Journal of Cellular Biochemistry 36:83–89 (1988)
Steroid Hormone Action 101–107

Role of Receptor Occupancy in the Transition From Responsive to Unresponsive States in Cultured Breast Tumor Cells

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Progression from a steroid sensitive to insensitive state is characteristic of breast tumors, but little is known about the molecular mechanisms involved. Changes in steroid receptor can be associated with the progression. This paper reviews the cell culture data pertaining to loss of response and concludes that loss of receptor is a consequence rather than a cause of insensitivity. This view is based on evidence that loss of all response parameters occurs despite the presence of fully functional receptors as determined by transfection experiments. The postreceptor defect appears to be at the level of the hormone response element of the responsive genes and may involve DNA methylation. The implications of the model for human breast cancer biology are discussed.

Key words: breast cancer, steroid sensitivity/insensitivity, receptor phenotypes, transfection, cell biology, tissue culture, mouse mammary tumor virus (MMTV), DNA methylation

Receptors for steroid hormones have rightly been assigned a prime role in the actions of these endocrine agents. Based on data indicating that appropriate receptors were present in responsive cells and absent in unresponsive cells, receptor analyses are now used to decide treatment of patients with breast and other steroid-related tumors. Implicit in most models of tumor receptor content and hormone sensitivity is the concept that loss of receptor is a fundamental event in the progression from the responsive to unresponsive state. That idea should now be questioned on the basis that loss of receptor may be a consequence and not a cause of insensitivity. We have been studying the transition from responsive to unresponsive state in cultured mammary tumor cell lines [1–3] and have obtained data indicating that, in at least some cases, insensitivity results from changes in transcription in the face of fully functional receptors. These data will be reviewed in the context of the points presented above.

Received February 19, 1987; revised and accepted July 20, 1987.

GENERAL BACKGROUND

We have mostly used the cell line (S115) derived from a spontaneous mouse mammary tumor. Androgens (testosterone) stimulate log-phase growth of these cells but also have major effects on many cell biological features such as density regulation, anchorage independence, and morphology. From these studies we have developed the model (Fig. 1) that, in cell biological terms, androgens convert S115 cells from a normal to transformed phenotype [1,3–6]. This may be a general phenomenon as compatible data have been published for estrogen/human breast cancer cells [7–9], 1α,25 dihydroxyvitamin D/mouse epidermal cells [10], and estrogen/human endometrial cancer HEC-11 cells [11].

The molecular mechanisms by which these effects occur are ill understood but are unlikely to be simple. At least three pathways can be envisaged (Fig. 1), alteration of membrane sensitivity to external agents, autocrine loops, and internal changes. We have been analysing the possibility that 16S mRNA coded mainly from the long terminal repeat (LTR) of mouse mammary tumor virus (MMTV) mediates at least some of these responses. It has been hypothesised that this message codes for a growth regulatory protein [12,13]. By comparing the androgen and glucocorticoid (dexamethasone) effects on cell proliferation, saturation density, anchorage-independent growth, and morphology with those on 16S mRNA induction we have concluded that this RNA could mediate steroid effects on density regulation and/or anchorage-independent growth but not log-phase proliferation in monolayer culture [14]. However, these data are correlative and we have as yet no direct evidence for a causative role for this mRNA. It is currently being cloned and characterised to further define its properties [15,16].

Regardless of whether or not this 16S mRNA is an intermediate in steroid-mediated growth, it is a sensitive marker of both androgen and glucocorticoid responsiveness of these cells [17,18]. Furthermore, both classes of steroid mediate their effects by receptor-related events [17,19] directly on the gene, as cycloheximide does not prevent inducibility of either the 16S mRNA [17] or RNA's transcribed from steroid-sensitive chimaeric genes stably transfected into S115 cells [20].

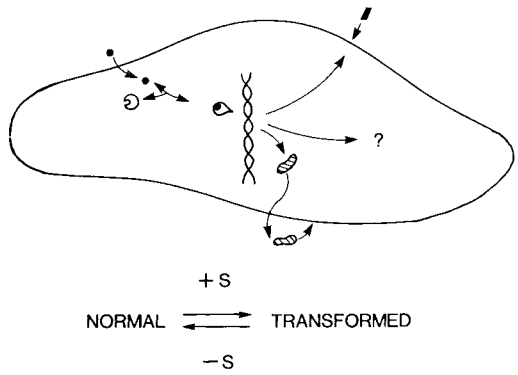

Fig. 1. Steroid effects on cultured cells. Steroids (S, ●), by combining with their specific intracellular receptors (ℰ), initiate multiple events that culminate in the generation of a transformed phenotype. These processes are reversed on removal of steroid. The changes in behaviour are mediated by autocrine or paracrine loops ( ), by altering membrane sensitivity to external agents ( ), and by other unknown mechanisms (?).

