Membrane-Associated Estrogen Receptor Signaling Pathways in Human Cancers

Richard J. Pietras and Diana C. Márquez-Garbán

Background

Hormonal therapy is a mainstay in the clinical management of patients with breast tumors that express estrogen receptor (ER). The antitumor efficacy of these endocrine treatments depends on tight regulation of breast tumor cell growth by estrogens and growth factors. However, as these cancers progress, they usually become resistant to antiestrogens, and most patients no longer respond to endocrine therapy. New options for antiestrogen treatment are urgently needed to enhance patient survival and quality of life. Improved therapies and treatment strategies may derive from the discovery that alternative pathways are involved in the modulation of estrogen signaling. Overexpression and activation of growth factor receptors often occurs in breast malignancies and tends to associate with the failure of antiestrogen therapy. Substantial data now show that both nuclear and extranuclear ERs interact in a cooperative fashion with transmembrane growth factor receptor signaling pathways in breast and other cancers that bear ERs, such as non–small cell lung tumors. This cross-communication between estrogen and growth factor receptors promotes downstream signaling for tumor cell proliferation, survival, and endocrine resistance. Defining the molecular interactions between estrogen and growth factor receptor signaling pathways that lead to tumor progression is essential to develop novel therapeutics that target this critical signal transduction axis.

Hormonal therapy was first used more than 100 years ago, marking the start of the current era of targeted antitumor treatment (1). This approach is based on blocking the activity of estrogens and their receptors, ERα and ERβ (2). The classic mechanism of hormone action involves estrogen binding to ER in the nucleus, thus promoting association with specific estrogen response elements in the promoters of target genes (3). However, ER also regulates the expression of many genes without direct binding to DNA. This occurs via protein-protein interactions with other transcription factors, such as activator protein-1, and with extranuclear signaling complexes that, in turn, modulate downstream gene expression (2, 4). A focus of this review will be extranuclear signaling that occurs in seconds to minutes, with initiation at the plasma membrane. As first reported 30 years ago (5–7), these rapid effects are mediated by ER in or tethered to the membrane. Acute signaling by estrogen in target cells, such as breast tumors and non–small cell lung cancer (NSCLC), is now widely reported and involves nuclear and extranuclear ERs acting in concert with growth factor receptor pathways to promote downstream signaling for cell proliferation and survival (see refs. 8–14). Defining these molecular interactions is critical for developing novel antitumor therapeutics targeting specific receptor actions.

Structure and activity of nuclear ER. In early concepts of estrogen action, both nuclear and extranuclear ERs were postulated, but later studies focused largely on nuclear ER and transcription (3, 11, 15). ERα and ERβ are products of different genes and have similar, but not identical, structure (see Fig. 1). On binding estradiol, ERα undergoes a change in conformation of the ligand-binding domain to form a novel hydrophobic surface that modulates binding of coactivator and corepressor proteins (2, 16–18). As a phosphoprotein, ER undergoes phosphorylation at serine and tyrosine residues after activation by ligand binding, and this contributes to receptor activity and DNA binding (11, 19–21). Ligand-bound ERs function as transcription factors by binding directly as homodimers to estrogen response elements (2, 17). However, about one third of estrogen-induced genes lack functional estrogen response elements, and estrogens indirectly regulate transcription of these genes by modulating activity of other transcription factors such as activator protein-1, Elk-1, serum response factor, cyclic AMP–responsive element binding protein, nuclear factor κB, and signal transducers and activators of transcription (4). Blockade of ER signaling by interfering with estrogen binding to ER is the basis of the hormonal agent tamoxifen, a partial agonist that limits estrogen-stimulated proliferation in breast. Fulvestrant is a novel ER antagonist that down-regulates cellular levels of ER and, unlike tamoxifen, has no agonist activity (22). Further, inhibitors of aromatase, anastrazole, letrazole, and exemestane, reduce estrogen biosynthesis in target tissues and show some advantages over tamoxifen as breast tumor therapies (23–25).

Nature and activity of extranuclear ERs. *ERα* gene codes for a major 66-kDa transcript and a minor 46-kDa isoform lacking portions of the NH_2-terminal region of full-length ERα (26, 27). Although most ERs localize in tumor cell nuclei, a significant pool of ERs occurs in extranuclear sites in cell lines and archival breast cancer and NSCLC cells (14, 28). ERα associated with membrane is detected by the use of controlled homogenization procedures with quantitative subcellular fractionation to limit extraction artifacts (11, 29) and by antibodies directed to different domains of nuclear ERα in intact breast (30–32), NSCLC (13, 14), and pituitary tumor cells (33), as well as in nonmalignant vascular cells (34). The 46-kDa ER also occurs in membranes of endothelial (27) and breast (35)

