

Molecular Mechanisms of Resistance to Tamoxifen Therapy in Breast Cancer

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Clinical data suggest that the use of adjuvant tamoxifen citrate (Nolvadex) for a minimum of 5 years, and possibly indefinitely, will result in maximal antitumor benefit. There is concern that long-term tamoxifen maintenance therapy may result in the induction of drug resistance. This article reviews the potential molecular mechanisms of resistance to tamoxifen and explores the possibility of tamoxifen-stimulated tumor growth.

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There are more than 4.5 million women years of experience with tamoxifen (Nolvadex) for the treatment of breast cancer. During the past two decades, the initial application of tamoxifen as a palliative therapy for the treatment of stage IV breast cancer has expanded to establish this antiestrogen as the endocrine treatment of choice for all stages of breast cancer. Indeed, the fact that adjunct tamoxifen produces a survival advantage in both node-positive and node-negative breast cancer and also reduces the incidence of second primary breast cancers by up to 40%¹ has increased enthusiasm to test the worth of tamoxifen to prevent breast cancer in normal women.²

Tamoxifen has a low incidence of side effects that have resulted in a tendency to administer therapy for more than 5 years. Tamoxifen also has some positive estrogen-like effects that maintain bone density³ and reduce the incidence of fatal myocardial infarction.⁴ Tamoxifen maintenance therapy can clearly be advantageous to patients with node-negative breast cancer as a hormone replacement therapy, but indefinite treatment of patients with stage I and II cancer

raises the specter of rapidly progressing disease when drug resistance develops.

By the end of the 20th century, between 400 000 and 500 000 women in the United States could be taking tamoxifen to treat or prevent breast cancer. On a worldwide basis, this could be millions of women. It is clearly time to review the potential mechanisms of drug failure so that women can be treated successfully on a longer treatment regimen. At present, we have no definitive data about the clinical expression of drug resistance to tamoxifen during indefinite therapy because the clinical trials have not been completed. It is therefore appropriate to focus attention on this aspect of the actions of tamoxifen so that suitable strategies can be developed to aid patient care.

This article will review the current theories about the various molecular mechanisms by which a responsive tumor could become either refractory or stimulated by tamoxifen.

POTENTIAL MECHANISM OF DRUG RESISTANCE

The mechanisms to be considered are illustrated in **Figure 1**, but only the molecular mechanisms will be discussed in detail. Since tamoxifen is a competitive inhibitor of estrogen action by blocking estradiol bind-

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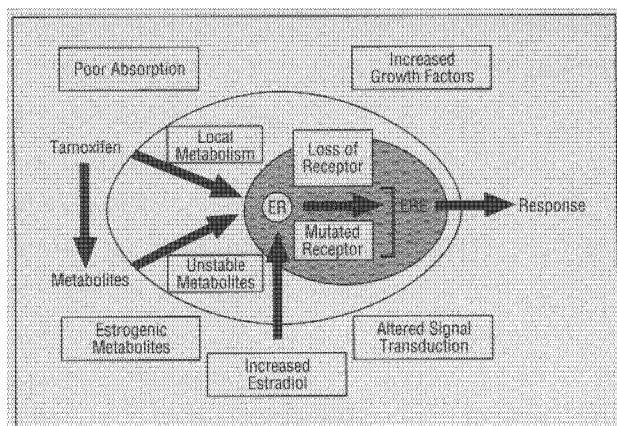


Figure 1. The potential mechanisms of drug resistance to tamoxifen in the breast cancer cell. Estrogen binds to the estrogen receptor (ER) to form a receptor complex that activates gene transcription through an estrogen response element (ERE) on the DNA. Tamoxifen and its metabolites block the competitive inhibition of estrogen binding to ER.

