Viewpoint

Amping up estrogen receptors in breast cancer

Amy M Fowler and Elaine T Alarid

Department of Oncology, University of Wisconsin-Madison, Madison, WI 53706, USA

Corresponding author: Elaine T Alarid, alarid@oncology.wisc.edu

Published: 16 August 2007

This article is online at http://breast-cancer-research.com/content/9/4/305

Breast Cancer Research 2007, **9:**305 (doi:10.1186/bcr1748)

Abstract

This article highlights a recent study by Holst *et al*. in *Nature Genetics* that finds estrogen receptor-alpha (ER-α) amplification in early benign lesions and more advanced invasive carcinomas of the breast, and discusses the potential implications to our present understanding of the role of ER-α in breast tumorigenesis.

Introduction

A key discovery that sparked bench-to-bedside breakthroughs in the field of breast cancer was the recognition of the hormone-dependence of many breast cancers. Observations made by Cooper and Beatson correlated the size of breast tumors with the phases of the menstrual cycle and showed that ovariectomy caused tumor regression and improved prognosis [1,2]. We now know that estrogen and its receptor, estrogen receptor-α (ERα), underlie these effects through the transcriptional regulation of genes involved in cell proliferation and differentiation. Understanding the mechanisms of estrogen and ERα action created the foundation for the design of therapies that interfere with estrogen signaling and block tumor growth. These include: reduction of endogenous estrogens via aromatase inhibitors (exemestane, anastrazole, letrozole) and/or ovariectomy; interference of ER-mediated transcriptional control via selective ER modulators (tamoxifen); and degradation of the receptor via selective ER downregulator compounds (fulvestrant). These approaches are generally successful at prolonging patient survival for those tumors expressing ERα and have less toxic side effects than chemotherapy. For instance, it was estimated that tamoxifen has saved the lives of 400,000 women since its introduction in the 1970s [3].

Has this achievement tempted us into complacency with regard to the extent of our understanding of ERα's role in the pathogenesis of breast cancer? Given the large, on-going research effort and number of publications devoted to estrogen in breast cancer (26,303 articles entered into PubMed as of June 2007), some individuals would say no. Yet, a recent report by Holst *et al*. [4] in *Nature Genetics* was the first to investigate whether a common mechanism of oncogene activation, gene amplification, occurred at the ERα gene locus during tumor progression. Their work is an important scientific contribution that expands upon prior studies demonstrating *ESR1* gene amplification in breast cancer cell lines and in some advanced tumors [5-7].

Causes of ERα overexpression in breast cancer

The pattern of ERα expression in normal breast tissue compared with precancerous and cancerous lesions is strikingly different. In normal breast tissue, ERα expression is restricted to a small proportion of non-proliferating luminal epithelial cells, typically at low to intermediate levels [8,9]. However, in more than half of premalignant lesions and carcinomas, this dissociation breaks down and the receptor is detected in proliferating cells, generally at higher levels [8]. Additionally, there is a striking increase in the intracellular amount of ERα protein [10]. A significant unknown in the field of breast cancer is what drives the change in ERα expression and distribution in breast lesions. Holst *et al.* [4] used fluorescence *in situ* hybridization (FISH) to probe a tissue microarray containing 2,222 invasive breast cancers and 295 normal, pre-malignant, and pre-invasive samples and found *ESR1* gene amplification in 358 samples (21%) of the 1,739 invasive breast carcinomas with analyzable FISH data. Virtually all (99%) cases with amplification exhibited correspondingly high ERα protein levels as measured by immunohistochemistry. Characterization of the *ESR1* amplicon at 6q25.1 by PCR-based methods found that it was relatively small and did not extend into any other genes. Furthermore, *ESR2*, which encodes a second ER, ERß, was not amplified. Amplification of other known oncogenes (*HER2/neu*, *MDM2*, *MYC*, *EGFR*) was detected in invasive cancer samples, although these were found to be independent of *ESR1* amplification. Interestingly, *ESR1* amplification was observed in proliferative benign breast lesions (36.4% of papillomas and 8.3% of usual ductal hyperplasia) and carcinomas *in situ* (35% ductal and 33% lobular) in addition to more advanced

ER = estrogen receptor; FISH = fluorescence *in situ* hybridization.

tumors [4]. While these studies require independent validation, the data provide evidence that amplification of ERα appears in early lesions and may contribute, in part, to the appearance of high levels of ERα in breast tumorigenesis.