Authors' Affiliation: Department of Medicine-Division of Hematology/Oncology, David Geffen School of Medicine, and Jonsson Comprehensive Cancer Center, University of California at Los Angeles, Los Angeles, California
Received 6/4/07; accepted 7/9/07.
Grant support: California Breast Cancer Research Program, U.S. Army Breast Cancer Research Program, Susan G. Komen Breast Cancer Research Program, Nina Kocsis Fund, Stiles Program in Integrative Oncology, and the National Cancer Institute Lung Cancer Specialized Program of Research Excellence.
Requests for reprints: Richard J. Pietras, Department of Medicine-Hematology/Oncology, Geffen School of Medicine, University of California at Los Angeles, 11-934 Factor Building, Los Angeles, CA 90095-1678. Phone: 310-825-9769; Fax: 310-825-6192; E-mail: rpietras@ucla.edu.

doi:10.1158/1078-0432.CCR-07-1373

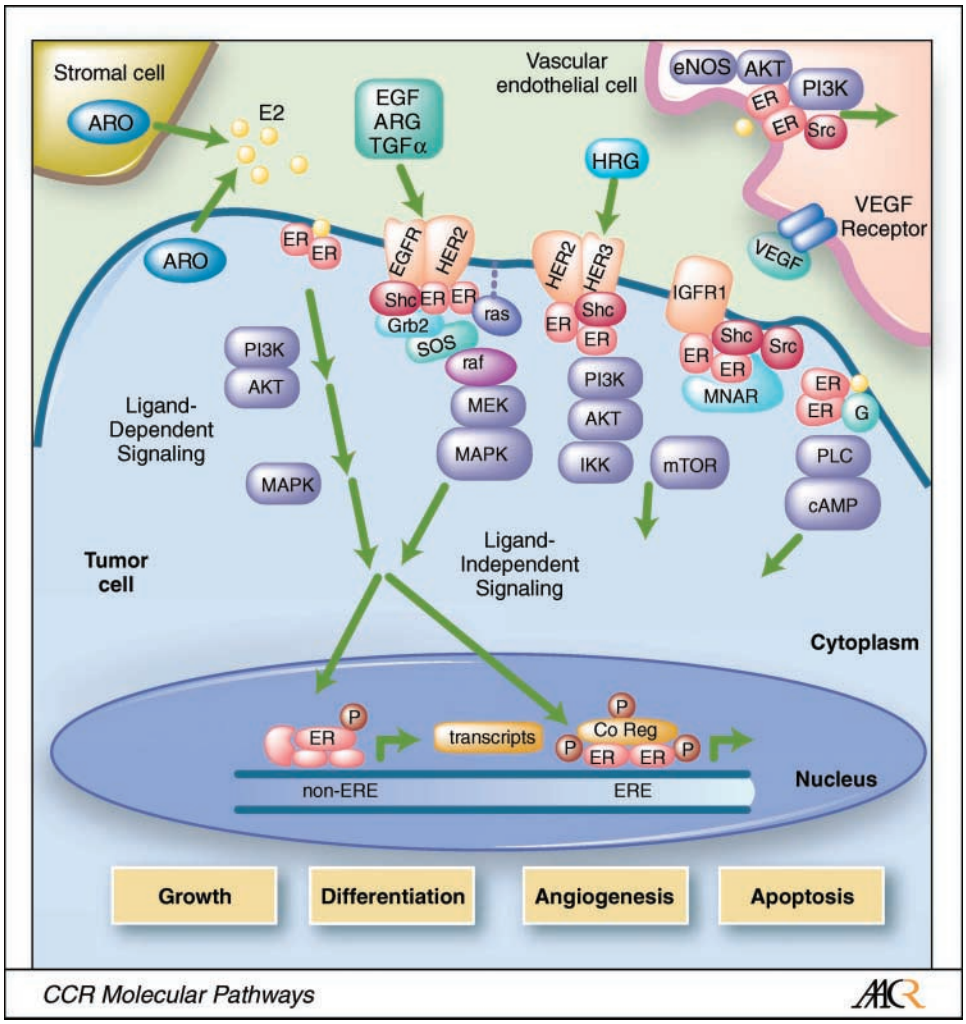

Fig. 1. Interactions of estrogen and growth factor receptor signaling in human tumors. The proliferation and survival of human breast cancer and NSCLC cells is closely regulated by growth factor receptors as well as the activity of estrogens (*E2*) and their receptors, ERα and ERβ (2). ERα has six major functional domains including an NH_2-terminal transactivation domain, an adjacent DNA-binding domain, and a portion involved in hormone-binding, receptor dimerization, and activity of a second transactivation region (16–18). In classic models of estrogen action, estrogen binds ER to promote dimerization and phosphorylation of the receptor (19). This allows direct binding of the ligand-ER complex with steroid receptor coactivators and estrogen response elements (*ERE*) in DNA, leading to changes in gene transcription that regulate growth, differentiation, apoptosis, and angiogenesis. These estrogen-dependent actions in the nucleus are modulated by ligand structure, receptor subtype (ERα, ERβ) or isoform (splice variants), the gene promoter, and the balance of coactivator and corepressor proteins that modulate the transcriptional response (2, 17). In addition, there are alternate pathways of estrogen action that involve protein-protein interactions and do not require direct ER binding to DNA (4). A subset of ERs associate with extranuclear sites, caveolae, or lipid raft domains in plasma membrane, and there interact with transmembrane growth factor receptors such as EGFR, HER2, and insulin-like growth factor receptor I (*IGFR1*) and other signaling molecules, including components of the ras-MAPK and phosphatidylinositol 3-kinase (*PI3K*)/AKT pathways, Shc, Src kinases, Janus-activated kinase/signal transducer and activator of transcription signaling, nitric oxide synthase (*NOS*), and G-proteins (see refs. 11, 12, 49). Membrane-associated ER may undergo posttranslational modification, such as palmitoylation, and/or associate with adaptor proteins, such as Shc, MNAR, or lipid raft proteins. Growth factor and ERs may form a structured complex for signal transduction to MAPK and/or phosphatidylinositol 3-kinase/AKT kinase that interacts, in turn, with nuclear ER and steroid receptor coactivators. Signaling for cell growth involves phosphorylation (*P*) of nuclear ER and coactivator