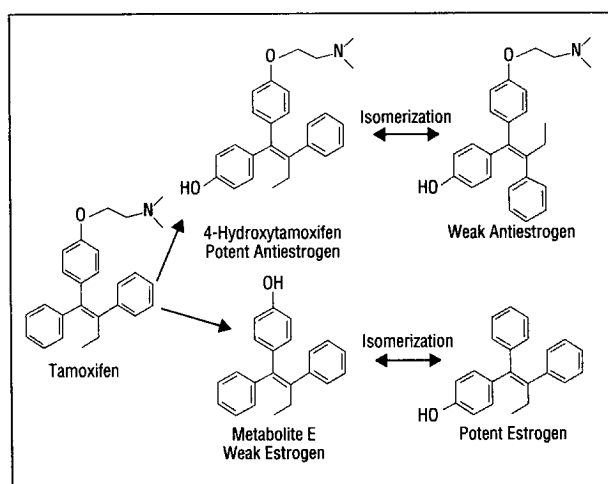


Figure 2. A proposed scheme for the metabolism of tamoxifen in breast tumors that could cause tamoxifen-stimulated growth. Tamoxifen could be converted to the potent antiestrogen 4-hydroxytamoxifen and the weak estrogen referred to as metabolite E. The key event in the hypothesis is the instability of the metabolites in the tumor cells to isomerize to a weak antiestrogen and a potent estrogen. Compounds that cannot isomerize have been shown to produce tumor-stimulated growth that makes this proposal unlikely to occur.

ing to the human estrogen receptor (ER),⁵ an increase in circulating estradiol could potentially reverse the antitumor action of tamoxifen. The administration of adjuvant tamoxifen to premenopausal women⁶ causes an increase in circulating estrogen levels; however, there is evidence that tamoxifen is effective in node-negative premenopausal women.¹

Nevertheless, patients with stage IV disease who initially respond to tamoxifen and subsequently experience drug failure can respond to oophorectomy.⁷ This suggests that ovarian steroids may eventually reverse the antitumor actions of tamoxifen. Clearly, tamoxifen will be more effective in a low estrogen environment, but consistently maintained levels (>100 ng/mL) of tamoxifen

should prove to be adequate to treat premenopausal women and avoid premature drug failure.

The pharmacokinetics and metabolism of tamoxifen have been extensively studied in patients.⁸⁻¹⁰ There is no evidence that poor absorption or systemic metabolism to estrogens contributes to drug resistance. However, recent laboratory studies have focused on the metabolism and stability of antiestrogenic metabolites within the tumor itself as a potential mechanism of tamoxifen-stimulated growth.

LOCAL METABOLISM

It is possible that the tumor cells, or the stromal component, could locally metabolize tamoxifen to potent estrogens that would stimulate tumor growth. In the laboratory, tamoxifen will stimulate the growth of human breast (MCF-7) or endometrial tumors transplanted into athymic mice.^{11,12} The tumors are ER positive and grow in response to estradiol, tamoxifen, and a variety of nonsteroidal antiestrogens.¹³ Since steroidal antiestrogens that have none of the estrogenlike properties of tamoxifen will block tamoxifen-stimulated tumor growth,¹⁴ it is reasoned that tamoxifen must be converted to estrogens that stimulate growth through the ER.

Tamoxifen is metabolized to 4-hydroxytamoxifen in the mouse.¹⁵ This metabolite is a potent antiestrogen that has been shown to have antitumor activity in the athymic mouse model.¹⁶ However, the potent antiestrogenic Z isomer is unstable and can convert to the weakly antiestrogenic E isomer.¹⁷ If the isomerization occurs locally, the net antiestrogenicity of tamoxifen will decrease, but this would not in itself account for increased tumor growth; an estrogenic stimulus is required. Minute amounts of metabolite E (tamoxifen without the dimethylaminoethane side chain) have been detected in human tumors during tamoxifen therapy.¹⁸ Fortunately, this metabolite of tamoxifen is too weakly estrogenic to promote tumor growth alone. Nevertheless, the metabolite is unstable and can isomerize to a potent estrogen.¹⁷ It is possible that if large quantities of this estrogenic metabolite accumulated in the tumors, this could account for tamoxifen-stimulated tumor growth by preferential binding of estrogenic ligands at the ER. This hypothesis¹⁹ is summarized in **Figure 2**.

We recently addressed the question of metabolite isomerization as the mechanism of tamoxifen-stimulated growth by determining the ability of tamoxifen derivatives that cannot isomerize to cause tumor growth. Since we have found that tumor growth is adequately supported by nonisomerizable derivatives of tamoxifen,²⁰ it is unlikely that local metabolite instability is responsible for tamoxifen-stimulated growth. It is perhaps more likely that clones of cells that are extremely sensitive to the intrinsic activity of tamoxifen as an estrogen are selected and gain a dominant growth advantage. Clearly, the mechanism of signal transduction that converts an antagonist to

an agonist is an area of great interest within the molecular biology community.

