Derailed Estrogen Signaling and Breast Cancer: An Authentic Couple

Bramanandam Manavathi, Oindrilla Dey, Vijay Narsihma Reddy Gajulapalli, Raghavendra Singh Bhatia, Suresh Bugide, and Rakesh Kumar

Molecular and Cellular Oncology Laboratory (B.M., O.D., V.N.R.G., R.S.B., S.B.), Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India; Cancer Research Program (R.K.), Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695014, India; and Department of Biochemistry and Molecular Biology (R.K.), The George Washington University, Washington, D.C. 20052

Estrogen or 17β-estradiol, a steroid hormone, plays a critical role in the development of mammary gland via acting through specific receptors. In particular, estrogen receptor-α (ERα) acts as a transcription factor and/or a signal transducer while participating in the development of mammary gland and breast cancer. Accumulating evidence suggests that the transcriptional activity of ERα is altered by the action of nuclear receptor coregulators and might be responsible, at least in part, for the development of breast cancer. In addition, this process is driven by various posttranslational modifications of ERα, implicating active participation of the upstream receptor modifying enzymes in breast cancer progression. Emerging studies suggest that the biological outcome of breast cancer cells is also influenced by the cross talk between microRNA and ERα signaling, as well as by breast cancer stem cells. Thus, multiple regulatory controls of ERα render mammary epithelium at risk for transformation upon deregulation of normal homeostasis. Given the importance that ERα signaling has in breast cancer development, here we will highlight how the activity of ERα is controlled by various regulators in a spatial and temporal manner, impacting the progression of the disease. We will also discuss the possible therapeutic value of ERα modulators as alternative drug targets to retard the progression of breast cancer. **(*Endocrine Reviews* 34: 1–32, 2013)**

ISSN Print 0163-769X ISSN Online 1945-7189
Printed in U.S.A.

doi: 10.1210/er.2011-1057 Received November 30, 2011. Accepted July 9, 2012.
First Published Online September 4, 2012

I. Introduction

Breast cancer is heterogeneous in nature that originates from the mammary epithelial cells. Despite advances made in the understanding of the molecular and cellular events that underlie the disease, it remains the leading cause of cancer deaths among females worldwide (1). A woman's risk of breast cancer is influenced by her reproductive history, *i.e.*, lifetime exposure to reproduc-

Abbreviations: AIB1, Amplified in breast cancer-1; AKT, serine/threonine protein kinase; ALDH, aldehyde dehydrogenase; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and rad3-related protein; BCAS3, breast carcinoma amplified sequence 3; BRCA1, breast cancer 1; BrCSC, breast cancer stem cell; BT-IC, breast tumor-initiating cell; CDK4, cyclin-dependent kinase; Ciz1, CDKN1A-interacting zinc finger protein 1; DACH1, dachshund homolog 1; DBC1, deleted in breast cancer 1; DNAPK, DNA-dependent protein kinase; E2, estrogen or 17β-estradiol; Efp, estrogen-responsive finger protein; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; ERE, estrogen response element; GPR30, G protein-coupled receptor 30; GREB1, growth regulation by estrogen in breast cancer 1; GSK3β, glycogen synthase kinase 3β; HAT, histone acetyl transferase; HDAC, histone deacetylase; HPIP, hematopoietic PBX-interacting protein 1; MaSC, mammary stem cell; miRNA, microRNA; MTA, metastasis-associated protein; MTA1s, MTA1 short form; NCOR1, nuclear receptor corepressor 1; NuRD, nucleosome remodeling and histone deacetylation complex; PAK1, serine/threonine p21-activated kinase; PELP1, proline, glutamic acid and leucine-rich protein; PHB, prohibitin; PI3K, phosphatidylinositol 3 kinase; PKA, protein kinase A; PR, progesterone receptor; PRMT1, protein arginine N-methyltransferase 1; REA, repressor of ER activity; SAFB, scaffold attachment factor B; SCID, severe combined immunodeficiency; SERM, selective ER modulator; SIRT1, sirtuin 1; S6K1, S6 kinase 1; SP, specificity protein; TFF1, trefoil factor 1; UTR, untranslated region.

tive hormone milieu, primarily estrogen (2). 17β-Estradiol or estrogen (we refer to both hereafter as E2), a steroid hormone that plays a significant role in mammary gland development, serves as one of the main risk factors for breast cancer development. E2 actions are mainly mediated by two receptors, estrogen receptor-α (ERα) and -β (ERβ). Experimental and clinical evidence suggests that ERα subtype is the major culprit for the development of the majority of the breast cancers (3–5). Therefore, antiestrogens that antagonize E2 binding to the receptor and E2 synthesis inhibitors (aromatase inhibitors) were developed to treat breast cancers (6).

The ERα is a ligand-dependent transcription factor that regulates genes involved in cell proliferation, differentiation, and migration (7). Therefore, deregulated actions of ERα signaling are associated with breast cancer development (8). In addition to the classical genomic actions exerted by ERα, emerging studies suggest that extranuclear signaling, coregulators, posttranslational modifications, and now microRNA (miRNA) add several levels of complexity to the action of ERα in breast cancer cells. Because deregulated expressions/actions of all of these modulators of ERα are found to correlate with breast cancer risk, the mammary epithelium is at high risk for transformation into a cancer cell. Therefore, the interrelationship between the ERα signaling and breast cancer development looks strong and attractive because the majority of breast cancers are ERα-dependent. In brief, breast cancer is a signaling disorder wherein deregulation of critical signaling pathways contributes to breast cancer pathogenesis. Here, we will summarize new insights into the E2-ERα signaling axis and its deregulation in breast cancer.

II. E2 Signaling in Mammary Gland Development

The human mammary gland undergoes several major developmental changes involving cell proliferation, differentiation, apoptosis, and morphogenesis in coordination with the influence of various endocrine and paracrine factors (9, 10). Using endocrine disruption and replacement studies in rodents, it was established that female reproductive hormones such as E2 and progesterone are key regulators of postnatal development of mammary gland (11). The mammary gland at birth is underdeveloped, but with the onset of puberty, E2 initiates the maturation of the mammary gland together with progesterone (9, 12). In particular, E2 triggers ductal elongation during puberty (13, 14). The precise role of E2-mediated actions in mammary gland came from receptor knockout studies in mice. Deletion of ERα, which mediates the E2 action in mice, results in a rudimentary ductal system that fails to branch out (15). Therefore, in ERα-null mice, mammary glands are normal before puberty (16). However, after the onset of puberty, terminal end buds remained absent, and ducts failed to invade into the fat pad beyond the nipple, indicating the strong influence of ERα in initiation of mammary gland development (17). Recent studies also established that ERα not only regulates ductal morphogenesis during puberty but is also involved in alveologenesis during pregnancy and lactation (18). By contrast, ERβ-null mice show no difference in morphology compared with the mammary glands of wild-type littermates, indicating that ERα (but not ERβ) regulates mammary gland development (19). These findings explicitly established the importance of ERα in mediating E2 actions in the development of mammary gland. Interestingly, ERβ seems to antagonize proliferative activity of ERα in breast cancer cells, suggesting that ERβ plays a tumor-suppressive role with respect to breast tumor development (20, 21).

III. ERα Genomic Signaling in Breast Cancer

A. ERα genomic action in breast cancer

The first link between steroid hormone signaling and breast cancer came from Beatson's observation in 1896 (22). He reported in *Lancet* that the metastatic breast cancer patients who underwent bilateral oophorectomy showed regression of tumors implying the rationale for hormone therapy for the treatment of breast cancer (22). Several decades later, O'Malley *et al.* (23) observed changes in transcriptional message upon E2 stimulation of the chick oviduct, suggesting the role of E2 in transcription regulation. Immediately after this finding, an extensive search for an ER was pioneered in 1971 by Jensen *et al.* (24). As a result, a specific ER was discovered that was present in breast tumors, and its expression level could correlate to endocrine disruptions, thereby establishing a link between cancer and E2 (24). Later on, overwhelming evidence showed the overexpression of ERα in 60–70% of breast cancers, and so this receptor has been treated as a therapeutic target for breast cancers (25–27).

The ERα (classified as NR_3A_1) is a ligand-dependent transcription factor that belongs to the nuclear receptor superfamily of proteins with defined functional domains that can both activate and repress the expression of genes (28). In the absence of ligand, ERα is sequestered in complex with an inhibitory heat shock protein in target cell nuclei. Upon ligand binding, the receptor detaches from the heat shock protein complex and undergoes dimerization (29). The interaction of ERα with target gene promoters can occur either directly, through specific estrogen response elements (ERE), or indirectly through contacts

with other DNA-bound transcription factors such as activation protein 1, specificity protein (SP) 1, or nuclear factor κ-light-chain-enhancer of activated B cells. Once tethered to DNA, the receptor can either positively or negatively regulate target gene transcription (30). ERα regulates many genes that are involved in mammary gland development, and their altered expression is associated with breast cancer progression (31). Initially, the single gene approach has identified few target genes for ERα. The egg-white proteins in chicken oviduct and X*enopus laevis* vitellogenin gene are among the first ERα target genes to be identified (32, 33). Later, pS2/trefoil factor 1 (TFF1), c-MYC, and cyclin D1 were identified as E2-responsive genes in breast cancer cells (34–36). The functions of pS2/TFF1 in breast cancer are not fully understood; however, a few reports show that ectopic expression of pS2/TFF1 in MCF7 cells is associated with increased cell proliferation, anchorage-independent growth, migration, and motility (37). pS2 is selectively expressed in breast cancers and positively correlates with the ERα status in different grades of breast tumors (38–40). In the recent past, many novel ERα target genes have been identified, *e.g.*, *FOXM1*, *Efp*, *PELP1*, *CIZ1*, *GREB1*, *etc.* Several of these gene expressions are associated with breast cancer progression. For instance, FOXM1 mediates mitogenic functions of E2, and its deregulation contributes to antiestrogen resistance (41). Estrogen-responsive finger protein (*Efp*) is an E2-responsive gene (42). Efp possesses a RING finger B-box coiled-coil (RBCC) motif and displays ubiquitin ligase activity. Efp promotes breast cancer cell growth by targeting 14-3-3δ, a p53 target gene that inhibits cell cycle progression, for proteasomal degradation (43). Efp expression is positively associated with lymph node status and ERα status, while negatively correlated with 14-3-3σ (44). Proline, glutamic acid, and leucine-rich protein (*PELP1*), which serves as a coactivator of ERα, is also a genomic target of E2; it is involved in ERα cross talk with the cell cycle machinery and mediates E2-induced breast cancer cell proliferation, and its overexpression confers tamoxifen resistance (45–47). Likewise, CDKN1A-interacting zinc finger protein 1 (*Ciz1*), a coactivator of ERα, is responsive to E2 and confers hypersensitivity to E2 in breast cancer cells upon its overexpression (48). Growth regulation by estrogen in breast cancer 1 (*GREB1*) is another ERα target gene that mediates E2-induced proliferation in breast cancer cells (49). Similarly, metastasis-associated protein 3 (*MTA3*) is activated by E2 and regulates epithelial-mesenchymal transition (EMT) and breast cancer metastasis (50, 51).