proteins, and such phosphorylation can occur in ligand-dependent as well as ligand-independent modes. Estrogen response element – dependent and alternate transcription sites may be activated. Further, estrogens are produced locally in breast cancer and NSCLC cells and in host supporting cells via the action of aromatase (*ARO*), and aromatase is regulated by both nuclear and extranuclear ERs and growth factor – mediated signaling. In addition, estrogens may regulate tumor-associated angiogenesis by direct interactions with vascular endothelial cells or by indirect stimulation of vascular endothelial growth factor (*VEGF*) secretion from tumors. Pathways derived from data previously published (21); refer to text for details.

cells where it may form part of a signaling complex. To assess the nature of membrane ER, nuclear *ERα* gene was transfected in ER-null Chinese hamster ovary cells, and this resulted in cellular expression of both membrane and nuclear ERs (36). Studies based on knockdowns of ERα by small interfering RNA (35) or ER antisense *in vitro* (37) and knockouts of both ERα and ERβ *in vivo* (38) support the hypothesis that membrane and nuclear ERs share a common origin. Further, membrane ERs do not occur in ER-negative MCF-7 breast tumor subclones that lack nuclear ER, and these cells, unlike ER-positive MCF-7 cells, do not show rapid estrogen-induced phosphorylation of steroid receptor coactivator AIB1 (35). Importantly, recent studies using mass spectroscopy provide evidence that peptides derived from ERα occur in membrane fractions prepared from breast tumor (39) and vascular endothelial cells (38). Together, these findings affirm that membrane-associated ER derives from the same gene as nuclear ERα (34–39). How ER associates with membranes is another challenging question. Although ERα has many hydrophobic regions on Kyte-Doolittle plots, there are no documented transmembrane,

glycosylphosphatidylinositol-anchor, or PDZ domains to foster membrane association (26). However, localization and activity of ER at plasma membrane is stimulated by posttranslational palmitoylation of Cys^{447} in the ligand-binding domain of ERα (14, 27, 40). Further, a highly conserved nine-amino-acid motif in the ligand binding domains of human ERα and ERβ seems to be necessary for mediation of receptor palmitoylation and subsequent steroid signaling (12). The membrane receptor may also form homodimers or couple with other proteins, such as caveolins, flotillins (12, 35), and coactivator protein modulator of nongenomic actions of the estrogen receptor (MNAR)/proline-, glutamic acid-, and leucine-rich protein-1 (PELP1; ref. 41). Transmembrane growth factor receptors may also provide docking sites (32, 42). Currently, most findings indicate that rapid and specific estrogen-induced membrane events are mediated predominantly by classic or variant ER forms (11, 12, 27, 34). However, other membrane estrogen-binding proteins, such as G protein–coupled receptor GPR30, or additional molecules may contribute to biological or promiscuous estrogen signaling in cells with or without ER expression (43, 44), but their role in regulating cell proliferation and survival is uncertain (38).

