Elusive Extranuclear Estrogen Receptors in Breast Cancer

Ellis R. Levin1,2,3

Estrogen receptors at the plasma membrane and cytoplasm have been difficult to detect in breast cancer specimens. New imaging approaches are needed to determine the percentage of cancers expressing extranuclear estrogen receptors and their impact on cancer biology and treatment. Clin Cancer Res; 18(1); 6–8. ©2012 AACR.

In this issue of Clinical Cancer Research, Welsh and colleagues report their approach for detecting cytoplasmic estrogen receptors (ER) in clinical breast cancer specimens (1).

Rapid effects of steroid hormones have been known for almost 60 years (2). Estrogen acting at specific binding sites at the membranes of endometrial cells triggers calcium flux in seconds, supporting a functional pool of receptors at this part of the cell (3). In 1986, Berthois and colleagues showed the binding of fluorescent estrogen conjugates to the plasma membrane of breast cancer cell lines (4). More recent work has strongly supported the idea that plasma membrane and nuclear ER-α is derived from the same gene and are the same protein in a variety of cells, including breast cancer (5, 6).

From animal studies and human cell models, much has been learned about the functions of extranuclear steroid receptors. Membrane ERs rapidly signal as G-protein–coupled receptors (Fig. 1) to generate calcium flux, stimulate cAMP and cGMP production, and trigger phosphoinositide 3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) pathway activation. ERs have also been identified in mitochondria and endosomes, and signaling from these sites must be integrated with nuclear ER action to produce the final functions of the steroid. Rapid signaling from the membrane ER pool has an impact on gene transcription and nongenomic functions (the latter altering the activity and localization of existing proteins). In all these ways, membrane ER signaling contributes to the many actions of this sex steroid in breast cancer cells (7). In a xenograft model of MCF-7 human breast cancer cells injected into nude mice, engagement of only the membrane receptor by an estrogenic compound failed to stimulate proliferation of the tumor (8). The results of this study indicate that communication between extranuclear and nuclear ERs is likely to be important to promote the growth of human breast tumors.

One limitation to investigating the functions of ERs outside the nucleus has been the inability to clearly identify such receptor pools in clinical breast cancer specimens. Typically, pathologists designate breast cancers as ER-positive or -negative based on the detection of the receptors in the nucleus. ER status in the tumor, of course, has an impact on the therapeutic decision about whether a patient is offered adjuvant endocrine therapies. A challenge for immunohistochemistry localization of extranuclear ERs in the tumor tissue is the often 10-fold lower membrane steroid receptor pool compared with the abundant nuclear receptor pool. Techniques previously used have been unable to accurately define ERs outside the nucleus consistently, leading to the questioning of both the presence and importance of such receptor pools.

With this in mind, Welsh and colleagues took on the Herculean task of determining cytoplasmic and/or membrane receptor density in nearly 3,200 patient breast cancer specimens (1). They used a quantitative fluorescent imaging approach that is based upon immunodetection by specific ER-α antibodies. The antibodies were first evaluated in breast cancer cell lines and then applied to the clinical specimens. Some antibodies that have been used by many investigators for staining cells and tissue specimens in animals and human organs were identified as being nonspecific and, therefore, not useful for the approaches taken here. The authors reported that only a small number of clinical specimens clearly showed cytoplasmic ER-α (~1.5%), using several antibodies to assess the clinical tissue specimens.

What conclusions can be drawn from this study? First, it is clear that in the clinical laboratory, antibodies need to undergo rigorous validation about specificity, both identifying the target protein and not detecting nonspecific proteins. Yet, the problem is complex in that virtually any antibody used for classic or fluorescent immunohistochemistry can detect nonspecific proteins if used in high

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Author’s Affiliations: 1Division of Endocrinology, Department of Medicine, and 2Department of Biochemistry, University of California, Irvine, Irvine; 3Department of Veterans Affairs Medical Center, Long Beach, Long Beach, California

Corresponding Author: Ellis R. Levin, Medical Service (111-I), Long Beach VA Medical Center, 5301 E. 7th Street, Long Beach, CA 90822. Phone: 562-826-5748; Fax: 562-926-5515; E-mail: ellis.levin@va.gov

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concentrations, for long duration, or at temperatures that promote binding to nonspecific proteins. It is possible that the discrepancies between the work reported here and the many publications from investigators using some of the antibodies discredited by Welsh and colleagues could reflect these important aspects of antibody use. A standard approach for all clinical laboratories using well-validated antibodies and conditions could be important to detect extranuclear ERs in tissue specimens. Another consideration is that, upon deparaffinizing archival specimens, the tissue may suffer degradation of tumor antigens, such as membrane ERs, that are necessary for detection using specific antibodies. Perhaps degraded ERs at the cell surface may have contributed to the inability of Welsh and colleagues to detect this steroid receptor at the membrane and/or cytoplasm of most specimens.

It is also possible that the number of human breast tumors that produce extranuclear ERs may be small and, thus, that the identification of relatively few such tumors reported here by Welsh and colleagues is accurate. However, extranuclear ERs have been shown in many ER-positive human breast cancer cell lines (6, 7) and in mammalian mammary gland epithelial cells (9), making this interpretation less likely. The trafficking of membrane ERs to the cell surface is a dynamic process (10), and this pool of receptors in breast cancer cells recycles to endosomes and the Golgi apparatus, possibly even to the nucleus. Thus, at any one time, a limited number of receptors can be reliably detected at the membrane–cytoplasm interface.

What conclusions drawn from the work of Welsh and colleagues (1) may be applicable to the identification of membrane and/or cytoplasmic ERs in breast cancer specimens? First, careful and standardized approaches must be taken as described. However, there may be an inherent sensitivity limitation to the immunofluorescent approaches taken to identify small receptor pools outside the nucleus. These issues are also germane to identifying membrane- and/or cytoplasmic-localized pools of progesterone receptors in breast cancer (11). Use of high-resolution microscopy with enhanced fluorescent probes (12) or other approaches may be needed to reliably determine the percentage of human breast cancers that express membrane-localized ERs. Upon accomplishing sensitive and reliable detection, we can then correlate the presence of this ER pool to the clinical parameters of patient outcomes and response to endocrine therapies. Such data could justify trials of selective inhibition of membrane ER functions in some tumors that highly express extranuclear sex steroid receptors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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