With successful completion of the human genome project and introduction of novel technologies, a plethora of novel targets of ERα has been identified. Using chromosomal walking and carboxy terminus of HSP70-binding protein, Brown and colleagues (52) revealed that only a minor fraction of ERα binding sites are located in promoter regions, whereas a vast majority is located at long distances from target genes. Similarly, using the circular chromosome conformation capture method, it has been shown that multiple ERα binding sites interact at classical ERα target genes of pS2/TFF1, GREB1, carbonic anhydrase 12 (CA12), and B-cell lymphoma 2 via looping to regulate transcription (53–55). Fullwood *et al.* (56) mapped the chromatin interaction network bound to ERα in the human genome by utilizing chromatin interaction analysis by paired end tag sequencing and discovered that most high-confidence ERα-binding sites are anchored at gene promoters through long-range chromatin interactions like looping (Fig. 1). Similar three-dimensional chromatin interaction studies in cancer patient samples revealed that the clinical outcome of the breast cancers is decided at the level of chromatin interaction by ERα (57). Furthermore, this study also demonstrates that drug-resistant breast cancers still recruit ERα to the chromatin but with different binding abilities, and such a differential ERα-binding pattern in patients with poor outcome is not due to the selection of a rare subpopulation of cells as previously thought, but is due to the FOXA1-mediated reprogramming of ERα binding. Thus, different clinical outcomes in breast cancer will derive from distinct combinations of cis-regulatory elements regulated by ERα in cancer cells.

B. ERα coregulators in breast cancer

Accumulating evidence shows that ERα target gene expression results from the coordinated actions of ERα and its coregulators, which include both coactivators and corepressors (58–60). Most of these coregulators contain a LXXLL motif (L, leucine; X, any amino acid) that interacts with the ligand binding domain of ERα. These coregulators more often are associated with various enzymatic properties, *e.g.*, acetyltransferase, deacetylases, methyltransferase, phosphokinase, ubiquitin ligase, and ATPases, that regulate chromatin remodeling, thereby directly or indirectly regulating target gene expression (60–62). For instance, coactivator p300, a histone acetyl transferase (HAT), acetylates histones on ERα's target gene chromatin, which facilitates opening of ER target chromatin and recruitment of transcription initiation complex that activates E2-responsive gene transcription (61). SWI/SNF (SWItch/Sucrose Non-Fermentable) complex, an ATP-dependent chromatin remodeling complex, is also known to regulate ERα transcriptional activity (63).

Figure 1.

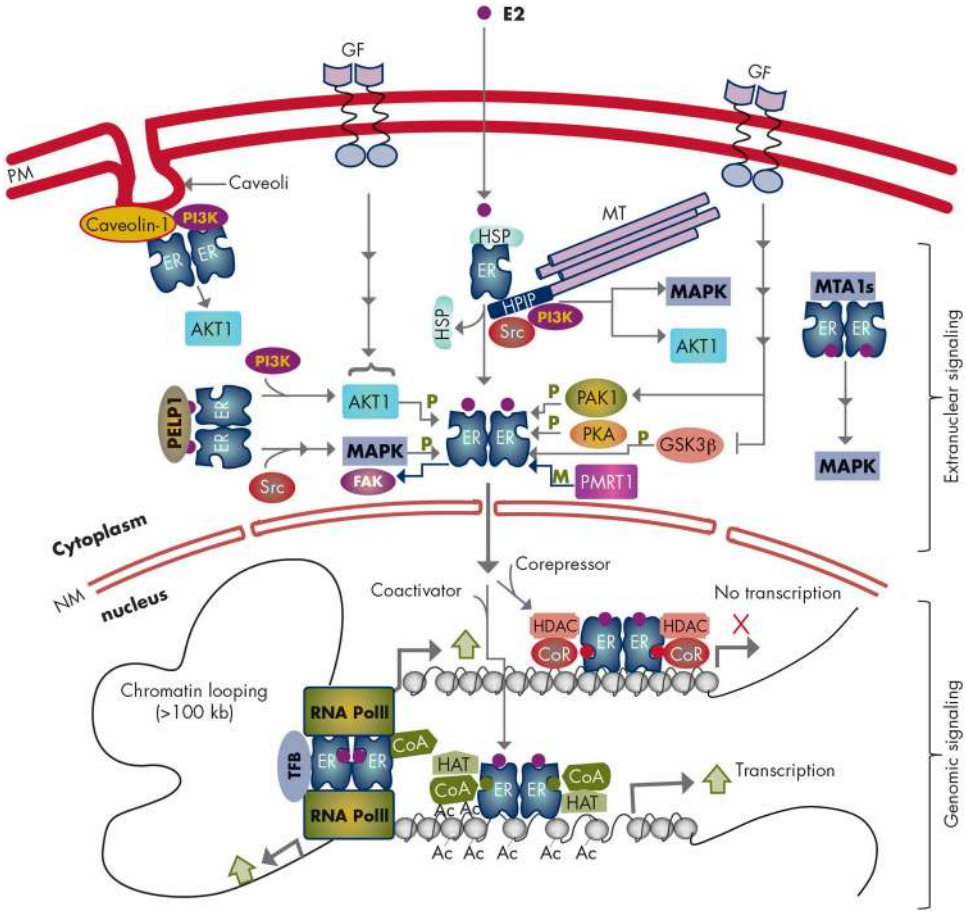

Figure 1. Hypothetical model illustrates the E2-ERα signaling pathway involving both genomic and extranuclear signaling pathways. *Extranuclear signaling of E2/ERα:* Several signaling proteins like PI3K, Src, HPIP, MTA1s, *etc.*, interact with ERα in the cytoplasm to activate E2 extranuclear signaling. Rapid E2 signaling promotes interaction of ERα with caveolin-1 in the caveoli and activates the PI3K/AKT pathway. Sequestration of ERα by HPIP in the cytoplasm through a microtubule scaffolding mechanism facilitates PI3K/Src recruitment, and activation of AKT/MAPK pathways occurs in response to rapid E2 signaling. MTA1s also activate E2 rapid signaling through cytoplasmic sequestration of the receptor. In response to rapid E2/ERα signaling, PELP1 could activate AKT and MAPK pathways by interaction with PI3K and Src kinase, respectively. Activation of downstream signaling kinases such as AKT, MAPK, PAK1, *etc.*, by growth factor signaling led to phosphorylation of ERα, which could further impact its nuclear activity. Phosphorylation of ERα by GSK3 also enhances ERα transcriptional activity. Methylation of ERα at arginine 260 by PRMT1 involves activation of FAK signaling in response to E2 rapid signaling. *Genomic signaling of E2/ERα:* Ligand binding to ERα ensures heat shock protein (HSP) dissociation and the receptor's nuclear entry. Upon nuclear translocation, ligand-bound receptor binds to its target genes to activate the transcription. If the HDAC complex is recruited to ERα chromatin, ERα-dependent transcription is repressed, whereas HAT complex recruitment activates ERα-dependent transcription. This model also illustrates that ERα can regulate the gene expression by extensive chromatin looping to bring genes together for coordinated transcriptional regulation. PM, Plasma membrane; NM, nuclear membrane; P, phosphorylation; M, methylation; GF, growth factor; MT, microtubules; CoA, coactivator; CoR, corepressor; ER, estrogen receptor α; HDAC, histone deacetyl transferase; RNA Pol II, RNA polymerase II; TFB, transcription factor binding proteins; FAK, focal adhesion kinase.

1. Coactivators in breast cancer

There is compelling evidence that deregulation of coregulator expression is associated with tumor progression, cancer cell migration, invasion, metastasis, and drug resistance (64–68). Table 1 summarizes the list of coregulators deregulated in breast cancer. According to ONCOMINE data, 38% of coregulators have shown deregulated expression in various diseases including cancer (98). Likewise, overexpression of coregulators like amplified in breast cancer-1 (AIB1)/SRC3, GRIP1, PELP1, MUC1, breast carcinoma amplified sequence 3 (BCAS3), Ciz1, SRA, *etc.*, has been shown to induce breast carcinogenesis (68, 98). AIB1/SRC3 and BCAS3 are both ERα coactivators known to be amplified, overexpressed, and associated with tamoxifen resistance in breast cancers (69, 70, 99, 100). A recent clinical study using 560 human breast tumor tissues found the AIB1 expression along with expression of genes involved in cell migration and invasion such as polyomavirus enhancer activator 3 and matrix metalloproteinases 2 and 9, suggesting a positive correlation of AIB1 expression with tumor metastasis

TABLE 1. List of deregulated ERα coregulators in human breast cancers

Coregulators deregulated in breast cancer	Ref.
Coactivator	
AIB1/SRC-3	69
BCAS3	70
BRG1	71
CARM1	72
CBP	64
CITED1	73
Cyclin D1	74
DBC1	75
E6-AP	76
GCN5L2	65
MUC1	77
p300	78
PELP1	79
SRA	80
SRC1/GRIP1	81, 82
SRC2/TIF2	83
Corepressor	
ATBF1	84
BRCA1	85
BRCA2	86
MTA1	87
MTA1s	88
MTA2	89
MTA3	51
NCOR1	65
NSD1	90
REA	91
RIP140	92
SAFB1/2	93
Smad4	94, 95
SMRT	96
DACH1	97

(101). The AIB1 coactivator activates ERα-dependent transcription by recruiting HAT such as p300 and P/CAF to ERα target gene chromatin (102). AIB1 interact with ERα in a ligand-dependent fashion, and such interaction and coactivator activity of AIB1 is potentiated by CK1δ and PKCς-mediated phosphorylation of AIB1 in breast cancer cells (103, 104). Because suppression of AIB1 levels leads to ERα stabilization in the presence of E2, a reduced recruitment of ERα to its target gene promoters was also reported (105). AIB1 thus plays a dual role in regulating ERα activity, one in recruiting HAT involved in chromatin remodeling and the other in regulating ERα protein degradation mediated by the ubiquitin-proteasome pathway. BCAS3 is an E2-inducible gene, and its overexpression confers impaired responses to tamoxifen in hormone receptor-positive premenopausal breast cancers (100). BCAS3 associate with a transcriptional complex comprised of ERα, histone H3, and HAT protein P/CAF (p300/CBP-associated factor) to activate ERα target genes. Nevertheless, BCAS3 coactivator functions are dependent on PELP1 protein, another ERα coactivator (70). It seems a transcriptional coactivator complex with PELP1 and P/CAF is recruited by ERα/BCAS3 complex to activate ERα-dependent transcription.

Deleted in breast cancer 1 (DBC1) is a recently identified novel coactivator of ERα (106). DBC1 potentiates ERα transcriptional activity by inhibiting the association of sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide-dependent deacetylase, with ERα and SIRT1-mediated deacetylation of ERα. DBC1 and SIRT1 expressions are also associated with distant metastatic relapse and shorter relapse-free survival in breast cancer patients (75). Ciz1 is a novel coactivator of ERα, known to participate in DNA replication and cell cycle regulation. Ciz1 regulate the activity of ERα by directly promoting the ligand bound receptor to ERα target genes (48). Interaction of CIZ1 with ERα enhances receptor sensitivity to E2 which impacts on breast cancer cell growth. Recently, actinin α 4, a cytoskeletal modulator, has been identified as a novel atypical ERα coactivator that regulates transcription networks to control cell growth. Actinin α 4 interacts with ERα through a functional LXXLL receptor interaction motif present in the coactivator and potentiates ERα gene expression in MCF7 cells (107). The DEAD-box RNA helicases p68 (DDX5) and p72 (DDX17), which are primarily involved in RNA splicing, also act as ERα coactivators in breast cancer cells. Although helicase activity is not required for their coactivator function, they act in synergy with SRC-1, another ERα coactivator (108). p72 interacts with ERα in a ligand-dependent manner in the nucleus. Therefore, p72 is important for ligand-dependent transcriptional activity of ERα and E2-dependent cell growth in breast cancer cells. Furthermore, p72 expression, but not p68 expression, is associated with an increased period of relapse-free and overall survival in ERα-positive primary breast cancers (108). MUC1, a transmembrane glycoprotein normally expressed on the apical borders of secretory mammary epithelia, is also a potent coactivator of ERα. A positive correlation between MUC1 and ERα levels in breast tumors is also established (77). MUC1 regulates ERα activity by directly binding to the DNA binding domain of ERα and stabilizes ERα by blocking its ubiquitination and degradation in breast cancer cells (109).