Estrogen and growth factor receptor synergy in malignant progression. Members of the *erbA* and *erbB* gene families cooperate in cell transformation (45, 46). Because ER is part of the *erbA* gene family, and epidermal growth factor receptor (EGFR)/HER-1 and HER2 are members of the *erbB* gene family, biological interactions between these receptors may be operative in human cancer progression. ER is an important prognostic factor in breast cancer that predicts response to endocrine therapy. However, resistance to endocrine therapy usually emerges, leading to tumor progression and poor patient survival. Cooperative interactions between growth factor receptor and ER signaling pathways have been identified in breast cancer and NSCLC, and growth factor–mediated pathways, notably those of EGFR, HER2, and insulin-like growth factor receptor I, are critical in development of antiestrogen resistance in breast tumors (47–49). About 20% to 25% of breast cancers have overexpression of HER2, and increased HER2 expression correlates with poor clinical outcome and resistance to endocrine therapy (47, 48). Similarly, overexpression of EGFR in ~50% of breast cancers also correlates with hormonal resistance (47, 50).

Ligand-dependent and ligand-independent extranuclear ER signaling. Growth factor receptors such as EGFR and HER2 often concentrate in specific domains of plasma membrane, termed caveolae or lipid rafts, together with other signal transduction molecules. Lipid rafts are moving cholesterol-rich platforms in membrane that provide a matrix for signal transduction (11, 12, 35, 51); caveolae and caveolae-like rafts are specialized forms of lipid rafts containing structural proteins, caveolin or flotillin, respectively (35, 51). Extranuclear ERs also localize in lipid rafts (11, 12, 35), thereby promoting activation and transactivation of EGFR and HER2 receptors (11, 32, 48) and interactions with other signaling molecules including insulin-like growth factor receptor I, the p85 regulatory subunit of phosphatidylinositol 3-kinase, G-proteins, Src, and Shc, a protein that may couple ER with growth factor receptors (11, 12, 42, 52). Coregulators, MNAR/PELP1 or metastasis-associated protein 1, also sequester ER in the extranuclear compartment to increase membrane action (41). Activation of these pathways by estrogen relays downstream proliferative and survival signals via mitogen-activated protein kinase (MAPK) and AKT. Further, MAPK stimulated by EGFR or/and HER2 signaling can, in turn, phosphorylate nuclear ER and receptor coactivators such as AIB1/SRC-3 (2, 35, 53, 54). These events can be triggered by estrogen (ligand-dependent signaling) or receptor kinases in the absence of estrogen (ligand-independent ER activation), with the latter process underlying many forms of endocrine resistance (52, 55).

Clinical-Translational Advances

Clinical significance of interactions between estrogen and growth factor signaling pathways. A major problem in breast cancer management is the conversion from estrogen-sensitive to hormone-resistant disease after starting antiestrogen therapy (17, 47). Emergence of estrogen-independent tumors is due, in part, to enhanced interactions between growth factor receptors and ER during cancer progression that elicit ligand-independent ER activation, thus negating responses to antiestrogens (47, 55, 56). ER^+ and/or PR^+, HER2-overexpressing tumors respond poorly to endocrine therapy (57–61), and a meta-analysis of seven clinical studies indicates that metastatic breast cancers with HER2 overexpression are tamoxifen resistant (62). In this setting, HER2 overexpression triggers signaling for increased ER phosphorylation (19, 48, 63) and loss of the inhibitory effect of tamoxifen on ER-mediated transcription (49, 61, 64–66). Recent clinical data on breast tumors also suggest that the ER^+/PR^- phenotype, in particular, associates with high expression of extranuclear ER and enhanced AKT signaling (28) and may identify tumors driven primarily by growth factor receptor signaling (47, 49, 67). It will be important to further examine the ER^+/PR^- phenotype to determine if it signals a greater likelihood to respond to growth factor receptor–targeted therapy in combination with hormonal treatment.

Tumor cells may also circumvent pharmacologic blockade of estrogen response pathways by enhanced expression of aromatase to increase estrogen biosynthesis. Aromatase induction occurs in HER2-overexpressing tumors (68, 69) and in those with active cross-talk between ER and growth factor–mediated signaling pathways (69). Laboratory models show that long-term estrogen deprivation that mimics low estrogen levels produced by aromatase inhibition eventually elicits adaptive changes in tumor cell behavior, with emergence of hypersensitivity to estrogen and increased aromatase activity (70, 71). In addition, estrogen hypersensitivity associates with acquired overexpression of growth factor receptors and enhanced estrogen binding to membrane-associated ERα, leading to interactions with Shc, insulin-like growth factor receptor I, and EGFR and activation of downstream signaling (42, 52, 71, 72). Together, these acquired changes allow tumors to be stimulated by much smaller amounts of estrogen, thus negating benefits of treatment with aromatase inhibitors. This complex modulation of estrogen sensitivity by tumors may support use of sequential endocrine therapy with agents having different mechanisms of action (22, 47).

