Cracking the Estrogen Receptor's Posttranslational Code in Breast Tumors

Muriel Le Romancer, Coralie Poulard, Pascale Cohen, Stéphanie Sentis, Jack-Michel Renoir, and Laura Corbo

Université de Lyon (M.L.R., C.P., P.C., S.S., L.C.), F-69000 Lyon, France; Université Lyon 1 (M.L.R., C.P., P.C., S.S., L.C.), F-69000, Lyon, France; Université Lyon 1 (P.C., S.S.), Institut des Sciences Pharmacologiques et Biologiques, Faculté de Pharmacie de Lyon, F-69000 Lyon, France; Equipe Labellisée 'La Ligue' (M.L.R., C.P., P.C., S.S., L.C.), Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 1052, Centre de recherche en Cancérologie de Lyon, F-69000 Lyon, France; Centre National de la Recherche Scientifique Unité Mixte de Recherche 5286 (M.L.R., C.P., P.C., S.S., L.C.), Centre de recherche en Cancérologie de Lyon, F-69000 Lyon, France; Centre Léon Bérard (M.L.R., C.P., P.C., S.S., L.C.), F-69000, Lyon, France; INSERM Unité 749 (J.-M.R.), Institut Gustave Roussy, 94805 Villejuif Cedex, France; and Université Paris-Sud (J.-M.R.), Orsay, F-91405, France

Estrogen signaling pathways, because of their central role in regulating the growth and survival of breast tumor cells, have been identified as suitable and efficient targets for cancer therapies. Agents blocking estrogen activity are already widely used clinically, and many new molecules have entered clinical trials, but intrinsic or acquired resistance to treatment limits their efficacy. The basic molecular studies underlying estrogen signaling have defined the critical role of estrogen receptors (ER) in many aspects of breast tumorigenesis. However, important knowledge gaps remain about the role of posttranslational modifications (PTM) of ER in initiation and progression of breast carcinogenesis. Whereas major attention has been focused on the phosphorylation of ER, many other PTM (such as acetylation, ubiquitination, sumoylation, methylation, and palmitoylation) have been identified as events modifying ER expression and stability, subcellular localization, and sensitivity to hormonal response. This article will provide an overview of the current and emerging knowledge on ER PTM, with a particular focus on their deregulation in breast cancer. We also discuss their clinical relevance and the functional relationship between PTM. A thorough understanding of the complete picture of these modifications in ER carcinogenesis might not only open new avenues for identifying new markers for prognosis or prediction of response to endocrine therapy but also could promote the development of novel therapeutic strategies. **(*Endocrine Reviews* 32: 597–622, 2011)**

I. Introduction

The steroid hormone estrogens play a critical role in various target tissues, including the reproductive tract and the central nervous, vascular, and skeletal systems, as well as in normal mammary gland development (1). This effect is mediated by two receptors, estrogen receptor (ER) α and ERβ, which function in the nucleus as ligand-dependent transcription factors. ERα is responsible for many of the effects of estrogens on normal and malignant breast tissues. Lifetime exposure to 17β-estradiol (E_2) or' estrogenic compounds constitutes a major risk factor for breast cancer development (2), through either transcriptional regulation (genomic action) (3) or involvement in membrane and cytoplasmic signaling cascades (nongenomic action) (4). In normal breast, only 10 to 20%

Abbreviations: AF-1, Activation function; AF-2, transactivation function; AI, aromatase inhibitor; AP-1, activator protein-1; CBP, CREB binding protein; Cdk, cyclin-dependent kinase; DBD, DNA-binding domain; E_2, 17β-estradiol; E6-AP, E6-associated protein; EGF, epidermal growth factor; EGFR, EGF receptor; ER, estrogen receptor; ERE, estrogen response element; GSK3, glycogen synthase kinase-3; HAT, histone acetyltransferase; HDAC, histone deacetylase; hER, human ER; IKK, IkB kinase; LBD, ligand-binding domain; LMP2, low molecular mass polypeptide 2; mER, mouse ER; PAK1, serine/threonine p21-activated kinase; PAT, palmitoyl acyltransferase; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PRMT1, protein arginine methyltransferase 1; PTM, posttranslational modification; RSK, ribosomal S6 kinase; SDF-1, stromal cell-derived factor-1; SERD, selective ER down-regulator; SERM, selective ER modulator; SRC, steroid receptor coactivator; SUMO, small ubiquitin-related modifier.

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of epithelial cells express ERα (5), but ERα-null mice only develop a rudimentary mammary gland, indicating that ERα expression is essential to mammary development (1). By contrast, the mammary glands of ERβ-null mice develop normally (1). Interestingly, in breast cells, ERβ seems to act as an antagonist of ERα activity, impairing the ability of estrogen to stimulate proliferation (6–8). Thus, ERβ may play a protective role in breast tumors by inhibiting proliferation and invasion (9–11).

There is compelling clinical and experimental evidence that sustained exposure to estrogens increases the breast cancer risk and promotes cancer progression by stimulating malignant cell proliferation (12). Although in the normal breast ERα and ERβ are expressed at low levels (13), 70% of breast cancers are ERα positive, underpinning the use of agents that suppress receptor function (antiestrogens) or estrogen synthesis (aromatase inhibitors) in breast cancer treatment (14). ERα is a well-established predictive marker for hormone sensitivity and a good prognostic marker in breast cancer; it helps identify tumors that are likely to respond to endocrine treatment. Unfortunately, about 40 to 50% of the women receiving endocrine therapy fail to respond (*de novo* resistance) or become resistant (acquired resistance) to the treatment (15). A focus on estrogen signaling could help understand its deregulations in patients prone to develop resistance to endocrine therapy. Cellular responses to estrogens are complex and highly controlled. They involve the regulation of ER functions through coactivators that stabilize ER binding to DNA, thus promoting gene transcription (genomic action) and the direct activation of signaling cascades (nongenomic action).

Further levels of complexity are added by the fact that several isoforms have been identified for each receptor (16), as well as mutations (17, 18) and numerous posttranslational modifications (PTM) (19) that regulate their function by influencing a number of interactions with other proteins including cytoplasmic signaling molecules (20). In addition to the long-known phosphorylation of ERα, other covalent additions such as acetylation, ubiquitination, sumoylation, and methylation have been recently described. The complexity of ER regulation is a rapidly growing issue because new PTM are continuously being identified. Reviews focusing on ER phosphorylation (21, 22) or acetylation (23) have been published, but there is a real need for more recent updated information about other ER-targeting PTM. The aim of this article is to summarize current and emerging knowledge on ER PTM, particularly on those that are deregulated in breast tumors; this could help to identify new prognostic biomarkers or biomarkers predictive of response to endocrine therapy and/or to circumvent endocrine-resistant tumor growth.

II. Biology of Estrogen Receptors and Breast Cancer

A. ERα and ERβ subtypes

ER belong to the superfamily of ligand-activated transcription factors (24). There are two known ER isoforms, ERα and ERβ. They arise from separate genes located on different chromosomes, but they share a high degree of overall homology, especially in their DNA binding domain (25), whereas they display different physiological functions. Like other members of this class of transcription factors, ER proteins have a characteristic modular structure. They are organized into five main domains: an N-terminal A/B region bearing the ligand-independent activation function (AF-1), a DNA-binding domain (DBD) composed of two zinc fingers, a hinge region containing the nuclear localization signal, and a domain E harboring the ligand-binding domain (LBD) and the ligand-dependent transactivation function (AF-2). The adjacent region F, located at the C terminus of the receptors, is a variable domain whose specific function has not yet been fully clarified (Fig. 1) (26). The classical 66-kDa ERα (27) and the 60-kDa ERβ (28, 29) have similar structures in their central DNA-binding region (98% identity), whereas they largely differ in their activation domains (less than 15% homology in their N termini), suggesting that they may recruit different coactivator proteins, thereby altering their specific transcriptional effects. Moreover, even if ERβ, like ERα, binds to E_2, ERβ has a markedly reduced transcriptional activation partially due to the lack of AF-1 activity (30). The two receptors share little homology (53%) in their LBD (31), which accounts for the differences in their affinity for various ligands. For example, both full-length ER bind to E_2 with high affinity, but they differ in their ability to bind other natural and synthetic ligands. In particular, ERβ has higher affinity for phytoestrogens like genistein, quercetin, or coumestrol than ERα (32, 33). Two distinct classes of synthetic ER ligands have been developed; by binding to ER, they give rise to distinct tissue-selective pharmacological profiles. Selective ER modulators (SERM) are a class of ER ligands, exemplified by tamoxifen and raloxifene, that inhibit AF-2 (but not AF-1)-dependent activation of ER (34). Tamoxifen acts as an antagonist in breast cells, in which ER activity is mainly dependent on AF-2, and as an agonist mimicking the action of estrogens in tissues like the uterus, where AF-1 activity is more important (35). Selective ER down-regulators (SERD), including the pure ER antagonist ICI 182,780 or Faslodex (also called fulvestrant), are antiestrogenic in all tissues (36, 37). They bind to ERα with 100-fold greater affinity than tamoxifen, thus inhibiting receptor dimerization and abrogating estrogen signaling. The development of knockout or transgenic mice with

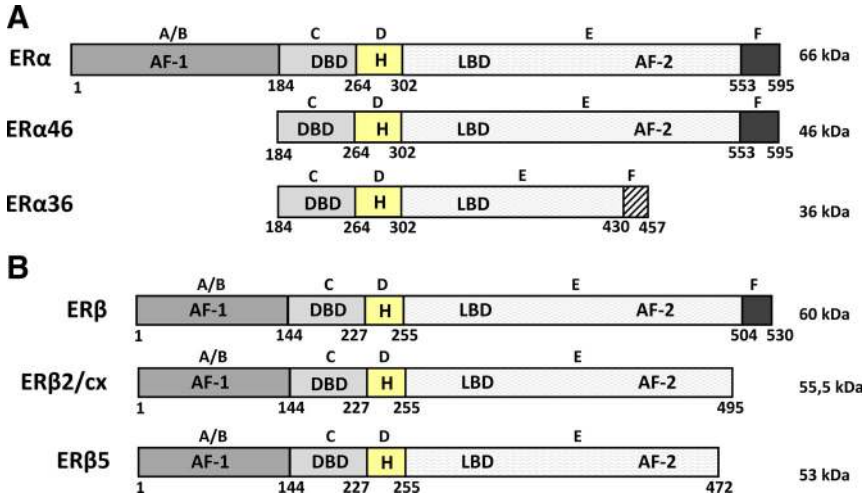

FIG. 1. Structure of ER isoforms for ERα (A) and ERβ (B). ER, like other members of the nuclear receptor family, display conserved functional domains: A/B, encompassing AF-1; C, containing the DBD; D, called hinge domain contains nuclear localization signals; E, containing the LDB and AF-2; and F, the agonist/antagonist regulator. A, ERα46, a truncated form of ERα lacking AF-1 is also shown. Another spliced variant lacking the AF-1 and AF-2 domains, ERα36, has been cloned in breast cells. B, ERβ2/cx and ERβ5 are two spliced variants of ERβ lacking the F domain.

disruptions, mutations, or overexpression of ER has increased our understanding of the relative roles and biological functions of ERα and ERβ (38–41). ERα is essential for proliferation, and in contrast, ERβ has an antiproliferative role (40, 42–44). The two receptors regulate different genes in response to E_2 as well as antiestrogens (42, 43, 45). They can form heterodimers that may contribute to differences in ER-dependent gene expression (46, 47). Finally, ERβ is known to inhibit both ERα-mediated transcription and E_2-induced proliferation in various cancer cells (6, 7, 48–51).

For both ER, the type of response elicited by ligand binding also depends on the cell type and on the promoters that "communicate" most of the mitogenic and survival stimuli of estrogens through direct or indirect effects on gene expression.

In addition to the classical transcripts of both receptors, numerous mRNA splice variants arising from alternative splicing or alternative promoters have been characterized. The full-length ERα66 (named ERα in this review) is produced from an mRNA transcribed from the ERα gene that contains at least seven upstream promoters (52). The ERα46 variant lacking the AF-1 domain (Fig. 1A) has been identified in MCF-7 breast cancer cells (53), osteoblasts (54), human macrophages (55), and endothelial cells (56). ERα46 represses the AF-1 activity of ERα66 (57) and inhibits the E_2-induced transcription of the *pS2* gene through recruitment of corepressors (58). In breast cell lines, this variant inhibits the action of wild-type ER in terms of both E_2 binding (59) and transcriptional response (60, 61), thus participating in endocrine resistance. The transfection of ERα46 in tamoxifen-resistant breast cancer cells inhibits ERα66 responses and enhances endocrine treatment (62). However, the expression of this isoform has not been evaluated in breast tumor samples.

A 36-kDa variant (ERα36) lacking both AF-1 and AF-2 domains has been found in breast cancer cell lines (Fig. 1A) (63). This isoform transduces membrane-initiated estrogen-dependent activation of the MAPK pathway in nongenomic E_2-mediated signaling (64, 65). Interestingly, patients who express ERα and high levels of ERα36 are less likely to respond to tamoxifen treatment (65, 66).

In addition to ERβ1, the full-length or wild-type receptor of ERβ, other variants resulting from differential splicing, namely ERβ2/cx (67, 68) and ERβ5 (25, 67, 69), have been identified in breast tumors (Fig. 1B). Their precise role in breast cancer is still poorly understood, but their implication in various forms of resistance is strongly suspected (70).

B. Genomic action of ER

The classical model of ER activation implies that, after ligand binding, a ligand-specific conformational change of the receptor occurs, allowing its dissociation from inhibitory heat shock protein complexes and subsequent phosphorylation (71–73). These modifications trigger homo- or heterodimerization of ER and binding to specific estrogen response elements (ERE) in target genes (Fig. 2, path A). The ligand-induced conformational change of the receptor facilitates the association/dissociation of components of coregulator complexes associated with enzymatic activities that regulate chromatin function, including nucleosome remodeling, histone acetyltransferase (HAT), or methyltransferase, as well as communication with the transcriptional machinery (reviewed in Refs. 74–76) (Fig. 2, path A).

In contrast to estrogens, the binding of estrogen antagonists from the SERM group induces a distinct conformation of ER that triggers their association with corepressors like NCoR and SMRT, thereby shutting off gene transcription (77). Tamoxifen, which aims to block ERα action, has been the mainstay of hormonal therapy in both early and advanced breast cancer patients for approximately three decades (78, 79). Interestingly, tamoxifen has mixed agonist/antagonist activity and may either stimulate or antagonize ER function in tissues and genes (35). As

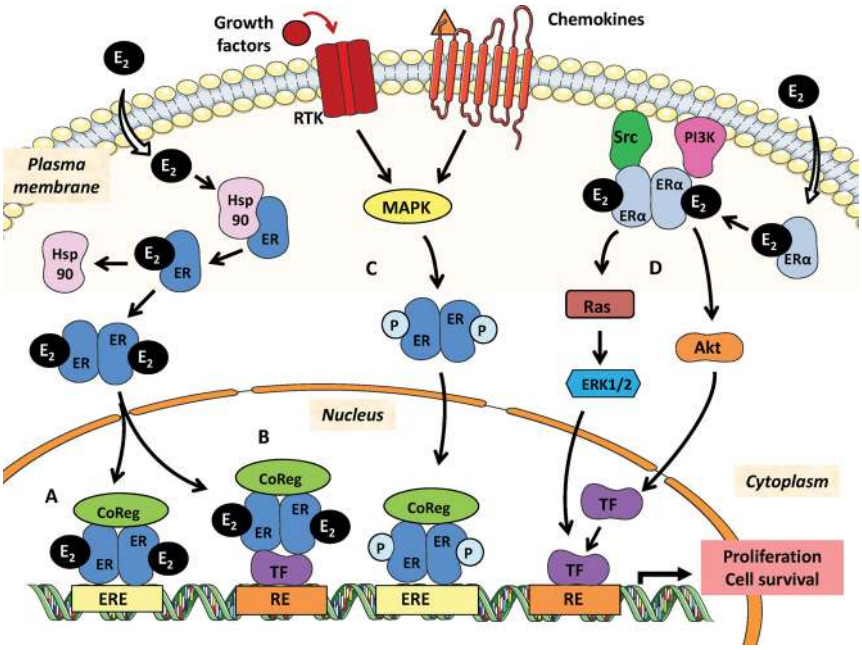

FIG. 2. ER signaling pathways. When pathways are common for ERα and ERβ, we designated ER; otherwise, ER subtype is specified. Four distinct pathways of estrogen signaling through ER are shown. Pathway A is called the classical genomic pathway; E_2-bound ER dimerize and, after a change of conformation, go to the nucleus and bind ERE and recruit coactivators that will activate the transcription of target genes. Pathway B, the nonclassical genomic pathway, involves ER interactions with other transcription factors like AP-1. Pathway C is E_2-independent and activates ER through phosphorylation induced by growth factors. Pathway D, the nongenomic pathway, involves a small pool of ER located close to the membrane that, through recruitment of protein kinases (Src and PI3K), activate signaling cascades (Akt, MAPK). All of these pathways converge to cell proliferation and survival. RE, Response elements to other transcription factors; P, phosphorylation; RTK, tyrosine kinase receptor; CoReg, coregulators; TF, transcription factor; Hsp90, heat shock protein 90.

previously demonstrated, the level of expression and/or activity of coregulatory proteins can lead to alterations in ER signaling. Several studies have reported that high expression of the ER coactivator steroid receptor coactivator (SRC)-3/AIB1 (for review, see Ref. 80) in patients receiving tamoxifen adjuvant therapy is associated with poor clinical outcome, which is indicative of tamoxifen resistance (81, 82).