LOSS OF THE ER

Estrogen responsiveness of tissues and tumors is correlated with the presence or absence of the ER. Breast cancer requires estrogen to promote the process of carcinogenesis, and it is generally accepted that tumors are initially ER positive but eventually lose the receptor, and growth becomes hormone independent.

It is an important goal of laboratory research to develop models of human breast cancer progression. The objective is to study the biological processes involved in the evolution of hormone dependency to find a strategy to prevent, or at least delay, hormone-independent growth. Regrettably, there are only a few hormone-dependent human breast cancer cell lines. Both ZR-75 and MCF-7 cell lines have been used to develop antiestrogen-resistant or estrogen-independent sublines, but invariably the tumor cells retain the ER. In contrast, T47D breast cancer cells that are ER positive and estrogen responsive for growth do lose the ER if the cells are maintained in an estrogen-free environment for many months.²¹ The cloned cells are insensitive to both estrogens and antiestrogens. We are currently using this new model system to devise ways to reactivate the ER gene to produce a functional receptor. During the 1980s, the gene for the ER was isolated (**Figure 3**) and the resulting complementary DNA (cDNA) studied extensively to determine the important domains on the protein.

Estrogen receptor genes have been transfected into receptor-negative animal and human cell lines with varying degrees of success.^{22,23} High levels of receptor result in a tidal effect from estrogen treatment.²⁴ In related experiments, we have transfected the ER gene into the ER-negative breast cancer cell line MDA-MB-231.²⁵ We chose to develop cell lines that contain levels comparable with those observed in hormone-responsive cells, ie, approximately 150 to 300 fmoL/mg of cytosol protein. Estradiol decreases the growth rate of transfected breast cancer cells, an effect that is blocked by pure antiestrogens. It is possible that the selective reactivation or transfection of cancer cells with steroid receptor could prove to be a novel therapeutic strategy to control previously refractory disease.

MUTATED ER

There is much interest in determining the biological relevance of mutated steroid hormone receptors. Laboratory models have demonstrated that specific mutations of the androgen²⁶ and progesterone receptors²⁷ can change the biological properties of antiandrogens and antiprogestins to full agonist molecules. It is therefore possible that mutations in the ER could change the pharmacology from

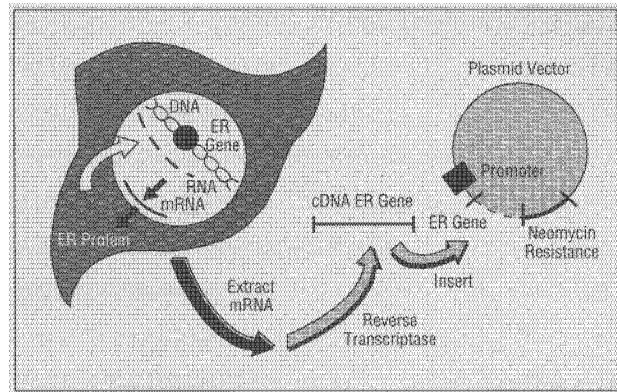


Figure 3. A diagrammatic representation of the isolation of the estrogen receptor (ER) complementary DNA (cDNA). The messenger RNA (mRNA) for ER is transcribed from the ER gene in a breast cancer cell, but it is then processed to cut out intervening sequences (introns) of the transcript to retain the exons that can be translated into the ER protein. The processed mRNA can be used as a template to produce the cDNA for the ER gene with the enzyme-reverse transcriptase (an enzyme identified from RNA-based oncogenic viruses). The cDNA can be spliced into a vector that will continuously transcribe the ER message from a cytomegaloviral promoter. The vector produces a polycistronic RNA of both the ER and an enzyme that confers neomycin resistance to transfected cells. Growth of cells in a normally lethal environment of antibiotic will select resistant clones that will also contain ER.