Approaches to overcome hormonal resistance. Gaps in our understanding of mechanisms of endocrine action and resistance remain. However, based on the notion that multiple signaling pathways activate ER, combination therapies with

both endocrine and nonendocrine agents that block different pathways are rational and testable. Thus, a strategy to treat patients with ER^+, $HER2^+$ tumors is dual blockade of HER2 and ER-dependent pathways that interact to promote growth. In preclinical work, combined treatment with HER2 receptor antibody, Herceptin, and tamoxifen enhanced antitumor effects in HER2-overexpressing cells with ER (48, 65, 73). Moreover, combination of Herceptin with fulvestrant is even more effective in blocking growth of tumors expressing HER2 and ER (65, 74). Fulvestrant down-regulates ER, resulting in reduced cell proliferation, and this action favors improved responses to growth factor–mediated ER activation (47). These dual treatment approaches are now being tested in the clinic (47, 75). Data from the TAnDEM trial in patients with metastatic breast cancer similarly indicate that treatment with Herceptin combined with anastrazole is superior to anastrazole alone (76), providing clinical confirmation for a working biological hypothesis.

There is also potential to use growth factor receptor tyrosine kinase inhibitors, alone or combined with antihormone agents, to treat endocrine-resistant breast cancer (47, 50, 73, 74). *In vitro* studies show that gefitinib, an EGFR kinase inhibitor, suppresses proliferation of breast cancer cells (77) and interferes with growth factor receptor and ER cross-communication (78, 79). Similarly, other inhibitors of downstream kinases, such as p42/p44 MAPK or p38 MAPK (47, 49), which are activated with tamoxifen resistance, may have future clinical utility.

In NSCLC, new research shows significant expression of ER forms that interact cooperatively with EGFR to stimulate tumor growth (13, 14). Preclinical studies reveal that either gefitinib or erlotinib, combined with fulvestrant, elicits marked inhibition of tumor progression. Results of a phase I clinical trial using fulvestrant together with gefitinib indicate that administration of this couplet is safe and shows antitumor efficacy in patients with advanced NSCLC (80), and a phase II trial with the combination of fulvestrant and erlotinib is under way (75).

EGFR and HER2 are responsible for some, but not all, states of endocrine resistance. Strategies to disrupt signal transduction by independent signaling pathways for cell proliferation and/or for blocking apoptosis are also being considered, including inhibition of insulin-like growth factor receptor I, Src, phosphatidylinositol 3-kinase, AKT, and mammalian target of rapamycin (see refs. 47, 52, 75, 81, 82).

Finally, the tumor itself is not the only therapeutic target. Cancer progression depends on formation of an adequate blood supply (83), and vascular endothelial growth factor promotes growth of breast cancer and NSCLC cells (83, 84). Vascular endothelial growth factor is increased by estradiol in breast cancers, and tumor angiogenesis is also driven by direct interaction of estradiol with vascular endothelial cells that contain ER and proliferate in response to estrogen (27, 85). Thus, it is reasonable to target estrogen signaling in both the tumor and the host vasculature, potentially offering more effective antitumor therapy than treating the tumor alone.

The Path Forward

Our understanding of the mechanisms of estrogen action and the biological basis of resistance to hormonal therapies is advancing rapidly. However, it is now clear that there are multiple routes by which ER-mediated pathways may be activated, and this complexity enables tumors to use adaptive mechanisms for sustained growth after initiation of endocrine therapy. As we go forward, it will be important to determine the clinical significance of ER subtypes and isoforms (12, 47) and to investigate the role of tumor stem cells that bear ER and function as progenitor cells in the etiology of hormone-dependent cancers (55, 86). Currently, combination therapies directed to extranuclear and nuclear ER as well as to growth factor signaling pathways are being assessed in the clinic, and hopefully, these new strategies will offer improved antitumor efficacy for patients afflicted with hormone receptor–positive tumors.