ER can also regulate the activity of other transcription factors through protein-protein interactions with coactivator proteins (83). Via this so-called "nonclassical" action, nuclear ER interact with specificity protein 1 (84, 85) or activator protein-1 (AP-1) (84, 86) or nuclear factor-κB (87) through a process referred to as transcription factor cross talk (84, 86) (Fig. 2, path B). However, there is an interesting difference between ERα and ERβ at AP-1 sites. In the presence of estrogens, ERα induces AP-1 driven reporter activity, whereas ERβ has no effect (88). Of interest, increased AP-1 and nuclear factor-κB transcriptional activities have been shown to be involved in endocrine resistance (89–91).

Finally, ER activities are also regulated by estrogen-independent pathways involving kinases that are activated through growth factor receptors like epidermal growth factor receptor (EGFR) (92, 93) (Fig. 2, path C). ERβ is also known to be phosphorylated by the chemokine receptor CXCR4 after its activation by binding to CXCL12/stromal cell-derived factor-1 (SDF-1) through Erk activation (94) (Fig. 2, path C). Very recently, this pathway has also been involved in acquired tamoxifen resistance (95).

C. Nongenomic action of ER

Estrogens also exert rapid stimulatory effects on a variety of signal transduction proteins. This pathway, largely studied for ERα, begins outside the nucleus and is independent of gene transcription. The identity of the receptors mediating this rapid signaling is still a matter of controversy (96–98). However, there is clear evidence that this activity is mediated, at least in part, by a small fraction of ERα that is localized near or at the plasma membrane (99). Dimers of ERα (100) activate multiple signal transduction cascades through direct interactions of ER with various proteins, including the tyrosine kinase Src, the phosphatidylinositol 3-kinase (PI3K), and adaptor proteins (101–105) (Fig. 2, path D). ERα localization to the membrane can also be mediated by interactions with the membrane adaptor protein Shc (106) and with a variety of proximal signaling molecules such as G proteins (107). ERα interactions with Src and PI3K are also activated by other accessory proteins such as the adaptor protein p130Cas, which regulates the activation of Src kinase in T47D human breast carcinoma cells (108). Other partners may be involved, like the caveolin-binding protein striatin, which targets ERα to the plasma membrane (109). Estradiol-dependent complex formation of ERα with Src and the PI3K subunit p85 activates two major pathways: the Src/ras/MAPK and the PI3K/Akt pathways.

The activation of kinases triggers biological responses that are either dependent or independent of transcription. For example, the activation of Src and PI3K leads to increased cell proliferation (103) and abrogation of apoptosis through inactivation of the proapoptotic protein BAD via phosphorylation (110). More recently, nongenomic signaling has been shown to be associated with other cel-

lular processes. E_2 activation of PI3K/Akt signaling, for instance, inhibits the Ataxia Telangiectasia-mutated and Rad3-related kinase pathway controlling cell cycle checkpoints and DNA repair (111) and the interaction of histone deacetylase (HDAC) 6 with membrane-localized ERα, causing rapid deacetylation of tubulin that potentially contributes to cell migration and to aggressiveness of ERα-positive breast cancer cells (112). Catalano *et al.* (113) have identified another role of nongenomic estrogen signaling, namely that E_2-stimulated Src increases aromatase activity, revealing the existence of an autocrine loop between E_2 and aromatase possibly involved in breast tumorigenesis. Even if little is known on ERβ nongenomic signaling, the presence of ERβ at the plasma membrane suggests that it must also be involved in extra nuclear actions (114) and particularly through its association with Src (115).

For convenience, we present the genomic and nongenomic pathways separately. However, these signaling pathways are not mutually exclusive but rather complementary, with many interactions between them. The idea is that nongenomic and genomic effects may integrate a unique mode of action of estrogens in which the nongenomic signaling pathways converge for transcriptional regulation (116).

D. Endocrine therapies in breast cancer

Approximately 70% of all breast cancers are dependent on estrogen and on a functional ERα for growth. Hence, ERα-positive breast cancer is usually treated with hormone reduction or antiestrogens (15). Treatment approaches include the blockade of ERα activity by SERM like tamoxifen (117) or the destabilization and degradation of ER by SERD like fulvestrant (118–121). The antineoplastic effects of endocrine drugs are largely mediated by the inhibition of ER transcriptional effects on gene expression. Tamoxifen, which binds to and antagonizes ER, only blocks the transcriptional activating function AF-2 and thus only partially inactivates ER-regulated transcription (122, 123). Fulvestrant, which blocks both AF-1 and AF-2 functions, induces complete abrogation of the transcription of ER-regulated genes (36, 37). In the past two decades, tamoxifen has been the mainstay of endocrine therapy for both early and advanced breast cancers and has significantly contributed to the decrease in breast mortality (124). However, approximately 50% of patients with advanced disease do not respond to first-line treatment with tamoxifen, and almost all patients with metastases relapse and die from the disease (15). A second endocrine therapy strategy has recently emerged. It consists of reducing the production of estrogen in peripheral tissues and within the tumor using aromatase inhibitors (AI) like anastrozole, letrozole, or exemestane (125, 126). AI inhibit tumor growth and have proven superior to tamoxifen in certain patient subsets (127).

However, whatever the endocrine treatment used, intrinsic or acquired resistance may occur. The molecular causes of endocrine resistance are multiple and not completely understood. Several mechanisms have been described, including the deregulation of ER expression and maturation, as well as the deregulation of PTM for both receptors and their cofactors. Moreover, increased Her2/NEU (Erb-B2) receptor tyrosine kinase signaling and deregulation of the cell cycle and apoptosis machineries have also largely been associated with resistance (for review, see Ref. 128).

Because only 1% of primary breast cancers carry ERα mutations (129), it seems more relevant to focus on ER signaling to elucidate the mechanisms responsible for resistance, to develop more specific biomarkers predictive of response to endocrine therapy, and to identify new therapeutic targets for endocrine-resistant disease.

III. Posttranslational Modifications of Estrogen Receptors

Because PTM are crucial to the physiological function of proteins, understanding the coding of these modifications is particularly important to elucidate the activity of a given protein.

ER and their coregulators are targets for multiple PTM (130). The synergic coupling between extranuclear and genomic actions of hormones is accompanied by highly specific PTM that serve not only as triggers but also as mediators and promoters of the signaling cascades. Deregulating both the phosphorylation cascades and the acetylation process, for example, can dramatically affect the fate of a number of factors involved in ER signaling (121, 131, 132) and impact cell survival. The development of site-specific antibodies recognizing only modified forms of ERα has greatly simplified the analysis of PTM and has led to rapid advances in mechanistic studies. More recently, mass spectrometry has facilitated the identification of additional PTM, and the catalog of ERα PTM now comprises modifications of approximately 22 sites throughout the molecule, including phosphorylation, methylation, acetylation, sumoylation, and ubiquitination. Much less information is available for ERβ. Most of our understanding of ERβ PTM is derived from the pioneering work of A. Tremblay and relates to phosphorylation (133). However, it seems that the phosphorylation of ERβ by protein kinases varies with the type of cell, the type of promoter, and the identity of the effector.

In this section, we will focus on well-documented PTM targeting ER (less well-documented PTM are de-

scribed only in Table 1). These modifications influence ER functions, integrating genomic and rapid action, as they act on protein stability, subcellular localization, dimerization, DNA binding, and interaction with coregulators. We will also describe the known interplay between modifications as well as their deregulation in breast cancer.

All modified residues and their functional data are listed in Table 1 for ERα and in Table 2, for ERβ. PTM are also presented on ER sequences to permit visualization of the domain where the modification occurs (Fig. 3).

A. PTM involved in ER genomic signaling

1. Phosphorylation of ERα on serine

It has been known since the early 1980s that ERα is a phosphoprotein whose ligand-binding activity is enhanced by phosphorylation (134) through the activation

TABLE 1. Modified sites of human ERα and their function

Amino acid	Modification	Activator	Enzyme involved	Function	Ref.
Y52	Phosphorylation	Ligand independent	c-Abl	Activates stability, transcription	262
S102	Phosphorylation	Constitutive, E_2	GSK3	Activates transcription	137
S104/106	Phosphorylation	Constitutive, E_2	GSK3	Activates transcription	137
		Constitutive, E_2, Tam	Cyclin A-Cdk2	Activates transcription	138
		E_2, Tam, ICI, PMA	MAPK	Activates transcription	136
		E_2	MAPK	Dimerization	140
S118	Phosphorylation	E_2, Tam, ICI	ND	Activates transcription	145
		EGF, IGF-I	MAPK	Activates transcription	92, 146
		E_2	Cdk7	Activates transcription	146, 263
		ND	MAPK	Activates RNA splicing	139
		E_2	GSK3	Activates transcription	137
		E_2	IKKα	Activates transcription	147, 148
		Prolactin	ND	Activates transcription	149
		ROS	MAPK	Down-regulation	264
		E_2	MAPK	Dimerization	140
S154	Phosphorylation	Constitutive, E_2, EGF	ND	ND	265
S167	Phosphorylation	Constitutive	Akt	Activates transcription	152
		ROS	Akt	Down-regulation	264
		EGF	p90 RSK	Activates transcription	151
		Insulin, PMA	S6K1	Activates transcription	153, 154
		ND	Akt	Activates stability	266
S236	Phosphorylation	Constitutive	PKA	Inhibits dimerization	155
Y219	Phosphorylation	ND	c-Abl	Activates: dimerization, DNA binding, stability, and transcription	262
R260	Methylation	E_2	PRMT1	Nongenomic signalling	227
K266	Acetylation	E_2	p300	Activates transcription	181
	Sumoylation	E_2, Tam	SUMO-1	Activates transcription, DNA binding,	206
K268	Acetylation	E_2	p300	Activates transcription	181
	Sumoylation	E_2, Tam	SUMO-1	Activates transcription, DNA binding	206
S282	Phosphorylation	E_2	CK2	Inhibits transcription	267
S294	Phosphorylation	E_2	ND	Activates transcription	267
K299	Acetylation	Constitutive	p300	Inhibits transcription	182
	Sumoylation	E_2, Tam	SUMO-1	Activates transcription, DNA binding	206
K302	Acetylation	Constitutive	p300	Inhibits transcription	182
	Sumoylation	E_2, Tam	SUMO-1	Activates transcription, DNA binding	206
	Ubiquitination	Constitutive, E_2, ICI	Ubiquitin	Proteasomal degradation	201
	Methylation	Constitutive	SET7	Activates stability	208
K303	Acetylation	Constitutive	p300	Inhibits transcription	182
	Sumoylation	E_2, Tam	SUMO-1	Activates transcription, DNA binding	206
	Ubiquitination	Constitutive, E_2, ICI	ubiquitin	Proteasomal degradation	201
S305	Phosphorylation	ND	PAK1	Activates transcription	158, 160
		ND	PKA	Activates transcription	157
		ND	Akt	Resistance to AI	163
T311	Phosphorylation	E_2	p38-MAPK	Nuclear localization	268
C447	Palmitoylation	Constitutive	PAT	Plasma membrane localization	215
Y537	Phosphorylation	E_2	Calf uterine kinase	E_2 binding	269
		Constitutive	Src	Dimerization, DNA binding	223, 270
		Constitutive	Src	E_2 binding	271
		EGF	EGFR	Proliferation	272
S559	Phosphorylation	Constitutive	CK2	Inhibits transcription	267

ND, Not determined; ICI, ICI 172 780; PAK1, p21-activated kinase-1; PMA, phorbol myristate acetate; ROS, reactive oxygen species; Tam, tamoxifen; CK2, casein kinase 2.

TABLE 2. Modified sites of mouse ERβ and their function

Amino acid	Modification	Activator	Enzymes involved	Functions	Ref.
S80 (S60)	Glycosylation	ND	ND	Inhibits ERβ degradation	169
	Phosphorylation	ND	ND	Stimulates ERβ degradation	169
S94 (S75)	Phosphorylation	Constitutive	MAPK	Stimulates ERβ degradation	170
S106 (S87)	Phosphorylation	EGF, Ras	MAPK	Enhances the recruitment of SRC-1	93
		Constitutive	MAPK	Stimulates ERβ degradation	170
		CXCR4/SDF-1	MAPK	Activates transcription	94
S124 (S105)	Phosphorylation	EGF, Ras	MAPK	Enhances the recruitment of SRC-1	93
		E_2, DPN, genistein	ND	ND	261
S255	Phosphorylation	Erb-B2, Erb-B3	Akt	Inhibits transcription	173
(C399)	Palmitoylation	ND	PAT	Plasma membrane localization & activation of proapoptotic cascades	219
Y507	Phosphorylation	Constitutive	Src	Activates transcription	174

Most studies have been performed using mouse transfected HA-tagged ERβ; residues in *parentheses* correspond to residues in human ERβ. ND, Not determined; CXCR4, (CXC motif) receptor 4; DPN, diarylpropionitrile.

of a Ca^{++}-dependent calmodulin kinase (135) possibly involved in ER nuclear translocation. Although pioneer experiments were carried out with partially purified calf uterine ERα and although they caused some controversy at the time they were published, the concept of the relationship between a nuclear hormone receptor activity and its phosphorylation-dependent activation was launched.

The target residues include serine, threonine, and tyrosine; phosphorylated serines are more concentrated within the AF-1 domain, resulting in ligand-independent regulation of receptor transactivation (21). There are so many targets for phosphorylation that we have chosen to focus on the most widely analyzed residues; for the others, information can be found in Table 1.

a. S104/106. These two sites, when phosphorylated, are involved in ERα activity because acidic amino acid substitution stimulates ERα activity as strongly as S118 substitution (136). The particularity of S104/106 phosphorylation is that it is induced exclusively by estrogen and not by growth factor pathways. So far, both glycogen synthase kinase-3 (GSK3) and cyclin-dependent kinase 2 (Cdk2) kinases were involved in this modification (137, 138). More recently, MAPK has also been shown to phosphorylate these residues (136). Of note, the two residues are required for the agonist activity of tamoxifen, in conjunction with S118, suggesting that these sites might contribute to tamoxifen resistance in breast cancer (136).

b. S118. S118 has been the most widely studied target site and is a good example of the complexity of ERα phosphorylation. S118 has been reported to be important for the dimerization of ERα and ERα-mediated RNA splicing (139, 140). In addition, phosphorylation at this site is also important for direct binding to and activation/repression of several ERα target genes (141). S118 phosphorylation facilitates ERα interactions with coactivators such as CREB binding protein (CBP)/p300 (142) and SRC-1 (143) and then mediates ligand-dependent as well as ligand-independent activation of the receptor (144, 145). In support of this statement, it has been documented that both estrogens and growth factors such as epidermal growth factor (EGF) and IGF-I can result in S118 phosphorylation on ERα (146). Growth factors trigger S118 phosphorylation through Ras-MAPK cascades (92). The S118A mutant is not activated by EGF because no enhancement of E_2-induced ERE reporter activity has been observed in transfected cells (144), demonstrating the independence from ligand and the phosphorylation requirement for ERα transcriptional activity. Several different kinases are responsible for the estrogen-dependent phosphorylation of S118. Cdk7 (146), IkB kinase (IKK)α (147), and GSK3 (137) have been identified as good candidates. However, a recent study performed in MCF-7 cells using specific inhibitors has shown that IKKα, but not Cdk7, is involved in estrogen-mediated phosphorylation at S118 (148). More recently, this residue has also been shown to be phosphorylated upon prolactin treatment, but the kinase responsible remains unidentified (149). This phosphorylation is also involved in ERα-mediated RNA splicing. This effect is mediated by the binding of ERα with a component of the spliceosome complex, the splicing factor SF3a, which is dependent on the MAPK-mediated phosphorylation of ERα at S118 (139).

c. S167. S167 is a major multiregulated phosphorylated residue in ERα (150). Mutation analysis of this site has demonstrated its involvement in the transcriptional activity of the receptor (151). Its phosphorylation was previously attributed to Akt and p90RSK (p90 ribosomal S6 kinase) (151, 152), but recent convincing data have shown that S6K1 is a physiological kinase for S167 that regulates ERα transcriptional activity, thus contributing to the pro-