2. Corepressors in breast cancer

In contrast to coactivators, corepressors recruit histone deacetylases (HDAC) to ERα target gene chromatin, which leads to chromatin condensation and inhibition of ERα target gene expression in breast cancer cells (110). The corepressors thus counterbalance the action of coactivators to control the magnitude of E2 responses, leading to inhibition of ERα target gene expression. Therefore,

loss of ERα corepressors promotes breast cancer (111). Many corepressors of ERα have been identified, and their activities associated with breast cancer were characterized (Table 1). For instance, metastasis associated protein 1 (MTA1) containing nucleosome remodeling and histone deacetylation complex (NuRD) suppresses ERα-mediated gene expression, resulting in an invasive breast cancer phenotype (112). Because the NuRD complex possesses HDAC activity, the MTA1-NuRD complex brings chromatin condensation by deacetylating ERα target chromatin, which leads to RNA polymerase II dissociation from target gene chromatin and loss of transcription. The tamoxifen-ERα complex has been shown to recruit the MTA1/NuRD chromatin-remodeling complex onto ERα target genes (96). MTA1 overexpression is associated with highly aggressive breast cancer types with poor survival rate (114). Similarly, repressor of ER activity (REA) plays an essential role in mammary gland morphogenesis and functional activities (115). REA suppresses ERα transcription activity by recruiting HDAC1 onto ERα target genes (116). Clinical evidence shows positive correlation between REA expression and ERα levels in 40 human breast tumor biopsies used for the study (91).

Nuclear receptor corepressor 1 (NCOR1) is another well-defined corepressor of ERα. It inhibits ERα transcriptional activity by binding to the ligand binding domain of ERα through a I/LXXI/VI motif (I, isoleucine; V, valine; X, any amino acid), also known as CoRNR (corepressor of nuclear receptor) box, which is similar to the NR box such as the LXXLL motif found in ERα coactivators (117). Low expression of NCOR1 is associated with significantly shorter relapse-free survival in breast cancer patients, implying that loss of NCOR1 enhances breast cancer development (65). Further decreased NCOR1 protein expression is correlated with acquired tamoxifen resistance in a mouse model of breast cancer (118). Both scaffold attachment factor B (SAFB) 1 and SAFB2 suppress ERα target gene expression in breast cancer cells by associating with NCOR1 (119). Similarly, low expression of scaffold attachment factors such as SAFB1 and SAFB2 is associated with poor overall survival in patients who did not receive adjuvant therapy (93). Dachshund homolog 1 (DACH1), a cell fate decision factor, is a novel corepressor of ERα (97). DACH1 represses ERα signaling by blocking of coactivator-receptor interactions, *i.e.*, PELP1-ERα interactions, which results in increasing the relative abundance of HDAC1 on ERα target genes to suppress the ER transcription. Expressions of ERα and DACH1 are also reported to be inversely correlated in human breast cancers (97). Depletion of endogenous prohibitin (PHB), a tumor suppressor, is shown to enhance the expression of ERα target genes in MCF7 breast cancer cells. Mice that are heterozygous for PHB null allele exhibit a hyperproliferative mammary gland phenotype, indicating that PHB absence causes breast cancer (120). From the above examples, it is clearly evident that coregulators, both coactivators and corepressors, modulate ERα transcriptional activity, and their expression is associated with breast cancer progression. Therefore, assessment of ERα coregulator status and activity is crucial to determine the role of ERα in breast cancer progression and to predict prognosis and response to therapy.

C. E2 signaling, BRCA, and breast cancer risk

Gain-of-function mutations in oncogenes or loss-of-function mutations in tumor suppressor genes are known to cause cancers. Both *BRCA1* (breast cancer 1) and *BRCA2* are tumor suppressor genes, and loss-of-function mutations in these two proteins are predisposed to breast cancer development because they are key components of the genome maintenance network (121). Several studies demonstrated that BRCA1 expression is absent or decreased in approximately 40% of sporadic breast cancers (122–124). The physiological link between BRCA1 and E2 was established through mouse models. *Brca1* knockout mice confer hypersensitivity to E2, and accelerated development of mammary hyperplasias, preneoplastic mammary lesions, and adenocarcinomas was observed (125). It is clearly established that *BRCA1* mutations and E2 use are risk factors for the development of breast cancer (126). Most sporadic breast cancers show reduced or absent BRCA1 expression due to promoter methylation, loss of one *BRCA1* allele, *etc.* (123, 124), but the majority of *BRCA1* mutant cancers are ERα- and progesterone receptor (PR)-negative (129). Nevertheless, several lines of evidence support the pivotal role played by steroid hormones and their receptors in the development of *BRCA1*-mutant cancers. For instance, *BRCA1* mutation carriers exhibited substantial reduction (about 50%) in breast cancer risk (130), and removal of ovaries reduced the incidence of mammary cancer in mice with a mammary-targeted deletion of full-length *Brca1* gene (131). Pregnancy appears to increase the risk of breast cancer in *BRCA1* carriers due to high circulating levels of E2 and progesterone, implying that steroid hormones may confer increased breast cancer risk in *BRCA1* carriers (132).

Interestingly, both *BRCA1* and *BRCA2* are E2-responsive genes, and BRCA1 in turn regulates ERα activity through posttranslational mechanisms (133, 134). For instance, BRCA1/BARD1 complex monoubiquitinate ERα in MCF7 cells, and thus, ubiquitinated ERα becomes transcriptionally inactive (134, 135). Because p300 acetylates ERα and BRCA1 inhibits p300 expression, ERα acetylation mutant is resistant to BRCA1-mediated repression

of ERα activity (134, 136, 137). E2-ERα influences BRCA1/2 expression, and BRCA inhibits ERα activity through a monoubiquitination mechanism, implying the existence of a negative feedback mechanism that regulates functional interaction between ERα and BRCA in breast cancer cells. Loss of BRCA1 expression also led to tamoxifen resistance. This is due to increased coactivator and decreased corepressor recruitment onto ERα-regulated gene promoters under BRCA1 silencing (138). In addition to its direct ubiquitin ligase activity on ERα, BRCA1 also regulates ERα gene, *i.e.*, ESR1 expression, in association with transcription factor Oct-1 (139). This mechanism may explain why most sporadic tumors express wild-type BRCA1 and are ERα-positive. Based on these considerations, a model has been proposed for BRCA1-mutant breast cancer formation (140). According to this model, ER/PR-positive mammary epithelial cells deficient for *BRCA1* are hypersensitive to endogenous E2 and progesterone and secrete growth factors that stimulate proliferation of nearby ER/PR-negative mammary epithelial cells. Thereby continual hormonal stimulation results in ER/PR-negative hyperplasia. In BRCA1 deficiency, these lesions eventually become autonomous and progress to invasive cancer.

D. E2 signaling on cell cycle machinery and breast cancer development

In general, loss of control over cell cycle progression results in cancer development. The cyclin proteins play a major role in G_1 to S phase transition during cell cycle progression and are critical components of endocrine and paracrine factor-induced mitogenesis in breast epithelial cells (141, 142). Of different cyclins, cyclin D1 is a target of E2 signaling (36). Mammary epithelial cell-specific overexpression of cyclin D1 leads to mammary carcinoma, whereas in cyclin D1-deficient mice, mammary gland development is arrested before lobuloalveolar development, implicating the significance of cyclin D1 in mammary gland (143). Cyclin D1 is encoded by *CCDN1* gene, which is located in chromosome 11q13—a region of the genome commonly amplified in a range of human carcinomas, including about 15% of breast cancers (74, 144).

Although cyclin D1 promoter lacks either ERE or ERE-like elements, E2-ERα regulates cyclin D1 expression by recruiting various transcription factors involving ATF-2 and c-Jun (36, 145). A recent report showed that hexamethylene bisacetamide inducible protein 1 inhibits ERα-mediated expression of cyclin D1 in mammary cells by curbing the recruitment of the transcription factor complex comprised of ERα, positive transcription elongation factor b, and serine 2-phosphorylated RNA polymerase II onto *CCDN1* promoter, implying that hexamethylene bisacetamide inducible protein 1 is a critical regulator of E2-induced cyclin D1 expression in breast cancer cells (146). Because cyclin D1 regulates cyclin-dependent kinase (CDK) 4 activity and retinoblastoma protein functionality, which decides the transcriptional activity of E2F transcription and S phase progression, it is expected that up-regulation of cyclin D1 gene expression in response to E2 promotes G_1 to S transition by activating CDK4 through cyclin D1 induction (147). Therefore, the treatment of breast cancer cells with antiestrogens is associated with an acute decline in cyclin D1 mRNA and protein expression accompanied by a decline in cyclin D1-CDK4 activity and decreased phosphorylation of retinoblastoma (148–151). Cyclin D1 can also interact with ERα in a CDK-independent manner through the cAMP/protein kinase A (PKA)-mediated pathway (152). Overexpression of cyclin D1 protein and mRNA correlates strongly with ERα synthesis in tumor tissues and relates inversely to the level of cyclin E1 (153). Consistent with this possibility, one small clinical study suggested that the duration of response to tamoxifen was significantly longer in ERα patients with low cyclin D1 than those with high cyclin E1 (154). E2 regulates cell cycle progression not only through the cyclin D1/CDK4 pathway but also by regulating CDK2 activity, another cell cycle regulatory protein, by repressing $p27^{KIP1}$, an inhibitor of CDK2, in MCF7 cells. This results in increased activity of cyclin A/CDK2 in the late G_1 phase of the cell cycle (155). In a recent clinical study, evaluation of $p27^{KIP1}$ in 328 breast cancers from premenopausal patients revealed that down-regulation of $p27^{KIP1}$ is associated with high proliferation and tamoxifen resistance (156). These facts suggest that E2/ERα-regulated cyclins can be considered key targets for developing ER-positive breast cancer therapies.

IV. E2 Extranuclear Signaling in Breast Cancer

In the last decade, extensive research on E2 signaling made a few interesting discoveries that could explain some novel pathophysiological anomalies associated with breast cancer. Although the majority of the ER is localized in the nucleus, several biochemical and microscopic analyses have suggested the existence of different pools of ERα in the cellular environment, including the plasma membrane, the mitochondria, and the endoplasmic reticulum (157). The cytoplasmic pool of ERα results in rapid actions of E2 via signal transduction pathways (157, 158) (Fig. 1). Palmitoylation at cysteine 447 localizes ERα to the plasma membrane and is responsible for the ligand-induced activation of MAPK and phosphatidylinositol 3 kinase (PI3K)/serine/threonine protein kinase (AKT) pathways in breast cancer cells

(159). Another mechanism proposed is that protein arginine N-methyltransferase 1 (PRMT1) methylates ERα at arginine 260 in the DNA-binding domain of the receptor mediating the extranuclear function of the receptor, which would then interact with Src/focal adhesion kinase and p85 and propagate the signal to downstream transduction cascades (160). It provides compelling evidence to support the existence of a functional extranuclear signaling pathway for E2 in breast cancer cells.

