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Perspectives and Commentaries

The Control of Hormone-dependent Breast Cancer Growth—Are We Talking About Estrogen Alone?

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(A COMMENT ON: Welshons WV, Jordan VC. Adaptation of estrogen-dependent MCF-7 cells to low estrogen (phenol-red-free) culture. *Eur J Cancer Clin Oncol* 1987, **23**, 1935–1939; Katzenellenbogen BS, Kendra KL, Norman MJ, Berthois Y. Proliferation, hormonal responsiveness and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogen. *Cancer Res* 1987, **47**, 4355–4360.)

THE high incidence of breast cancer in Western women, and the realization that combination chemotherapy cannot cure metastatic breast cancer, has forced the re-evaluation of treatment strategies. There was an early appreciation that some advanced breast cancers are hormone dependent [1, 2] but the availability of relatively nontoxic antiestrogen drugs [3] has focused new efforts in the laboratory and the clinic to understand the control of hormone-dependent disease and to prevent the change to hormone-independent growth.

Endocrine therapy of breast cancer appears to be a paradox with endocrine ablation producing similar response rates to high dose estrogen (usually diethylstilbestrol) [2, 4] or antiestrogen therapy [5]. It almost seems that any perturbation of the endocrine milieu is sufficient to affect the growth of a sensitive breast tumor. These tumors can, however, be identified by their estrogen receptor content, i.e. receptor rich tumors are more likely to respond to endocrine therapy than receptor poor tumors [6, 7].

The identification of estrogen receptors within breast tumors reinforced the idea that estrogen directly caused tumor growth and acted as a stimulus to use antiestrogenic drugs like tamoxifen to treat breast cancer.

The efficacy and low toxicity of tamoxifen observed during the treatment of stage IV disease has facilitated its application as an adjuvant therapy in stage II disease. The laboratory demonstration that tamoxifen is a tumoristatic agent [8, 9] has provided a basis for long-term or indefinite adjuvant therapy [10–12]. Obviously, a true tumoristatic antihormonal therapy could prevent cell replication; however, the fact that tamoxifen has some estrogen-like actions [5] may ultimately lead to drug failure. Tamoxifen is currently being evaluated as a chemosuppressive agent in stage I disease and there is a growing debate about whether to evaluate the use of tamoxifen in women only at risk for breast cancer to prevent the development of the disease [13, 14]. The application is based upon the assumption that the promotional phases of breast cancer development are hormone dependent, or at least a higher proportion of early disease might be hormone responsive.

Thus, the long-term control of hormone responsive cells with an antiestrogen seem to be a reasonable and effective strategy. It is important, therefore, to focus research efforts to investigate the conversion of cells from a hormone-dependent state to a hormone-independent state. Clearly if the process could be subverted then the disease could be controlled for a longer period with antihormones.

Whilst it is almost axiomatic that loss of hormone-dependence occurs with a loss of the estrogen receptor, there are virtually no data that document the molecular biology of the evolutionary process within the cell. (This is distinct from the transfection of MCF-7 cells with the ras^H oncogene [15] to produce an estrogen receptor positive hormone-

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independent cell.) Indeed, if breast cancer cells are initially dependent upon estrogen for proliferation how does the mutant that is ER negative survive? These questions were raised originally by Sluyser and Mester [16] who proposed that the DNA binding portion of the receptor without a steroid binding domain might be overproduced by cells. Evidence for this attractive hypothesis has not been forthcoming although the cDNA is available to prepare the probes [17].

Culture of hormone-dependent breast cancer cells would appear to offer a controlled environment to study the shift to hormone-independent growth. However, the demonstration of estrogen stimulated growth *in vitro* has, until recently, been inconsistent and somewhat controversial. In contrast, antiestrogens could be shown to slow cell replication in control cultures but the effect could be reversed with additional estradiol ('estrogen rescue') [18]. The discovery by Berthois *et al.* [19] that manufacturers of media include high concentrations of the estrogenic indicator phenol red (or an impurity? [20]) has provided an explanation for the high growth rate of MCF-7 cells 'in the absence of added estradiol'. Clearly continuous culture of MCF-7 breast cancer cells in an estrogen has caused a selection to preserve hormone dependency. Interestingly, cell replication in the absence of phenol red appears to be more sensitive to estrogen stimuli than progesterone receptor induction (Fig. 1). This differential sensitivity explains why MCF-7 cells grown in phenol red-containing media respond minimally to estrogen with growth (the cells are already replicating maximally) but there is reserve to induce more progesterone receptor [21].

