Short report

Effect of transforming growth factor β_2 on oestrogen metabolism in the MCF-7 breast cancer cell line

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Abstract

Transforming growth factor β (TGF-β) is a multifunctional regulator of cellular growth and differentiation in many cell types and has a growth inhibitory effect on mammary epithelial cells. The TGF-β_2 isoform has been shown to be present in high concentrations in breast cyst fluid and might have a protective role in breast cancer. In addition, oestrogens play an important role in breast cancer development, and oestrone sulphate (E_1S) might be the main source of active oestrogens in the breast. The aim of this study was to assess the effect of TGF-β_2 on oestrogen synthesis in an attempt to understand the mechanism by which TGF-β_2 may exert a protective effect in breast cancer. In this study, higher concentrations of TGF-β_2 significantly inhibited the conversion of E_1S to oestrone (E_1) and the conversion of E_1 to the potent oestrogen, oestradiol (E_2). TGF-β_2 did not have any effect on MCF-7 cell growth or on E_2 to E_1 conversion. In conclusion, TGF-β_2 might exert a protective role in breast cancer by reducing the amount of active oestrogens present in the breast.

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Keywords: transforming growth factor β_2; oestrone sulphatase; oestradiol-17β hydroxysteroid dehydrogenase; breast cancer

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Transforming growth factor β (TGF-β) is involved in the regulation of cell growth and function, particularly during development and repair.[1] Studies showing the distribution of both TGF-β and its receptor in normal tissues have indicated that TGF-β is a crucial regulatory molecule in many physiological processes. It has potent proliferative and antiproliferative effects, depending on the cell type, and it modulates expression of cell function in a wide variety of in vitro systems.[1] Moreover, its growth inhibitory effect on epithelial cells suggests a potential protective role in breast cancer.[2]

Oestrogens have been shown to be closely associated with breast cancer development and their concentrations are much higher in breast tumour tissue than in blood.[3] The conversion of oestrone sulphate (E_1S) to oestrone (E_1) is regulated by oestrone sulphatase, whereas conversion of E_1, to the more potent oestrogen, oestradiol (E_2), is regulated by oestradiol-17β hydroxysteroid dehydrogenase (E_2DH). The aromatase enzyme complex converts androstenedione to E_1. E_1S is present in high concentrations in breast tissue and might act as an important source of active oestrogens in the breast.[4]

We have shown previously that the TGF-β_2 isoform is present in much higher concentrations (ranging from 0.3 to 125 μg/litre) than the TGF-β_1 isoform (ranging from < 0.34 to 25 μg/litre) in breast cyst fluid.[5]

The aim of our present study was to determine the effect of TGF-β_2 on the growth of MCF-7, an oestrogen receptor positive breast cancer cell line, and to test its effect on oestrogen metabolism, in an attempt to understand the protective role of this growth factor in oestrogen responsive breast cancers.

Methods

CELL CULTURE

The hormone dependent MCF-7 breast cancer cell line was a gift from Professor MJ Reed (unit of metabolic medicine, St Mary's Hospital Medical School, University of London).

MCF-7 human breast cancer cells were maintained in EMEM (3 ml), containing 20 mM HEPES, 5% foetal bovine serum (FBS), 2 mM L-glutamine, non-essential amino acids, and 0.075% $NaHCO_3$ and grown in 25 cm^2 tissue culture flasks. For the experiments, 25 cm^2 tissue culture flasks were seeded with approximately 1×10^5 cells/flask using the medium described above. The cells were allowed to grow for three days at 37°C in a 5% CO_2 incubator. The medium was then removed, and several different concentrations (0.5 ml) of TGF-β_2 and fresh EMEM (2.5 ml) containing 5% dextran charcoal stripped FBS, 20 mM HEPES, 2 mM L-glutamine, non-essential amino acids, and 0.075% $NaHCO_3$ were added. All treatments were performed in triplicate. MCF-7 cells were exposed to the treatment for 48 hours at 37°C in a 5% CO_2 incubator. At the end of the treatment period

the monolayers were assayed for E_1S or E_2DH activity and the number of cell nuclei counted on a coulter counter.