In breast cancer cells, rapid E2 actions stimulate various growth factor receptors such as IGF-I receptor and epidermal growth factor receptor (EGFR) and activation of effector molecules such as Src and PI3K through adaptor protein, SHC-transforming protein 1 and AKT, and MAPK (46, 161). The cross talk between E2 and growth factor signaling suggests that adaptor proteins play a key role in the extranuclear actions of ERα. For instance, the mammalian target of rapamycin/S6 kinase 1 has been found crucial for IGF-I receptor and ERα cross talk (162). The 40 S ribosomal S6 kinase 1 (S6K1) phosphorylates ERα at serine 167, and so inhibition of S6K1 kinase activity abrogates IGF-I-stimulated S6K1/ERα association and ERα target gene transcription (163). This leads to the suppression of IGF-induced colony formation and breast cancer cell proliferation. S6K1 overexpression is associated with poor prognosis of ER-positive breast cancers, implying that the cross talk between ERα and the IGF-I/S6K signaling pathway is crucial for development of breast cancers (163).

Extranuclear actions have a profound impact on breast cancer cell proliferation, migration, drug resistance, and apoptosis blockade (164, 165). Rapid E2 actions lead to the activation of MAPK through kinase (166). This study has shown that MAPK blockers inhibit breast cancer cell proliferation and tumor growth, indicating that the rapid E2-activated ERα/Src/MAPK pathway is functional in breast cancer cells. Similarly, integrin-linked kinase also participates in extranuclear signaling of E2 through the PI3K pathway and regulates breast cancer cell migration (167). PI3K inhibitors such as LY294002 also blocked PI3K/integrin-linked kinase/ERα-mediated breast cancer cell migration (167). Another recent finding shows that ERα regulates deacetylation of tubulins in association with HDAC6 through the E2 extranuclear signaling pathway and promotes breast cancer cell migration (168). In another report tamoxifen is shown to induce tubulin deacetylation implying that extranuclear signaling through tubulin deacetylation conferring endocrine resistance in breast cancer cells. In addition, Fernando and Wimalasena (169) have shown that E2 induces Bcl-2-associated death promoter phosphorylation through both the Ras/PI3K/AKT and the Ras/ERK/p90RSK1 pathways, suggesting that functional activation of the PI3K/AKT pathway may be required for E2 to block apoptosis induced by TNF, hydrogen peroxide, and serum withdrawal. This model suggests the antiapoptotic activity of E2 extranuclear rapid action to support the survival of the breast cancer cell.

Emerging evidence suggests that various genomic coregulators of ERα can also act as extranuclear coregulators and can integrate genomic and extranuclear signaling pathways (170). ERα coregulators such as PELP1, MTA1 short form (MTA1s), hematopoietic PBX-interacting protein 1 (HPIP), and p130Cas are known to influence both functions of ERα. PELP1 was originally identified as Src homolog 2 domain-interacting proteins (45, 171). PELP1 contains 10 LXXLL motifs that participate in interaction with nuclear receptors and three proline-rich motifs that could participate in interaction with SH3 domain-containing proteins. PELP1 can act as an extranuclear adaptor protein between ERα and Src, thereby allowing E2-dependent activation of Src and the downstream ERK/MAPK signaling cascade (46). Interestingly, this pathway confers tamoxifen resistance in breast cancer cells through the activation of both PI3K/AKT and Src/MAPK pathway (46). In fact, PELP1-trangenic mice, which express cytoplasmic PELP1 in mammary gland-formed tumors, displayed tamoxifen resistance, suggesting that extranuclear actions are responsible for such drug resistance (172). PELP1 has also been implicated in aromatase regulation in breast cancer cells by involving short extranuclear autocrine loop between E2 and aromatase expression (173). This supports that extranuclear signaling of E2 indeed regulates aromatase activity (174).

Another protein that is known to integrate extranuclear signaling of ERα into genomic signaling is HPIP. HPIP, also known as pre-B-cell leukemia homeobox interaction protein (PBXIP1), is a microtubule-binding protein that interacts with ERα (175). HPIP binds to ERα through the LXXLL motif located on its C-terminus part of the protein. HPIP localizes predominantly to cytoplasm and interacts with cell survival signaling proteins such as PI3K and Src. E2 stimulates the formation of a signalosome consisting of ERα, Src, PI3K, and HPIP on the microtubules network to activate AKT/MAPK pathways in breast cancer cells (175). Treatment of breast cancer cells with nocodazole (a microtubule-depolymerizing agent) or HPIP silencing by HPIP-specific small interfering RNA enhanced ERE-dependent transcription, whereas paclitaxel (a microtubule-polymerizing agent) suppressed ERE-dependent transcription (ERE-Luciferase assay), suggesting the sequestration of the steroid receptor through the HPIP-microtubule network. A contradictory finding showed that E2-ERα/HPIP-activated Src/PI3K pathways can also integrate into genomic func-

tions of ERα by enhancing the receptor phosphorylation at serine 167 (176). This discrepancy could be due to different functional assays used by two groups. HPIP is also highly overexpressed in infiltrative ductal carcinoma of breast and confers taxol resistance to breast cancer cells (B. Manavathi, personal communication).

MTA1s is a frameshift-derived shorter form of MTA1 protein. The LXXLL motif, located on the C terminus of the protein, participates in interaction with ERα, and this interaction is enhanced in response to E2. Lack of nuclear localization signal ensures MTA1s cytoplasmic localization and sequestration of ERα in the cytoplasm that enhances extracellular functions of the receptor in the target cells while impairing genomic functions (88). In this context, E2 activates casein kinase I-γ2 transcription, which in turn phosphorylates MTA1s in breast cancer cells, thus enhancing the MTA1s ability to restrict ERα to the cytoplasm (177). The MTA1s expression is associated with human breast tumors with no (or low) nuclear ERα. Another protein that regulates both genomic and extranuclear activities of ERα is p130Cas. The p130Cas (Crk-associated substrate) is an adaptor protein and is a prime substrate of the Src kinase. Being an adaptor, p130Cas links the actin cytoskeleton signaling to the extracellular matrix during cell migration and cell invasion (178). p130Cas interacts with ERα in the cytoplasm, which leads to hyperstimulation of the Src/MAPK pathway and cyclin D1 induction in breast cancer cells (179). Thus, p130Cas-mediated E2 extranuclear signaling regulates E2-dependent cell cycle progression by modulating cyclin D1 expression.

E2 rapid signaling also participates in DNA damage response. In general, if damaged DNA is not repaired, genomic integrity can be compromised, and unrestrained proliferation of aberrant cells may occur. Inhibition of normal DNA repair signaling may simulate genetic loss of DNA damage response signaling molecules such as ataxia telangiectasia mutated (ATM), ataxia telangiectasia and rad3-related protein (ATR), DNA-dependent protein kinase (DNAPK), BRCA1 and -2, p53, and Chk2 and predispose normal cells to acquire transforming mutations (180). A recent report showed that in ER-positive breast cancer cells, DNA damaging agents including UV, ionizing radiation, and hydroxyurea rapidly activate ATR-dependent phosphorylation of endogenous p53 and Chk1 (181). Interestingly, this pathway involves extranuclear actions of E2 via plasma membrane-localized ERα and activation of PI3K and AKT signaling pathway. E2 delays DNA repair and increases chromosomal damage by regulating ATR and Chk1 activation in breast cancer epithelial cells. Ligand bound ERα regulates ATR activity by potentiating AKT-mediated phosphorylation of DNA topoisomerase 2-binding protein 1 at serine 1159, which prevents binding of topoisomerase 2-binding protein 1 with ATR after DNA damage. Since the association of Chk1 with Claspin is important for Chk1 activity, E2-ERα regulates Chk1 activity via AKT-mediated phosphorylation of Chk1 which prevents its association with Claspin and signaling to the G_2/M checkpoint (181). Because ATM protein expression is found to be aberrantly reduced more frequently among BRCA1- and BRCA2-expressing tumors than in non-BRCA1 and -2 tumors, reduced ATM expression was found more often in ERα- and PR-negative breast cancer, indicating loss-of-function interaction among these molecules (182). This explains how E2 signaling can also affect DNA repair systems to delay the repair mechanism to support breast cancer cell growth.

V. ERα Posttranslational Modification and Its Impact on Breast Cancer Progression

Posttranslational modifications such as phosphorylation, methylation, ubiquitination, sumoylation, also regulate ERα activity and is shown to have potential implications in breast cancer development and drug resistance (183) (Fig. 2). Several kinases including MAPK, AKT, glycogen synthase kinase 3β (GSK3β), serine/threonine p21-activated kinase (PAK1), and PKA are known to phosphorylate ERα at distinct sites located in particular at the N-terminal region (183). For example, MAPK has been shown to phosphorylate ERα at serine 118, located in the activation function-1 domain of the receptor (184, 185). Phosphorylation at serine 118 directs gene-specific recruitment of ERα and its coregulators on ERα target promoters (186). The presence of ERα phosphorylation at serine 118 in human breast tumors further implies a clinical relevance to this modification with the disease (187). Likewise, growth factor-activated signaling kinases, such as PAK1 or PKA, phosphorylate ERα at serine 305 located in the transactivation function-2 domain and promote transactivation functions in the absence of ligand (188, 189). Transgenic mice expressing ERα serine 305E mutant gene in the mammary gland exhibit mammary hyperplasia (188). Furthermore, it has been shown that this phosphorylation is sufficient to activate the cyclin D1 in breast cancer cells (190). The serine 305 site in ERα has also been implicated in modifying the action of tamoxifen in breast cancer cells by regulating ERα phosphorylation at serine 118 (191). ERα serine 305 phosphorylation levels in advanced breast cancers indeed associate with sensitivity to tamoxifen in breast tumors (192). A recent report shows that prolactin also contributes to ligand-independent activation of ERα through activation of receptor phosphor-

Figure 2.