If breast cancer cells have adapted to grow maximally in phenol red-containing media then removal of the stimulus may provide an interesting model to describe the adaptation to estrogen independent

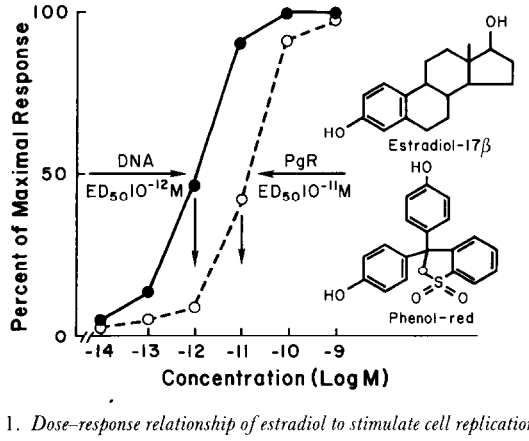

Fig. 1. Dose–response relationship of estradiol to stimulate cell replication (DNA) and progesterone receptor (PgR) in cells cultured in the absence of phenol red. The 6 day assay causes a 4-fold increase in DNA and a 10-fold increase in PgR.

growth, i.e. estrogen receptor negative. Two laboratories simultaneously, yet independently, performed this experiment [22, 23]. Interestingly, and bearing in mind that there are so many varieties of MCF-7 cells that have developed during the past 15 years of culture, the results were remarkably similar. Prolonged (several months) withdrawal of the estrogenic stimulus resulted in the selection of more rapidly growing cells that are insensitive to the growth stimulating effects of estrogen. Progesterone receptor is induced in response to estrogen and antiestrogens decreased the growth rate of control cultures. This is an apparent return to the situation observed in the presence of phenol red, *except* progesterone receptors are absent in control cultures. It is therefore unlikely that estrogen contamination is responsible for the findings in both laboratories.

The most intriguing finding is that cells grown under phenol red-free conditions increase their estrogen receptor concentration at about the same time that the growth rate increases in control cultures [22]. There could be several reasons to explain this phenomenon. Perhaps in an estrogen deprived medium a population of cells survive with a supersensitivity to estrogen for growth. The receptor population is expanded to scavenge for estrogen but the amounts are below detection and below the levels required to induce progesterone receptor. However, the growth rate is rapid and antiestrogens are less effective at controlling growth than when the wild type MCF-7 are used in phenol red containing media.

Another possibility is that the cells may not require the ligand to stimulate growth. The population of cells capable of surviving in the estrogen-deprived medium may use the excess of unoccupied receptors (possibly altered in some way to facilitate binding to promoter sites on DNA) to regulate gene expression. Antiestrogens, which can potentially alter the receptor conformation to decrease gene expression [24], may be able to facilitate the changes necessary to encourage the production of transforming growth factor β to slow cell replication [25].

Alternatively, the increase in growth rate and the increase in unoccupied receptors may be unconnected events. The cells may have become adapted to other factors in the medium. Clearly, it would be important to discover the identity of the factor(s) that substitute for estrogen (if this is the dominant hormone of dependence) once estrogen is withdrawn. Additional experimentation may provide evidence to support any, all or none of these alternatives.

In the light of these observations it is important to determine whether the new generation of non-estrogenic antiestrogens [26] will be able to prevent the drift to hormone independent growth or whether the partial estrogen-like action of tamox-

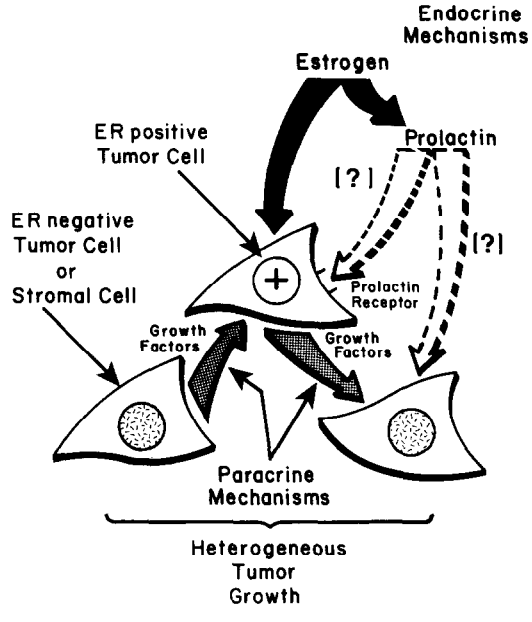

Fig. 2. Possible endocrine and autocrine factors to be considered in the growth of a heterogenous breast tumor. An imbalance of these forces may result in the development of estrogen independent growth.

ifen is a beneficial part of the antitumor mechanism.

What is clear, though, is that the expected drift from an ER+ cell to an ER− cell did not occur in the MCF-7 breast cancer cell line when estrogen was withdrawn. Perhaps years of culture will provoke the required change or alternatively our view of hormone-dependent cancer is too simplistic and the culture model is unable to replicate adquately the conditions experienced *in vivo* as a tumor evolves through endocrine and paracrine influences. A case in point is the additional revelation that human prolactin can stimulate the growth of some types of MCF-7 cells in phenol red-containing medium [27] and 2% stripped (bovine prolactin free) serum. Perhaps this is another example of cell culture technology subverting our view of hormone dependence as bovine prolactin (present in serum) blocks the action of human prolactin added to the medium. However, these results obviously require confirmation from other laboratories using a broad range of breast cancer cells to determine the extent of the phenomenon and whether it has any overall relevance to the hormone dependency of human cancer.

Thus, an integrated view of hormone-dependent growth (Fig. 2) may become essential to understand the important slip towards hormone-independent growth.

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