OESTRONE SULPHATASE ASSAY

For the oestrone sulphatase assay, intact monolayers of MCF-7 cells in triplicate 25 cm^2 tissue culture flasks were washed with phosphate buffered saline (PBS; 2.5 ml) and [^{3}H]-E_1S (5 pmol; 90 Ci/mmol) was added to flasks in 2.5 ml of phenol red free medium. Blank incubations, in which labelled steroid was added to cell free flasks, were carried out in parallel. After incubating the cells for four hours at 37°C, 1 ml of medium was pipetted into separate tubes containing [^{14}C]-E_1 (5×10^3 disintegrations/minute (dpm)), which were used for recovery correction. The mixture was shaken with toluene (5 ml) for 30 seconds. An aliquot (2 ml) of the organic phase was removed and both ^{3}H and ^{14}C activity were measured by liquid scintillation spectrometry. The mass of E_1S hydrolysed was calculated from the ^{3}H counts obtained, corrected for the volumes of the medium and organic phase used, and for recovery of [^{14}C]-E_1, and the specific activity of the substrate. Results were expressed as fmol/4 hour/10^6 cells.

E_2DH ASSAY

Cells were grown to at least 10^6 cells/flask before being assayed for E_2DH activity. Intact cell monolayers of MCF-7 cells in triplicate 25 cm^2 tissue culture flasks were washed with PBS at the end of the treatment period. E_2DH activity in both the reductive (E_1 to E_2) and oxidative (E_2 to E_1) direction was measured in the intact monolayers. [^{3}H]-E_1 (for reductive activity) or [^{3}H]-E_2 (for oxidative activity) was added in serum free medium (2.5 ml) to each flask (7×10^5 dpm) and incubated for four hours at 37°C. Medium (2 ml) was then removed from each flask and added to glass tubes containing 50 µl [^{14}C]-E_2 (for reductive activity) or [^{14}C]-E_1 (for oxidative activity) recovery labels (5×10^3 dpm) and unlabelled E_2 and E_1, respectively, at a concentration of 2 mg/ml in ethanol, as carrier steroids for thin layer chromatography and for visualisation of the position of the steroid product under UV light. The oestrogens were extracted into ether (3 ml), dried down under nitrogen, and separated by thin layer chromatography using dichloromethane/ethyl acetate (4/1 vol/vol) as solvent. The positions of the product (E_1 or E_2) were located by viewing under UV light. The product together with recovery label were eluted with methanol (0.5 ml) from strips cut from the thin layer chromatography plate. ^{3}H and ^{14}C activities were measured by liquid scintillation spectrometry. Results were expressed as fmol/4 hour/10^6 cells.

CELL COUNTING

MCF-7 cells were counted by release of cell nuclei. After washing cell monolayers with PBS, cells were incubated with HEPES buffer (20 mM containing 1.5 mM $MgCl_2$) and a lytic agent, Zap-oglobin (0.2 ml). After 10 minutes, the released cell nuclei were diluted with isotonic solution, Isoton, and an aliquot counted in the coulter counter. One flask in each batch was used to assess cell viability using the trypan blue exclusion method, and 95–98% of the cells were found to be viable.

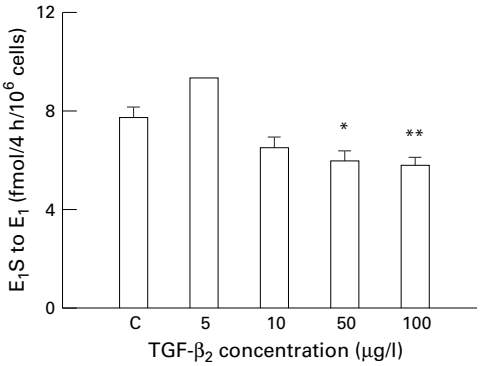

*Figure 1 Effect of transforming factor β_2 (TGF-β_2) on oestrone sulphate (E_1S) to oestrone (E_1) conversion in the MCF-7 breast cancer cell line. Values shown are means of triplicate determinations plus 1 SD. **$p < 0.01$; *$p < 0.05$. The standard deviation at 5 µg/litre was quite large (not shown in figure) and the E_1S to E_1 conversion at this concentration was not significantly different from the control.*