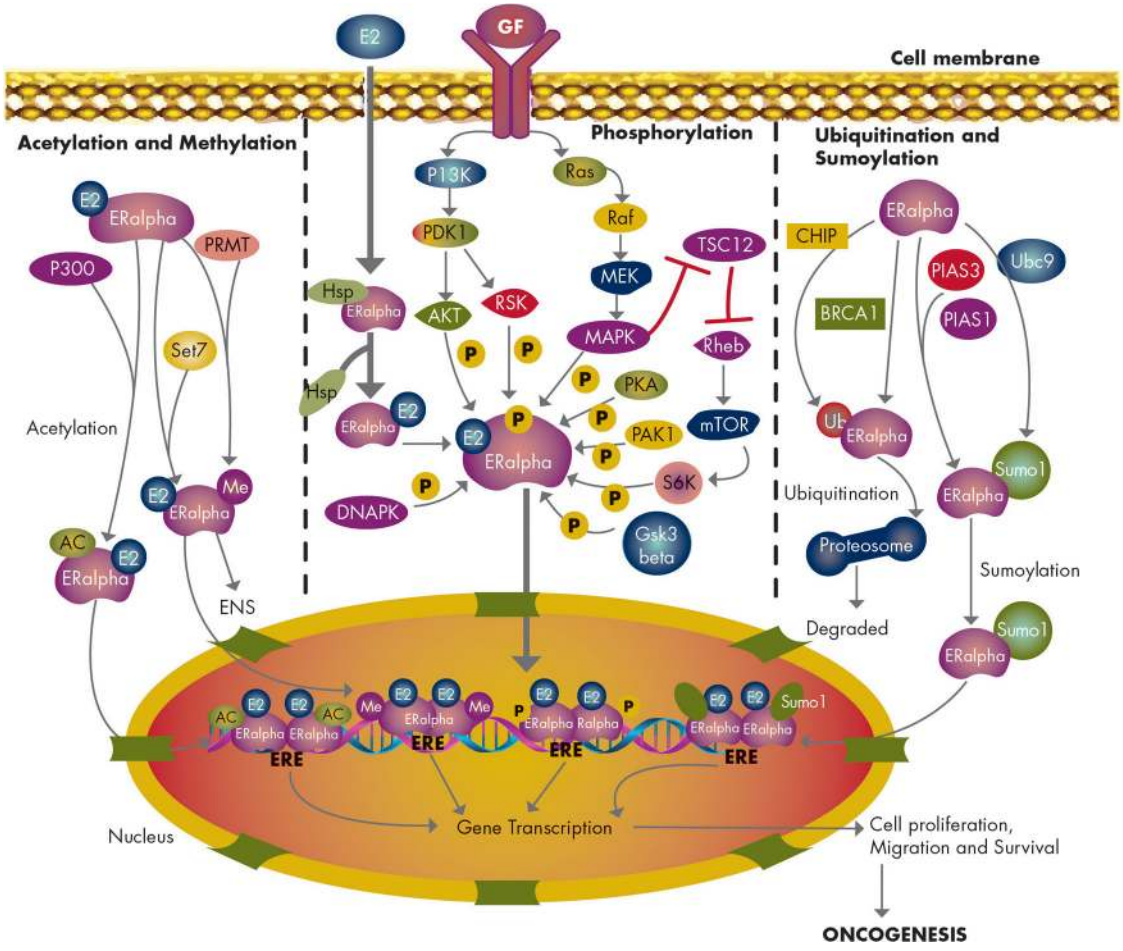

Figure 2. Posttranslational modifications of ERα. Most of the known growth factor responsive kinases like AKT, RSK, MAPK, PKA, PAK1, and S6K phosphorylate ERα at specific serine or threonine residues and are known to activate its transcriptional activity. Phosphorylation of ERα by DNAPK and GSK3β inhibits its proteasomal degradation and thereby enhances its nuclear activity. Acetylation of ERα at lysines 266, 268, 302, and 303 by p300 enhances transcriptional activity of ERα. Methylation of ERα at arginine 260 by PRMT1 promotes E2 extranuclear signaling (ENS), whereas ERα methylation at lysine 302 by SET7 methyltransferase led to transcriptional activation of ERα. PIAS1 and PIAS3, in association with Ubc9, sumoylate ERα and promote its transcriptional activity. CHIP regulates polyubiquitination of ERα, which leads to proteasomal degradation of the receptor, whereas BRCA1 monoubiquitinates ERα and reduces its transcriptional activity. RSK, Ribosome S6 kinase; Ubc9, SUMO-conjugating enzyme; CHIP, carboxy terminus of HSP70-binding protein.

ylation at serine 118 (193). AKT1, another serine/threonine kinase that acts downstream to growth factor signaling, also phosphorylates ERα at serine 167 through growth factor signaling activation (194). Clinical studies using patients treated with tamoxifen showed a positive relationship among AKT activation, ERα phosphorylation at serine 167, and tamoxifen resistance (194–196). These studies provided additional evidence for the role of PI3K/AKT-mediated tamoxifen resistance in breast cancers. Interestingly, a recent clinical study found an improved survival rate in ER-positive breast cancer patients who showed low phosphorylation of ERα serine 118 and high phosphorylation of ERα serine 167 (197). This suggested that we can distinguish the patients who are likely to benefit from endocrine therapy alone from those who are not. GSK3β, also a serine/threonine kinase that opposes AKT1 functions, has been shown to phosphorylate ERα at serines 102, 103, 106, and 118 and to stabilize ERα from proteasome degradation, thereby enhancing ERα transcriptional activity (198). Similarly, DNAPK, a DNA repair enzyme, also phosphorylates ERα but only at serine 118. Phosphorylation results in stabilization of ERα because inhibition of DNAPK resulted in its proteasomal degradation (199). In addition to the above well-defined sites, a recent proteomic approach identified a few new phosphorylation sites in ERα, which include serine at 212, 294, 554, and 559 (200). However, the functional consequences of these residues on ERα activity are not known

yet. The ERα phosphorylation at ligand binding domain (AF2) Tyr 537 by Src and MAPK also promotes cell proliferation and hormone-independent activation of ERα (201–203). Phosphorylation of ERα at Thr 311 (located in ligand binding domain) by p38 protein kinase and MAP kinase kinase kinase, regulates nuclear export of ERα, and also inhibits the interaction with of the receptor with p160 (204). However, the relevance of these phosphorylation sites in ERα with breast cancer is yet to be analyzed. A detailed list of ERα modifications and enzymes involved in the modification is reviewed in Ref. 183.

ERα also subjected to acetylation modification and its functional significance has been well documented (205). It has been shown that ERα is directly acetylated by the coactivator p300 at the well-conserved lysine residues at 266, 268, 302, and 303 (137, 206). Particularly, acetylation of lysine at 302 and 303, which are located in the hinge region of ERα appears to play a key role in enhancing ligand sensitivity and subsequently ERα's transcriptional activity (206). Interestingly, clinical studies determined that the lysine residue is in a mutation-susceptible site in breast cancers. In 34% of atypical breast hyperplasia samples, a Lys-to-Arg substitution was found at residue 303 (K303R) of the ERα (208). This mutant also confers hypersensitivity to E2 and induces resistance to an aromatase inhibitor via the PI3K/AKT kinase pathway (209). Because acetylation is a reversible process, it indicates the existence of an ERα deacetylase in cells. SIRT1, along with nicotinamide adenine dinucleotide, is shown to deacetylate ERα and inhibit E2-dependent cell proliferation (210). Interestingly, lysine 302 of ERα is also a site for monoubiquitination by BRCA1 (135). Down-regulation of BRCA1 activates ERα because of the absence of monoubiquitination, whereas overexpression of BRCA1 down-regulates ERα activity, indicating that ERα activity is controlled by the relative degree of acetylation *vs.* ubiquitination of ERα at 302 (134, 211). Monoubiquitination at K302 and K303 is associated with reduced ERα transcriptional activity, phosphorylation at serine 118, AKT activation, and ERα-induced cell proliferation (212). Furthermore, lysine 266 and 268 of ERα are also sites for sumoylation. Sumoylation at these sites appears to enhance ERα transcriptional activity in breast cancer cells (213, 214). In addition to lysine 266 and 268, lysines 299, 302, and 303 of ERα also seem to be sumoylated; therefore, mutation of these sites further reduces transcriptional activity of ERα (213). ERα also undergoes methylation at arginine 260 by PRMT1 in the cytoplasm (215). In addition, methylation at lysine 302 by SET7 methyltransferase was reported to stabilize ERα and efficient recruitment of the ERα to its target genes (216). ERα is methylated in normal epithelial breast cells and is hypermethylated in a subset of breast cancers, indicating that ERα methylation could serve as a prognostic marker to a subset of breast cancers (215). Altogether, these observations indicate that ERα posttranslational modifications regulate ERα activity in breast cancer cells, implying that their deregulation is also responsible for breast carcinogenesis.

VI. Cross Talk between miRNA and E2 Signaling in Breast Cancer

miRNA are a class of endogenous short noncoding RNA of 22–24 nucleotides in length and capable of regulating the expression of protein-coding genes at the posttranscriptional level by cleaving target mRNA and/or repressing their translation (217). More than 50% of human miRNA genes are located at chromosomal regions with high frequencies of amplification or deletion that are genetically altered in cancers (218). Emerging studies show that miRNA function as oncogenes or tumor suppressors to modulate multiple oncogenic cellular processes, including cell proliferation, apoptosis, invasion, and migration by targeting various important cell regulators including p53, Her2, Myc, *etc.* (219–221). It has also been shown that miRNA, *e.g.*, miR-101a, miR-126–3p, miR-212, and miR-132 regulate mammary gland development, indicating the importance of these miRNA in normal physiology (222). Because ERα is one of the major culprits for endocrine-related breast cancer development, it naturally creates a curiosity to look for miRNA that target ERα.

A. E2 signaling on miRNA expression

In the past few years, several genome-wide profiling studies have been made to characterize E2-dependent miRNA in breast cancer cell lines and biopsies (223–226). In a recent study to understand the effect of E2 on miRNA expression in both MCF7 and ZR75 cells, 172 miRNA were identified to be up- or down-regulated by ERα, of which 52 are similarly regulated in both of the cell models (225). The most consistently deregulated miRNA on E2 treatment are miR-206, miR-125a/b, miR-17–5p, miR-34a; some members of the let-7 family that act as tumor suppressor genes; and miR-21, miR-155, and miR-10b, which are usually overexpressed in breast cancer and may act as oncogenes (225). The miR-21 expression is found to be higher in ER-positive breast cancer tumors than negative. E2 induced down-regulation of miR-21 in MCF7 cells and concomitantly induced overexpression of miR-21 target genes, bcl2 and PTEN (227). On the contrary, Bhat-Nakshatri *et al.* (228) have reported an increase in miR-21 expression on E2-mediated induction of

MCF7 cells. This discrepancy can be explained by the biphasic regulation of E2, *i.e.*, induction followed by repression of miR-21. A recent study has demonstrated that re-expression of miR-21 results in migration and invasion by activating the EMT process and enhancing the characteristics of cancer stem cells in MCF7 cells (229). Maillot *et al.* (230) identified a set of 23 miRNA (including miR-181a, miR-21, miR-181b, miR-26a, miR-200c, miR-26b, miR-27b, miR-23b) to be down-regulated by E2 in various ER-containing human cell lines. Several pri-miRNA of these miRNA, particularly pri-miRNA-21 and pri-miRNA-181a~b-1, are primary targets of ERα transcriptional repression. miR-26a and miR-181a oppose the E2-dependent increase in cell proliferation through a global deregulation of genes (*e.g.*, PR) involved in the control of cell growth (230). Another study reported that E2 significantly induced B-cell lymphoma 2, cyclin D1, and survivin expression by suppressing the levels of miR-16, miR-143, and miR-203 in MCF7 cells, and these miRNA are highly expressed in triple positive breast cancers (231). In a genome-wide microarray approach, the miRNA that were up-regulated by E2 were identified as members encoded by the paralogous transcripts, pri-mir-17-92 and pri-mir-106a-363a. c-MYC, an ERα transcriptional target, seems to regulate miR-17-92 expression by directly binding to its promoter in response to E2 treatment. However, the miRNA (miR-18a, miR-19b, and miR-20b) derived from these pri-miRNA are again involved in an inhibitory loop and down-regulate ERα (232). E2 also down-regulates the promoter activity of miR-34b gene through the interaction between ERα and p53 (233). From these studies, one can derive the possible mechanisms by which E2 regulates miRNA expression by directly binding to the regulatory sites of miRNA, by inducing mRNA-encoding genes that harbor miRNA genes in their introns, by regulating transcription factors that in turn regulate miRNA expression, or by regulating miRNA processing machinery, *e.g.*, E2 can induce Dicer (228) and Ago2 (234). In another instance, the human vascular endothelial growth factor transcript bears several target sequences for E2-regulated miRNA like miR-16 in its 3′ untranslated region (UTR). In response to E2 treatment, half-lives of the human vascular endothelial growth factor transcripts were stabilized (235). The activated ERα attenuates the processing of primary miRNA into pre-miRNA through E2-dependent association with the Drosha complex, resulting in stabilization of the transcript of an ERα target gene through its 3′ UTR. The miRNA that are known to be modulated by E2 are listed in Fig. 3A. In addition to the miRNA listed above, many more miRNA that are yet to be discovered may be targets for E2.

Figure 3.