Gene amplification alone, however, cannot explain all cases involving high ERα protein levels. Only 54% of cancers with high ERα expression also had gene amplification [4]. The remaining 46% showed high ERα expression without gene amplification [4]. This suggests that other mechanisms contribute to high ERα protein levels, such as altered regulation of *ESR1* transcription, mRNA stability, or ERα protein turnover. For example, recent studies have demonstrated that disruption of caveolin-1 and micro-ribonucleic acid 206 can increase ERα levels [11,12]. How such upstream factors regulate the ERα gene and protein is not well understood and needs further attention.

Role of misregulated ERα expression in breast tumorigenesis

A significant point raised by the finding of *ESR1* amplification in early lesions is whether high levels of ERα expression are a cause or consequence of malignant transformation. Studies of *HER2/neu* provide a clear example where overexpression of an amplified gene product is oncogenic [13]. Could this also be the case for ERα? *ESR1* gene amplification was identified in several benign proliferative breast lesions, which increase a patient's risk of cancer [14]. Studies have shown that high levels of ERα are present in benign epithelium of women with breast cancer compared to controls and there is an inability to downregulate the receptor in response to estrogen in these cases, supporting a potential role for ERα overexpression in breast cancer risk [15,16]. Transgenic mouse models also indicate that overexpression of ERα is sufficient for the development of ductal hyperplasia, lobular hyperplasia, and ductal carcinoma *in situ* [17].

How high ERα levels might contribute to tumorigenesis is less understood. The simplest explanation is that the presence of additional receptors supports a more robust response to estrogen. An alternative and intriguing possibility comes from analogy of studies conducted on ErbB2/Her2-neu. Proteomic analysis of ErbB2 protein interactions showed that elevated concentrations of ErbB2 lead to promiscuous interactions and promote activation of distinct signaling pathways [18]. In this model, overexpression of the oncoprotein, resulting from amplification or other processes, could lead to an expansion of its regulatory role by permitting protein interactions that activate non-canonical signaling pathways. Similar findings have been reported for ERα in a breast cancer cell model system of ERα overexpression in which the mechanism of transactivation and target gene regulation differ when ERα protein levels are elevated [19,20]. These studies of ErbB2 and ERα overexpression raise the interesting scenario that perhaps ERα in normal breast epithelium is maintained at restrictive levels that are necessary to promote differentiation. When the ERα protein concentration increases during tumorigenesis, promiscuous interactions with coregulatory proteins or DNA could lead to the activation of proliferative signaling pathways, which, at normal levels of expression, would be too weak to occur. This scenario would predict that amplification or overexpression of ERα would be causally related to the high proliferative capacity of ER+ cells. This possibility remains to be tested.

Clinical implications

Classification of tumors into subtypes helps predict therapeutic responses and patient survival. Categorizing breast tumors as either ERα positive or negative by immunohistochemistry has proved clinically useful in determining which patients would benefit from endocrine therapy. More recently, microarray analysis has further refined the groupings of breast tumors on the basis of distinct gene expression profiles: basal-like, HER2+/ER-, normal breast-like, luminal A, and luminal B [21]. The latter has clearly shown that the ER positive cohort is not a single group of patients. Both luminal A and B subtypes are ER+; however, patients with luminal B tumors have poorer outcomes. The ER+ cohort can also be subdivided into IE and IIE subtypes. The group IIE tumors are similar to subtype B and express more proliferative genes [22]. The same proliferative gene signature was shown by Dai *et al.* [23] to be a marker of poor outcome in patients with tumors expressing high levels of ERα for their age. Although it is currently standard practice to offer hormonal therapy to all patients categorized as ER+, these and other studies demonstrate marked heterogeneity within this group in terms of gene expression profiles and patient survival.