TRANSITION FROM RESPONSIVE TO UNRESPONSIVE STATE

When cultured in the absence of androgen, the S115 cells rapidly lose their sensitivity to both androgens and glucocorticoids [1,18]. The addition of either androgen (Fig. 2) or glucocorticoid (Fig. 3) prevents this transition [18]. These changes occur so rapidly that it would appear to be an epigenetic phenomenon. Preliminary data with the estrogen-sensitive human, ZR-75-1 breast cancer cell line indicate that a similar phenomenon may occur with these cells [7, and data not shown].

All of the cell biological features we have studied (proliferation, saturation density, morphology, anchorage independence) lose their steroid sensitivity in a reproducible and ordered sequence [3–5]. Two types of unresponsive state, a facultative and constitutive form, can be distinguished based on the ability to reverse the S115 cells to a hormone-sensitive state (Fig. 2). The facultative state can be reversed to full steroid sensitivity by prolonged exposure to androgens, whereas the constitutively unresponsive cells will not so change [3]; 16S mRNA inducibility follows the same pattern (Fig. 4). The facultatively unresponsive cells grow more slowly than their responsive antecedents (minus testosterone), but transition to constitutive unresponsiveness is associated with more rapid proliferation [3].

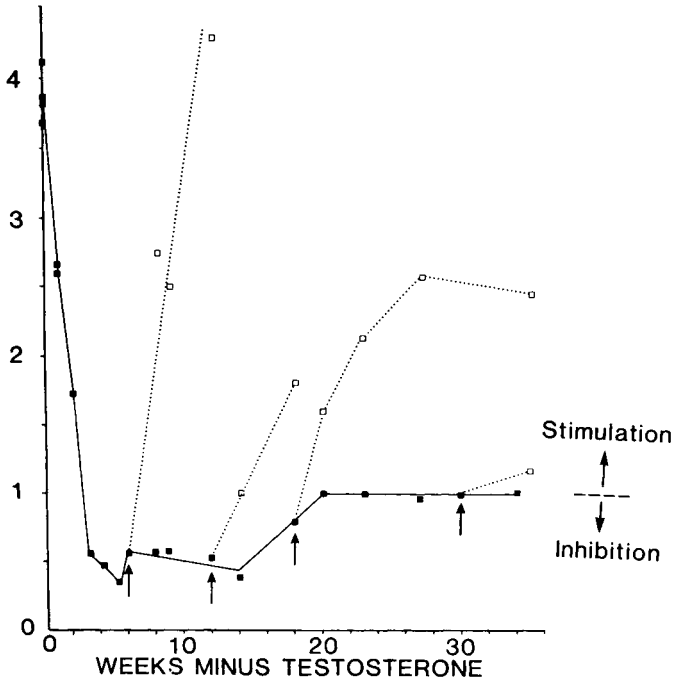

Fig. 2. Loss of steroid sensitivity of S115 mouse mammary tumor cells following prolonged withdrawal of androgen (testosterone). Effects on saturation density are presented. For other parameters consult Darbre and King [3] and Yates and King [4]. Stock cells were deprived of testosterone for the time stated in the ordinate, and at appropriate times (■) an aliquot of cells was tested for androgen sensitivity by a 1-wk growth curve in the presence and absence of testosterone. Results are expressed as a ratio of cell number plus (+) and minus (−) testosterone (T) (solid line). In some cases (dotted line), stock cells were reexposed to androgen for varying periods before testing for androgen sensitivity (□). Adapted from Darbre and King [3].