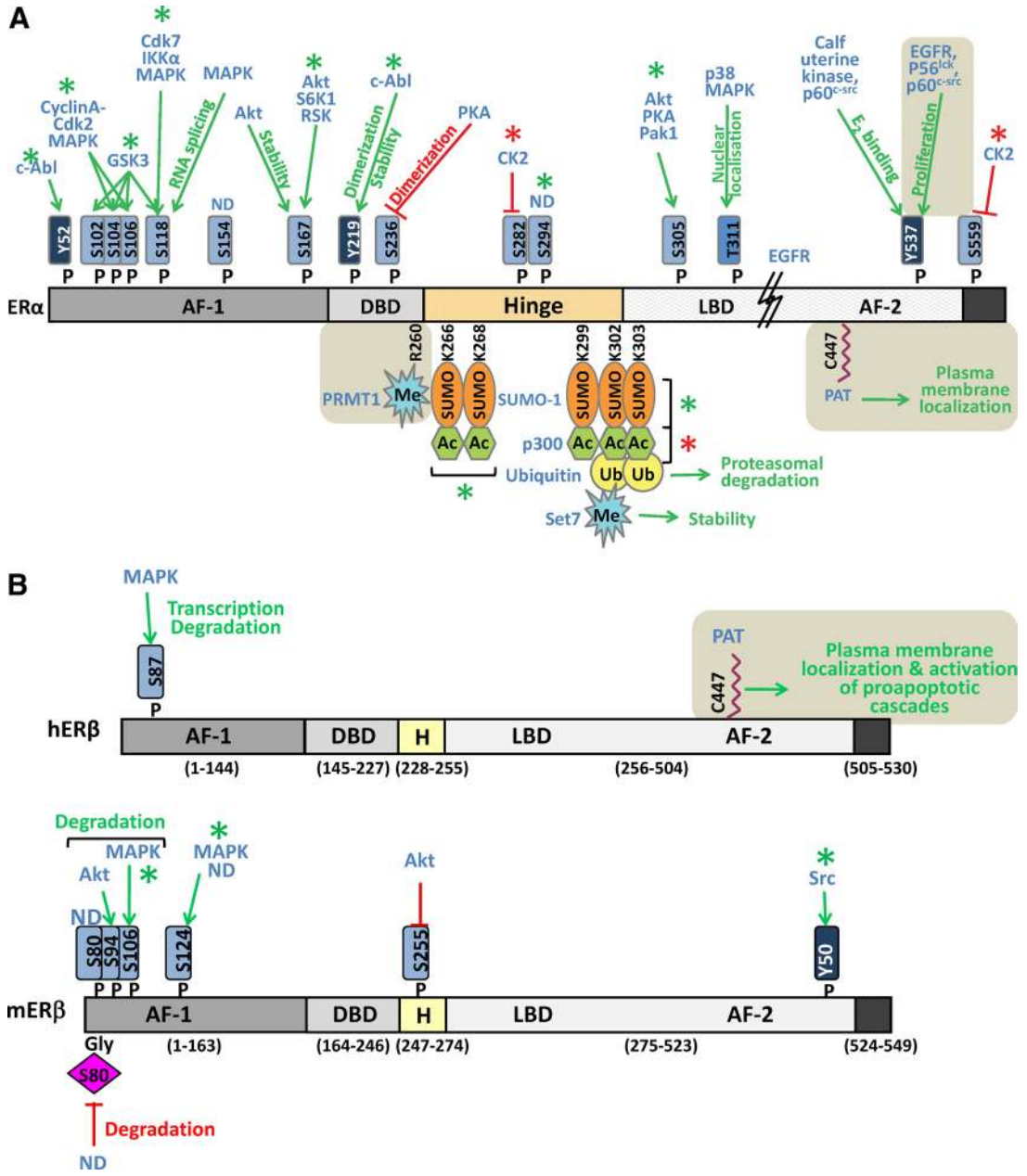

FIG. 3. Amino acids modified in ER are presented in panel A for ERα and in panel B for ERβ. All modified residues and kinases implied are represented on ER sequences. A, For ERα, the hinge domain was highly modified by PTM. B, For ERβ, because most of the sites have been described on the mouse receptor, we present both the human (hERβ) and the mouse (mERβ) isoforms. Modifications are designated as follows: Me, methylation; P, phosphorylation; Ac, acetylation; Ub, ubiquitination; Sumo, sumoylation; Gly, glycosylation. Palmitoylated sites are represented as ⟿. Functions of modifications are presented in *green* for activation and *red* for inhibition. *, Transcription activity. PTM involved in nongenomic pathways are shaded in *gray*. ND, Not determined.

liferation of ER-positive breast cancer cells (153). In fact, this phosphorylation is regulated by a temporal combination of two pathways: the MAPK/RSK and the mammalian target of rapamycin 1/S6K1 pathways (154). Interestingly, pS167 is associated with tamoxifen resistance and agonist activity in endometrium cancer cells (143).

d. S236. *In vitro*, in the absence of estrogen, protein kinase A (PKA) can phosphorylate S236, located in the DNA binding domain. S236 phosphorylation participates in ERα dimerization as a mutation of serine to glutamic acid prevents DNA binding by inhibiting dimerization. In addition, this mutation reduces E_2 and 4-hydroxy-tamoxifen activity in both the presence and absence of PKA (155). Other works have shown that S236 phosphorylation protects ERα from ligand-mediated proteasome degradation (156). This is in agreement with previous data showing that inhibition of PKA decreases ERα stability in both the presence and absence of ligands (121).

e. S305. A large body of information on S305 phosphorylation in ERα has been gathered by Michalides *et al.* (157),

who have shown that this site is phosphorylated by PKA *in vitro* and thus participates in ERα transcriptional activity. An ERα S305E mutant, mimicking constitutive phosphorylation, has been shown to increase receptor dimerization and fixation to promoters of target genes in the presence of estrogen (158). Interestingly, ERα activation at S305 is sufficient to up-regulate the expression of several ER-regulated genes like cyclin D1, a major ER-regulated gene involved in the E_2-induced progression of breast cancer (159). S305 phosphorylation and activation of ERα transcriptional activity have also been evidenced in a murine model expressing a constitutively activated serine/threonine p21-activated kinase (PAK1) (160). These mice have developed mammary gland hyperplasia, revealing a role for the PAK1-ERα pathway in promoting tumorigenesis in mammary epithelium (160). Michalides' team has shown that the down-regulation of PKA-RIα, a negative regulator of PKA, triggers PKA hyperactivation leading to S305 phosphorylation, converting tamoxifen from an ERα inhibitor into a growth stimulator (157). These authors have also pointed out the prime importance of phosphorylation at S305 for interactions of ERα with coactivators like SRC-1 and activation of gene transcription upon tamoxifen treatment (161). Interestingly, despite its ability to induce tamoxifen resistance, pS305 ERα alone does not modify the response to another endocrine molecule like the SERD fulvestrant, and the additional overexpression of cofactors like cyclin D1 and SRC-1 is required (157).

However, recent data obtained using an antibody specific for pS305 ERα have demonstrated that in a cellular context, PKA (and not PAK1) can induce this phosphorylation (162).

Recently, Fuqua's team (163) has shown that this residue is also phosphorylated by Akt through a cross talk between the IGF-I and ERα pathways. They have also demonstrated that when the K303R ERα mutant is overexpressed in breast cancer cells, S305 is constitutively phosphorylated, leading to resistance to tamoxifen (164) but also to the AI anastrozole (163, 165).

2. Phosphorylation of ERβ

Much less is known about the phosphorylation of ERβ. Although the intracellular kinases involved have not yet been precisely identified, residues, mostly localized in the AF-1 domain, have been observed, whether in the presence or in the absence of ligand binding to the receptor. The modulation of several kinase activities could activate unliganded ERβ-mediated transcription (166).

Contrary to the situation for ERα, for ERβ most of the modified residues have been identified in the mouse receptor; the corresponding amino acids in human will be given in *parentheses*. It appears that there is a discrepancy in ERβ amino acid nomenclature due to the discovery of a longer transcript at the 5′ end of the molecule (166, 167).

a. S80 (S60). The phosphorylation of mouse S80 has been identified by mass spectrometry (168). The same study has reported that this site could also be modified through O-GlcNAcylation (Table 2). S80 is located in a PEST region enriched with proline, glutamine, serine and threonine, responsible for the rapid degradation of proteins, suggesting that modification of this residue could be involved in ERβ stability (169). Studies using mutants of mouse ERβ (mERβ) S80E mimicking phosphorylation have clearly demonstrated that phosphorylation of S80 results in rapid degradation of the receptor (169).

b. S94 (S75). In mouse, phosphorylated S94 regulates the nuclear mobility of ERβ through a proteasome-dependent pathway implicating the E6-associated protein (E6-AP) ubiquitin ligase (170). This observation suggests that the AF-1 domain of ERβ is involved not only in E6-AP-dependent transactivation but also in ERβ stability through phosphorylations via MAPK pathways.

c. S106 (S87) and S124 (S105). Both EGF and the oncogene Ras are also able to activate ERβ through MAPK-directed phosphorylation of mouse S106 and S124 (corresponding to human S87 and S105), favoring the recruitment of SRC-1 and HAT CBP to the AF-1 domain (93, 171). Additionally, these two phosphorylated sites are partially responsible for the Erb-B2/Erb-B3-mediated inhibition of liganded ER signaling (172).

Finally, mouse ERβ S106 has been shown to be phosphorylated by MAPK in response to CXCL12/SDF-1, the ligand of the chemokine receptor CXCR4. S106 phosphorylation leads to enhanced ER transcriptional activity and expression of ER target genes involved in cancer cell growth, including SDF-1 (94). This phosphorylation enables ERβ responsiveness at AP-1 sites in an autocrine/paracrine feedback loop, even when the receptor is complexed with a SERM like tamoxifen.

Other sites have been shown to be phosphorylated (Table 2). S255 (equivalent to human S234), for instance, is phosphorylated via the PI3K/Akt pathway through the phosphorylation-dependent release of CBP (173), whereas Y507 in mouse (corresponding to human Y488) is involved in the ligand-independent activation of ERβ (174), which is suppressed by antiestrogens. Mutation of this Y488 [which corresponds to Y537 for human ERα (hERα)] in arginine is involved in the ligand-independent activation of ERβ (174).

Although it has been shown that inhibitors of protein phosphatases regulate ERα signaling (175, 176), little information is available regarding the identities of the ER phosphatases involved. There is only one paper describing that the serine/threonine phosphatase PP5 targets the dephosphorylation of S118 in ERα, mediating an inhibition of its transcriptional activity (177). Efforts should be made to identify more of these phosphatases and to modulate the level of phosphorylation of ERα.

3. Acetylation of ERα

Acetylation of proteins plays an important role in the regulation of their activity. In addition to histones, a broad range of other proteins, including transcription factors, are acetylated, resulting in enhanced or reduced activity (23, 178). The HAT p300/CBP has been described as a coactivator for ERα through interaction with proteins to form "enhanceosomes" or acetylation of specific lysines in the N terminus of histones (179, 180). ERα is acetylated at multiple sites because five lysines have been reported to be acetylated by p300: K266, K268, K299, K302, and K303 (181, 182) (Table 1). Interestingly, the effect of acetylation on ERα activity varies with the target lysine; acetylation of K299, K302, and K303 inhibits the transcriptional activity of the receptor (182), whereas acetylation of K266 and K268 triggers a stimulatory effect (181). Acetylation of K299, K302, and K303 is constitutive, whereas acetylation of K266 and K268 is estrogen-dependent. However, antibodies specific for acetylated lysines are not available to address the relevance of this modification *in vivo*. Little is known about the mechanisms regulating ERα acetylation, but recent data show that BRCA1 could be a potent inhibitor of ERα activity, partly through impairment of p300-mediated ERα acetylation (183). The enzyme responsible for the reversal of this modification remains to be identified, even if deacetylase sirtuin 1 has emerged as a good candidate in *in vitro* experiments (181). For ERβ, no acetylation has been described so far.

Interestingly, the degradation of native ERα after HDAC inhibitor exposure of ER-positive breast cancer cells has been shown to occur through the proteasome pathway, an important tool to reduce estradiol-mediated cell growth (132, 184). On the contrary, the stability of ERβ seems to be unaffected by HDAC inhibitors (185).

4. Ubiquitination of ERα

All steroid receptors are subjected to ubiquitination, and several of the enzymes involved in this PTM have been identified (19). In most cases, however, the exact site of modification is uncertain, possibly due to the instability of polyubiquitinated proteins.

It has long been known that the regulation of ERα cellular level and transcriptional activity involves the ubiquitin-26S proteasome system (118–120). Although both basal (*e.g.*, ligand-independent) and ligand-induced ERα degradations are mediated by this proteolysis pathway (121, 186), the regulation of receptor degradation at the molecular level is highly dependent upon the physiological state and nature of the cellular stimuli. Ubiquitin-dependent degradation of E_2-bound receptor is thought to be critical for promoter clearance and additional rounds of transcription (187). Distinct mechanisms are involved in the down-regulation of ERα and the promotion of lysine polyubiquitination and subsequent proteasome-mediated receptor degradation. ERα is degraded via three distinct pathways in breast cancer cells.

First, unliganded ERα is very stable, with a half-life of up to 5 d (188), and it is targeted for degradation by dynamic interactions with heat-shock proteins, cochaperones, and the ubiquitin E3 ligase carboxy terminus of Hsp70-interacting protein (189). This degradation pathway is important for the quality control of ERα because it maintains appropriate steady-state receptor levels in the cytoplasm and is responsible for the clearance of misfolded ERα. Recent data demonstrate that the oncogene E3 ubiquitin ligase MDM2 (mouse double minute-2) could be involved in this ligand-independent turnover of ERα (190).

Second, in the presence of E_2, the ERα half-life drops dramatically to 3–5 h (188, 191), and the receptors are targeted for degradation through a transcription-coupled pathway requiring new protein synthesis. During each binding cycle, ERα recruits specific ubiquitin-proteasome components to the target gene promoter (118–120, 187, 192, 193). Evidence from several studies shows that the inhibition of ubiquitin-proteasome activity blocks both ERα cycling and the activation of target gene expression despite increased ERα abundance obtained by immobilizing ERα on the nuclear matrix, as determined by fluorescence recovery after photobleaching (186). Métivier *et al.* (194) report that the initial steps of transcription are linked to monoubiquitination of the liganded ERα, and this process may enhance receptor interaction with DNA or coactivators. As transcription progresses, ERα is degraded as a consequence of the recruitment of several proteins implicated in the proteasome system. The proteins putatively involved are: 1) ERα E3 ubiquitin ligases like E6-AP (118, 195), MDM2 (187, 190, 196), and EFP (estrogen-responsive finger protein) (197); 2) the low molecular mass polypeptide 2 (LMP2) subunit of the 26S proteasome (193) and the 26S protease regulatory subunit 8 (PRS8/SUG1) in the 19S regulatory cap of the proteasome (187); and 3) the coactivators SRC-1 and SRC-3. SRC-1

interacts directly with LMP2, and this recruitment of LMP2 by SRC coactivators is necessary for cyclic association of ER-regulated transcription complexes on ER targets (193). SRC-3 has been shown to be required for the E_2-dependent turnover of ERα through interaction with the ubiquitin-E3 ligase EFP (198).

And third, in the presence of fulvestrant or other SERD like RU58668, ERα is targeted to degradation independently of transcriptional activity and new protein synthesis (120, 121, 199). Fulvestrant induces ERα degradation by dissociating the Hsp90-ERα complex and immobilizing ERα to the nuclear matrix by inducing its association with CK8 and CK18, two members of the family of nuclear matrix intermediate filament structural proteins, for subsequent degradation (200).

Although ERα ubiquitination was well documented, the target K302 and K303 have only recently been identified. Both lysines protect ERα from basal degradation and are necessary for efficient E_2- and fulvestrant-induced turnover in breast cancer cells (201). As previously demonstrated by *in vitro* ubiquitination assays, in the presence of E_2, K302 and K303 are monoubiquitinated by the ubiquitin ligase BRCA1/BARD1 (183, 202). But the impact of K302 monoubiquitination on ERα stability is unknown, and the possible existence of other ubiquitination sites has not been ruled out.

A recent publication has shown that the protein OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) deubiquitinates the receptor and represses its transcriptional activity. OTUB1 also regulates endogenous ERα level by stabilizing the protein on the chromatin (203).

5. Ubiquitination of ERβ

The carboxy terminus of Hsp70-interacting protein binds to the N-terminal domain of the receptor, which is ubiquitinated to induce ERβ degradation and the cessation of transcription (204). Suppressor of Gal 1, a regulatory subunit of the 26S proteasome, is also involved in ERβ degradation in a ligand-dependent manner (205), but the target lysines have not been identified so far.

6. Sumoylation of ERα

Although other steroid receptors contain clearly identifiable ψKXE small ubiquitin-related modifier (SUMO) acceptor sites, neither ERα nor ERβ matches this consensus sequence (19). Nevertheless, ERα has been described to be sumoylated by SUMO-1 (206). This sumoylation, which occurs in overexpressed and endogenous ERα and is dependent on the presence of ERα ligands like E_2 or tamoxifen, targets five lysines located in the hinge region: K266, K268, K299, K302, and K303. In addition, ERα sumoylation is stimulated in the presence of the SUMO E3 ligases PIAS1 and PIAS3 (protein inhibitor of activated signal transducer and activator of transcription). Because mutations that prevented SUMO modification impaired ERα-induced transcription through decreased receptor binding to DNA without affecting ERα nuclear localization, ERα sumoylation results in enhanced ERα transcriptional activity (206). The sumoylation of ERβ has not yet been described, but it would be of interest to determine whether this modification could be involved in the regulation of ERβ activity.

7. Lysine methylation of ERα

Although lysine methylation is commonly attributed to histones, methylation has recently been described in nonhistone proteins such as p53 or DNA methyltransferase. Methylation on K residues is a complex process because a lysine can be mono-, di-, or trimethylated (207). Monomethylation of ERα at K302 is mediated by SET7 lysine methyltransferase; this modification acts on ERα stability and facilitates the recruitment of ERα to its target genes for their transactivation (208). However, the timing of this modification has not been studied in detail because the endogenous modified form of ERα has been undetectable.