References

1. Beatson G. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases. Lancet 1896;ii:104–7, 162–5.
2. O'Malley B. A life-long search for the molecular pathways of steroid hormone action. Mol Endocrinol 2006;19:1402–11.
3. Jensen E, Greene G, Closs L, et al. Receptors reconsidered: a 20-year perspective. Recent Prog Horm Res 1982;38:1–40.
4. Bjornstrom L, Sjoberg M. Mechanisms of estrogen receptor signaling: Convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 2005;19:833–42.
5. Pietras R, Szego C. Endometrial cell calcium and oestrogen action. Nature 1975;253:357–9.
6. Pietras R, Szego C. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. Nature 1977;265:69–72.
7. Szego C, Davis J. Adenosine 3′,5′-monophosphate in rat uterus: acute elevation by estrogen. Proc Natl Acad Sci U S A 1967;58:1711–8.
8. Migliaccio A, Di Domenico M, Castoria G, et al. Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells. EMBO J 1996;15:1292–300.
9. Nemere I, Farach-Carson M. Membrane receptors for steroid hormones: a case for specific cell surface binding sites for vitamin D metabolites and estrogens. Biochem Biophys Res Commun 1998;248:443–9.
10. Kelly M, Levin E. Rapid actions of plasma membrane estrogen receptors. Trends Endocrinol Metab 2001;12:152–6.
11. Pietras R, Nemere I, Szego C. Steroid hormone receptors in target cell membranes. Endocrine 2001;14:417–27.
12. Pedram A, Razandi M, Sainson R, et al. A conserved mechanism for steroid receptor translocation to the plasma membrane. J Biol Chem. Epub 2007 May 29.
13. Stabile L, Davis A, Gubish C, et al. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor α and β and show biological responses to estrogen. Cancer Res 2002;62:2141–50.
14. Pietras R, Marquez D, Chen H-W, et al. Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. Steroids 2005;70:372–81.
15. Welshons W, Grady L, Judy B, et al. Subcellular compartmentalization of MCF-7 estrogen receptor synthesis and degradation. Mol Cell Endocrinol 1993;94:183–94.
16. Green S, Chambon P. Nuclear receptors enhance our understanding of transcription regulation. Trends Genet 1988;4:309–14.
17. Katzenellenbogen BS. Estrogen receptors: bioactivities and interactions with cell signaling pathways. Biol Reprod 1996;54:287–93.
18. White R, Parker M. Molecular mechanisms of steroid hormone action. Endocr Relat Cancer 1998;5:1–14.
19. Weigel N. Steroid hormone receptors and their regulation by phosphorylation. Biochem J 1996;319:657–67.
20. Yudt M, Vorojeikina D, Zhong L, et al. Function of estrogen receptor tyrosine 537 in hormone binding, DNA binding, and transactivation. Biochemistry 1999;38:14146–56.
21. Gruber C, Tschugguel W, Schneeberger C, Huber J. Mechanisms of disease: production and actions of estrogens. N Engl J Med 2002;346:340–52.
22. Dowsett M, Nicholson R, Pietras R. Biological characteristics of the pure antiestrogen fulvestrant: overcoming endocrine resistance. Breast Cancer Res Treat 2005;93 Suppl 1:S11–8.
23. Coombes RC, Hall E, Gibson LJ, et al.; the Intergroup Exemestane Study. A randomized trial of exemestane after two or three years of tamoxifen therapy in postmenopausal women with primary breast cancer. N Engl J Med 2004;350:1081–92.
24. Howell A, Cuzick J, Baum M, et al.; the ATAC Trialists' Group. Results of the ATAC (Arimidex, tamoxifen, alone or in combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet 2005;365:60–2.