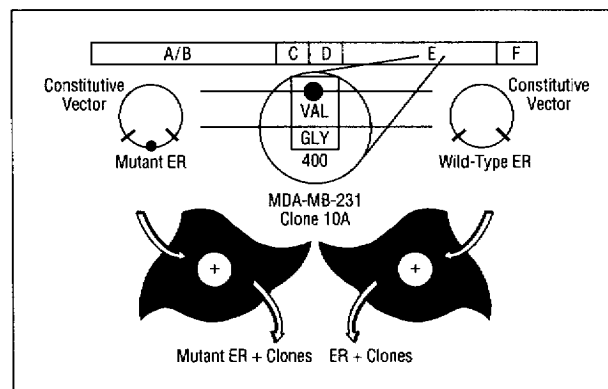


Figure 4. The human estrogen receptor (ER) has been cloned and the complementary DNA (cDNA) is available for molecular biological studies of gene transfection. The ER cDNA is divided into different areas indicated at the top of the figure. The C region is the DNA-binding domain that is essential to interact with the estrogen response element on the genome (Figure 1). The DNA-binding domain is exposed when estradiol binds in the steroid-binding domain E. Both the wild-type and a mutant cDNA for the ER (ie, with a point mutation that now produces a protein with a valine [VAL] rather than a glycine [GLY] at position 400 in the steroid-binding domain) have been spliced into a vector that can be transfected into an ER-negative breast cancer cell line (MDA-MB-231) so that the effects of estrogen on the resulting cell lines can be compared and contrasted.

antiestrogens to estrogens and explain tamoxifen-stimulated growth in tumors.

Screening of clinical tumor material has resulted in the identification of several mutations of the ER,²⁸ but the biological relevance of the findings is unclear. However, it is possible to examine the impact of point mutations of the ER on the pharmacology of antiestrogens under laboratory conditions. If MDA-MB-231 cells are transfected with either a wild-type ER gene or an ER gene with a glycine to valine mutation at amino acid 400, the result-

ing transfectants (**Figure 4**) will respond to estrogen by decreasing the growth rate.²⁵ This then becomes a laboratory model to determine the degree of estrogenicity expressed by a test molecule under controlled conditions. Pure antiestrogens prevent the inhibitory effect of estradiol in both wild-type and mutant transfectants.²⁵

In contrast, the antiestrogens 4-hydroxytamoxifen²⁹ and RU39411,³⁰ which are partial estrogens with antiestrogenic properties in the wild-type transfectants, only express estrogenic activity in the mutant transfectants. Clearly, these data indicate that the pharmacology of antiestrogen can be changed to express fully estrogenic properties. Should mutations of the ER be found in clinical specimens that are suspected of playing a role in the drug resistance to tamoxifen, the cDNA could be transfected into receptor-negative cells in the laboratory to study the actions of the translated mutant receptor.

ALTERED SIGNAL TRANSDUCTION

It is possible that hormone-independent cells could still synthesize a normal ER, but either the local environment or additional subcellular factors have changed. This would prevent the hormone (or antihormone) receptor complex from either binding with other transcription factors or preventing the complex binding adequately to estrogen response elements.

EARLY STUDIES with drug resistance to the antiestrogen LY117018 demonstrated that an ER-positive clone of MCF-7 cells could continue to grow in an antiestrogenic environment.³¹ The receptor was shown to have the same sequence as the wild-type hormone-responsive MCF-7 cell line.³² Similarly, we have described³³ an ER-positive clone of MCF-7 cells that does not respond to either estrogens or antiestrogens for growth. Estradiol does not stimulate progesterone receptor production, but the ER sequence is not mutated. Clearly, there is a fundamental alteration in the signal transduction mechanism that controls replication, but a vestigial receptor still remains. An intervention that could resolve the aberrant control mechanism might potentially become a valuable new treatment strategy.

The local environment of growth factors can alter hormone and antihormone responsiveness. Epidermal growth factor can stimulate cell replication and potentially reverse the inhibitory effects of antiestrogen on estrogen-stimulated growth.^{34,35} Indeed, the increased local concentration of growth factors within a heterogeneous tumor may be the reason why some ER-positive tumors (that are progesterone receptor negative) do not respond to tamoxifen or other antihormonal therapy.³⁶

COMMENT

The ubiquitous use of tamoxifen for the treatment of breast cancer has not only provided the clinical community with a safe and effective therapy but also has provided an insight into the molecular mechanisms of hormone-dependent tumor growth.

However, a fundamental piece of information is missing that might be obtained by the research strategies currently being investigated in the laboratory. We do not know about the precise and specific control mechanisms that regulate the activation of the ER gene. The current experiments on the drift of hormone-dependent growth to independent growth through the controlled loss of the ER are an important start to find critical steps in the biochemistry that might respond to therapeutic modulation.

