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Sounding Board

Cellular Distribution Patterns of Estrogen Receptors in Human Breast Cancer

JOHANNES P. VAN NETTEN,* JANE B. ARMSTRONG,* SHEILA J. CARLYLE,* NANCY L. GOODCHILD,† IAN G. THORNTON,† MALCOLM L. BRIGDEN,‡ PETER COY‡ and CHRISTINE FLETCHER*

**Department of Pathology, Royal Jubilee Hospital, Greater Victoria Hospital Society, †Department of Biological Sciences, University of Victoria, and ‡Cancer Control Agency of British Columbia, Victoria Cancer Clinic, Victoria, British Columbia, Canada*

In a previous publication [1] we reported on the existence of a group of breast tumors with a rather uniform low/moderate estrogen receptor (ER) level as demonstrated through multiple site analysis of breast tumors. We suggested that this type of tumor could represent an intermediate stage between ER-positive and -negative variants and might theoretically consist of either a 'checkerboard' of ER-positive and -negative cells or a homogeneous population of cells with truly intermediate ER levels. Using a purely biochemical method we were unable to distinguish between these two alternative hypotheses.

With the availability of specific monoclonal antibodies directed against ER, we have developed a combined biochemical/immunohistochemical multiple microsample technique designed to provide both the ER levels and to illustrate the distribution pattern in different areas of the same tumor [2].

Twenty-one tumors have been analyzed at multiple sites [2] and the data indicate that many tumors with an intermediate ER distribution pattern can indeed be subdivided into two basic types as we had hypothesized. These are: (1) tumors in which all cells express ER but at a reduced level (Type II) and (2) tumors that consist of a mosaic of ER-positive and -negative cells throughout (Type III). Although the number of tumors in this series is rather small it appears that, of the two, Type III is the more common form.

In addition to these patterns, we also detected tumors with a predominant focal ER expression where ER-positive and -negative cell populations exist in clusters (Type I). This type of expression usually occurs in combination with a Type III pattern. In fact, four tumors expressed both Type I and Type III distribution, one expressed a Type II pattern and seven tumors showed a Type III 'checkerboard' pattern.

We now propose that instead of the two pathways suggested before [1] there are at least three possible avenues for transition between tumors with a homogeneously high ER concentration throughout to tumors in which the ER system is not expressed. There is little evidence in the human that tumors follow such pathways in only one direction (high ER level to low ER level). For instance, many cases have been noted where there is an increase in receptor level when tumors are rebiopsied after a period of time. In fact, repeat routine biochemical ER analyses on 83 breast cancers (range 0.5–8 years) have indicated wide fluctuations in ER levels within individual breast tumors over time [van Netten, unpublished observations]. This further suggests that fluctuations in ER level may occur in some tumors in either direction. The various possibilities are summarized in Fig. 1.

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Correspondence and reprint requests to: Dr. J.P. van Netten, Department of Pathology, Royal Jubilee Hospital, 1900 Fort Street, Victoria, British Columbia, Canada, V8R 1J8.
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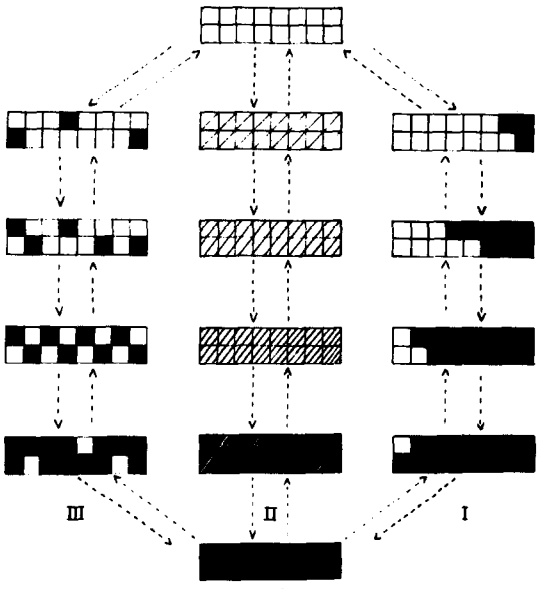

Fig. 1. Diagrammatic presentation of possible pathways of transition between receptor positive and receptor negative breast tumors. ■ Receptor positive (high level); squares with progressively reduced shading, cells with graded intermediate ER staining intensity; □ receptor negative cell. Pathway I represents intermediate stages arising from a 'focal' change within a tumor, pathways II and III represent intermediate stages arising from a 'field' change within a tumor.

Samples from tumors that express a Type I ER pattern should have a very different ER level when biochemically analyzed at multiple sites. This type of tumor could have resulted from a mutational event giving rise to an ER-negative cell population in an otherwise positive tumor. Depending on the selective advantage at any given time of the tumor's life cycle, either one or the other population could increase in size due to an increase in division rate. This growth pattern would result in a very heterogeneous receptor distribution throughout the tumor mass.

It is difficult to see how point mutations, even if occurring at multiple sites, could account for ER distribution patterns of Type II and Type III in tumors. It is possible that such patterns of ER expression could result from the gradual reduction, or random shedding, of a redundant receptor system within a tumor that has already progressed to the autonomous state [3] through a mutational event and subsquent clonal expansion. Such a growth pattern would result in a gradual decrease in receptor levels throughout the tumor over time. In many cases such as decrease is not observed.

The 'checkerboard' type of ER expression in tumor cells could reflect variations in the cell cycle. This seems unlikely, however, in view of the observation that in some tumors nearly all cells display ER specific staining unless all cells are in the same stage of the cell cycle.

An alternative possibility is that Type II and Type III patterns of receptor expression result from an intrinsic change throughout both tumor types, a so-called 'field' effect. This would allow for modulation of receptor expression within tumor cells. Such fields may involve connective tissue components within a tumor mass. For instance, the extracellular matrix of tumors has been shown to have considerable influence on cellular growth control mechanisms [4–10].

There is also evidence that during embryonic development steroid hormone receptor systems are induced in target organs with considerable input from nearby specific connective tissue cells (mesenchymal cells) [11–13]. It is therefore possible that hormone receptor systems, irrespective of their presence in normal epithelium or tumor cells, remain under the influence of connective tissue components. Thus, changes in the connective tissue (field) of such a growing tumor could indirectly influence its progression to autonomy. Recognition of such a potential mechanism would be important since the sensitivity of tumor cells within such cancers may vary between the hormone dependent state and autonomy depending on the environmental conditions influencing this field at any given time.

So far, there is little more than circumstantial evidence that such field effects exist. Algard [14–16], using organotypic vs. cell cultures, showed that hormonally induced animal tumors appear to modulate between the hormone-dependent and autonomous state according to the type of culture conditions employed, as well as on the growth conditions *in vivo*.

Shafie [17] has shown a lability of hormone responsiveness in human MCF-7 breast cancer cells depending on growth conditions in athymic nude mice and in tissue culture. Other investigators have addressed the lability of the steroid receptor system itself. Both van den Berg *et al.* [18] and Sica *et al.* [19] have shown that interferon can increase the level of receptors in tumor cells, *in vitro*. In one instance [18], there was a concomitant increase in the sensitivity of tumor cells to the antiproliferative effect of tamoxifen.

Although such receptor modulation may be difficult to detect in the human, its presence may identify those breast tumors whose hormone sensitivity might be modulated by new treatment modalities.

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