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SHORT COMMUNICATION

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Tamoxifen-induced estrogen receptor up-regulation in mammary tumor cells is not related to growth inhibition

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Abstract Treatment of estrogen receptor (ER)-positive mammary tumors with tamoxifen produces a dramatic accumulation of ER in the cell nucleus. We investigated whether this phenomenon might be related to the antitumor activity of the drug. Five tamoxifen derivatives for which an influence on MCF-7 cell growth had previously been established were tested for that purpose; two of them inhibited growth, one was growth-stimulatory, and the remaining two were without significant effect. At 1 μ*M* all compounds up-regulated ER in the cell nucleus after 3 days of culture, suggesting that the ER accumulation does not predict the response to tamoxifen treatment. An analysis of a tamoxifen-resistant clone (RT×6 cells) under similar experimental conditions led to the same conclusion: the ER level significantly increased in the presence of tamoxifen and its 4-hydroxy metabolite.

Key words Mammary tumor · Estrogen receptor level · Tamoxifen · MCF-7 and RT×6 cells

Introduction

Considerable attention has been given to understanding the mechanism of action of tamoxifen due to its ability to reduce the likelihood of recurrence in operable breast cancer patients, especially when their tumors are estrogen-receptor (ER)-positive. Our own studies on MCF-7 cells have revealed that tamoxifen as well as its metabolite 4-hydroxytamoxifen accumulate the receptor in the cell nucleus [5, 7] by increasing its half-life [2] (the balance between ER synthesis and degradation seems to be in favor of the former). Interestingly, such an ER up-regulation as detected by immunoenzymatic measurement of the receptor has also been noted in a few clinical studies [10, 12]; this led us to investigate whether the detection of high ER levels during tamoxifen therapy might predict the response to the drug. ER accumulation may indeed alter the transcription of target genes involved in growth control (squelching).

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Materials and methods

Tamoxifen derivatives

Tamoxifen and its 4-hydroxy metabolite were obtained from Zeneca (Macclesfield, UK). Five other tamoxifen derivatives (for formulas, see Table 1) were provided by Prof. M. Jarman (Institute of Cancer Research, Sutton, UK). Their influence on MCF-7 cell growth has previously been established in our laboratory [1, 4, 9]. Two of them inhibited growth (compounds 2 and 3) as did tamoxifen; one had a stimulatory action (compound 4); and the remaining two were without significant effect (compounds 5 and 6). The relative binding affinity (RBA) for ER of most of these five compounds was similar to that of tamoxifen; only compound 6 displayed a ~10-fold lower binding affinity.

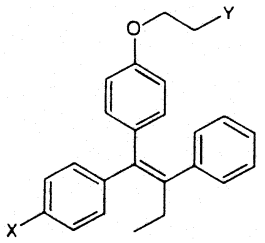
Cell and culture materials

MCF-7 cells (from the Michigan Cancer Foundation, Detroit, Mich.) are maintained in our laboratory in monolayer culture at 37 °C in Earle's base minimum essential medium (MEM) containing 10% heat-inactivated fetal calf serum (56 °C, 1 h), L-glutamine, penicillin, streptomycin, and gentamicin at the usual concentrations (all materials were obtained from Gibco, Gent, Belgium). Tamoxifen-resistant RT×6 cells (an MCF-7 variant clone obtained from Dr. J. C. Faye, INSERM U 168, Toulouse, France) are maintained under the same conditions in RPMI medium in the presence of 1 μ*M* tamoxifen, which is removed before each experiment.

ER assays

The effect of tamoxifen and its derivatives on ER levels was assessed in MCF-7 cells by measurement of cytosolic and nuclear receptor concentrations after 3 days of culture in their presence as previously described [7]. ER concentrations measured by enzyme immunoassay (ER-EIA, Abbott) were expressed as the percentage of the value established for untreated cells; control [^{3}H]-E_2-binding assays were run in parallel [7, 8].