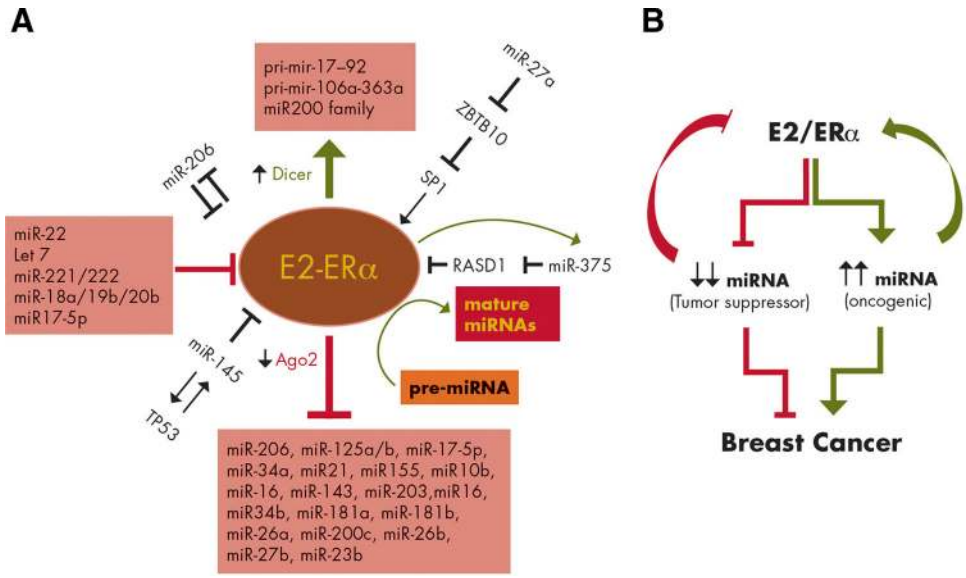

Figure 3. Reciprocal regulation of E2/ERα signaling and miRNA. A, Figure represents the miRNA that are targets of E2-ERα signaling and vice versa in breast cancer cells. E2-ERα signaling also modulates enzymes involved in miRNA processing such as Dicer and Ago2. ERα not only regulates miRNA expression but also controls miRNA maturation. miRNA375, a target of E2-ERα signaling, also regulates ERα levels through a positive feedback mechanism by repressing ERα's inhibitor, RASD1. miR-206 and ERα mutually repress each other's expression. miR-145 and TP53, which depend on each other for their activation, repress ERα levels. B, Hypothetical model illustrates that E2 may activate oncogenic miRNA while affecting the expression of miRNA that show tumor suppressor activity to ensure breast cancer development.

B. miRNA that target ERα in breast cancer cells

The ERα mRNA has putative binding sites for several miRNA. These miRNA act by reducing ERα mRNA stability or translation. The miRNA that target ERα are shown in Fig. 3A. Ectopic expression of miR-206 into MCF7 cells has been shown to reduce ERα levels and also the basal expression levels of ERα target genes such as PR, cyclin D1, and pS2, resulting in decreased cell proliferation (236). miR-206 expression was found to be low in ERα-positive human breast cancer tumors and MCF7 cells, whereas there were increased levels in ER-negative MB-MDA-231 cells. This indicates the presence of a negative feedback loop between miR-206 and ERα (236, 237). Recently, a cell-based screen using a cotransfection assay with luciferase reporter plasmid carrying a 4.7-kb 3′ UTR of ERα mRNA and a synthetic miRNA expression library has identified miR-22 as a potential ERα-targeting miRNA (238). miR-22 is frequently down-regulated in ERα-positive human breast cancer cell lines, and clinical samples indicating miR-22 could play a pivotal role in the pathogenesis of breast cancer (239). Several reports have shown that the let-7 family of miRNA is down-regulated in breast cancer tissues (240). The let-7 miRNA are down-regulated in breast tumor-initiating cells (BT-IC) and increased during BT-IC differentiation. Restoration of let-7 in BT-IC reduced cell proliferation, mammosphere formation, *in vitro* tumor formation, and metastasis in nonobese diabetic/severe combined immunodeficiency (SCID) mice (241). Introduction of let-7 miRNA in the MCF7 cell line negatively regulated ERα activity, whereas attenuating the ERα signaling by let-7 miRNA inhibited cell proliferation and subsequently triggered apoptosis in MCF7 cells (229). In fact, screening of the entire let-7 family of miRNA by *in situ* hybridization revealed let-7g as a unique member of the let-7 miRNA family, whose nullification can induce metastasis in otherwise nonmetastatic mammary carcinoma cells via preferential targets, Grb2-associated binding protein 2 and fibronectin 1, and consequent activation of p44/42 MAPK and specific matrix metalloproteinases (242). Cochrane *et al.* (243) have reported that Dicer expression is low in ER-negative breast cancers because these cells express high levels of miR-221/222 and miR-29a, which in turn targets and represses Dicer. miRNA-221/222 also targets ERα and confers tamoxifen and fulvestrant resistance in breast cancer cells (244, 245). In another study, mammosphere culture conditions were used to induce EMT in MCF7 cells, an ER-positive breast cancer cell line. This EMT was associated with increased cancer stem cell-like properties and reduced ERα expression, which correlated with suppression of miR-200c, miR-203, and miR-205 and overexpression of miR-222 and miR-221, further suggesting that ERα is the target of miR-221/222 in breast cancer cells (246). Intriguingly, miR-375 is found to be involved in a positive feedback loop with ERα in breast cancer cells by repressing RASD1, a GTPase activator protein, which inhibits ERα (247). In ER-positive MCF7 cells, ERα is also regulated indirectly by miR-27a through suppression of ZBTB10, a SP repressor (249). Because ERα expression is dependent on SP1 transcription factor, miR-27a-mediated suppression of ZBTB10 results in the expression of SP1 and its target gene such as ERα and subsequently establishing hormone responsiveness in breast cancer cells (249). miR-145, another important tumor suppressor miRNA, down-regulates ERα expression and exerts a proapoptotic effect in breast cancer cells in a TP53-dependent manner. TP53 activation in turn stimulates miR-145 expression, thereby Tp53 is involved in a death-promoting loop with miR-145 (248). A novel protein lysate microarray-based study identified miR-18a, miR-18b, miR-193b, miR-206, and miR-302c as ERα repressors. This is further confirmed by the high expression levels of miR-18a and miR-18b in ER-negative as compared with ER-positive clinical tumors (250). Because coregulators modulate ERα functions, miRNA that regulate coregulators also influence ERα functions in breast cancer cells. miR-17-5p, for instance, represses the translation of AIB1 mRNA, whereby it blocks ERα-mediated cell proliferation in MCF7 cells (251). With the above examples, we can understand that E2 may activate oncogenic miRNA, whereas affecting the expression of tumor suppressor miRNA to promote breast cancer development (Fig. 3B). Future research awaits whether these new blossoms (miRNA) in the garden of E2 signaling will serve as potential therapeutic targets for breast cancer treatment.

VII. Deregulated Expression of ERα in Breast Cancer

It is well documented that the activity of ERα is regulated at multiple levels in breast cancer cells. Extensive research over the last two decades established that various chemicals, hormones, hormone receptor modulators, transcription factors, and epigenetic modulators regulate ERα expression in breast cancer cells (reviewed in Refs. 252 and 253). The human ERα gene is located on chromosome 6 and is extensively methylated in ER-negative breast cancer cell lines and tumors, but not in ER-positive breast cancers, implying that methylation suppresses ERα expression in ER-negative cells (254, 255). This is one of the reasons that ER-negative tumors show poor response to tamoxifen treatment (256). Methylation of promoter by DNA methyltransferases hinders the binding of transcrip-

tion factors and RNA polymerases to reduce the target gene transcription. In association with methyltransferases, HDAC repress ERα promoter activity by deacetylating histones H3 and H4, which further ensures the compact nucleosome structure and suppression of ERα transcription. Therefore, treatment of ER-negative breast cancer cells with trichostatin-A, an HDAC inhibitor, induces ERα re-expression (257). Furthermore, a synergistic effect on ERα expression is observed in the treatment of cells with both trichostatin A and 5-aza-2-deoxy cytidine, an antimethylating agent, indicating the inactivation of ERα gene expression by both methylation and deacetylation (258). Several other novel modulators of ERα expression are identified. Table 2 summarizes the effects of various mod-

TABLE 2. Modulators of ERα expression in breast cancer cells

Agent	Mode of action	Cell type	ERα protein levels	Ref.
Chemicals				
Arsenite	Decreased mRNA expression	MCF7	−	259
Artemicinin	Decreased mRNA expression	MCF7	−	260
Cadmium	Decreased mRNA expression	MCF7	−	261
Celastrol	Decreased mRNA expression	MCF7 and T47D	−	262
Polyamines	Decreased mRNA expression	MCF7 and T47D	−	263
Shikonin	Posttranslational regulation	MCF7	−	264
Taxol	Inhibiting mRNA translation	MCF7	−	265
TPA	Posttranscriptional-destabilization of mRNA	MCF7	−	266
Valproic acid	HDAC inhibition	MDAMB 231	+	267
Epigenetic factors				
5-AZAC	Demethylating agent	MCF7	+	258
LBH589	HDAC inhibition	MDAMB 231	+	269
Trichostatin A	HDAC inhibition	MDAMB 231	+	257
Growth factors				
EGF	Growth factor signaling	MCF7	−	271
IGF-I	Insulin/IGF-I signaling	MCF7	−	272
TGFβ2	GSK3β/SNAIL signaling	MCF7 and T47D	−	273
Hormone receptor modulators				
Bazedoxifene	SERM, posttranslational destabilization of protein	MCF7	−	274
EB-1089	Vitamin D agonist	MCF7	−	275
ICI 182 780	Estrogen antagonist	MCF7	−	276
KH-1069	Vitamin D agonist	MCF7	−	275
ORG 2058	Progesterone agonist	T47D	−	277
R5020	Progesterone agonist	T47D and MCF7	−	277
Raloxifene	SERM	MCF7	−	278
Ro 23-7553	Vitamin D agonist	MCF7	−	275
Ro 27-0574	Vitamin D agonist	MCF7	−	275
RU486	Progesterone agonist	T47D	+	277
Tamoxifene	SERM	MCF7	ne	278
Hormones				
$1,25(OH)_2D_3$	Transcriptional regulation	MCF7	−	275
E2	Posttranslational regulation	MCF7	−	279
hCG	Gonadotropin/PKA	MCF7	−	280
Insulin	Insulin/IGF-I	MCF7	+	281
Hypoxia inducers				
Cobalt chloride	Hypoxia/HIF1α	MCF7	−	282
Ligands				
Wnt-5a	Wnt signaling	MCF7 and MDAMB 231	+	283
Pathway blockers				
Celecoxib	PI3K/Akt kinase	MCF7 and ZR75	+	284
Green tea polyphenol-epigallocatechin-3 gallate	PI3K/Akt kinase	MCF7 and ZR75	+	284
Wortmannin	PI3K/Akt kinase	MCF7 and ZR75	+	284
Transcription factors				
FoxM1	ERK 1/2	MCF7 and ZR-75	+	285
FOXO3a	PI3K/Akt kinase	MCF7 and ZR-75	+	284
GATA3	Binding to cis-regulating element	MCF7 and T47D	+	286

FOXO3a, Forkhead box transcription factor 3a; hCG, human chorionic gonadotropin; $1,25(OH)_2D3$, 1,25 dihydroxyvitamin D3; ORG2058, 16α-ethyl-21-hydroxy-19-norpregn-4-ene-3,20-dione; RU486, 17β-hydroxy-11β-(4-dimethylamino-phenyl)-17α-(1-propynyl)-estra-4,9-dien-3-one; R5020, 17,21-dimethyl-19-norpregna-4,9-dien-3,20-dione; EGF, epidermal growth factor; 5-AZAC, 5-azacytidine; FoxM1, forkhead box protein M1; HIF-1α, hypoxia-inducible factor 1α; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; +, increase; −, decrease; ne, no effect.

ulators on ERα expression in breast cancer cells (259–286). The reason for the broad interest in studying the ERα reexpression is mainly because ER-positive breast cancers can be treated with selective ER modulator (SERM) therapies.