Holst *et al.* [4] analyzed the clinical utility of classifying tumors based on *ESR1* amplification. Phenotypes associated with *ESR1* amplification included low tumor grade and lack of lymph node metastases, both positive prognostic indicators. Furthermore, tumors with *ESR1* amplification were associated with longer survival in patients treated with adjuvant tamoxifen compared with non-*ESR1* amplified and ER-negative tumors. However, there was no statistically significant difference in survival for patients with cancers having *ESR1* amplification compared to patients with non-*ESR1* amplified cancers containing the highest level of ERα protein ($P = 0.09$). Thus, the classification of tumors based on *ESR1* amplification does not yield more clinical information than does the current method of tumor characterization based on ERα protein levels.

While all breast cancers are analyzed for the expression of ERα, steroid receptor status is not routinely measured for benign breast lesions. Depending on the level of suspicion, biopsy-proven benign lesions can either be surgically excised or followed with imaging. One histological group whose management is currently under debate comprises benign papillary lesions, which includes papilloma [24]. Holst *et al.* [4] showed that *ESR1* amplification occurs in 8 of 22 (36%)

benign papilloma samples. Furthermore, elevated ERα protein levels have been demonstrated for papillomas and are associated with increased proliferation [25]. Measurement of *ESR1* gene amplification or ERα protein levels for papillary lesions may be potentially useful since the presence of amplification or overexpression would argue in favor of surgical excision instead of follow-up imaging.

Conclusion

Over 100 years have passed since the discovery of the importance of estrogen and, later, ERα to the growth of breast tumors. Since that time, tremendous advances have been made in our understanding of the molecular mechanisms of ERα activity and in the application of this knowledge to the development of therapies for the prevention and treatment of breast cancer. The recent discovery of ERα amplification in early breast lesions by Holst *et al.* is an important reminder that, despite our perception that we understand how ERα contributes to pathogenesis, there are still major questions that remain unanswered and breakthroughs to be made. Major clinical dilemmas still revolve around how better to predict response to hormonal therapy and how to fight endocrine resistance. Thus, in 2007, the question, “How does ER contribute to breast cancer?” remains one worth asking.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We thank the many investigators who have contributed to our understanding of ERα and breast cancer and apologize to those whom we could not include due to limitations in the numbers of references.