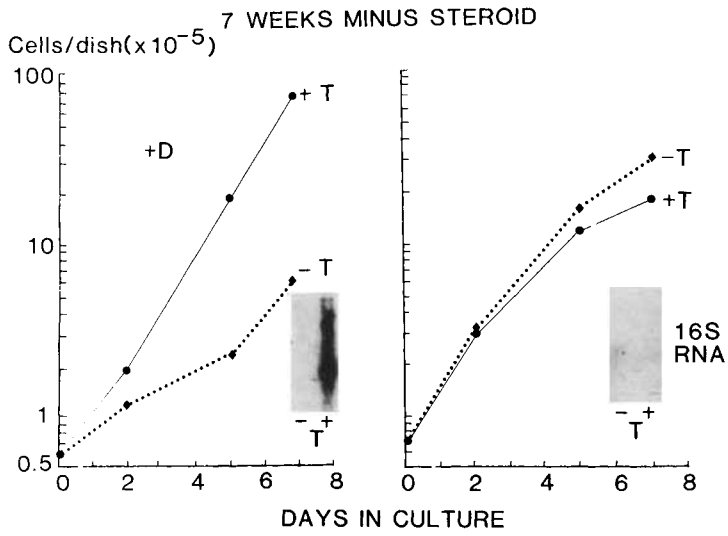

Fig. 3. Glucocorticoids (dexamethasone, D) prevent the loss of androgen (testosterone, T) responsiveness of S115 cells. Responsive cells were grown without added steroid (right-hand panel) or with dexamethasone (left-hand panel). Growth curves were then measured plus and minus testosterone. LTR-related 16S RNA was assayed at the same time (inset). Data from Darbre and King [18].

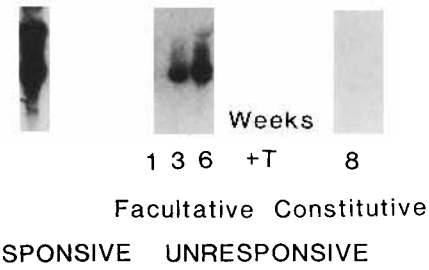

Fig. 4. Testosterone (T) inducibility of LTR-related 16S RNA. RNA was analysed from responsive cells grown continually in the presence of testosterone (responsive) and from responsive cells that had been deprived of testosterone for 10 wk (facultative) or 30 wk (constitutive) to generate the unresponsive state. Testosterone was added back for the stated number of weeks (+T). Data from Darbre and King [3] and Darbre et al [17].

The generation of unresponsiveness does not involve changes of receptor number or functionality. Androgen [19] and glucocorticoid [20] receptor numbers are approximately the same in responsive and unresponsive cells. These data were based on ligand binding assays, which gave no information as to whether or not the receptors are functional. However, transfection of constitutively unresponsive S115 cells with a marker gene (C3(1) gene from rat prostate) controlled by regulatory and promotor sequences from MMTV clearly show that both the androgen and glucocorticoid receptors remain functional in the face of continuing unresponsiveness of the growth and 16S mRNA response (Fig. 5). If these transfected cells are deprived of steroid, the transfected genes become insensitive. As some clones of transfected cells contain many hundred copies of chimaeric genes, it follows that all gene copies are subject to the deactivating mechanism. The idea that many steroid-sensitive genes are concomitantly desensitised is also compatible with the picture seen in the untransfected cells.

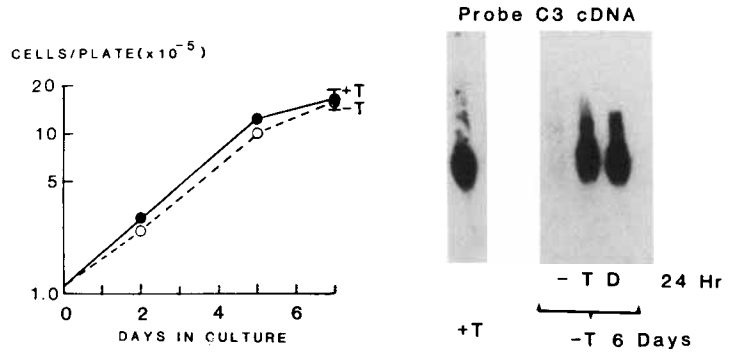

Fig. 5. Testosterone (T) and dexamethasone (D) receptor function in constitutively unresponsive S115 cells. Long-term deprived cells were transfected with LTR-C3-pSV2gpt vector and mycophenolic acid resistant clones selected and amplified. Proliferation remained insensitive to testosterone (left-hand panel), whereas the transfected gene plus its hormone response element was sensitive to both steroids (right-hand panel).