Table 1 MCF-7 cells – absence of a relationship between ER up-regulation and growth inhibition



X	Y	y	RBA[a] (E_2 = 100)	ER level[b] (% of control)		MCF-7 cell growth[a]
				Cytosol	Nuclear extract	
1	– H	– NMe_2	1	96	663	Inhibition
2	– SMe	– NMe_2	3	102	927	Inhibiton
3	– H	– $Me_2N \rightarrow O$	1	94	508	Inhibition
4	– SH	– NMe_2	1	132	382	Stimulation
5	– H	– $N(Me)COCH_3$	1	92	664	No effect
6	– H	– $N(Me)COCF_3$	≤0.1	64	425	No effect

[a] RBA values and growth-inhibition data were taken from previous studies (1, 4, 9)
[b] Control ER concentration (fmol/mg protein) established by enzyme immunoassay: cytosol = 562, nuclear extract = 528

Results and discussion

Table 1 shows that at the concentration of 1 μ*M*, all compounds tested up-regulated ER in the cell nucleus (nuclear extracts), as did tamoxifen (compound 1). As expected [8], cytosol from treated cells showed a decrease in specific [^{3}H]-E_2-binding capacity (data not shown). The up-regulation efficiency varied among compounds without any relationship to their ability to regulate growth. Hence, ER accumulation did not appear to predict the response to tamoxifen.

The results of tamoxifen treatment of RT× 6 tamoxifen-resistant cells [3] went in the same direction. ER levels significantly increased after 3 days of culture in the presence of the drug or its 4-hydroxy metabolite, albeit to a lesser extent than in control MCF-7 cells (Table 2). Long-term maintenance of RT×6 cells with 1 μ*M* tamoxifen, which is required to sustain their resistance, may explain this difference (removal of tamoxifen just before experiments); the nuclear ER level found in untreated cells exceeded that measured in control MCF-7 cells (nuclear extract/cytosol ratio: RT×6 5.44, MCF-7 1.16).

These data clearly indicate the absence of a relationship between ER up-regulation and in vitro growth blockade during tamoxifen treatment. This conclusion should be applicable for steroidal analogues of tamoxifen (i. e., RU 39411, RU 45144 [11]), which also accumulate ER in MCF-7 cells [6]. Although up-regulation may not have any influence on mammary tumor growth in cell culture, the estrogen sensitivity of these treated cells may be altered in such a way that tumor development under in vivo conditions may occur. Experiments conducted on mammary-tumor-bearing animals as well as clinical correlation studies may be informative in this regard.

Table 2 UP-regulation of ER in MCF-7 and RT×6 tamoxifen-resistant variant cells[a]

Drug	EIA in MCF-7 cells (fmol/mg protein)		EIA in RT×6 cells (fmol/mg protein)	
	Cytosol	Nuclear extract	Cytosol	Nuclear extract
None (CTR)	666	773	219	1,192
OH-Tamoxifen (0.1 μ*M*)	1,117	12,615	164	2,927
Tamoxifen (1 μ*M*)	819	7,593	145	1,852

[a] Control DNA contents after 5 days of culture in the presence or absence of tamoxifen at 1 μ*M* (μg DNA/culture well) – MCF-7: CTR 19.7 ± 0.9, Tamoxifen 9.8 ± 0.8; RT×6: CTR 10.8 ± 1.9, Tamoxifen 10.4 ± 0.8

Retrospective analysis of our data revealed that the nuclear ER up-regulation observed during tamoxifen treatment varied slightly among experiments (MCF-7 cells: 5.9- to 10.0-fold increase, $\bar{x}$ = 8.1-fold increase, n = 8; RT×6 cells: 1.5- to 3.0-fold increase, $\bar{x}$ = 2.3-fold increase, n = 4). Such a property should logically hold for all compounds recorded in Table 1 since it was also detected in the presence of hydroxytamoxifen. The origin of this variation as well as its potential biological (endocrinological) significance is unknown. Our present data indicate solely the absence of a relationship between up-regulation of ER and in vitro growth regulation.

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