VIII. Role of E2 Signaling in Breast Cancer Stem Cells—Beginning of a New Concept

As reviewed in the previous section, the adult mammary gland undergoes massive epithelial tissue remodeling during reproductive cycles. Over recent years, accumulated evidence has shown that mammary epithelium has a hierarchical organization. Using a fluorescence-activated cell sorting-based approach, two groups recently identified a subpopulation of murine mammary cells with $lin^- CD29^{hi}CD24^+$ and $CD49f^{hi}CD29^{hi}CD24^{+/mod}$ to have properties of mammary stem cells (MaSC) that could recapitulate into an entire mammary epithelial tree on transplantation into an epithelium-free mammary fat pad (287–289). However, these MaSC show a receptor-negative phenotype for ERα, PR, and ErbB2 (290). Despite the lack of steroid hormone receptors, ovariectomy of mice significantly reduced MaSC number and tumor-forming potential *in vivo*, whereas MaSC activity increased in mice treated with E2 plus progesterone (291). This indicates an increased risk of breast cancer associated with pregnancy; however, the molecular mechanism of such response still remains unclear and requires further investigation.

Because the lobular epithelium in the mammary gland is also the site for most breast tumors, evidence suggests the existence of a hierarchical organization for breast tumorigenesis similar to that of mammary gland development (292). A small population of tumor cells termed cancer stem cells is able to initiate tumor formation and undergo self-renewal. The most accepted model is that adult stem cells which are slow-dividing and long-lived, with a high proliferative capacity, accumulate multiple mutations and undergo transformation to generate these cancer stem cells (292–295). However, few research groups hold the idea that the cell of origin, the normal cell that acquires the first cancer-promoting mutation, need not necessarily be related to the cancer stem cell (296). In that line, Al-Hajj *et al.* (297) engrafted cells obtained from human breast cancer tumors into nonobese diabetic/SCID mice and found only a few to have the potential to generate new tumors. These were identified as a small population of breast cancer-initiating cells based on their cell surface markers ($CD44^+CD24^{-/low}$) that exclusively retained tumorigenic activity and display stem cell-like properties (297). In addition, Dontu *et al.* (298) reported that cells expressing high aldehyde dehydrogenase (ALDH) have stem/progenitor properties both in normal and neoplastic human breast epithelium, and expression of ALDH1 is correlated with poor prognosis of breast cancer (294, 298–300). Stem cells expressing high ALDH and $CD44^+CD24^{-/low}$ signature showed enhanced malignant and metastatic ability (301). However, the relation between the hormone signaling and the expression of surface markers in mammary cancer stem cells is unknown.

Although the role of E2 signaling in mammary gland development and breast cancer progression is well documented, the role of E2 and ERα status, molecular characteristics, and clinical significance in breast cancer stem cells (BrCSC) are still a matter of debate. Recently, it has been reported that E2 reduces the stem cell population in both normal mammary gland and breast cancer, whereas overexpression of stem cell genes OCT4 (octamer-binding transcription factor 4), SOX2 (sex-determining region Y-box 2), and NANOG reduces ERα expression and increases the number of stem cells and their capacity for invasion, properties that are associated with tumorigenesis and poor prognosis (302). On the contrary, another report reveals that E2 signaling expands the pool of functional BrCSC through a paracrine fibroblast growth factor/fibroblast growth factor receptor/Tbx3 signaling pathway (303). In one investigation, tumor-initiating mammospheres derived from ER-positive breast cancer cell lines show significantly reduced ERα expression and down-regulation of ERα target genes compared with the parent cell line, although ERα mRNA levels were not considerably down-regulated (304). Evidence from a number of investigations supports that $CD44^+$ BrCSC are ER-negative, although they were isolated from human ER^+ tumors (300, 305, 306). This can justify the failure of ER-targeted endocrine therapy in breast cancer. However, there are other reports of BrCSC derived from ER-positive MCF7 cells that can induce tumors when cells as low as 10^3 are injected into the mammary fat pad of an SCID mouse, indicating the existence of distinct ER^+ BrCSC (307). The "side population" cells obtained from mammospheres that effluxed Hoechst dye expressed high levels of ERα, p21(CIP1) and Msi1 genes (308). Because the role of ERα in BrCSC and tumor progression remains ambiguous, one fundamental question that still needs to be addressed immediately is whether or not breast cancers with different ERα status are derived from different MaSC. Current opinion is that ERα status of MaSC correlates with the ERα expression of BrCSC, *i.e.*, ER-positive breast cancers arise through ER-positive stem cells, and ER-negative breast cancers arise from the most ER-negative stem

Figure 4.

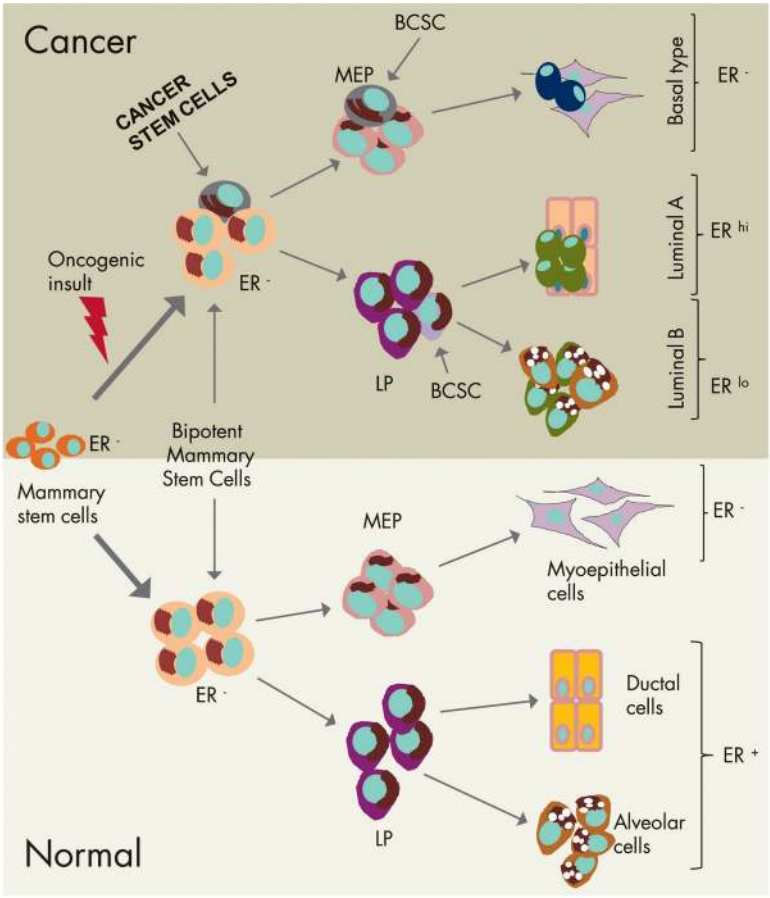

Figure 4. Hypothetical model illustrating the hierarchy of breast normal and cancer stem cells with ERα status. MaSC differentiate into bipotent stem cells that are ER-negative, and the differentiated progeny arising from this population is also ER-negative. Luminal type of mammary tissue that arises from its progenitor shows high ERα status, whereas myoepithelial cells arising from its progeny are ER-negative. In response to an oncogenic insult, rare population of MaSC become cancerous and give rise to three types of breast cancers such as basal type, which are ERα-negative; luminal A, which are high ERα; and luminal B, which show low ERα. BCSC, Breast cancer stem cells; LP, luminal progenitor; MEP, myoepithelial progenitor.

cells (294, 309) (Fig. 4). Another view about ERα status in BrCSC is that the normal mammary gland contains stem cells with basal phenotype that are ERα negative, so the BrCSC are endocrine-resistant, making the SERM therapy ineffective to treat breast cancers (310). More research in this area is warranted to know the expression status and precise role of ERα in BrCSC.

IX. Estrogen Receptor Subtypes in Breast Cancer

Until recently, there were two known classical receptors that mediate E2 action, ERα and ERβ. In the last decade, several isoforms of ERα and other related receptors have been identified. Of these, the role of ERβ in mammary gland development and its inverse relation with breast cancer progression is well characterized (311). In contrast to the positive correlation seen between ERα expression and breast cancer risk, various studies have shown that decreased expression of ERβ mRNA and protein levels in tumors compared with normal tissues (312). The loss of chromosome 14q, which encodes ERβ, was observed in some breast cancers, indicating the inverse correlation between ERβ and breast cancer risk (313, 314). It is clear that overexpression of ERβ in different breast cancer cell lines results in a decrease of proliferation and motility and promotes apoptosis (315, 316). ERβ appears to reduce the cell proliferation in response to E2 by inhibiting cyclin D1, cyclin E, and cdc25A expression, key factors that control cell division (317). Although ERβ also palmitoylated and targeted to plasma membrane like ERα, due to the lack of its interaction with PELP1/MNAR and Src kinase, ERβ does not stimulate the E2 extranuclear signals important for cell cycle progression (*i.e.*, ERK/MAPK and PI3K/AKT) and cyclin D1 transcription (318). ERβ2, an isoform of ERβ (also called ERβcx), was found to have no affinity for E2 and cannot activate transcription of ERE-responsive genes, but it was shown to negatively regulate the ERα transactivation in human breast cancer cells (319, 320). These findings suggest a tumor-suppressive function of ERβ.

Previous observations that E2 modulates the expression of several genes in ERα knockout mice and also specific aberrations in uteri of ERβ null mice in response to E2 suggested the presence of a novel receptor for E2 (321, 322). A newly identified G protein-coupled receptor (GPR30) was thought to mediate these functions. Indeed, GPR30 was shown to bind E2 both *in vitro* and *in vivo*, and it plays a key role in nongenomic signaling of E2 (323-327). GPR30 mediates E2-induced proliferation of ER-negative breast cancer cells through the rapid activation of MAPK (323, 328, 329). GPR30 signaling by E2 also promotes cell proliferation and migration in ER-negative breast cancer cells via induction of connective tissue growth factor (330). Immunohistochemistry analysis of a large number of breast carcinomas showed that half of these tumors, which are negative for ERα, are GPR30-positive, suggesting that these tumors may respond to E2 through GPR30 (331). Further overexpression of GPR30

is shown to associate with tumor size and Her2 expression, indicating that GPR30 may serve as a prognostic factor for aggressive breast cancers. Although these studies indicate that GPR30 mediates E2 actions in breast cancer cells and GPR30 expression correlates with aggressive breast cancers, controversies about its binding to E2 and E2-mediated GPR30 signaling remain. For instance, unlike ERα null mice, *Gpr30* null mice show no abnormalities in reproductive organs and, therefore GPR30 is dispersible in mediation of E2 effects in organs like the uterus and mammary gland (332). In addition, Levin's group demonstrated that silencing of *GPR30* by *GPR30*-specific siRNA had no effect on E2 nongenomic signaling in MCF7 cells (ER-positive) (333). Despite these controversies, accumulated evidence indicates that GPR30 is involved in breast cancer cell proliferation, tumor formation, migration, metastasis, and drug resistance (334–337). For example, heregulin-induced GPR30 promotes migration and invasion potential of the ER-negative breast cancer cell line SKBR3 through the activation of the ERBB2-ERBB3/MAPK pathway (338). The IGF-I-GPR30 axis was also shown to regulate MCF7 cell migration (339). Together, these studies suggest that GPR30 promotes tumor development through the activation of growth factor signaling independent of ERα.