8. Other modifications

Other modifications have been identified in ERα, although they have not been studied in details. These include S-nitrosylation, which occurs in nonidentified ER cysteine residues and results in inhibition of DNA binding at specific ERE (209). ERα glycosylation has also been reported, but the targeted residues have been identified only for murine receptors (210, 211). Recently, a systematic study of ERα PTM by tandem mass spectrometry has identified new sites, but their function has not been clarified (212).

B. PTM involved in ER nongenomic signaling

Although E_2 rapid effects are well documented, the molecular mechanisms involved remain elusive. It is not clear how the signals are initiated. Recently, some ERα PTM have been shown to be involved in nongenomic signaling, which gives new insight into this pathway.

1. Palmitoylation of ERα and ERβ

Palmitoylation is the attachment of a long chain of fatty acids that targets proteins to membrane microdomains (lipid rafts and caveolae). ERα was long ago reported to be located at the plasma membrane (213). Cycles of palmitoylation and depalmitoylation affect protein activation and allow protein movements within membrane subdomains. The small population of ERα localized at the plasma membrane is known to be palmitoylated on Cys447 by palmitoyl acyltransferase (PAT) (214). Upon

E_2 binding, ERα undergoes depalmitoylation and dissociates from caveolin-1, which facilitates ERα association with signaling molecules like Src and PI3K. S-Palmitoylation is a critical step for E_2-induced rapid events because the unpalmitoylable ERα mutant does not induce E_2 proliferative effects (106, 215). The plant-derived flavonoid naringenin is known to inhibit estrogen nongenomic signaling through a rapid depalmitoylation of the receptor (216). Targeting this modification could be a good strategy for antagonizing estrogen proliferative effects.

ERβ has also been shown to localize at the plasma membrane through palmitoylation (217). The site of palmitoylation has not been clearly identified, but C399 is a good candidate because it is conserved with ERα binding (218). In ERβ, contrary to observation in ERα, E_2-decreased palmitoylation increases receptor association with caveolin-1. The outcome effect is also totally different because ERβ palmitoylation is necessary for the p38-dependent activation of proapoptotic cascades (219).

2. Phosphorylation of ERα on tyrosine

The phosphorylation of Y537 was first reported to regulate E_2 binding in MCF-7 cells and the ability of ERα to interact with kinases containing SH2 domains (220–222). *In vitro* data suggest that Src tyrosine kinases ($p56^{lck}$ and $p60^{c\text{-}src}$) could be responsible for this phosphorylation (223). Auricchio *et al.* (224) recently obtained interesting results using peptides containing phosphoY537. They have shown that the phosphorylated peptide inhibits G_1/S transition by preventing ERα/Src interactions induced by E_2 in MCF-7 cells. This peptide also inhibits the growth of mammary cancer cell xenografts, supporting its potential therapeutic interest (224, 225).

Finally, *in vitro* results from Katzenellenbogen's lab (226) strongly indicate that Src phosphorylates ERα on other tyrosine residues.

3. Arginine methylation of ERα

Recently, our team reported a novel paradigm of ERα regulation through arginine methylation by protein arginine methyltransferase 1 (PRMT1), which transiently methylates R260 within the ERα DBD (227). The methylated ERα is localized exclusively in the cytoplasm of breast epithelial cells. This methylation event is required for mediating the extranuclear function of the receptor by triggering its interaction with the p85 subunit of PI3K, Src, and the focal adhesion kinase, and thereby propagating the signal to downstream transduction cascades via Akt activation (228). The transient methylation of the receptor suggests that a putative arginine demethylase could be involved in the regulation of this process.

Rapid down-regulation of ERα methylation and/or Src activity may serve to control rapid physiological responses to estrogen, inducing the dissociation of the complex and ultimately the repression of downstream kinase activation.

In conclusion, for ERα, although phosphorylation sites are scattered throughout the protein, the other modifications are highly concentrated around the hinge region (Fig. 3) and sometimes even on the same residue (K302, for instance, is targeted for multiple modifications), which facilitates cross talks between modifications. It is important to decipher the interplay between ER PTM and to determine precisely which PTM will be impacted if a given enzyme is targeted.

C. Molecular events affecting ER PTM

1. ER mutations

Although the number of mutations affecting ERα in breast tumors is relatively low (129), the impact of PTM on ER signaling is indirectly supported by ERα mutations affecting PTM target sites of the receptor. For example, the Y537N mutant (phosphorylated site) identified in tumors of metastatic breast cancer patients has been shown to confer a constitutive transactivation function to the receptor (229).

It has also been shown that the K303R mutation (a multimodified residue) associated with breast cancer modifies K302 methylation both *in vitro* and *in vivo* (208). The K303R mutant leads to hyperphosphorylation of ERα by PKA (230) and by Akt-dependent pathways (165) at residue 305, suggesting a cross talk between ERα acetylation and phosphorylation. Moreover, the K303R mutation has been shown to result in altered recruitment of coactivators or corepressors like calmodulin or BRCA1 (183, 231). Interestingly, the K303R mutation of ERα has been found associated with predictors of unfavorable outcome and poor prognosis like lymph node positivity or enlarged tumor size (232). Altogether, these observations suggest that events affecting K303 (PTM or mutations) might seriously affect the functional role of ERα through a deregulation of ERα PTM.

2. Cross talk between ER PTM

A protein can be modified by more than one type of PTM or by the same PTM at different residues. The occurrence of several PTM, acting sequentially and/or in concert, seems to be a very efficient mechanism to initiate, terminate, or fine-tune the outcome of signaling pathways.

Although the implications of individual ER modifications such as phosphorylation, acetylation, and methylation have been relatively well documented, interactions between these modifications remain to be elucidated. It

appears that one modification may be coupled to the enhancement or suppression of another one at the same or a nearby residue.

In this context, we will try to discuss the known interplay between PTM and to investigate other potential ones.

3. Phosphorylation and phosphorylation

The first cross talk between two phosphorylation sites, S118 and S305, in ERα has been described by the group of Kumar (233). They have found that ERα phosphorylation by PAK1 on S305 influences the activation status of S118 and that the S305-associated ERα transactivation activity requires a functional S118 (233). This cross talk has not been analyzed in clinical studies, but it has been shown that transgenic mice expressing a kinase-active PAK1 exhibit both activated ERα-305 and ERα-S118.

Although less well-documented, a positive cross talk also exists between S104, S106, and S118 (136).

4. Phosphorylation and acetylation

K303 resides adjacent to a phosphorylation site, S305, and a negative cross talk has been found between S305 phosphorylation and K303 acetylation (230). Mutation of S305 to E305 mimicking constitutive phosphorylation blocks K303 acetylation and generates an enhanced transcriptional response. This cross talk could partially explain the link between the phosphorylation of S305 and tamoxifen resistance in breast tumors. On the contrary, the phosphorylation of S118 and S167 positively regulates ER acetylation (183).

5. Phosphorylation and ubiquitination

Multiple agents regulating phosphorylation also impact ERα proteolysis (183, 234). A direct link between ERα phosphorylation and ubiquitination has been established. S118 plays an important role in E_2-mediated ERα degradation by regulating differential recruitment of factors mediating proteolysis (183, 235, 236).

A recent study by the group of Tremblay has shown that ERβ phosphorylation serves as a signal for receptor ubiquitination and degradation (170). The recruitment of the E3 ubiquitin ligase E6-AP to mouse ERβ is induced by Erk phosphorylation at S94 (conserved at S75 in hERβ) and S106 (equivalent to hS87), but the target lysines have not been identified.

6. Phosphorylation and glycosylation

It appears that reciprocal occupancy of S80 (conserved at S60 in hERβ) by either O-phosphate or O-GlcNAc modulates the degradation and activity of mERβ. In fact, the saccharide may act by blocking the addition of phosphate, which itself targets the protein for rapid degradation (169). This interplay is another good example of coordinated modifications responsible for regulating ERβ activity.

7. Acetylation and methylation

Because ERα K302 can be either acetylated or methylated, one could imagine a competition between the two modifications. *In vitro* experiments have shown that a previously acetylated ERα peptide is a poor substrate for SET7-induced methylation (208). Given the fact that lysine methylation stabilizes ERα, this negative cross talk may destabilize the receptor mediating the inhibitory effect of K303 acetylation on transcription.

8. Methylation and ubiquitination

Estrogen-stimulated ubiquitination and subsequent degradation by the proteasome play an important role in ER transcriptional activity (187). Given that K302 methylation can stabilize ERα, this modification could be antagonistic to ubiquitination because of direct competition for the same lysine residue. Because methylated lysine residues can serve as docking sites for binding proteins through bromo- or chromodomains, the formation of new complexes would thereby limit ERα ubiquitination and degradation. Calmodulin would be a good candidate because K302 falls within a calmodulin-binding motif, and its binding to ERα prevents ubiquitination by E6-AP and degradation of the receptor. Interestingly, K302 mutation has been shown to reduce interactions between the two proteins (237). As a consequence, targeting K302 methylation could help to increase the degradation of ERα. In addition, K302 methylation could recruit a methyl lysine-binding protein that would, in turn, modulate ERα degradation through regulating the recruitment of E3 ubiquitin ligases.

In conclusion, several interactions between PTM have been described *in vitro*, but one must be careful in interpreting these results because most studies have been performed with mutants. One cannot exclude that the observed effects are due to conformational changes of the receptors rather than to a change in the modification. To integrate these findings for a better understanding of their significance, the next step will be to study the correlations between PTM *in vivo* (knock-in mice and patient tumors).

Finally, it is reasonable to assume that ongoing studies should clarify the combinatorial relationships between the different PTM and help to unravel ER-dependent gene transcription and to improve response to endocrine therapy.

D. ER PTM deregulated in breast cancer

The presence or absence of ERα is a well-established prognostic marker in breast cancer (at least in the early years after diagnosis) and a predictive marker for endo-

crine therapy (238–240). Because ER PTM have been shown to affect hormone sensitivity and localization, receptor stability, and/or ER transcription activity, it is tempting to speculate that immunohistochemical detection and quantification of these PTM could be an important new prognostic or predictive biomarker for tumor evaluation. Studies with antibodies specific to PTM have shown that several ERα modifications are deregulated in breast tumors, although it is so far unclear whether these deregulations are a cause or a consequence of the disease. Three phosphorylation sites of ERα (S118, S167, and S305) have received particular attention, especially because they have been implicated in ligand-independent activation of ERα. This section will focus mainly on ERα because almost nothing is known yet about ERβ PTM in breast tumors. A list of the PTM found to be deregulated in breast tumors and the corresponding survival outcome are given in Table 3.

1. S118 of ERα

Immunohistochemical studies of pS118-ERα expression have found a significant positive correlation with active p-MAPK (241–245), active p-Akt (244, 246), and p-p90-RSK (246), suggesting that these kinases may be involved, directly or indirectly, in the S118 phosphorylation of ERα *in vivo*. High pS118-ERα expression levels have been associated in breast tumors with more differentiated phenotype, lower grade, and other markers of good prognosis such as ERα or progesterone receptor (241–244, 247–249). Concerning its predictive value for response to tamoxifen, the clinical studies yielded conflicting results. In fact, four studies do not associate S118 phosphorylation with tamoxifen response (243, 244, 250, 251), although other groups found this phosphorylation associated with better disease outcome in women treated with tamoxifen (242, 248, 252), and higher levels are found in ERα-positive cancer patients who respond to tamoxifen than in those who progress. These discrepancies probably result from the use of different antibodies in small nonrandomized patient series. Recently, a randomized study of 239 patients has confirmed that pS118-ERα is associated with tamoxifen response (248), and Generali *et al.* (253) have described this modification as an independent factor for endocrine responsiveness in patients receiving AI (letrozole)-based treatment, confirming that when this residue is phosphorylated, patients are more likely to respond to endocrine therapy.

2. S167 of ERα

In ER-positive primary breast cancers, pS167-ERα has been shown to be positively correlated with p-MAPK (244, 246), p-p90-RSK (246), and p-Akt (244, 246). pS167-ERα is also associated with good prognostic factors (such as lower tumor grade or lymph node negativity) and with increased relapse-free and overall survival (246). pS167-ERα has been found to be predictive of response to endocrine therapy (250). These data strongly suggest that phosphorylation of ERα at S167 could be a useful marker for selecting the patients who are most likely to benefit from endocrine therapy.

3. S305 of ERα

A number of *in vitro* studies have indicated that phosphorylation of S305 by PKA and/or PAK1 in ERα resulted

TABLE 3. ER PTM deregulated in breast cancer

Amino acid	Status in breast tumors	Survival outcome	Marker	References
ERαS104/106	Hyperphosphorylation	Good	ND	249
ERαS118	Hyperphosphorylation	Good	Tam response	242
		Good	Tam response	248
		Poor	Not for Tam response	250
		Good	ND	241
		Good	Good prognosis	243
		Good	ND	249
	Hypophosphorylation	Good	Tam response	244
ERαS167	Hyperphosphorylation	Good	Good prognosis	246
		Good	Tam response	250
		Good	Good prognosis	244
		Good	ND	249
ERαR260	Hypermethylation	ND	ND	227
ERαS282	Hyperphosphorylation	Good	ND	249
ERαK303	Mutated to arginine	Poor	Poor prognosis	232
ERαS305	Hyperphosphorylation after PKA activation	Poor	Tam resistance	157
	Hyperphosphorylation	Poor	Tam resistance	162, 257
ERαT311	Hyperphosphorylation	Poor	ND	249
ERαS559	Hyperphosphorylation	Good	ND	249
ERβS105	Hyperphosphorylation	Good	ND	261

ND, Not determined; Tam, tamoxifen.

in enhanced transactivation of ER (158, 161) and was associated with tamoxifen resistance (157, 161).

A link between PKA expression, ERα phosphorylation on S305, and resistance to tamoxifen has been demonstrated in breast cancer patients (157), consistent with early data from clinical studies correlating deregulation of PKA with tamoxifen resistance (254). Other studies have associated tamoxifen resistance with nuclear expression or amplification of PAK1 (255, 256).

A recent clinical study of a large series of tumors from node-negative postmenopausal breast cancer patients has established that the concomitant expression of nuclear PAK1 and pS305ERα correlates with reduced response to tamoxifen, suggesting a putative importance of this signaling pathway for treatment prediction. Furthermore, lack of nuclear pS305ERα or of nuclear PAK1 expression results in tamoxifen-improved response in postmenopausal breast cancers (257).

Although validating the correlation between PAK1 expression and tumor progression after tamoxifen treatment, the work by Michalides and colleagues (162) shows no direct link between PAK1 expression and pS305ERα. This analysis concludes that both PAK1 and PKA/pS305ERα levels are associated with sensitivity to tamoxifen in breast tumors, and the combination of these variables is predictive of tamoxifen benefit (162). Furthermore, a gene expression analysis has shown an enrichment of the PKA pathway in pS305ERα-positive tumors, but not of the PAK1-related pathway.

The discrepancy between these studies can be explained by the use of distinct anti-phosphoantibodies and differences between patients: premenopausal breast cancer (257) *vs.* postmenopausal (162) patients.

In conclusion, phosphorylation of ERα at S305 could be a new predictive marker for tamoxifen response, and targeting PKA to block S305 ERα phosphorylation represents a promising new strategy to prevent or to reverse endocrine resistance.

4. S282 and T311 of ERα

Phosphorylation of S282 is associated with better disease outcome (longer relapse-free survival) and phosphorylation of T311 with poor clinical outcome (decreased relapse-free survival) in women treated with tamoxifen (249).

5. R260 of ERα

The use of an antibody specific for ERα methylated on R260 has shown that this modification is relatively rare in normal epithelial breast tissue and that its expression is deregulated in breast tumors. ERα methylation status varies between tumors. In fact, ERα is hypermethylated in about 55% of invasive breast tumors (227). This deregulation could be due to an overexpression of the arginine methyltransferase PRMT1 because isoforms of the enzyme have been shown to be overexpressed in breast tumors (258). So far, no correlation with clinical outcome or clinical parameters has been evidenced, and whether this modification is a cause or a consequence of the disease is still under investigation.

6. K303 of ERα

K303, a hot spot for PTM, was first shown to be mutated to arginine in ductal hyperplasia (259) and more recently in invasive breast tumors (232, 260). This mutation has been significantly associated with aggressive biological behavior and has been shown to be a marker of poor prognosis associated with reduced relapse-free survival (232). K303 mutation triggers ERα hypersensitivity to estrogen and resistance to tamoxifen and aromatase inhibitors (163). This hyperactivity is partly due to the hyperphosphorylation of the adjacent S305. Given that K303 can be modified by sumoylation, acetylation, or ubiquitination, and that it regulates K302 lysine methylation (208), we can speculate that this mutation disrupts one or more of these modifications, thereby inducing ERα hypersensitivity. For example, the mutation impeding acetylation triggers S305 phosphorylation and ERα hyperactivation. This is a striking example of the importance of understanding the complex relationship between ER PTM for deciphering the mechanisms of endocrine resistance and circumventing this major problem in breast cancer treatment.

7. S105 of hERβ (equivalent to S124 of mERβ)

An antibody specific for ERβ phosphorylated on the S105 residue has been used to analyze its expression in tamoxifen-treated breast tumors. S105 phosphorylation is associated with better survival, even in tamoxifen-resistant cases (261). However, these results need to be confirmed on other cohorts of patients before S105 can be considered as a potential new marker.