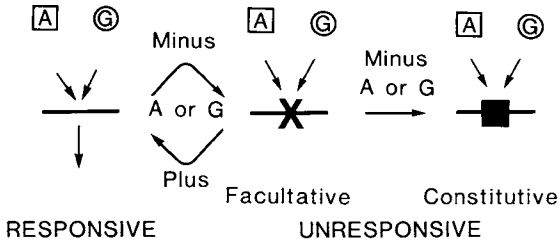

Fig. 6. Model showing how androgen (A) and glucocorticoid (G) could influence sensitivity to either steroid through their specific receptors (□, ○). Initial changes in the transcriptional sensitivity (X) are reversible, whereas later changes (■) are not.

All of the cell biological features are desensitised despite the fact that more than one gene must be involved (see above).

As the receptors are functional, whereas an induction involving direct interaction of receptor with endogenous hormone response elements of genes are not [20,21], it is possible to pinpoint the defect in the unresponsive cells to hormone response elements. We do not know if all such elements are inactive but there must be some specificity about the desensitising mechanism otherwise all genes would be switched off and the cells would die. In fact, the converse is true for the constitutively unresponsive cells as they grow faster than their responsive counterparts in the absence of androgen [3].

The transition to constitutive unresponsiveness is accompanied by increased methylation of LTR sequences as judged by isoschizomeric restriction enzyme analysis. However, this relatively insensitive method of assessing methylation patterns detected no differences between LTR's from responsive and facultatively unresponsive cells [3].

Our working model to explain the S115 data is presented in Figure 6. Either androgen or glucocorticoid can maintain the fully responsive state. There are several ways in which this might be achieved. The two most obvious being (a) the inactivating mechanism is switched off or reversed by a steroid-sensitive process and (b) the

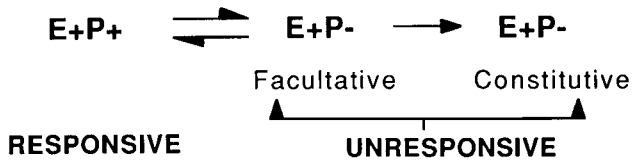

Fig. 7. Hypothetical model, based on Figure 6, for estradiol (E) and progesterone (P) receptor phenotypes of human breast tumors.

binding of steroid receptor complex to the hormone response element protects that site in a way that the receptor minus steroid cannot achieve.

HUMAN BREAST CANCER

It is important that the ideas outlined in the preceding section are tested for their validity in human breast cancer systems because important practical consequences ensue. Our preliminary data with the ZR-75-1 cells (see above) plus reports that other human breast cancer cell lines exhibit features of hormone insensitivity when cultured without estrogen [7–9] suggest that the model does have relevance beyond that of murine mammary tumors. The fact that loss of response of human cells in estrogen-depleted medium has not been identified previously may be due to the presence of the weak estrogen phenol red in all the media.

Based on the model shown in Figure 6, the three major estradiol and progesterone receptor phenotypes of human breast tumors could be redefined as shown in Figure 7. The main points are that there is a progression from one phenotype to another, there are two categories of ER+PR− tumors.

Tumors with an ER+PR− phenotype are known to be heterogeneous and have an approximately 30% response rate to hormone therapy [22]. Furthermore, some of these tumors can be converted to the ER+PR+ category by estrogen treatment [23]. This has been interpreted as being due to stimulation of PR by standard transcriptional/translational mechanisms but is equally compatible with the idea of gene reactivation as occurs with the S115 cells.

We have not demonstrated loss of receptor in our experimental systems but would speculate that loss of this gene product(s) could occur as a further step in the progression pathway.

If, as this model predicts, receptor loss is a consequence of other changes, it has interesting implications at both the laboratory and clinical level. By focusing attention on hormone response elements of the genome as the site of post-receptor defects, it narrows the area of investigation. Furthermore, defective receptor proteins or mRNAs would not necessarily be found if receptor changes are not a central event for the transition to insensitivity in these tumors; modifications at the DNA level may well occur. Characterisation of the biochemical events involved in desensitisation of the genome should help to better define the clinical responsiveness of the ER+PR− tumors and might indicate potential therapeutic routes for reverting unresponsive tumors to the responsive state.

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