25. Thürlimann B, Keshaviah A, Coates AS, et al.; the Breast International Group (BIG) 1-98 Collaborative Group. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med 2005;353:2747–57.
26. Green S, Walter P, Kumar V, et al. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature 1986;320:134–9.
27. Li L, Haynes M, Bender J. Plasma membrane localization and function of the estrogen receptor α variant (ER46) in human endothelial cells. Proc Natl Acad Sci U S A 2003;100:4807–12.
28. Kim R, Kaneko M, Arhiro K, et al. Extranuclear expression of hormone receptors in primary breast cancer. Ann Oncol 2006;17:1213–20.
29. Pietras R, Szego C. Estrogen receptors in uterine plasma membrane. J Steroid Biochem 1979;11: 1471–83.
30. Nenci I, Marchetti E, Marzola A, Fabris G. Affinity cytochemistry visualizes specific estrogen binding sites on the plasma membrane of breast cancer cells. J Steroid Biochem 1981;14:1139–46.
31. Pappas T, Gametchu B, Watson C. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. FASEB J 1995; 9:404–10.
32. Marquez D, Pietras R. Membrane-associated binding sites for estrogen contribute to growth regulation of human breast cancer cells. Oncogene 2001;20: 5420–30.
33. Norfleet A, Clarke C, Gametchu B, Watson C. Antibodies to the estrogen receptor-α modulate rapid prolactin release from rat pituitary tumor cells through plasma membrane estrogen receptors. FASEB J 2000;14:157–65.
34. Chambliss K, Yuhanna I, Mineo C, et al. Estrogen receptor α and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. Circ Res 2000;87:E44–52.
35. Marquez D, Chen H-W, Curran E, et al. Estrogen receptors in membrane lipid rafts and signal transduction in breast cancer. Mol Cell Endocrinol 2006;246: 91–100.
36. Razandi M, Pedram A, Greene G, Levin E. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERα and ERβ expressed in Chinese hamster ovary cells. Mol Endocrinol 1999;13:307–19.
37. Norfleet A, Thomas M, Gametchu B, Watson C. Estrogen receptor-α detected on the plasma membrane of aldehyde-fixed GH3/B6/F10 rat pituitary tumor cells by enzyme-linked immunocytochemistry. Endocrinology 1999;140:3805–14.
38. Pedram A, Razandi M, Levin E. Nature of functional estrogen receptors at the plasma membrane. Mol Endocrinol 2006;20:1996–2009.
39. Xiang R, Shi Y, Dillon D, et al. 2D LC/MS analysis of membrane proteins from breast cancer cell lines MCF7 and BT474. J Proteome Res 2004;3:1278–83.
40. Acconcia F, Ascenzi P, Bocedi A, et al. Palmitoylation-dependent estrogen receptor α membrane localization: regulation by 17β-estradiol. Mol Biol Cell 2005;16:231–7.
41. Acconcia F, Kumar R. Signaling regulation of genomic and nongenomic functions of estrogen receptors. Cancer Lett 2005;238:1–14.
42. Song RX. Membrane-initiated steroid signaling action of estrogen and breast cancer. Semin Reprod Med 2007;25:187–97.
43. Prossnitz ER, Arterburn JB, Sklar LA. GPR30: a G protein-coupled receptor for estrogen. Mol Cell Endocrinol 2007;265–6:138–42.
44. Thomas P, Pang Y, Filardo E, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology 2005;146: 624–32.
45. Beug H, Graf T. Cooperation between viral oncogenes in avian erythroid and myeloid leukaemia. Eur J Clin Invest 1989;19:491–501.
46. Bishop J. Oncogenes as hormone receptors. Nature 1986;321:112–3.
47. Pietras R. Biologic basis of sequential and combination therapies for hormone-responsive breast cancer. Oncologist 2006;11:704–17.
48. Pietras R, Arboleda J, Reese D, et al. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 1995;10:2435–46.
49. Osborne C, Schiff R. Estrogen-receptor biology: continuing progress and therapeutic implications. J Clin Oncol 2005;23:1616–22.
50. Nicholson R, Hutcheson I, Harper M, et al. Modulation of epidermal growth factor receptor in endocrine-resistant, oestrogen receptor-positive breast cancer. Endocr Relat Cancer 2001;8:175–82.
51. Smart E, Graf G, McNiven M, et al. Caveolins, liquid-ordered domains, and signal transduction. Mol Cell Biol 1999;19:7289–304.
52. Fan P, Wang J, Santen R, Yue W. Long-term treatment with tamoxifen facilitates translocation of estrogen receptor α out of the nucleus and enhances its interaction with EGFR in MCF-7 breast cancer cells. Cancer Res 2007;67:1352–60.
53. Font de Mora J, Brown M. AIB1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor. Mol Cell Biol 2000;20:5041–7.
54. Osborne C, Bardou V, Hopp T, et al. Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. J Natl Cancer Inst 2003;95:353–61.
55. Weinberg O, Marquez-Garban D, Pietras R. New approaches to reverse resistance to hormonal therapy in human breast cancer. Drug Resist Updat 2005;8: 219–33.
56. Johnston S, Martin L, Head J, et al. Aromatase inhibitors: combinations with fulvestrant or signal transduction inhibitors as a strategy to overcome endocrine resistance. J Steroid Biochem Mol Biol 2005;95:173–81.
