM at low doses may have a direct effect on TGF-β_1 translation. In the present study higher doses of TAM (10 μM) increased TGF-β_1 protein and mRNA expression in MCF-7 and MDA-231 cells. Thus, the effects of TAM on TGF-β_1 expression may be dose dependent as well as time dependent.

The precise mechanism whereby TAM effects TGF-β_1 expression is unknown. TAM has been shown to have a variety of effects on cellular genes which are gene specific, dose dependent, and tissue specific (Lau *et al.*, 1991). TAM has

been shown to have effects on several immediate early response genes such as *fos* and *jun* (Lau *et al.*, 1991; Nephew *et al.*, 1993), whose products bind to the control regions of genes containing activator protein 1 (AP-1) specific sequences. TAM has also been shown to inhibit growth factor induced AP-1-dependent transcriptional activity (Phillips *et al.*, 1993). AP-1 binds to phorbol ester-responsive elements which have been identified in the potential TGF-β_1 regulatory domain (Scotto *et al.*, 1990; Nutt *et al.*, 1991). In the present study TAM induction of TGF-β_1 mRNA was not inhibited by cycloheximide, suggesting that at least some of the effects of TAM on TGF-β_1 may be direct, rather than indirectly mediated through effects on other genes.

The results of this study along with our previous work suggest that TGF-β_1 may be an important mediator of TAM-induced cell cycle kinetic changes, cytostasis and apoptosis. The effects of TAM on TGF-β_1 expression appear to be independent of ER status and may be due to a direct regulatory effect on TGF-β_1 transcription. Whether these results will hold true in other models *in vitro* or *in vivo* remains to be determined. Understanding the mechanisms whereby TAM exerts anti-tumour activity may lead to the development of novel and potentially useful approaches for treating cancer.

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