Differential Antiestrogenic Effects of TCDD with Early And Late Passage T47D Human Breast Cancer Cells Cultured in Estrogen-Rich and Estrogen-Deprived Conditions

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Abstract

T47D human breast cancer cells express the estrogen and aryl hydrocarbon (Ah) receptors and, in low passage cells, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits the estrogen-induced proliferation of these cells. However, the long-term maintenance of T47D cells in different culture media resulted in cell populations with altered receptor levels and responsiveness to 17ß-estradiol, TCDD, and their combination. For example, T47D cells cultured for 6 to 12 months in 5% fetal bovine serum (FBS) and alpha-minimal essential medium (alpha-MEM) in the presence or absence of 17ß-estradiol (E_2) expressed relatively low levels of the estrogen receptor (ER) (36 to 155 fmol/mg) and elicited a time-dependent decrease in Ah receptor (AhR) levels (96 to 25 fmol/mg). The responsiveness of these late passage cells and early passage cells to E_2 was similar; however, TCDD was not an antiestrogen in the late passage cells. In T47D cells maintained on defined media (3% controlled processed serum replacement and 1% FBS in the absence of estrogens), there was a dramatic increase in ER binding (1095 fmol/mg) but this was not accompanied by an increase in estrogen responsive element (ERE) binding to nuclear extracts in a gel mobility shift assay. E_2 was not a mitogen in these cells and TCDD stimulated E_2-induced cell proliferation. When the cells were transiently transfected with an estrogen responsive plasmid (vit-CAT), TCDD only inhibited E_2-induced CAT activity in cells grown under normal conditions.

Introduction

Endocrine therapy has been extensively used for the treatment of breast cancer[1]; however, one of the major problems associated with this approach has been the development of hormone resistance[2-3]. In many cases, patients that are diagnosed with ER-positive tumors and are initially responsive to antiestrogens, later become refractory to such treatment. To circumvent such failures, antiestrogenic therapy mediated through a non-steroidal receptor, the AhR, has been proposed. This pathway surfaced from recent studies which reported that TCDD and related compounds exhibit a broad spectrum of antiestrogenic responses in the rodent uterus and human breast cancer cells in culture[4-7]. This suggests an interaction between the AhR and ER signal transduction pathways. Since breast cancer cells often become insensitive to endocrine therapy as they progress

towards hormone resistance, it was of interest to ascertain whether similar problems could arise with antiestrogenic responses mediated through the AhR.

T47D human breast cancer cells were chosen as the model cell line since they are highly enigmatic and have been reported to progress to a hormone resistant state[8-11]. By growing these cells under normal (5% FBS), estrogen-rich (5% FBS + 1 nM E_2) and estrogen-deprived (stripped serum; phenol red-free) conditions, different phenotypes of cells with altered characteristics were selected. The objective of this study was to determine whether TCDD would elicit the same antiestrogenic effects in the new T47D phenotypes as were observed in low passage cells grown under normal conditions.

Materials and Methods

Establishing Different Cell Models T47D human breast cancer cells were grown as monolayer cultures and passaged 1:2 every 3 to 5 days, using alpha-MEM supplemented with $NaHCO_3$, antibiotics and 5% FBS (Med-1). An aliquot of these cells were seeded into two separate growth media, an estrogen-rich media comprised of Med-1 plus 1 nM E_2 (Med-3) and an estrogen-deficient media comprised of DME/F12 media without phenol red, plus 0.01g/L apo-transferrin, 0.2 g/L BSA, 3% controlled processed serum replacement (CPSR-2) and 1% FBS (Med-2). Both sera were previously stripped twice with dextran-coated charcoal.

Nuclear Receptor Assays Nuclear estrogen, progesterone (PR) and aryl hydrocarbon receptor levels were quantitated by sucrose density gradient analysis as described[6].

Gel Retardation Analysis DNA binding of hER to complementary strands of synthetic oligonucleotides containing the ERE 5'-GTCCAAAGTCAGGTCACAGTGACCT GATCAAAGTT-3' was performed as previously described[12].

Cell Proliferation Assay Growth studies were performed as previously described[7].

Transient Gene Expression and CAT Assay The plasmid construct pA2(-821/+14)CAT, (vit-CAT), was a generous gift from Dr. G. Ryffel. T47D cells ($2x10^6$) were seeded in 10-cm plates using their respective maintenance media. After 14 h, the plates were washed and refed with Med-2. CAT assays were performed as described[13]. Cells were treated with either vehicle alone (0.2% EtOH), 10^{-8}M E_2, 10^{-8}M TCDD, or E_2 + TCDD for 48 h and harvested by scraping.

Statistics and Controls The statistical differences between different treatment groups were determined by ANOVA and the data were expressed as means ± standard deviation.

Results and Conclusions

The T47D cells were grown in culture for over a year using normal (Med-1), estrogen-deficient (Med-2) and estrogen-rich (Med-3) media. The results in Table 1 summarize the effects of long term growth of T47D cells in different media on nuclear ER, PR, and AhR binding activity. Low passage (L) and high passage (H) cells maintained in Med-1 or Med-3 (H[E+]) expressed relatively low levels of ER. In contrast, T47D cells grown in estrogen-deprived media (H[E-]) expressed 10x more ER. Under all growth conditions, extended culture of the cells resulted in a slight increase in PR levels and a decrease in AhR levels.