IV. Discussion

Over the last several years, numerous studies have reported that ER, particularly ERα, are subject to a plethora of PTM in response to different stimuli and that these PTM differentially regulate the functions of these receptors, adding another layer of complexity to the regulation of estrogen signaling.

Some of these PTM are deregulated in breast cancer, even if we ignore whether they are just a consequence of the disease or they actually play a role in breast tumorigenesis. However, literature data show that they could at

least constitute new prognosis and/or predictive markers for the disease.

ERα phosphorylation, the most extensively studied PTM to date, has been shown to play important roles in most, if not all, estrogen-regulated pathways. In particular, a number of *in vitro* studies have shown that some ERα phosphorylation sites are clearly involved in endocrine therapy responsiveness. To validate the significance of these observations, several groups have investigated the relevance of various ERα phosphorylation sites by immunohistochemical detection in breast tumors. Many studies have focused on the phosphorylation of S118, S167, and S305. The obtained results illustrate the complexity of identifying a specific marker of the emergence of endocrine resistance. Indeed, contrary to what had been expected from *in vitro* observations, pS118-ERα and pS167-ERα do not seem to be associated *in vivo* with *de novo* endocrine resistance. Indeed the presence of pS118-ERα or pS167-ERα has been associated with better outcome in patients on endocrine therapy (162, 242, 245, 246, 252, 253), suggesting that these two ERα PTM could rather indicate good prognosis and responsiveness to endocrine therapy. Conversely, *in vitro* and clinical studies have validated that pS305-ERα modification is strongly associated with endocrine resistance because high pS305-ERα expression level is a predictive biomarker for endocrine therapy responsiveness. However, contradictory *in vivo* data from other groups (243, 244, 250, 251) have highlighted the complexity of such evaluation. The controversial results published could reflect differences in the antibodies used, in the stability of the phosphoepitopes during tumor collection and storage, in the size of the study cohorts, in the pre- or postmenopausal status of the patients, in the type of molecule used (ER ligand or AI) for endocrine therapy, and/or in the type of treatment (short-term neoadjuvant *vs.* long-term adjuvant endocrine therapy).

The limitation of the clinical studies described above can arise from the analysis of a single biomarker to distinguish between responders and nonresponders to endocrine therapy. Indeed, each phosphorylation could alter the receptor function in a distinctive manner, individually or in combination. So, it would be important to develop new scores combining several PTM and allowing more accurate identification of the patients likely to benefit from endocrine therapy.

In this context, recent studies from the laboratory of L. C. Murphy (252) have demonstrated the presence of multiple phosphorylated isoforms of ERα (S104/106, S118, S167, S282, S294, T311, and S559) in breast tumors. Interestingly, they have shown that any individual phosphoepitope is positively correlated with at least two others, and pS559 is positively correlated with all other epitopes (252). They have also investigated the relationship between these phosphorylated forms of ERα and clinical outcome after tamoxifen therapy in 300 patients with ERα-positive breast tumors (249). Interestingly, they have found that phosphoepitopes clustered in the N terminus of ERα (S104/106, S118, S167, S282, S294) are predictors of good outcome, whereas other epitopes clustered toward the C terminus (T311 and S559) are predictors of bad outcome. Accordingly, the authors propose a "phospho-score" that, by taking into account seven phospho-sites on ERα, integrates both "good" and "bad" sites. This score has been shown to be a statistically significant independent predictor of overall survival and relapse-free survival in tamoxifen-treated ER-positive patients (249). Along this line, the FRET analysis published by Zwart *et al.* (30) has illustrated that the combinations of ERα phosphomodifications lead to different conformational changes upon each antiestrogen treatment. In consequence, we can speculate on the existence of a specific code for each type of endocrine therapy.

Additional complexity has been introduced with a recent study describing that the hyperphosphorylation of ERβ on the S105 residue is associated with improved disease-free and overall survivals in breast tumor samples (261), suggesting that ERβ modification should be included in the "phospho-score."

Investigations of the relationship between modifications of ERα and resistance to hormone therapy have mainly focused on phosphorylation. As discussed above, ERα also undergoes several other modifications affecting multiple sites. However, these modifications are less well characterized, and because of the unavailability of specific antibodies, their *in vivo* significance is poorly understood. Little or no *in vivo* studies have been published about other ER PTM, and the preliminary data obtained need to be validated on independent cohorts.

Given the complexity described above, assessing the expression of the different molecules involved in ER modifications might allow more accurate selection of patients with different responses to endocrine therapy. The enzymes triggering the PTM have not yet been clearly identified, and very little is known about the enzymes responsible for reversing these modifications. Actually, in breast tumor samples, a significant positive correlation has been found between the expression of p-MAPK or p-Akt and pS167- or pS118-ERα and between p-p90-RSK and pS118-ERα, indicating that these kinases participate directly or indirectly in these modifications (241–246). In addition, two recent studies have shown that combinations of pS305ERα and nuclear PAK1 (257) or PKA/pS305ERα and PAK1 are more powerful in predicting response to tamoxifen (162).

Altogether, these data suggest that assessing the PTM code of ERα, either individually or in association with the expression and/or activity of the enzymes responsible for these PTM, could provide an efficient personalized antiestrogen therapy.

V. Future Directions

At present, ERα status is the only factor used routinely for prediction of response to endocrine therapy and patient selection in breast cancer. As we have largely discussed above, PTM play a pivotal role in the regulation of ERα functions, and their deregulation is clearly involved in phenomena of resistance to endocrine therapies in breast cancer patients.

Before we can elucidate the mechanisms by which breast cancers evade antiestrogen-mediated growth arrest, we need to understand how PTM regulate ER actions. Undoubtedly, our understanding of these dynamic modifications is still at an early stage due to the lack of appropriate tools to monitor modifications of endogenous proteins. Future studies should investigate how a specific modification regulates a subset of ER target genes to determine specificity and how these different modifications act, sequentially and/or in concert, under physiological and pathological conditions. Using proteomic approaches, including developing site- and modification-specific antibodies and employing mass spectrometry techniques, would allow a better understanding of the regulation of these dynamic modifications *in vivo*. Furthermore, genetic approaches, such as the generation of modification-deficient ER knock-in animals, would provide new insights into the possible physiological functions of these modifications. Important efforts should also be made to better define the actors involved in PTM regulation, notably the enzymes responsible for reversing ER PTM.

New treatments targeting the molecules responsible for modifying or reversing PTM could be used in combination with endocrine therapies or after treatment failure or acquired resistance. However, specifically targeting PTM in breast cancer cells is a prerequisite to any efficient therapy because most of the enzymes involved in such modifications are present in both the tumor and healthy tissues and treatment may cause a wide range of unwanted side effects. Important advances have been made in this direction with the development of nanocarriers loaded with cytotoxic drugs (stealth liposomes, for example) characterized by a prolonged blood circulation time when injected iv [for review, see Urbinati *et al.* (273)].

Many approaches and avenues remain unexplored in developing targeted drugs for effective blockade of ERα signaling. We can anticipate that in the future, thanks to the ER posttranslational code, a molecular subtyping of individual tumors will help to define the antihormonal treatment for each patient.

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Address all correspondence and requests for reprints to: Muriel Le Romancer, Centre de Recherche en Cancérologie de Lyon, Centre Léon Bérard, Bâtiment Cheney D, 28 rue Laennec, 69373 Lyon Cedex 08, France. E-mail: leroman@lyon.fnclcc.fr.

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References

1. **Couse JF, Korach KS** 1999 Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev 20:358–417
2. **Santen RJ, Boyd NF, Chlebowski RT, Cummings S, Cuzick J, Dowsett M, Easton D, Forbes JF, Key T, Hankinson SE, Howell A, Ingle J** 2007 Critical assessment of new risk factors for breast cancer: considerations for development of an improved risk prediction model. Endocr Relat Cancer 14:169–187
3. **McDonnell DP, Norris JD** 2002 Connections and regulation of the human estrogen receptor. Science 296:1642–1644
4. **Levin ER** 2009 Membrane oestrogen receptor α signalling to cell functions. J Physiol 587:5019–5023
5. **Russo J, Ao X, Grill C, Russo IH** 1999 Pattern of distribution of cells positive for estrogen receptor α and progesterone receptor in relation to proliferating cells in the mammary gland. Breast Cancer Res Treat 53:217–227
6. **Pettersson K, Delaunay F, Gustafsson JA** 2000 Estrogen receptor β acts as a dominant regulator of estrogen signaling. Oncogene 19:4970–4978
7. **Ström A, Hartman J, Foster JS, Kietz S, Wimalasena J, Gustafsson JA** 2004 Estrogen receptor β inhibits 17β-estradiol-stimulated proliferation of the breast cancer cell line T47D. Proc Natl Acad Sci USA 101:1566–1571
8. **Treeck O, Lattrich C, Springwald A, Ortmann O** 2010 Estrogen receptor β exerts growth-inhibitory effects on human mammary epithelial cells. Breast Cancer Res Treat 120:557–565
9. **Behrens D, Gill JH, Fichtner I** 2007 Loss of tumourigenicity of stably ERβ-transfected MCF-7 breast cancer cells. Mol Cell Endocrinol 274:19–29

10. **Lazennec G, Bresson D, Lucas A, Chauveau C, Vignon F** 2001 ER β inhibits proliferation and invasion of breast cancer cells. Endocrinology 142:4120–4130
11. **Williams C, Edvardsson K, Lewandowski SA, Ström A, Gustafsson JA** 2008 A genome-wide study of the repressive effects of estrogen receptor β on estrogen receptor α signaling in breast cancer cells. Oncogene 27:1019–1032
12. **Colditz GA** 1998 Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. J Natl Cancer Inst 90:814–823
13. **Crandall DL, Busler DE, Novak TJ, Weber RV, Kral JG** 1998 Identification of estrogen receptor β RNA in human breast and abdominal subcutaneous adipose tissue. Biochem Biophys Res Commun 248:523–526
14. **Harvey JM, Clark GM, Osborne CK, Allred DC** 1999 Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 17:1474–1481
15. **Ali S, Coombes RC** 2002 Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev Cancer 2:101–112
16. **Taylor SE, Martin-Hirsch PL, Martin FL** 2010 Oestrogen receptor splice variants in the pathogenesis of disease. Cancer Lett 288:133–148
17. **Barone I, Brusco L, Fuqua SA** 2010 Estrogen receptor mutations and changes in downstream gene expression and signaling. Clin Cancer Res 16:2702–2708
18. **Herynk MH, Fuqua SA** 2004 Estrogen receptor mutations in human disease. Endocr Rev 25:869–898
19. **Faus H, Haendler B** 2006 Post-translational modifications of steroid receptors. Biomed Pharmacother 60:520–528
20. **Hall JM, McDonnell DP** 2005 Coregulators in nuclear estrogen receptor action: from concept to therapeutic targeting. Mol Interv 5:343–357
21. **Lannigan DA** 2003 Estrogen receptor phosphorylation. Steroids 68:1–9
22. **Ward RD, Weigel NL** 2009 Steroid receptor phosphorylation: assigning function to site-specific phosphorylation. Biofactors 35:528–536
23. **Popov VM, Wang C, Shirley LA, Rosenberg A, Li S, Nevalainen M, Fu M, Pestell RG** 2007 The functional significance of nuclear receptor acetylation. Steroids 72:221–230
24. **Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM** 1995 The nuclear receptor superfamily: the second decade. Cell 83:835–839
25. **Zhao C, Dahlman-Wright K, Gustafsson JA** 2008 Estrogen receptor β: an overview and update. Nucl Recept Signal 6:e003
26. **Tsai MJ, O'Malley BW** 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486
27. **Tora L, White J, Brou C, Tasset D, Webster N, Scheer E, Chambon P** 1989 The human estrogen receptor has two independent nonacidic transcriptional activation functions. Cell 59:477–487
28. **Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA** 1996 Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93: 5925–5930
29. **Mosselman S, Polman J, Dijkema R** 1996 ER β: identification and characterization of a novel human estrogen receptor. FEBS Lett 392:49–53
30. **Zwart W, de Leeuw R, Rondaij M, Neefjes J, Mancini MA, Michalides R** 2010 The hinge region of the human estrogen receptor determines functional synergy between AF-1 and AF-2 in the quantitative response to estradiol and tamoxifen. J Cell Sci 123:1253–1261
31. **Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA** 2001 Mechanisms of estrogen action. Physiol Rev 81:1535–1565
32. **Magee PJ, Rowland IR** 2004 Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. Br J Nutr 91:513–531
33. **Miodini P, Fioravanti L, Di Fronzo G, Cappelletti V** 1999 The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. Br J Cancer 80:1150–1155
34. **Jordan VC, O'Malley BW** 2007 Selective estrogen-receptor modulators and antihormonal resistance in breast cancer. J Clin Oncol 25:5815–5824
35. **Smith CL, O'Malley BW** 2004 Coregulator function: a key to understanding tissue specificity of selective receptor modulators. Endocr Rev 25:45–71
36. **Dowsett M, Nicholson RI, Pietras RJ** 2005 Biological characteristics of the pure antiestrogen fulvestrant: overcoming endocrine resistance. Breast Cancer Res Treat 93(Suppl 1):S11–S18
37. **Wakeling AE, Dukes M, Bowler J** 1991 A potent specific pure antiestrogen with clinical potential. Cancer Res 51: 3867–3873
38. **Dahlman-Wright K, Cavailles V, Fuqua SA, Jordan VC, Katzenellenbogen JA, Korach KS, Maggi A, Muramatsu M, Parker MG, Gustafsson JA** 2006 International Union of Pharmacology. LXIV. Estrogen receptors. Pharmacol Rev 58:773–781
39. **Hewitt SC, Harrell JC, Korach KS** 2005 Lessons in estrogen biology from knockout and transgenic animals. Annu Rev Physiol 67:285–308
40. **Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA, Smithies O** 1998 Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc Natl Acad Sci USA 95: 15677–15682
41. **Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O** 1993 Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci USA 90:11162–11166
42. **Tee MK, Rogatsky I, Tzagarakis-Foster C, Cvoro A, An J, Christy RJ, Yamamoto KR, Leitman DC** 2004 Estradiol and selective estrogen receptor modulators differentially regulate target genes with estrogen receptors α and β. Mol Biol Cell 15:1262–1272
43. **Monroe DG, Getz BJ, Johnsen SA, Riggs BL, Khosla S, Spelsberg TC** 2003 Estrogen receptor isoform-specific regulation of endogenous gene expression in human osteoblastic cell lines expressing either ERα or ERβ. J Cell Biochem 90:315–326
44. **Shim GJ, Wang L, Andersson S, Nagy N, Kis LL, Zhang Q, Mäkelä S, Warner M, Gustafsson JA** 2003 Disruption of the estrogen receptor β gene in mice causes myeloprolifer-