Clearly, it must be a goal of laboratory research to elucidate the cascade of events that subverts effective transcriptional control through the ER. Conversely, it may be equally productive to discover precise ways to maintain receptor control. Cell-specific receptor reactivation could become a powerful tool for the molecular biologist to apply to therapeutic research. The clues obtained from understanding receptor mechanisms in breast cancer could become an important first step in developing strategies to treat all cancers.

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REFERENCES

1. Early Breast Cancer Trialists Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic or immune therapy. *Lancet*. 1992;339:1-15, 71-85.
2. Fisher B. The evolution of paradigms for the management of breast cancer: a personal perspective. *Cancer Res*. 1992;52:2371-2383.
3. Love RR, Mazess RB, Barden HC, et al. Effects of tamoxifen bone mineral density in postmenopausal women with breast cancer. *N Engl J Med*. 1992;326:852-856.
4. MacDonald CC, Stewart HJ. Fatal myocardial infarction in the Scottish adjuvant tamoxifen trial. *BMJ*. 1991;303:435-437.
5. Jordan VC, Koerner S. Tamoxifen (ICI46,474) and the human tumour 8S oestrogen receptor. *Eur J Cancer*. 1975;11:205-206.
6. Jordan VC, Fritz NF, Langan Fahey S, Thompson M, Tormey DC. Alteration of endocrine parameters in premenopausal women with breast cancer during long-term adjuvant tamoxifen monotherapy. *J Natl Cancer Inst*. 1991;83:1488-1491.
7. Sawka CA, Pritchard KI, Paterson DJA, et al. Role and mechanism of action of tamoxifen in premenopausal women with metastatic breast cancer. *Cancer Res*. 1986;46:3152-3156.
8. Lien EA, Solheim E, Kvinnsland S, Veland PM. Identification of 4-hydroxy-N-desmethyltamoxifen as a metabolite of tamoxifen in human bile. *Cancer Res*. 1988;48:2304-2308.