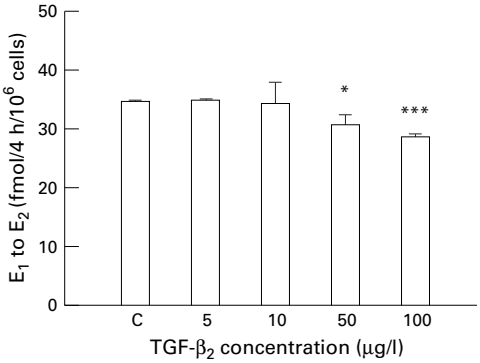

*Figure 2 Effect of transforming factor β_2 (TGF-β_2) on oestrone (E_1) to oestradiol (E_2) conversion in the MCF-7 breast cancer cell line. Values shown are means of triplicate determinations plus 1 SD. ***$p < 0.001$; *$p < 0.05$.*

STATISTICAL ANALYSIS

The effects of TGF-β_2 on cell growth and enzyme activities were compared with controls using the unpaired student's *t* test. Results were considered to be significant when $p < 0.05$.

Results

All cell culture experiments assessing the effect of TGF-β_2 on cell growth and oestrogen metabolism in the MCF-7 cell line were performed in triplicate. The higher concentrations of TGF-β_2 (50 and 100 µg/litre) significantly inhibited E_1S to E_1 (fig 1) and E_1 to E_2 conversion (fig 2) in the MCF-7 breast cancer cell line. TGF-β_2 did not have any effect on MCF-7 cell growth or on E_2 to E_1 conversion over the range of concentrations tested. Similar results were obtained when the experiments were repeated a further two times.

Discussion

Oestrogens have been shown to regulate the production of TGF-β in breast cancer cell lines. Treatment of oestrogen receptor positive

breast cancer cell lines with oestradiol resulted in decreased amounts of mRNA for TGF-β_2 and TGF-β_3, but did not affect values of mRNA for TGF-β_1.[6] In vitro studies have shown that tamoxifen, an antioestrogen, can induce autocrine secretion of TGF-β in human breast cancer cell lines, in which it acts as an inhibitor of growth.[7] Tamoxifen has also been shown to induce the secretion of active TGF-β_1 from human fibroblasts, despite the demonstrated absence of oestrogen receptors within these cells in vitro and in vivo.[8]

In our study, high concentrations of TGF-β_2 inhibited E_1S to E_1 and E_1 to E_2 conversion in the MCF-7 breast cancer cell line. TGF-β has been shown to have a growth inhibitory effect on the oestrogen receptor positive MCF-7 breast cancer cell line and oestrogen receptor negative breast cancer cell lines.[9] TGF-β_1 has been shown to be at least 10 times more potent than TGF-β_2 at inhibiting the growth of MCF-7 cells.[6] We found no significant effect of TGF-β_2 on MCF-7 cell growth over the concentrations tested. This difference might be the result of differences in the MCF-7 cell line used in different laboratories.

We found recently that TGF-β_1 inhibited MCF-7 cell growth in a dose dependent manner over the range of concentrations tested (0.1 to 8 μg/litre).[10] TGF-β_1 significantly stimulated the conversion of E_1S to E_1 over the range of concentrations tested (0.1 to 8 μg/litre) and significantly stimulated the conversion of E_1 to E_2 from 2 to 8 μg/litre. TGF-β_1 did not have any effect on E_2 to E_1 conversion. The effect of TGF-β_1 on E_1S to E_1 conversion and E_1 to E_2 conversion are opposite to those of TGF-β_2, and these effects of TGF-β_1 occur at lower concentrations than those of TGF-β_2. The TGF-β isoforms have been shown to be differentially regulated by E_2.[6] Conversely, we have shown that the TGF-β isoforms might have different effects on oestrogen synthesis.

The inhibitory effect of TGF-β_2 on E_1S to E_1 and on E_1 to E_2 conversion is a novel finding, and suggests that TGF-β_2 might reduce the amount of the potent oestrogen, E_2, present in the breast. In view of the role that oestrogens might have in the development of breast cancer, this action of TGF-β_2 could conceivably protect against the development of breast cancer.

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