ative disease resembling chronic myeloid leukemia with lymphoid blast crisis. Proc Natl Acad Sci USA 100:6694–6699
45. **Vivar OI, Zhao X, Saunier EF, Griffin C, Mayba OS, Tagliaferri M, Cohen I, Speed TP, Leitman DC** 2010 Estrogen receptor β binds to and regulates three distinct classes of target genes. J Biol Chem 285:22059–22066
46. **Cowley SM, Hoare S, Mosselman S, Parker MG** 1997 Estrogen receptors α and β form heterodimers on DNA. J Biol Chem 272:19858–19862
47. **Papoutsi Z, Zhao C, Putnik M, Gustafsson JA, Dahlman-Wright K** 2009 Binding of estrogen receptor α/β heterodimers to chromatin in MCF-7 cells. J Mol Endocrinol 43:65–72
48. **Gougelet A, Mueller SO, Korach KS, Renoir JM** 2007 Oestrogen receptors pathways to oestrogen responsive elements: the transactivation function-1 acts as the keystone of oestrogen receptor (ER)β-mediated transcriptional repression of ERα. J Steroid Biochem Mol Biol 104:110–122
49. **Hall JM, McDonnell DP** 1999 The estrogen receptor β-isoform (ERβ) of the human estrogen receptor modulates ERα transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 140:5566–5578
50. **Liu MM, Albanese C, Anderson CM, Hilty K, Webb P, Uht RM, Price Jr RH, Pestell RG, Kushner PJ** 2002 Opposing action of estrogen receptors α and β on cyclin D1 gene expression. J Biol Chem 277:24353–24360
51. **Paruthiyil S, Parmar H, Kerekatte V, Cunha GR, Firestone GL, Leitman DC** 2004 Estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. Cancer Res 64:423–428
52. **Kos M, Reid G, Denger S, Gannon F** 2001 Minireview: genomic organization of the human ERα gene promoter region. Mol Endocrinol 15:2057–2063
53. **Trivedi S, Piccart M, Muquardt C, Gilot N, Hadiy S, Patel D, Leclercq G** 1996 Tamoxifen aziridine labeling of the estrogen receptor-potential utility in detecting biologically aggressive breast tumors. Breast Cancer Res Treat 40:231–241
54. **Denger S, Reid G, Kos M, Flouriot G, Parsch D, Brand H, Korach KS, Sonntag-Buck V, Gannon F** 2001 ERα gene expression in human primary osteoblasts: evidence for the expression of two receptor proteins. Mol Endocrinol 15:2064–2077
55. **Murphy AJ, Guyre PM, Wira CR, Pioli PA** 2009 Estradiol regulates expression of estrogen receptor ERα46 in human macrophages. PLoS One 4:e5539
56. **Li L, Haynes MP, Bender JR** 2003 Plasma membrane localization and function of the estrogen receptor α variant (ER46) in human endothelial cells. Proc Natl Acad Sci USA 100:4807–4812
57. **Flouriot G, Brand H, Denger S, Metivier R, Kos M, Reid G, Sonntag-Buck V, Gannon F** 2000 Identification of a new isoform of the human estrogen receptor-α (hER-α) that is encoded by distinct transcripts and that is able to repress hER-α activation function 1. EMBO J 19:4688–4700
58. **Penot G, Le Péron C, Mérot Y, Grimaud-Fanouillère E, Ferrière F, Boujrad N, Kah O, Saligaut C, Ducouret B, Métivier R, Flouriot G** 2005 The human estrogen receptor-α isoform hERα46 antagonizes the proliferative influence of hERα66 in MCF7 breast cancer cells. Endocrinology 146:5474–5484
59. **Garcia T, Lehrer S, Bloomer WD, Schachter B** 1988 A variant estrogen receptor messenger ribonucleic acid is associated with reduced levels of estrogen binding in human mammary tumors. Mol Endocrinol 2:785–791
60. **Fuqua SA, Fitzgerald SD, Allred DC, Elledge RM, Nawaz Z, McDonnell DP, O'Malley BW, Greene GL, McGuire WL** 1992 Inhibition of estrogen receptor action by a naturally occurring variant in human breast tumors. Cancer Res 52:483–486
61. **McGuire WL, Chamness GC, Fuqua SA** 1991 Estrogen receptor variants in clinical breast cancer. Mol Endocrinol 5:1571–1577
62. **Klinge CM, Riggs KA, Wickramasinghe NS, Emberts CG, McConda DB, Barry PN, Magnusen JE** 2010 Estrogen receptor α 46 is reduced in tamoxifen resistant breast cancer cells and re-expression inhibits cell proliferation and estrogen receptor α 66-regulated target gene transcription. Mol Cell Endocrinol 323:268–276
63. **Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF** 2005 Identification, cloning, and expression of human estrogen receptor-α36, a novel variant of human estrogen receptor-α66. Biochem Biophys Res Commun 336:1023–1027
64. **Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF** 2006 A variant of estrogen receptor-α, hER-α36: transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signaling. Proc Natl Acad Sci USA 103:9063–9068
65. **Zhang XT, Kang LG, Ding L, Vranic S, Gatalica Z, Wang ZY** 2011 A positive feedback loop of ER-α36/EGFR promotes malignant growth of ER-negative breast cancer cells. Oncogene 30:770–780
66. **Shi L, Dong B, Li Z, Lu Y, Ouyang T, Li J, Wang T, Fan Z, Fan T, Lin B, Wang Z, Xie Y** 2009 Expression of ER-α36, a novel variant of estrogen receptor α, and resistance to tamoxifen treatment in breast cancer. J Clin Oncol 27:3423–3429
67. **Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL, Su JL, Kliewer SA, Lehmann JM, Willson TM** 1998 Cloning and characterization of human estrogen receptor β isoforms. Biochem Biophys Res Commun 247:75–78
68. **Saunders PT, Millar MR, Macpherson S, Irvine DS, Groome NP, Evans LR, Sharpe RM, Scobie GA** 2002 ERβ1 and the ERβ2 splice variant (ERβcx/β2) are expressed in distinct cell populations in the adult human testis. J Clin Endocrinol Metab 87:2706–2715
69. **Davies MP, O'Neill PA, Innes H, Sibson DR, Prime W, Holcombe C, Foster CS** 2004 Correlation of mRNA for oestrogen receptor β splice variants ERβ1, ERβ2/ERβcx and ERβ5 with outcome in endocrine-treated breast cancer. J Mol Endocrinol 33:773–782
70. **Skliris GP, Leygue E, Curtis-Snell L, Watson PH, Murphy LC** 2006 Expression of oestrogen receptor-β in oestrogen receptor-α negative human breast tumours. Br J Cancer 95:616–626
71. **Gougelet A, Bouclier C, Marsaud V, Maillard S, Mueller SO, Korach KS, Renoir JM** 2005 Estrogen receptor α and β subtype expression and transactivation capacity are differentially affected by receptor-, hsp90- and immunophi-

lin-ligands in human breast cancer cells. J Steroid Biochem Mol Biol 94:71–81
72. **Jensen EV** 1991 Steroid hormone receptors. Curr Top Pathol 83:365–431
73. **Sabbah M, Redeuilh G, Secco C, Baulieu EE** 1987 The binding activity of estrogen receptor to DNA and heat shock protein (Mr 90,000) is dependent on receptor-bound metal. J Biol Chem 262:8631–8635
74. **Perissi V, Jepsen K, Glass CK, Rosenfeld MG** 2010 Deconstructing repression: evolving models of co-repressor action. Nat Rev Genet 11:109–123
75. **Lonard DM, O'Malley BW** 2006 The expanding cosmos of nuclear receptor coactivators. Cell 125:411–414
76. **Robyr D, Wolffe AP, Wahli W** 2000 Nuclear hormone receptor coregulators in action: diversity for shared tasks. Mol Endocrinol 14:329–347
77. **Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M** 2000 Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843–852
78. **Early Breast Cancer Trialists' Collaborative Group (EBCTCG)** 2005 Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365: 1687–1717
79. **Gradishar WJ** 2005 The future of breast cancer: the role of prognostic factors. Breast Cancer Res Treat 89(Suppl 1): S17–S26
80. **York B, O'Malley BW** 2010 Steroid receptor coactivator (SRC) family: masters of systems biology. J Biol Chem 285: 38743–38750
81. **Kirkegaard T, McGlynn LM, Campbell FM, Müller S, Tovey SM, Dunne B, Nielsen KV, Cooke TG, Bartlett JM** 2007 Amplified in breast cancer 1 in human epidermal growth factor receptor-positive tumors of tamoxifen-treated breast cancer patients. Clin Cancer Res 13:1405–1411
82. **Osborne CK, Bardou V, Hopp TA, Chamness GC, Hilsenbeck SG, Fuqua SA, Wong J, Allred DC, Clark GM, Schiff R** 2003 Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. J Natl Cancer Inst 95:353–361
83. **Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, Uht RM, Webb P** 2000 Estrogen receptor pathways to AP-1. J Steroid Biochem Mol Biol 74:311–317
84. **O'Lone R, Frith MC, Karlsson EK, Hansen U** 2004 Genomic targets of nuclear estrogen receptors. Mol Endocrinol 18:1859–1875
85. **Saville B, Wormke M, Wang F, Nguyen T, Enmark E, Kuiper G, Gustafsson JA, Safe S** 2000 Ligand-, cell-, and estrogen receptor subtype (α/β)-dependent activation at GC-rich (Sp1) promoter elements. J Biol Chem 275:5379–5387
86. **Björnström L, Sjöberg M** 2004 Estrogen receptor-dependent activation of AP-1 via non-genomic signalling. Nucl Recept 2:3
87. **Quaedackers ME, van den Brink CE, van der Saag PT, Tertoolen LG** 2007 Direct interaction between estrogen receptor α and NF-κB in the nucleus of living cells. Mol Cell Endocrinol 273:42–50
88. **Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, Scanlan TS** 1997 Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. Science 277:1508–1510
89. **Johnston SR, Lu B, Scott GK, Kushner PJ, Smith IE, Dowsett M, Benz CC** 1999 Increased activator protein-1 DNA binding and c-Jun NH2-terminal kinase activity in human breast tumors with acquired tamoxifen resistance. Clin Cancer Res 5:251–256
90. **Schiff R, Reddy P, Ahotupa M, Coronado-Heinsohn E, Grim M, Hilsenbeck SG, Lawrence R, Deneke S, Herrera R, Chamness GC, Fuqua SA, Brown PH, Osborne CK** 2000 Oxidative stress and AP-1 activity in tamoxifen-resistant breast tumors in vivo. J Natl Cancer Inst 92: 1926–1934
91. **Zhou Y, Yau C, Gray JW, Chew K, Dairkee SH, Moore DH, Eppenberger U, Eppenberger-Castori S, Benz CC** 2007 Enhanced NFκB and AP-1 transcriptional activity associated with antiestrogen resistant breast cancer. BMC Cancer 7:59
92. **Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P** 1995 Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270:1491–1494
93. **Tremblay A, Tremblay GB, Labrie F, Giguère V** 1999 Ligand-independent recruitment of SRC-1 to estrogen receptor β through phosphorylation of activation function AF-1. Mol Cell 3:513–519
94. **Sauvé K, Lepage J, Sanchez M, Heveker N, Tremblay A** 2009 Positive feedback activation of estrogen receptors by the CXCL12-CXCR4 pathway. Cancer Res 69:5793–5800
95. **Rhodes LV, Short SP, Neel NF, Salvo VA, Zhu Y, Elliott S, Wei Y, Yu D, Sun M, Muir SE, Fonseca JP, Bratton MR, Segar C, Tilghman SL, Sobolik-Delmaire T, Horton LW, Zaja-Milatovic S, Collins-Burow BM, Wadsworth S, Beckman BS, Wood CE, Fuqua SA, Nephew KP, Dent P, Worthylake RA, Curiel TJ, Hung MC, Richmond A, Burow ME** 2011 Cytokine receptor CXCR4 mediates estrogen-independent tumorigenesis, metastasis, and resistance to endocrine therapy in human breast cancer. Cancer Res 71:603–613
96. **Hammes SR, Levin ER** 2007 Extranuclear steroid receptors: nature and actions. Endocr Rev 28:726–741
97. **Olde B, Leeb-Lundberg LM** 2009 GPR30/GPER1: searching for a role in estrogen physiology. Trends Endocrinol Metab 20:409–416
98. **Pappas TC, Gametchu B, Watson CS** 1995 Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. FASEB J 9:404–410
99. **Levin ER** 2009 Plasma membrane estrogen receptors. Trends Endocrinol Metab 20:477–482
100. **Razandi M, Pedram A, Merchenthaler I, Greene GL, Levin ER** 2004 Plasma membrane estrogen receptors exist and functions as dimers. Mol Endocrinol 18:2854–2865
101. **Acconcia F, Manavathi B, Mascarenhas J, Talukder AH, Mills G, Kumar R** 2006 An inherent role of integrin-linked kinase-estrogen receptor α interaction in cell migration. Cancer Res 66:11030–11038
102. **Björnström L, Sjöberg M** 2005 Mechanisms of estrogen receptor signaling: convergence of genomic and non-genomic actions on target genes. Mol Endocrinol 19:833–842

103. **Castoria G, Migliaccio A, Bilancio A, Di Domenico M, de Falco A, Lombardi M, Fiorentino R, Varricchio L, Barone MV, Auricchio F** 2001 PI3-kinase in concert with Src promotes the S-phase entry of oestradiol-stimulated MCF-7 cells. EMBO J 20:6050–6059
104. **Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK** 2000 Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature 407:538–541
105. **Song RX, Zhang Z, Santen RJ** 2005 Estrogen rapid action via protein complex formation involving ERα and Src. Trends Endocrinol Metab 16:347–353
106. **Song RX, Barnes CJ, Zhang Z, Bao Y, Kumar R, Santen RJ** 2004 The role of Shc and insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor α to the plasma membrane. Proc Natl Acad Sci USA 101: 2076–2081
107. **Kumar P, Wu Q, Chambliss KL, Yuhanna IS, Mumby SM, Mineo C, Tall GG, Shaul PW** 2007 Direct interactions with G α i and G βγ mediate nongenomic signaling by estrogen receptor α. Mol Endocrinol 21:1370–1380
108. **Cabodi S, Moro L, Baj G, Smeriglio M, Di Stefano P, Gippone S, Surico N, Silengo L, Turco E, Tarone G, Defilippi P** 2004 p130Cas interacts with estrogen receptor α and modulates non-genomic estrogen signaling in breast cancer cells. J Cell Sci 117:1603–1611
109. **Lu Q, Pallas DC, Surks HK, Baur WE, Mendelsohn ME, Karas RH** 2004 Striatin assembles a membrane signaling complex necessary for rapid, nongenomic activation of endothelial NO synthase by estrogen receptor α. Proc Natl Acad Sci USA 101:17126–17131
110. **Fernando RI, Wimalasena J** 2004 Estradiol abrogates apoptosis in breast cancer cells through inactivation of BAD: Ras-dependent nongenomic pathways requiring signaling through ERK and Akt. Mol Biol Cell 15:3266–3284
111. **Pedram A, Razandi M, Evinger AJ, Lee E, Levin ER** 2009 Estrogen inhibits ATR signaling to cell cycle checkpoints and DNA repair. Mol Biol Cell 20:3374–3389
112. **Azuma K, Urano T, Horie-Inoue K, Hayashi S, Sakai R, Ouchi Y, Inoue S** 2009 Association of estrogen receptor α and histone deacetylase 6 causes rapid deacetylation of tubulin in breast cancer cells. Cancer Res 69:2935–2940
113. **Catalano S, Barone I, Giordano C, Rizza P, Qi H, Gu G, Malivindi R, Bonofiglio D, Andò S** 2009 Rapid estradiol/ERα signaling enhances aromatase enzymatic activity in breast cancer cells. Mol Endocrinol 23:1634–1645
114. **Chambliss KL, Yuhanna IS, Anderson RG, Mendelsohn ME, Shaul PW** 2002 ERβ has nongenomic action in caveolae. Mol Endocrinol 16:938–946
115. **Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, Barone MV, Ametrano D, Zannini MS, Abbondanza C, Auricchio F** 2000 Steroid-induced androgen receptor-oestradiol receptor β-Src complex triggers prostate cancer cell proliferation. EMBO J 19:5406–5417
116. **Ordóñez-Morán P, Muñoz A** 2009 Nuclear receptors: genomic and non-genomic effects converge. Cell Cycle 8:1675–1680
117. **MacGregor JI, Jordan VC** 1998 Basic guide to the mechanisms of antiestrogen action. Pharmacol Rev 50:151–196
118. **Nawaz Z, Lonard DM, Dennis AP, Smith CL, O'Malley BW** 1999 Proteasome-dependent degradation of the human estrogen receptor. Proc Natl Acad Sci USA 96:1858–1862
119. **Lonard DM, Nawaz Z, Smith CL, O'Malley BW** 2000 The 26S proteasome is required for estrogen receptor-α and coactivator turnover and for efficient estrogen receptor-α transactivation. Mol Cell 5:939–948
120. **Wijayaratne AL, McDonnell DP** 2001 The human estrogen receptor-α is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 276: 35684–35692
121. **Marsaud V, Gougelet A, Maillard S, Renoir JM** 2003 Various phosphorylation pathways, depending on agonist and antagonist binding to endogenous estrogen receptor α (ERα), differentially affect ERα extractability, proteasome-mediated stability, and transcriptional activity in human breast cancer cells. Mol Endocrinol 17:2013–2027
122. **McDonnell DP, Dana SL, Hoener PA, Lieberman BA, Imhof MO, Stein RB** 1995 Cellular mechanisms which distinguish between hormone- and antihormone-activated estrogen receptor. Ann NY Acad Sci 761:121–137
123. **Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB, Pike JW, McDonnell DP** 1994 Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. Mol Endocrinol 8:21–30
124. **Jensen EV, Jordan VC** 2003 The estrogen receptor: a model for molecular medicine. Clin Cancer Res 9:1980–1989
125. **Johnston SR, Dowsett M** 2003 Aromatase inhibitors for breast cancer: lessons from the laboratory. Nat Rev Cancer 3:821–831
126. **Jordan VC** 2004 Aromatase inhibitors that regulate estrogen target tissues selectively? Bone 34:372–375
127. **Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, Castiglione M, Tu D, Shepherd LE, Pritchard KI, Livingston RB, Davidson NE, Norton L, Perez EA, Abrams JS, Cameron DA, Palmer MJ, Pater JL** 2005 Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA. 17. J Natl Cancer Inst 97:1262–1271
128. **Musgrove EA, Sutherland RL** 2009 Biological determinants of endocrine resistance in breast cancer. Nat Rev Cancer 9:631–643
129. **Roodi N, Bailey LR, Kao WY, Verrier CS, Yee CJ, Dupont WD, Parl FF** 1995 Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J Natl Cancer Inst 87:446–451
130. **O'Malley BW, Qin J, Lanz RB** 2008 Cracking the coregulator codes. Curr Opin Cell Biol 20:310–315
131. **Li X, Lonard DM, Jung SY, Malovannaya A, Feng Q, Qin J, Tsai SY, Tsai MJ, O'Malley BW** 2006 The SRC-3/AIB1 coactivator is degraded in a ubiquitin- and ATP-independent manner by the REGγ proteasome. Cell 124:381–392
132. **Margueron R, Duong V, Castet A, Cavaillès V** 2004 Histone deacetylase inhibition and estrogen signalling in human breast cancer cells. Biochem Pharmacol 68:1239–1246
133. **Sanchez M, Picard N, Sauvé K, Tremblay A** 2010 Challenging estrogen receptor β with phosphorylation. Trends Endocrinol Metab 21:104–110