57. Wright C, Nicholson S, Angus B, et al. Relationship between c-erbB-2 protein product expression and response to endocrine therapy in advanced breast cancer. Br J Cancer 1992;65:118–24.
58. Borg A, Baldetorp B, Ferno M, et al. ErbB2 amplification is associated with tamoxifen resistance in steroid-receptor positive breast cancer. Cancer Lett 1994;81:137–43.
59. Leitzel K, Teramoto Y, Konrad K, et al. Elevated serum c-erbB-2 antigen levels and decreased response to hormone therapy of breast cancer. J Clin Oncol 1995; 13:1129–35.
60. Benz C, Scott G, Sarup J, et al. Estrogen-dependent, tamoxifen-resistent tumorigenic growth of MCF-7 cells transfected with HER2/neu. Breast Cancer Res Treat 1993;24:85–92.
61. Lipton A, Ali S, Leitzel K, et al. Elevated serum Her-2/neu level predicts decreased response to hormone therapy in metastatic breast cancer. J Clin Oncol 2002;20:1467–72.
62. De Laurentis M, Arpino G, Massarelli E, et al. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. Clin Cancer Res 2005;11: 4741–8.
63. Kato S, Endoh H, Masuhiro Y, et al. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 1995;270: 1491–4.
64. Stoica G, Franke T, Wellstein A, et al. Estradiol rapidly activates Akt via the ErbB2 signaling pathway. Mol Endocrinol 2003;17:818–30.
65. Pietras RJ, Marquez DC, Chen H-W, et al. Improved antitumor therapy with Herceptin and Faslodex for dual targeting of HER-2 and estrogen receptor signaling pathways in human breast cancers with overexpression of HER-2/neu gene. Breast Cancer Res Treat 2003;82:12–3.
66. Ellis M, Coop A, Singh B, et al. Letrozole inhibits tumor proliferation more effectively than tamoxifen independent of HER1/2 expression status. Cancer Res 2003;63:6523–31.
67. Finn R, Dering J, Ginther C, et al. ER^+ PR^- breast cancer defines a unique subtype of breast cancer that is driven by growth factor signaling and may be more likely to respond to EGFR targeted therapies. J Clin Oncol ASCO Ann Meeting Proc 2006;24:514S.
68. Subbaramaiah K, Howe L, Port E, et al. HER-2/neu status is a determinant of mammary aromatase activity *in vivo*: evidence for a cyclooxygenase-2-dependent mechanism. Cancer Res 2006;66:5504–11.
69. Kinoshita Y, Chen S. Induction of aromatase (CYP19) expression in breast cancer cells through a nongenomic action of estrogen receptor α. Cancer Res 2003;63:3546–55.
70. Masamura S, Santner SJ, Heitjan DF, et al. Estrogen deprivation causes estradiol hypersensitivity in human breast cancer cells. J Clin Endocrinol Metab 1995;80: 2918–25.
71. Santen RJ, Song RX, Zhang Z, et al. Adaptive hypersensitivity to estrogen: mechanism for sequential responses to hormonal therapy in breast cancer. Clin Cancer Res 2004;10:337–45S.
72. Song R, Santen R. Membrane initiated estrogen signaling in breast cancer. Biol Reprod 2006;75: 9–16.
73. Witters I, Kumar R, Chinchilli V, Lipton A. Enhanced antiproliferative activity of the combination of tamoxifen plus HER-2-neu antibody. Breast Cancer Res Treat 1997;42:1–5.
74. Kunisue H, Kurebayashi J, Otsuki T, et al. Anti-HER-2 antibody enhances the growth inhibitory effect of anti-oestrogen on breast cancer cells expressing both oestrogen receptors and HER-2. Br J Cancer 2000;82:46–51.
75. Márquez-Garbán D, Chen HW, Fishbein M, et al. Estrogen receptor signaling pathways in human non-small cell lung cancer. Steroids 2007;72: 135–43.
76. Mackey JR, Kaufman B, Clemens M, et al. Trastuzumab prolongs progression-free survival in hormone-dependent and HER2-positive metastatic breast cancer. Breast Cancer Res Treat 2006;100:abstract 3.
77. Ciardiello F, Caputo R, Bianco R, et al. Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. Clin Cancer Res 2001;7:1459–65.
78. Shou J, Massarweh S, Osborne C, et al. Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. J Natl Cancer Inst 2004;96:926–35.
79. Nicholson R, Gee J, Knowlden J, et al. The biology of antihormone failure in breast cancer. Breast Cancer Res Treat 2003;80:S29–34.
80. Dubey S, Siegfried J, Traynor A. Non-small-cell lung cancer and breast carcinoma: chemotherapy and beyond. Lancet Oncol 2006;7:416–24.
81. Ellis M. Overcoming Endocrine Therapy Resistance by Signal Transduction Inhibition. Oncologist 2004;9: 20–6.
82. Peralba J, DeGraffenried L, Friedrichs W, et al. Pharmacodynamic evaluation of CCI-779, an inhibitor of mTOR, in cancer patients. Clin Cancer Res 2003;9: 2887–92.
83. Ferrara N. VEGF as a therapeutic target in cancer. Oncology 2005;69 Suppl 3:11–6.
84. Pietras RJ. Interactions between estrogen and growth factor receptors in human breast cancers and the tumor-associated vasculature. Breast J 2003;9: 361–73.
85. Mendelsohn M, Karas R. The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999;340:1801–11.
86. Wicha M, Liu S, Dontu G. Cancer stem cells: an old idea—a paradigm shift. Cancer Res 2006;66: 1883–90; discussion 1895–6.