9. Jordan VC, Bain RR, Brown RR, Gosden B, Santos MA. Determination and pharmacology of a new hydroxylated metabolite of tamoxifen observed in patient sera during therapy for advanced breast cancer. *Cancer Res.* 1983;43:1446-1450.
10. Langan Fahey SM, Tormey DC, Jordan VC. Tamoxifen metabolites in patients on long-term adjuvant therapy for breast cancer. *Eur J Cancer.* 1990;26:883-888.
11. Gottardis MM, Jordan VC. Development of tamoxifen-stimulated growth on MCF-7 tumors in athymic mice after long-term antiestrogen administration. *Cancer Res.* 1988;48:6183-6187.
12. Satyaswaroop PG, Zaino RJ, Mortel R. Estrogen-like effects of tamoxifen on human endometrial carcinoma transplanted into nude mice. *Cancer Res.* 1984;44:4006-4010.
13. Gottardis MM, Ricchio ME, Satyaswaroop PG, Jordan VC. Effect of steroidal and nonsteroidal antiestrogens on the growth of a tamoxifen-stimulated human endometrial carcinoma (EnCa101) in athymic mice. *Cancer Res.* 1990;50:3189-3192.
14. Gottardis MM, Jiang SY, Jeng MH, Jordan VC. Inhibition of tamoxifen-stimulated growth of an MCF-7 variant in athymic mice by novel steroidal antiestrogens. *Cancer Res.* 1989;49:4090-4093.
15. Robinson SP, Langan Fahey SM, Johnson DA, Jordan VC. Metabolites, pharmacodynamics and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. *Drug Metab Dispos Biol Fate Chem.* 1991;19:36-43.
16. Gottardis MM, Robinson SP, Jordan VC. Estradiol stimulated growth of MCF-7 tumors implanted in athymic mice: a model to study the tumorigenic action of tamoxifen. *J Steroid Biochem Mol Biol.* 1988;20:311-314.
17. Murphy CS, Langan-Fahey SM, McCague R, Jordan VC. Structure-function relationships of hydroxylated metabolites of tamoxifen that control the proliferation of estrogen responsive T47D breast cancer cells in vitro. *Mol Pharmacol.* 1990;38:737-743.
18. Wiebe VJ, Osborne CK, McGuire WL, DeGregorio M. Identification of estrogenic tamoxifen metabolite(s) in tamoxifen-resistant human breast tumors. *J Clin Oncol.* 1992;10:990-994.
19. Osborne CK, Coronado E, Allred DC, Weibe VJ, DeGregorio M. Acquired tamoxifen (TAM) resistance: correlation with reduced breast tumor levels of tamoxifen and isomerization of trans-4-hydroxytamoxifen. *J Natl Cancer Inst.* 1991;83:1477-1480.
20. Wolf DM, Langan-Fahey SM, Parker CJ, McCague R, Jordan VC. Investigation of the mechanisms of tamoxifen-stimulated breast tumor growth with non-isomerizable analogues of tamoxifen and metabolites. *J Natl Cancer Inst.* 1993;85:806-812.
21. Murphy CS, Pink JJ, Jordan VC. Characterization of a receptor-negative, hormone non-responsive clone derived from T47D human breast cancer cell line kept under estrogen free conditions. *Cancer Res.* 1990;50:7285-7292.
22. Watts CK, Parker MG, King RJ. Stable transfection of the estrogen receptor gene into a human osteosarcoma cell line. *J Steroid Biochem Mol Biol.* 1989;34:483-490.
23. Touitou I, Mathieu M, Rochefort H. Stable transfection of the estrogen receptor cDNA into HELA cells induces estrogen responsiveness of endogenous cathepsin D-gene but not of growth. *Biochem Biophys Res Commun.* 1990;169:109-115.
24. Kushner PJ, Hort E, Shine J, Baxter JD, Greene GL. Construction of cell lines that express high levels of the human estrogen receptor and are killed by estrogens. *Mol Endocrinol.* 1990;4:1465-1473.
25. Jiang SY, Jordan VC. Growth regulation of estrogen receptor negative breast cancer cells transfected with cDNAs for estrogen receptor. *J Natl Cancer Inst.* 1992;84:580-591.
26. Veldscholte J, Ris-Stalpers C, Kuiper GGJM, et al. A mutation in the ligand domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to antiandrogens. *Biochem Biophys Res Commun.* 1990;173:534-540.
27. Vegeto E, Allan GF, Schrader WT, Tsai MJ, McDonnell DP, O'Malley BW. The mechanism of RU486 antagonism is dependent on the conformation of the carboxyterminal tail of the human progesterone receptor. *Cell.* 1992;69:703-713.
28. Murphy LC. Estrogen receptor variants in human breast cancer. *Mol Cell Endocrinol.* 1990;74:C83-C86.
29. Jiang SY, Langan-Fahey SM, Stella AL, McCague R, Jordan VC. Point mutation of the estrogen receptor (ER) in the ligand binding domain changes the pharmacology of antiestrogens in ER-negative breast cancer cells stably expressing cDNA's for ER. *Mol Endocrinol.* 1992;6:2167-2174.
30. Jiang SY, Parker CJ, Jordan VC. A model to describe how a point mutation of the estrogen receptor alters the structure function relationship of antiestrogens. *Breast Cancer Res Treat.* In press.
31. Bronzert DA, Greene GL, Lippman ME. Selection and characterization of a breast cancer cell line resistant to the antiestrogen LY117018. *Endocrinology.* 1985;117:1409-1415.
32. Mullick A, Chambon P. Characterization of the estrogen receptor in two antiestrogen resistant cell lines LY2 and T47D. *Cancer Res.* 1990;50:333-338.
33. Jiang SY, Wolf DM, Yingling JM, Chang C, Jordan VC. An estrogen receptor positive MCF-7 clone that is resistant to antiestrogens and estradiol. *Mol Cell Endocrinol.* 1992;90:77-80.
34. Konga M, Sutherland RL. Epidermal growth factor partially reverses the inhibitory effects of antiestrogens on T47D human breast cancer growth. *Biochem Biophys Res Commun.* 1987;146:739-745.
35. Cormier EM, Jordan VC. Contrasting ability of antiestrogens to inhibit MCF-7 growth stimulated by estradiol or epidermal growth factor. *Eur J Cancer Clin Oncol.* 1989;25:57-63.
36. Cormier EM, Wolf MF, Jordan VC. Decrease in estradiol-stimulated progesterone receptor production in MCF-7 cells by epidermal growth factor and possible clinical implications for paracrine regulated breast cancer growth. *Cancer Res.* 1989;49:576-580.