134. **Auricchio F, Migliaccio A** 1980 In vitro inactivation of oestrogen receptor by nuclei: prevention by phosphatase inhibitors. FEBS Lett 117:224–226
135. **Auricchio F, Migliaccio A, Castoria G, Rotondi A, Lastoria S** 1984 Direct evidence of in vitro phosphorylation-dephosphorylation of the estradiol-17β receptor. Role of Ca2+-calmodulin in the activation of hormone binding sites. J Steroid Biochem 20:31–35
136. **Thomas RS, Sarwar N, Phoenix F, Coombes RC, Ali S** 2008 Phosphorylation at serines 104 and 106 by Erk1/2 MAPK is important for estrogen receptor-α activity. J Mol Endocrinol 40:173–184
137. **Medunjanin S, Hermani A, De Servi B, Grisouard J, Rincke G, Mayer D** 2005 Glycogen synthase kinase-3 interacts with and phosphorylates estrogen receptor α and is involved in the regulation of receptor activity. J Biol Chem 280:33006–33014
138. **Rogatsky I, Trowbridge JM, Garabedian MJ** 1999 Potentiation of human estrogen receptor α transcriptional activation through phosphorylation of serines 104 and 106 by the cyclin A-CDK2 complex. J Biol Chem 274:22296–22302
139. **Masuhiro Y, Mezaki Y, Sakari M, Takeyama K, Yoshida T, Inoue K, Yanagisawa J, Hanazawa S, O'malley BW, Kato S** 2005 Splicing potentiation by growth factor signals via estrogen receptor phosphorylation. Proc Natl Acad Sci USA 102:8126–8131
140. **Sheeler CQ, Singleton DW, Khan SA** 2003 Mutation of serines 104, 106, and 118 inhibits dimerization of the human estrogen receptor in yeast. Endocr Res 29:237–255
141. **Cheng J, Zhang C, Shapiro DJ** 2007 A functional serine 118 phosphorylation site in estrogen receptor-α is required for down-regulation of gene expression by 17β-estradiol and 4-hydroxytamoxifen. Endocrinology 148:4634–4641
142. **Dutertre M, Smith CL** 2003 Ligand-independent interactions of p160/steroid receptor coactivators and CREB-binding protein (CBP) with estrogen receptor-α: regulation by phosphorylation sites in the A/B region depends on other receptor domains. Mol Endocrinol 17:1296–1314
143. **Shah YM, Rowan BG** 2005 The Src kinase pathway promotes tamoxifen agonist action in Ishikawa endometrial cells through phosphorylation-dependent stabilization of estrogen receptor (α) promoter interaction and elevated steroid receptor coactivator 1 activity. Mol Endocrinol 19:732–748
144. **Bunone G, Briand PA, Miksicek RJ, Picard D** 1996 Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. EMBO J 15:2174–2183
145. **Ali S, Metzger D, Bornert JM, Chambon P** 1993 Modulation of transcriptional activation by ligand-dependent phosphorylation of the human oestrogen receptor A/B region. EMBO J 12:1153–1160
146. **Chen D, Washbrook E, Sarwar N, Bates GJ, Pace PE, Thirunuvakkarasu V, Taylor J, Epstein RJ, Fuller-Pace FV, Egly JM, Coombes RC, Ali S** 2002 Phosphorylation of human estrogen receptor α at serine 118 by two distinct signal transduction pathways revealed by phosphorylation-specific antisera. Oncogene 21:4921–4931
147. **Park KJ, Krishnan V, O'Malley BW, Yamamoto Y, Gaynor RB** 2005 Formation of an IKKα-dependent transcription complex is required for estrogen receptor-mediated gene activation. Mol Cell 18:71–82
148. **Weitsman GE, Li L, Skliris GP, Davie JR, Ung K, Niu Y, Curtis-Snell L, Tomes L, Watson PH, Murphy LC** 2006 Estrogen receptor-α phosphorylated at Ser118 is present at the promoters of estrogen-regulated genes and is not altered due to HER-2 overexpression. Cancer Res 66:10162–10170
149. **González L, Zambrano A, Lazaro-Trueba I, Lopéz E, González JJ, Martín-Pérez J, Aranda A** 2009 Activation of the unliganded estrogen receptor by prolactin in breast cancer cells. Oncogene 28:1298–1308
150. **Arnold SF, Obourn JD, Jaffe H, Notides AC** 1994 Serine 167 is the major estradiol-induced phosphorylation site on the human estrogen receptor. Mol Endocrinol 8:1208–1214
151. **Joel PB, Smith J, Sturgill TW, Fisher TL, Blenis J, Lannigan DA** 1998 pp90rsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167. Mol Cell Biol 18:1978–1984
152. **Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H** 2001 Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor α: a new model for anti-estrogen resistance. J Biol Chem 276:9817–9824
153. **Yamnik RL, Digilova A, Davis DC, Brodt ZN, Murphy CJ, Holz MK** 2009 S6 kinase 1 regulates estrogen receptor α in control of breast cancer cell proliferation. J Biol Chem 284:6361–6369
154. **Yamnik RL, Holz MK** 2010 mTOR/S6K1 and MAPK/RSK signaling pathways coordinately regulate estrogen receptor α serine 167 phosphorylation. FEBS Lett 584:124–128
155. **Chen D, Pace PE, Coombes RC, Ali S** 1999 Phosphorylation of human estrogen receptor α by protein kinase A regulates dimerization. Mol Cell Biol 19:1002–1015
156. **Tsai HW, Katzenellenbogen JA, Katzenellenbogen BS, Shupnik MA** 2004 Protein kinase A activation of estrogen receptor α transcription does not require proteasome activity and protects the receptor from ligand-mediated degradation. Endocrinology 145:2730–2738
157. **Michalides R, Griekspoor A, Balkenende A, Verwoerd D, Janssen L, Jalink K, Floore A, Velds A, van't Veer L, Neefjes J** 2004 Tamoxifen resistance by a conformational arrest of the estrogen receptor α after PKA activation in breast cancer. Cancer Cell 5:597–605
158. **Tharakan R, Lepont P, Singleton D, Kumar R, Khan S** 2008 Phosphorylation of estrogen receptor α, serine residue 305 enhances activity. Mol Cell Endocrinol 295:70–78
159. **Balasenthil S, Barnes CJ, Rayala SK, Kumar R** 2004 Estrogen receptor activation at serine 305 is sufficient to upregulate cyclin D1 in breast cancer cells. FEBS Lett 567:243–247
160. **Wang RA, Mazumdar A, Vadlamudi RK, Kumar R** 2002 P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-α and promotes hyperplasia in mammary epithelium. EMBO J 21:5437–5447
161. **Zwart W, Griekspoor A, Berno V, Lakeman K, Jalink K, Mancini M, Neefjes J, Michalides R** 2007 PKA-induced resistance to tamoxifen is associated with an altered ori-

entation of ERα towards co-activator SRC-1. EMBO J 26: 3534–3544
162. **Kok M, Zwart W, Holm C, Fles R, Hauptmann M, Van't Veer LJ, Wessels LF, Neefjes J, Stål O, Linn SC, Landberg G, Michalides R** 2011 PKA-induced phosphorylation of ERα at serine 305 and high PAK1 levels is associated with sensitivity to tamoxifen in ER-positive breast cancer. Breast Cancer Res Treat 125:1–12
163. **Barone I, Iacopetta D, Covington KR, Cui Y, Tsimelzon A, Beyer A, Andò S, Fuqua SA** 2010 Phosphorylation of the mutant K303R estrogen receptor α at serine 305 affects aromatase inhibitor sensitivity. Oncogene 29:2404–2414
164. **Giordano C, Cui Y, Barone I, Ando S, Mancini MA, Berno V, Fuqua SA** 2010 Growth factor-induced resistance to tamoxifen is associated with a mutation of estrogen receptor α and its phosphorylation at serine 305. Breast Cancer Res Treat 119:71–85
165. **Barone I, Cui Y, Herynk MH, Corona-Rodriguez A, Giordano C, Selever J, Beyer A, Andò S, Fuqua SA** 2009 Expression of the K303R estrogen receptor-α breast cancer mutation induces resistance to an aromatase inhibitor via addiction to the PI3K/Akt kinase pathway. Cancer Res 69:4724–4732
166. **Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguère V** 1997 Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor β. Mol Endocrinol 11:353–365
167. **Bhat RA, Harnish DC, Stevis PE, Lyttle CR, Komm BS** 1998 A novel human estrogen receptor β: identification and functional analysis of additional N-terminal amino acids. J Steroid Biochem Mol Biol 67:233–240
168. **Cheng X, Cole RN, Zaia J, Hart GW** 2000 Alternative O-glycosylation/O-phosphorylation of the murine estrogen receptor β. Biochemistry 39:11609–11620
169. **Cheng X, Hart GW** 2001 Alternative O-glycosylation/O-phosphorylation of serine-16 in murine estrogen receptor β: post-translational regulation of turnover and transactivation activity. J Biol Chem 276:10570–10575
170. **Picard N, Charbonneau C, Sanchez M, Licznar A, Busson M, Lazennec G, Tremblay A** 2008 Phosphorylation of activation function-1 regulates proteasome-dependent nuclear mobility and E6-associated protein ubiquitin ligase recruitment to the estrogen receptor β. Mol Endocrinol 22:317–330
171. **Tremblay A, Giguère V** 2001 Contribution of steroid receptor coactivator-1 and CREB binding protein in ligand-independent activity of estrogen receptor β. J Steroid Biochem Mol Biol 77:19–27
172. **St-Laurent V, Sanchez M, Charbonneau C, Tremblay A** 2005 Selective hormone-dependent repression of estrogen receptor β by a p38-activated ErbB2/ErbB3 pathway. J Steroid Biochem Mol Biol 94:23–37
173. **Sanchez M, Sauvé K, Picard N, Tremblay A** 2007 The hormonal response of estrogen receptor β is decreased by the phosphatidylinositol 3-kinase/Akt pathway via a phosphorylation-dependent release of CREB-binding protein. J Biol Chem 282:4830–4840
174. **Tremblay GB, Tremblay A, Labrie F, Giguère V** 1998 Ligand-independent activation of the estrogen receptors α and β by mutations of a conserved tyrosine can be abolished by antiestrogens. Cancer Res 58:877–881
175. **Auricchio F, Di Domenico M, Migliaccio A, Castoria G, Bilancio A** 1995 The role of estradiol receptor in the proliferative activity of vanadate on MCF-7 cells. Cell Growth Differ 6:105–113
176. **Power RF, Mani SK, Codina J, Conneely OM, O'Malley BW** 1991 Dopaminergic and ligand-independent activation of steroid hormone receptors. Science 254:1636–1639
177. **Ikeda K, Ogawa S, Tsukui T, Horie-Inoue K, Ouchi Y, Kato S, Muramatsu M, Inoue S** 2004 Protein phosphatase 5 is a negative regulator of estrogen receptor-mediated transcription. Mol Endocrinol 18:1131–1143
178. **Singh BN, Zhang G, Hwa YL, Li J, Dowdy SC, Jiang SW** 2010 Nonhistone protein acetylation as cancer therapy targets. Expert Rev Anticancer Ther 10:935–954
179. **Chen H, Lin RJ, Xie W, Wilpitz D, Evans RM** 1999 Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell 98: 675–686
180. **Kim MY, Hsiao SJ, Kraus WL** 2001 A role for coactivators and histone acetylation in estrogen receptor α-mediated transcription initiation. EMBO J 20:6084–6094
181. **Kim MY, Woo EM, Chong YT, Homenko DR, Kraus WL** 2006 Acetylation of estrogen receptor α by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activities of the receptor. Mol Endocrinol 20:1479–1493
182. **Wang C, Fu M, Angeletti RH, Siconolfi-Baez L, Reutens AT, Albanese C, Lisanti MP, Katzenellenbogen BS, Kato S, Hopp T, Fuqua SA, Lopez GN, Kushner PJ, Pestell RG** 2001 Direct acetylation of the estrogen receptor α hinge region by p300 regulates transactivation and hormone sensitivity. J Biol Chem 276:18375–18383
183. **Ma Y, Fan S, Hu C, Meng Q, Fuqua SA, Pestell RG, Tomita YA, Rosen EM** 2010 BRCA1 regulates acetylation and ubiquitination of estrogen receptor-α. Mol Endocrinol 24: 76–90
184. **Urbinati G, Marsaud V, Plassat V, Fattal E, Lesieur S, Renoir JM** 2010 Liposomes loaded with histone deacetylase inhibitors for breast cancer therapy. Int J Pharm 397: 184–193
185. **Duong V, Licznar A, Margueron R, Boulle N, Busson M, Lacroix M, Katzenellenbogen BS, Cavaillès V, Lazennec G** 2006 ERα and ERβ expression and transcriptional activity are differentially regulated by HDAC inhibitors. Oncogene 25:1799–1806
186. **Stenoien DL, Patel K, Mancini MG, Dutertre M, Smith CL, O'Malley BW, Mancini MA** 2001 FRAP reveals that mobility of oestrogen receptor-α is ligand- and proteasome-dependent. Nat Cell Biol 3:15–23
187. **Reid G, Hübner MR, Métivier R, Brand H, Denger S, Manu D, Beaudouin J, Ellenberg J, Gannon F** 2003 Cyclic, proteasome-mediated turnover of unliganded and liganded ERα on responsive promoters is an integral feature of estrogen signaling. Mol Cell 11:695–707
188. **Nirmala PB, Thampan RV** 1995 Ubiquitination of the rat uterine estrogen receptor: dependence on estradiol. Biochem Biophys Res Commun 213:24–31
189. **Fan M, Park A, Nephew KP** 2005 CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor-α. Mol Endocrinol 19:2901–2914
190. **Duong V, Boulle N, Daujat S, Chauvet J, Bonnet S, Neel H,**

Cavaillès V 2007 Differential regulation of estrogen receptor α turnover and transactivation by Mdm2 and stress-inducing agents. Cancer Res 67:5513–5521
191. **Alarid ET, Bakopoulos N, Solodin N** 1999 Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. Mol Endocrinol 13: 1522–1534
192. **Laïos I, Journé F, Nonclercq D, Vidal DS, Toillon RA, Laurent G, Leclercq G** 2005 Role of the proteasome in the regulation of estrogen receptor α turnover and function in MCF-7 breast carcinoma cells. J Steroid Biochem Mol Biol 94:347–359
193. **Zhang H, Sun L, Liang J, Yu W, Zhang Y, Wang Y, Chen Y, Li R, Sun X, Shang Y** 2006 The catalytic subunit of the proteasome is engaged in the entire process of estrogen receptor-regulated transcription. EMBO J 25:4223–4233
194. **Métivier R, Penot G, Hübner MR, Reid G, Brand H, Kos M, Gannon F** 2003 Estrogen receptor-α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell 115:751–763
195. **Li L, Li Z, Howley PM, Sacks DB** 2006 E6AP and calmodulin reciprocally regulate estrogen receptor stability. J Biol Chem 281:1978–1985
196. **Saji S, Okumura N, Eguchi H, Nakashima S, Suzuki A, Toi M, Nozawa Y, Saji S, Hayashi S** 2001 MDM2 enhances the function of estrogen receptor α in human breast cancer cells. Biochem Biophys Res Commun 281:259–265
197. **Nakajima A, Maruyama S, Bohgaki M, Miyajima N, Tsukiyama T, Sakuragi N, Hatakeyama S** 2007 Ligand-dependent transcription of estrogen receptor α is mediated by the ubiquitin ligase EFP. Biochem Biophys Res Commun 357:245–251
198. **Shao W, Keeton EK, McDonnell DP, Brown M** 2004 Coactivator AIB1 links estrogen receptor transcriptional activity and stability. Proc Natl Acad Sci USA 101:11599–11604
199. **El Khissiin A, Leclercq G** 1999 Implication of proteasome in estrogen receptor degradation. FEBS Lett 448:160–166
200. **Long X, Nephew KP** 2006 Fulvestrant (ICI 182,780)-dependent interacting proteins mediate immobilization and degradation of estrogen receptor-α. J Biol Chem 281:9607–9615
201. **Berry NB, Fan M, Nephew KP** 2008 Estrogen receptor-α hinge-region lysines 302 and 303 regulate receptor degradation by the proteasome. Mol Endocrinol 22:1535–1551
202. **Eakin CM, Maccoss MJ, Finney GL, Klevit RE** 2007 Estrogen receptor α is a putative substrate for the BRCA1 ubiquitin ligase. Proc Natl Acad Sci USA 104:5794–5799
203. **Stanisiæ V, Malovannaya A, Qin J, Lonard DM, O'Malley BW** 2009 OTU Domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) deubiquitinates estrogen receptor (ER) α and affects ERα transcriptional activity. J Biol Chem 284:16135–16145
204. **Tateishi Y, Sonoo R, Sekiya Y, Sunahara N, Kawano M, Wayama M, Hirota R, Kawabe Y, Murayama A, Kato S, Kimura K, Yanagisawa J** 2006 Turning off estrogen receptor β-mediated transcription requires estrogen-dependent receptor proteolysis. Mol Cell Biol 26:7966–7976
205. **Masuyama H, Hiramatsu Y** 2004 Involvement of suppressor for Gal 1 in the ubiquitin/proteasome-mediated degradation of estrogen receptors. J Biol Chem 279:12020–12026
206. **Sentis S, Le Romancer M, Bianchin C, Rostan MC, Corbo L** 2005 Sumoylation of the estrogen receptor α hinge region regulates its transcriptional activity. Mol Endocrinol 19:2671–2684
207. **Pradhan S, Chin HG, Estève PO, Jacobsen SE** 2009 SET7/9 mediated methylation of non-histone proteins in mammalian cells. Epigenetics 4:383–387
208. **Subramanian K, Jia D, Kapoor-Vazirani P, Powell DR, Collins RE, Sharma D, Peng J, Cheng X, Vertino PM** 2008 Regulation of estrogen receptor α by the SET7 lysine methyltransferase. Mol Cell 30:336–347
209. **Garbán HJ, Márquez-Garbán DC, Pietras RJ, Ignarro LJ** 2005 Rapid nitric oxide-mediated S-nitrosylation of estrogen receptor: regulation of estrogen-dependent gene transcription. Proc Natl Acad Sci USA 102:2632–2636
210. **Cheng X, Hart GW** 2000 Glycosylation of the murine estrogen receptor-α. J Steroid Biochem Mol Biol 75:147–158
211. **Jiang MS, Hart GW** 1997 A subpopulation of estrogen receptors are modified by O-linked N-acetylglucosamine. J Biol Chem 272:2421–2428
212. **Atsriku C, Britton DJ, Held JM, Schilling B, Scott GK, Gibson BW, Benz CC, Baldwin MA** 2009 Systematic mapping of posttranslational modifications in human estrogen receptor-α with emphasis on novel phosphorylation sites. Mol Cell Proteomics 8:467–480
213. **Pietras RJ, Szego CM** 1977 Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. Nature 265:69–72
214. **Acconcia F, Ascenzi P, Fabozzi G, Visca P, Marino M** 2004 S-palmitoylation modulates human estrogen receptor-α functions. Biochem Biophys Res Commun 316:878–883
215. **Acconcia F, Ascenzi P, Bocedi A, Spisni E, Tomasi V, Trentalance A, Visca P, Marino M** 2005 Palmitoylation-dependent estrogen receptor α membrane localization: regulation by 17β-estradiol. Mol Biol Cell 16:231–237
216. **Galluzzo P, Ascenzi P, Bulzomi P, Marino M** 2008 The nutritional flavanone naringenin triggers antiestrogenic effects by regulating estrogen receptor α-palmitoylation. Endocrinology 149:2567–2575
217. **Pedram A, Razandi M, Sainson RC, Kim JK, Hughes CC, Levin ER** 2007 A conserved mechanism for steroid receptor translocation to the plasma membrane. J Biol Chem 282:22278–22288
218. **Marino M, Ascenzi P** 2008 Membrane association of estrogen receptor α and β influences 17β-estradiol-mediated cancer cell proliferation. Steroids 73:853–858
219. **Galluzzo P, Caiazza F, Moreno S, Marino M** 2007 Role of ERβ palmitoylation in the inhibition of human colon cancer cell proliferation. Endocr Relat Cancer 14:153–167
220. **Auricchio F, Migliaccio A, Castoria G, Di Domenico M, Pagano M** 1990 Phosphorylation of uterus estradiol receptor on tyrosine. Prog Clin Biol Res 322:133–155
221. **Migliaccio A, Rotondi A, Auricchio F** 1986 Estradiol receptor: phosphorylation on tyrosine in uterus and interaction with anti-phosphotyrosine antibody. EMBO J 5:2867–2872
222. **Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram MD, Ramos L, Gorman CM, Parker MG, Sliwkowski MX, Slamon DJ** 1995 HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 10: 2435–2446
223. **Arnold SF, Obourn JD, Jaffe H, Notides AC** 1995 Phos-