The nuclear ER levels were also characterized by their binding to a [^{32}P]ERE in a gel mobility shift assay. The amount of cpm incorporated into the retarded ER-ERE band and the relative complex formation from E_2-treated samples were: H cells, 229.2±5.51

(57.5%); H[E-] cells, 118.7±8.42 (29.8%); H[E+] cells, 112.8±13.6 (28.3%); L cells, 398.8±23.8 (100%). The highest levels of ERE binding by nuclear extracts were from L cells whereas the formation of the retarded band was significantly decreased in high passage cells grown in estrogen-rich or estrogen-deprived media.

The effects of E_2, TCDD, and their combination were evaluated in a 7-day cell proliferation assay and the results are summarized in Table 2. E_2 significantly increased cell proliferation in L, H, and H[E+] cells, but had no effect on H[E-] cells. TCDD only inhibited E_2 induced growth in low passage cells. Surprisingly, there was an enhancement of growth in H[E-] cells cotreated with TCDD plus E_2.

The functional activity of E_2, TCDD and their combination was also investigated in T47D cells transfected with the vit-CAT plasmid which contains the estrogen-inducible vitellogenin 5'-flanking region and a CAT reporter gene. The results in Table 3 indicate that 10 nM E_2 significantly induced CAT activity in low and high passage cells whereas TCDD had no significant effects on CAT activity. In cotreatment studies, TCDD significantly inhibited E_2-induced CAT activity only in H and L cells grown in unstripped FBS.

This study suggests that the antiestrogenic effects mediated by TCDD varied with changes in the characteristics of the cells. After extended culture, the cellular AhR levels decreased, as did their TCDD-responsiveness. The mechanism through which TCDD stimulated cell growth in late passage cells requires further investigation. (Supported by the National Institute of Health, ES04176)

Table 1. Nuclear ER, PR, and AhR Levels in Early and Late Passage T47D Cells Cultured in Different Media

Cell Groups (culture media)	~ Months in Culture	Receptor Levels (fmol/mg) ER	PR	AhR
L --> H (Med-1)	3-6	81±3.4	637±118	55±8
	9-12	155±16	1036±7.6	39±4
H[E-] (Med-2)	3-6	1106±65	979±114	66±10
	9-12	1229±103	1444±176	30±5
H[E+] (Med-3)	3-6	36±5	798±148	96±13
	9-12	83±16	1095±194	25±9

Table 2. Effects of 17ß-Estradiol, TCDD and their Combination on the Proliferation of Early and Late Passage T47D Cells

Treatments	T47D Cell Groups (mo. in culture) L (0.5)	H (23.5)	H[E+] (22.5)	H[E-] (23)
	% control	% control	% control	% control
.2% EtOH	100±2.55	100±3.09	100±1.52	100±4.56
10 nM TCDD	111± 10.2	200±13.8*	140±7.31*	137±4.18*
10 nM E_2	244±8.25*	281±2.58*	260±16.2*	67±2.74*
E_2 + TCDD	205±1.93^	328±5.05^	280±8.73^	145±2.08^

* Significantly different ($p<0.5$) than control cells
^ Significantly different ($p<0.5$) than E_2-treated cells

Table 3. Effects of 17ß-Estradiol, TCDD and their Combination on CAT Activity in Early and Late Passage T47D Cells Transiently Transfected the VitA2 Plasmid

Treatments	T47D Cell Groups (Mo. in culture)			
	L (4)	H (21)	H[E+] (20)	H[E-] (20.5)
	% control	% control	% control	% control
.2% EtOH	100±27.1	100±25.8	100±20.4	100±25.6
10 nM TCDD	56±19.7*	59.7±12.1	70.5±19.9	122±33
10 nM E_2	272±34*	262±16.7*	229±60	485±175*
E_2 + TCDD	148±21.7^	172±44.7^	201±77.3	428±150

* Significantly different ($p<0.5$) than control cells
^ Significantly different ($p<0.5$) than E_2-treated cells

References

1. Muss HB. *Breast Cancer Res Treat* 1992;21:15-26.
2. McGuire WL, Lippman ME, Osborne CK, Thompson EB. *Breast Cancer Res Treat* 1987;9:165-173.
3. Clarke R, Brenner N, Katzenellenbogen BS, Thompson EW, Normal MJ, Koppi C, Paik S, Lippman ME, Dickson RB. *Proc Natl Acad Sci USA* 1989;86:3649-3653.
4. Gallo MA, Hesse EJ, MacDonald GJ, Umbreit TH. *Toxicol Lett* 1986;32:123-132.
5. Gierthy JF, Lincoln DW, Gillespie MB, Seeger JI, Martinez HL, Dickerman HW, Kumar SA. *Cancer Res* 1987;47:6198-6203.
6. Harris M, Zacharewski T, Safe S. *Cancer Res* 1990;50:3579-3584.
7. Fernandez P and Safe S. *Toxicol Lett* 1992;61:185-197.
8. Murphy CS, Pink JJ, Jordan VC. *Cancer Res* 1990;50:7285-7292.
9. Graham ML, Krett NJ, Miller LA, Leslie KK, Gordon DF, Wood WM, Wei LL, Horwitz KB. *Cancer Res* 1990;50:6208-6217.
10. Murphy CS, Meisner LF, Wu SQ, Jordan VC. *Eur J Canc Clin Oncol* 1989;25:1777-1788.
11. Daly RJ and Darbe PD. *Cancer Res* 1990;50:5808-5875.
12. Kumar V and Chambon P. *Cell* 1988;55:145-156.
13. Gorman CM, Moffat LF, Howard BH. *Molecular and Cell Biol* 1982;2:1044-1051.