References

1. Beatson GT: **On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases.** *Lancet* 1896, **2:**104-107.
2. Cooper AP: *The Principles and Practice of Surgery. Volume 1*. London; 1836.
3. Jensen EV, Jordan VC: **The estrogen receptor: a model for molecular medicine.** *Clin Cancer Res* 2003, **9:**1980-1989.
4. Holst F, Stahl PR, Ruiz C, Hellwinkel O, Jehan Z, Wendland M, Lebeau A, Terracciano L, Al-Kuraya K, Janicke F, *et al*.: **Estrogen receptor alpha (*ESR1*) gene amplification is frequent in breast cancer.** *Nat Genet* 2007, **39:**655-660.
5. Nessling M, Richter K, Schwaenen C, Roerig P, Wrobel G, Wessendorf S, Fritz B, Bentz M, Sinn H-P, Radlwimmer B, *et al*.: **Candidate genes in breast cancer revealed by microarray-based comparative genomic hybridization of archived tissue.** *Cancer Res* 2005, **65:**439-447.
6. Hicks J, Krasnitz A, Lakshmi B, Navin NE, Riggs M, Leibu E, Esposito D, Alexander J, Troge J, Grubor V, *et al*.: **Novel patterns of genome rearrangement and their association with survival in breast cancer.** *Genome Res* 2006, **16:**1465-1479.
7. Schuur ER, Weigel RJ: **Monoallelic amplification of estrogen receptor-α expression in breast cancer.** *Cancer Res* 2000, **60:** 2598-2601.
8. Clarke RB, Howell A, Potten CS, Anderson E: **Dissociation between steroid receptor expression and cell proliferation in the human breast.** *Cancer Res* 1997, **57:**4987-4991.
9. Petersen OW, Høyer PE, van Deurs B: **Frequency and distribution of estrogen receptor-positive cells in normal, nonlactating human breast tissue.** *Cancer Res* 1987, **47:**5748-5751.
10. Fabris G, Marchetti E, Marzola A, Bagni A, Querzoli P, Nenci I: **Pathophysiology of estrogen receptors in mammary tissue by monoclonal antibodies.** *J Steroid Biochem* 1987, **27:**171-176.
11. Adams BD, Furneaux H, White BA: **The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-α (ERα) and represses ERα messenger RNA and protein expression in breast cancer cell lines.** *Mol Endocrinol* 2007, **21:**1132-1147.
12. Li T, Sotgia F, Vuolo MA, Li M, Yang WC, Pestell RG, Sparano JA, Lisanti MP: **Caveolin-1 mutations in human breast cancer: functional association with estrogen receptor α-positive status.** *Am J Pathol* 2006, **168:**1998-2013.
13. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: **Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene.** *Science* 1987, **235:**177-182.
14. Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, *et al*.: **Benign breast disease and the risk of breast cancer.** *N Engl J Med* 2005, **353:**229-237.
15. Khan SA, Rogers MA, Obando JA, Tamsen A: **Estrogen receptor expression of benign breast epithelium and its association with breast cancer.** *Cancer Res* 1994, **54:**993-997.
16. Khan SA, Sachdeva A, Naim S, Meguid MM, Marx W, Simon H, Halverson JD, Numann PJ: **The normal breast epithelium of women with breast cancer displays an aberrant response to estradiol.** *Cancer Epidemiol Biomarkers Prev* 1999, **8:**867-872.
17. Frech MS, Halama ED, Tilli MT, Singh B, Gunther EJ, Chodosh LA, Flaws JA, Furth PA: **Deregulated estrogen receptor α expression in mammary epithelial cells of transgenic mice results in the development of ductal carcinoma *in situ*.** *Cancer Res* 2005, **65:**681-685.
18. Jones RB, Gordus A, Krall JA, MacBeath G: **A quantitative protein interaction network for the ErbB receptors using protein microarrays.** *Nature* 2006, **439:**168-174.
19. Jiang X, Ellison SJ, Alarid ET, Shapiro DJ: **Interplay between the levels of estrogen and estrogen receptor controls the level of the granzyme inhibitor, proteinase inhibitor 9 and susceptibility to immune surveillance by natural killer cells.** *Oncogene* 2007, **26:**4106-4114.
20. Fowler AM, Solodin NM, Valley CC, Alarid ET: **Altered target gene regulation controlled by estrogen receptor-α concentration.** *Mol Endocrinol* 2006, **20:**291-301.
21. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, *et al*.: **Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.** *Proc Natl Acad Sci USA* 2001, **98:**10869-10874.
22. Oh DS, Troester MA, Usary J, Hu Z, He X, Fan C, Wu J, Carey LA, Perou CM: **Estrogen-regulated genes predict survival in hormone receptor-positive breast cancers.** *J Clin Oncol* 2006, **24:**1656-1664.
23. Dai H, van’t Veer L, Lamb J, He YD, Mao M, Fine BM, Bernards R, van de Vijver M, Deutsch P, Sachs A, *et al*.: **A cell proliferation signature is a marker of extremely poor outcome in a subpopulation of breast cancer patients.** *Cancer Res* 2005, **65:**4059-4066.
24. Hall FM, Mercado CL, Cangiarella J: **Papillary lesions of the breast.** *Radiology* 2007, **243:**300-301.
25. Shoker BS, Jarvis C, Clarke RB, Anderson E, Munro C, Davies MPA, Sibson DR, Sloane JP: **Abnormal regulation of the oestrogen receptor in benign breast lesions.** *J Clin Pathol* 2000, **53:**778-783.