phorylation of the human estrogen receptor on tyrosine 537 *in vivo* and by src family tyrosine kinases *in vitro*. Mol Endocrinol 9:24–33

224. **Auricchio F, Migliaccio A, Castoria G** 2008 Sex-steroid hormones and EGF signalling in breast and prostate cancer cells: targeting the association of Src with steroid receptors. Steroids 73:880–884
225. **Varricchio L, Migliaccio A, Castoria G, Yamaguchi H, de Falco A, Di Domenico M, Giovannelli P, Farrar W, Appella E, Auricchio F** 2007 Inhibition of estradiol receptor/Src association and cell growth by an estradiol receptor α tyrosine-phosphorylated peptide. Mol Cancer Res 5:1213–1221
226. **Likhite VS, Stossi F, Kim K, Katzenellenbogen BS, Katzenellenbogen JA** 2006 Kinase-specific phosphorylation of the estrogen receptor changes receptor interactions with ligand, deoxyribonucleic acid, and coregulators associated with alterations in estrogen and tamoxifen activity. Mol Endocrinol 20:3120–3132
227. **Le Romancer M, Treilleux I, Leconte N, Robin-Lespinasse Y, Sentis S, Bouchekioua-Bouzaghou K, Goddard S, Gobert-Gosse S, Corbo L** 2008 Regulation of estrogen rapid signaling through arginine methylation by PRMT1. Mol Cell 31:212–221
228. **Le Romancer M, Treilleux I, Bouchekioua-Bouzaghou K, Sentis S, Corbo L** 2010 Methylation, a key step for non-genomic estrogen signaling in breast tumors. Steroids 75:560–564
229. **Zhang QX, Borg A, Wolf DM, Oesterreich S, Fuqua SA** 1997 An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. Cancer Res 57:1244–1249
230. **Cui Y, Zhang M, Pestell R, Curran EM, Welshons WV, Fuqua SA** 2004 Phosphorylation of estrogen receptor α blocks its acetylation and regulates estrogen sensitivity. Cancer Res 64:9199–9208
231. **Herynk MH, Hopp T, Cui Y, Niu A, Corona-Rodriguez A, Fuqua SA** 2010 A hypersensitive estrogen receptor α mutation that alters dynamic protein interactions. Breast Cancer Res Treat 122:381–393
232. **Herynk MH, Parra I, Cui Y, Beyer A, Wu MF, Hilsenbeck SG, Fuqua SA** 2007 Association between the estrogen receptor α A908G mutation and outcomes in invasive breast cancer. Clin Cancer Res 13:3235–3243
233. **Rayala SK, Talukder AH, Balasenthil S, Tharakan R, Barnes CJ, Wang RA, Aldaz CM, Khan S, Kumar R** 2006 P21-activated kinase 1 regulation of estrogen receptor-α activation involves serine 305 activation linked with serine 118 phosphorylation. Cancer Res 66:1694–1701
234. **Calligé M, Kieffer I, Richard-Foy H** 2005 CSN5/Jab1 is involved in ligand-dependent degradation of estrogen receptor α by the proteasome. Mol Cell Biol 25:4349–4358
235. **Valley CC, Métivier R, Solodin NM, Fowler AM, Mashek MT, Hill L, Alarid ET** 2005 Differential regulation of estrogen-inducible proteolysis and transcription by the estrogen receptor α N terminus. Mol Cell Biol 25:5417–5428
236. **Valley CC, Solodin NM, Powers GL, Ellison SJ, Alarid ET** 2008 Temporal variation in estrogen receptor-α protein turnover in the presence of estrogen. J Mol Endocrinol 40:23–34
237. **García Pedrero JM, Del Rio B, Martínez-Campa C, Muramatsu M, Lazo PS, Ramos S** 2002 Calmodulin is a selective modulator of estrogen receptors. Mol Endocrinol 16:947–960
238. **Fernö M, Stål O, Baldetorp B, Hatschek T, Källström AC, Malmström P, Nordenskjöld B, Rydën S** 2000 Results of two or five years of adjuvant tamoxifen correlated to steroid receptor and S-phase levels. South Sweden Breast Cancer Group, and South-East Sweden Breast Cancer Group. Breast Cancer Res Treat 59:69–76
239. **Henry JA, Nicholson S, Farndon JR, Westley BR, May FE** 1988 Measurement of oestrogen receptor mRNA levels in human breast tumours. Br J Cancer 58:600–605
240. **Pichon MF, Broet P, Magdelenat H, Delarue JC, Spyratos F, Basuyau JP, Saez S, Rallet A, Courriere P, Millon R, Asselain B** 1996 Prognostic value of steroid receptors after long-term follow-up of 2257 operable breast cancers. Br J Cancer 73:1545–1551
241. **Bergqvist J, Elmberger G, Ohd J, Linderholm B, Bjohle J, Hellborg H, Nordgren H, Borg AL, Skoog L, Bergh J** 2006 Activated ERK1/2 and phosphorylated oestrogen receptor α are associated with improved breast cancer survival in women treated with tamoxifen. Eur J Cancer 42:1104–1112
242. **Murphy LC, Niu Y, Snell L, Watson P** 2004 Phosphoserine-118 estrogen receptor-α expression is associated with better disease outcome in women treated with tamoxifen. Clin Cancer Res 10:5902–5906
243. **Sarwar N, Kim JS, Jiang J, Peston D, Sinnett HD, Madden P, Gee JM, Nicholson RI, Lykkesfeldt AE, Shousha S, Coombes RC, Ali S** 2006 Phosphorylation of ERα at serine 118 in primary breast cancer and in tamoxifen-resistant tumours is indicative of a complex role for ERα phosphorylation in breast cancer progression. Endocr Relat Cancer 13:851–861
244. **Yamashita H, Nishio M, Toyama T, Sugiura H, Kondo N, Kobayashi S, Fujii Y, Iwase H** 2008 Low phosphorylation of estrogen receptor α (ERα) serine 118 and high phosphorylation of ERα serine 167 improve survival in ER-positive breast cancer. Endocr Relat Cancer 15:755–763
245. **Zoubir M, Mathieu MC, Mazouni C, Liedtke C, Corley L, Geha S, Bouaziz J, Spielmann M, Drusche F, Symmans WF, Delaloge S, Andre F** 2008 Modulation of ER phosphorylation on serine 118 by endocrine therapy: a new surrogate marker for efficacy. Ann Oncol 19:1402–1406
246. **Jiang J, Sarwar N, Peston D, Kulinskaya E, Shousha S, Coombes RC, Ali S** 2007 Phosphorylation of estrogen receptor-α at Ser167 is indicative of longer disease-free and overall survival in breast cancer patients. Clin Cancer Res 13:5769–5776
247. **Gee JM, Robertson JF, Gutteridge E, Ellis IO, Pinder SE, Rubini M, Nicholson RI** 2005 Epidermal growth factor receptor/HER2/insulin-like growth factor receptor signalling and oestrogen receptor activity in clinical breast cancer. Endocr Relat Cancer 12(Suppl 1):S99–S111
248. **Kok M, Holm-Wigerup C, Hauptmann M, Michalides R, Stål O, Linn S, Landberg G** 2009 Estrogen receptor-α phosphorylation at serine-118 and tamoxifen response in breast cancer. J Natl Cancer Inst 101:1725–1729
249. **Skliris GP, Nugent ZJ, Rowan BG, Penner CR, Watson PH, Murphy LC** 2010 A phosphorylation code for oestrogen receptor α predicts clinical outcome to endocrine therapy in breast cancer. Endocr Relat Cancer 17:589–597
250. **Yamashita H, Nishio M, Kobayashi S, Ando Y, Sugiura H,**

Zhang Z, Hamaguchi M, Mita K, Fujii Y, Iwase H 2005 Phosphorylation of estrogen receptor α serine 167 is predictive of response to endocrine therapy and increases postrelapse survival in metastatic breast cancer. Breast Cancer Res 7:R753–R764

251. **Yamashita H, Takahashi S, Ito Y, Yamashita T, Ando Y, Toyama T, Sugiura H, Yoshimoto N, Kobayashi S, Fujii Y, Iwase H** 2009 Predictors of response to exemestane as primary endocrine therapy in estrogen receptor-positive breast cancer. Cancer Sci 100:2028–2033
252. **Skliris GP, Rowan BG, Al-Dhaheri M, Williams C, Troup S, Begic S, Parisien M, Watson PH, Murphy LC** 2009 Immunohistochemical validation of multiple phospho-specific epitopes for estrogen receptor α (ERα) in tissue microarrays of ERα positive human breast carcinomas. Breast Cancer Res Treat 118:443–453
253. **Generali D, Buffa FM, Berruti A, Brizzi MP, Campo L, Bonardi S, Bersiga A, Allevi G, Milani M, Aguggini S, Papotti M, Dogliotti L, Bottini A, Harris AL, Fox SB** 2009 Phosphorylated ERα, HIF-1α, and MAPK signaling as predictors of primary endocrine treatment response and resistance in patients with breast cancer. J Clin Oncol 27:227–234
254. **Miller WR, Hulme MJ, Bartlett JM, MacCallum J, Dixon JM** 1997 Changes in messenger RNA expression of protein kinase A regulatory subunit iα in breast cancer patients treated with tamoxifen. Clin Cancer Res 3:2399–2404
255. **Bostner J, Ahnström Waltersson M, Fornander T, Skoog L, Nordenskjöld B, Stål O** 2007 Amplification of CCND1 and PAK1 as predictors of recurrence and tamoxifen resistance in postmenopausal breast cancer. Oncogene 26:6997–7005
256. **Holm C, Rayala S, Jirström K, Stål O, Kumar R, Landberg G** 2006 Association between Pak1 expression and subcellular localization and tamoxifen resistance in breast cancer patients. J Natl Cancer Inst 98:671–680
257. **Bostner J, Skoog L, Fornander T, Nordenskjöld B, Stål O** 2010 Estrogen receptor-α phosphorylation at serine 305, nuclear p21-activated kinase 1 expression, and response to tamoxifen in postmenopausal breast cancer. Clin Cancer Res 16:1624–1633
258. **Goulet I, Gauvin G, Boisvenue S, Côté J** 2007 Alternative splicing yields protein arginine methyltransferase 1 isoforms with distinct activity, substrate specificity, and subcellular localization. J Biol Chem 282:33009–33021
259. **Fuqua SA, Wiltschke C, Zhang QX, Borg A, Castles CG, Friedrichs WE, Hopp T, Hilsenbeck S, Mohsin S, O'Connell P, Allred DC** 2000 A hypersensitive estrogen receptor-α mutation in premalignant breast lesions. Cancer Res 60:4026–4029
260. **Conway K, Parrish E, Edmiston SN, Tolbert D, Tse CK, Geradts J, Livasy CA, Singh H, Newman B, Millikan RC** 2005 The estrogen receptor-α A908G (K303R) mutation occurs at a low frequency in invasive breast tumors: results from a population-based study. Breast Cancer Res 7:R871–R880
261. **Hamilton-Burke W, Coleman L, Cummings M, Green CA, Holliday DL, Horgan K, Maraqa L, Peter MB, Pollock S, Shaaban AM, Smith L, Speirs V** 2010 Phosphorylation of estrogen receptor β at serine 105 is associated with good prognosis in breast cancer. Am J Pathol 177:1079–1086
262. **He X, Zheng Z, Song T, Wei C, Ma H, Ma Q, Zhang Y, Xu Y, Shi W, Ye Q, Zhong H** 2010 c-Abl regulates estrogen receptor α transcription activity through its stabilization by phosphorylation. Oncogene 29:2238–2251
263. **Chen D, Riedl T, Washbrook E, Pace PE, Coombes RC, Egly JM, Ali S** 2000 Activation of estrogen receptor α by S118 phosphorylation involves a ligand-dependent interaction with TFIIH and participation of CDK7. Mol Cell 6:127–137
264. **Weitsman GE, Weebadda W, Ung K, Murphy LC** 2009 Reactive oxygen species induce phosphorylation of serine 118 and 167 on estrogen receptor α. Breast Cancer Res Treat 118:269–279
265. **Britton DJ, Scott GK, Schilling B, Atsriku C, Held JM, Gibson BW, Benz CC, Baldwin MA** 2008 A novel serine phosphorylation site detected in the N-terminal domain of estrogen receptor isolated from human breast cancer cells. J Am Soc Mass Spectrom 19:729–740
266. **Park S, Song J, Joe CO, Shin I** 2008 Akt stabilizes estrogen receptor α with the concomitant reduction in its transcriptional activity. Cell Signal 20:1368–1374
267. **Williams CC, Basu A, El-Gharbawy A, Carrier LM, Smith CL, Rowan BG** 2009 Identification of four novel phosphorylation sites in estrogen receptor α: impact on receptor-dependent gene expression and phosphorylation by protein kinase CK2. BMC Biochem 10:36
268. **Lee H, Bai W** 2002 Regulation of estrogen receptor nuclear export by ligand-induced and p38-mediated receptor phosphorylation. Mol Cell Biol 22:5835–5845
269. **Castoria G, Migliaccio A, Green S, Di Domenico M, Chambon P, Auricchio F** 1993 Properties of a purified estradiol-dependent calf uterus tyrosine kinase 1. Biochemistry 32:1740–1750
270. **Arnold SF, Vorojeikina DP, Notides AC** 1995 Phosphorylation of tyrosine 537 on the human estrogen receptor is required for binding to an estrogen response element. J Biol Chem 270:30205–30212
271. **Arnold SF, Melamed M, Vorojeikina DP, Notides AC, Sasson S** 1997 Estradiol-binding mechanism and binding capacity of the human estrogen receptor is regulated by tyrosine phosphorylation. Mol Endocrinol 11:48–53
272. **Márquez DC, Lee J, Lin T, Pietras RJ** 2001 Epidermal growth factor receptor and tyrosine phosphorylation of estrogen receptor. Endocrine 16:73–81
273. **Urbinati G, Marsaud V, Plassat V, Renoir JM** 2011 Nanocarriers targeting breast cancers to deliver modulators of estrogen receptor. In: Souto EB, ed. Lipid nanocarriers in cancer diagnosis and therapy. Akron, Ohio: iSmithers Creative Publishing Solutions 10:279–330