In addition to the above-mentioned E2-related receptors, recently a number of ERα splice variants have been reported to be important for breast cancer development. The ERα variants such as ERα36 and ERα46 initiate their transcription from exon 2 of the ERα gene. ERα46 lacks the first coding exon of ERα (AF1) and acts as an inhibitor for ERα functions, whereas ERα36 lacks both transcriptional activation domains (AF1 and AF2) but retains the DNA binding domain and the ligand binding domain (340). ERα36 is predominantly located in plasma membrane and in the cytoplasm and can be detected in both ER-positive and ER-negative breast cancers (341). Down-regulation of ERα36 mRNA correlated with local progression, lymph node metastasis, and advanced cancer stages, indicating its involvement in breast cancer progression (342). It appears that ERα36 mediates extranuclear and mitogenic E2 signaling in ER-negative breast cancer cells like MDA-MB-231 and MDA-MB-436 through the EGFR/Src/ERK signaling pathway, implying that ERα36 may play an important role in the malignant growth of ER-negative breast cancers (342). ERα36 is also known to be involved in tamoxifen resistance in breast cancer cells. For instance, rapid phosphorylation of ERK1/2 and AKT was detected in ERα36-overexpressed MCF7 cells treated with tamoxifen (341, 343). These subtypes of ERα pose an additional layer of complexity to breast cancer development and require attention for further research.

X. Therapeutic Targeting of ERα Pathway—A Cure for ER-Positive Breast Cancers

Breast cancer is the fifth leading cause of all cancer deaths worldwide (1). It is well documented that within breast cancers, ER-positive breast cancers account for two thirds of cases (3). To extirpate the ER-sensitive breast cancers, multiple options were explored to target ERα signaling pathways. Endocrine therapy was the first targeted therapy used in the oncology field, long before the therapeutic agents were known (344). After the discovery and characterization of ERα and ERβ, attention was focused on developing new strategies and drugs that specifically target the ERα pathway. In pursuit of this, many SERM and selective ER down-regulators were developed and are still in use for breast cancer therapy (345). About two thirds of breast tumors that express ERα respond well to tamoxifen, an antiestrogen; however, prolonged treatment with tamoxifen resulted in resistance to the drug (346). Alternatively, several other related SERM such as arzoxifene and raloxifene are also available to treat breast cancer. Both anastrozole and letrozole, which block E2 synthesis by inhibiting aromatase, are considered as first-line treatment of advanced breast cancer in postmenopausal women with ER-positive breast cancer on par with tamoxifen (347). Fulvestrant, a potent antiestrogen that targets and degrades ERα, was approved for treatment of hormone receptor-positive breast cancer in postmenopausal women with disease relapse after antiestrogen therapy (348).

Although the majority of breast cancers are ER-positive, about 30% of invasive breast cancers are hormone independent because they lack ERα expression due to hypermethylation of ER promoter (254, 255). Attempts were made to re-express ERα in ER-negative breast cancers because ER-positive breast cancers respond to SERM therapies. Indeed, such efforts were successful on cell lines. For instance, treatment with epigenetic modulators such as DNA methyltransferase inhibitors and/or HDAC inhibitors induces ERα expression and restores tamoxifen sensitivity in ER-negative breast cancer cell lines (349, 350). Valproic acid, an HDAC inhibitor, also induces ERα and FoxA1 expression in MDA231 cells (ER-negative breast cancer cell line) and restores E2 sensitivity to these cells (267). Several HDAC inhibitors (*e.g.*, trichostatin A, vorinostat, de-

citabine, *etc.*) either alone or in combination with other drugs were used in phase I or phase II clinical trials (351–355). Although disease stabilization was observed using vorinostat in relapsed or refractory breast cancer patients, no consistent response was seen (351). In a similar phase II trial using vorinostat but in advanced and metastatic tumors, stable disease was observed in a limited number of patients. However, the lack of a complete or partial response led to termination of the study (352). A phase II trial using vorinostat combined with tamoxifen exhibited an encouraging response in reversing hormone therapy-resistant breast cancers (354). Similarly, a good clinical response was also observed in metastatic breast cancer patients when a combination of vorinostat with paclitaxel and bevacizumab was used (353). EZH2, a histone H3 Lys 27 (H3K27) methyltransferase and polycomb group protein, is reported to be down regulated in association with up-regulation of ERα in breast cancer cells. As a result, growth of ER-negative breast cancer requires EZH2 expression. Thus, suppression of EZH2 expression which ensures ERα reexpression, provides an option for better response to antiestrogens in ER-negative breast cancers (356, 357).

Because peptide drugs have more target-specific activity and coregulators influence ERα transcriptional activity through LXXLL motifs, attempts are being made to generate small peptide molecules that mimic these motifs to target ERα (358, 359). A group of linear and cyclic peptides that inhibit interaction between coactivator–steroid receptor were synthesized. Of these, pentapeptide, a short cyclic peptide containing a copy of the LXXLL nuclear receptor box, exhibited strong binding abilities and selectively interacted with ERα, with a K_i (inhibitory constant) of 25 nM (360). Because dimerization is key for ERα nuclear translocation and activity, "dimer-interface" oligopeptides, called I-box peptides, were synthesized. The I-box peptide exerted ERα inhibitory action by promoting aggregation and precipitation of both ligand bound and unbound receptor (361).

Extensive studies revealed the direct functional interaction of the ERα signaling pathway with several growth factor signaling pathways, which include PI3K/AKT, Src/MAPK, mammalian target of rapamycin, EGFR, *etc.* Previous reports show that E2 activates the PI3K/AKT signaling pathway, and inhibition by its inhibitors such as wortmannin and LY294002 has been shown to suppress cellular proliferation and transforming activity of breast cancer cells (169, 362). Hence, several drugs that target the PI3K/AKT pathway in multiple levels are in clinical trials; most of them are competitive inhibitors for ATP or mimetic of ATP. For example, BKM120, XL-147, PX-866, CAL-101, INK-1117, and BYL719 specifically inhibit PI3K, whereas GDC-0068, GSK690693, and MK-2206 specifically inhibit AKT (363). Because E2 also activates Src kinase by extranuclear signaling and kinase activity of Src is linked to E2/ERα-dependent cell proliferation and transformation, Src inhibitors such as dasatinib, bosutinib, and PD180970 were also developed to treat breast cancers and have shown potential in the clinical setting (113, 364). Data from phase II trials with dasatinib have shown limited activity in hormone receptor-positive breast cancer patients (364). Therefore, clinical trials using a combination of dasatinib with other drugs are ongoing.

Because ERβ exhibits tumor-suppressive actions in breast, in recent years several ERβ agonists have been synthesized. For example, genistein, a natural compound of the isoflavone family, binds to ERβ by 26-fold higher affinity over ERα and activates ERβ by 7-fold greater potency than ERα (127). Structurally modified derivatives of genistein have also exerted better anticancer activity in breast cancer cells (128). Similarly, 2,3-bis(4-hydroxyphenyl)propionitrile (DPN) binds to ERβ by 72-fold higher affinity over ERα and activates ERβ by 80-fold greater potency than ERα (207). DPN has shown an antiproliferative effect and also inhibitory action on cellular transformation (268). Recently, in a virtual screening based on a structure optimized through molecular dynamics and bioassay approach, 18 potent ligands of ERβ were discovered (270). Some of these compounds could be novel SERM of the future that could benefit the therapy of ER-positive breast cancer.

XI. Conclusions and Future Prospects

ER-positive breast cancer constitutes a major proportion of breast cancer types. Therefore, breathtaking research has been carried out in the last three decades to understand ERα function and its relevance with breast cancer. As a result, many novel mechanisms of E2/ERα-mediated breast cancer development were discovered, including the identification of hundreds of ERα coregulators and their association with breast cancer development. Particularly in the last decade, extensive research on E2 extranuclear signaling led to the discovery of many novel signal transduction pathways associated with breast cancer growth and behavior, which are being explored as therapeutic targets. In addition, many

posttranslational modifications for ERα were identified, and their functional significance in disease progression was well studied. However, cross talk between multiple signaling pathways poses a barrier for such approaches. In such instances, combination therapy involving drugs that target both nongenomic signaling and posttranslational modifications should be developed. Despite all of these advancements, the survival rate has not improved greatly enough. Therefore, a detailed understanding of decoding the mystery behind the E2 signaling and breast tumor growth is important.

Antiestrogens are the current choice of endocrine therapy in the treatment of ER-positive breast cancers. However, drug resistance poses a major hurdle in the usage of antiestrogen therapy. Future studies should be focused on the newly discovered E2 signaling pathways to make use of them in new therapeutics against breast cancers. Especially, many well-characterized coregulators with the potential to influence the ERα-mediated breast cancers should be targeted. To this end, expression profiling data of all coregulators in the form of a "code" should be available for all subtypes of breast cancers. This "coregulator code" eventually may help in patient diagnosis and treatment. In addition, recent studies on the miRNA-ERα axis revealed novel mechanisms of breast cancer development. But more clinical studies are needed to better understand the role of miRNA on ERα actions and breast cancer status. Future research awaits on whether the novel gene therapy of using miRNA in breast cancer treatment will be a powerful tool for breast cancer treatment or just an addition to the existing drugs. The current theories on stem cells indicate that stem cells are important for initiation and maintenance of a tumor; very little is known about E2 signaling in MaSC functions. Therefore, it is certainly no exaggeration that exciting years of research on the role for E2 in mammary/breast stem cell maintenance are coming up to invite a new arena in the E2 signaling.

Acknowledgments

We thank all members of the Manavathi lab for their helpful discussions.

Address all correspondence and requests for reprints to: Dr. Bramanandam Manavathi, Assistant Professor, Department of Biochemistry, School of Life Sciences, Gachibowli, Prof. CR Rao Road, University of Hyderabad, Hyderabad 500046, India. E-mail: manavathibsl@uohyd.ernet.in.

This work was supported by Department of Biotechnology, India, Grant BT/PR11114/BRB/10/635/2008; Council for Scientific and Industrial Research, India, Grant 37 (1359)/09/EMR-II; Innovative Young Biotechnologist Award, Department of Biotechnology, India, Grant BT/01/IYBA/2009 (to B.M.); and National Institutes of Health Grant CA09823 (to R.K.).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D** 2011 Global cancer statistics. CA Cancer J Clin 61:69–90
2. **Brisken C, O'Malley B** 2010 Hormone action in the mammary gland. Cold Spring Harb Perspect Biol 2:a003178
3. **Fisher ER, Osborne CK, McGuire WL, Redmond C, Knight 3rd WA, Fisher B, Bannayan G, Walder A, Gregory EJ, Jacobsen A, Queen DM, Bennett DE, Ford HC** 1981 Correlation of primary breast cancer histopathology and estrogen receptor content. Breast Cancer Res Treat 1:37–41
4. **Khan SA, Rogers MA, Obando JA, Tamsen A** 1994 Estrogen receptor expression of benign breast epithelium and its association with breast cancer. Cancer Res 54:993–997
5. **Ali S, Coombes RC** 2000 Estrogen receptor α in human breast cancer: occurrence and significance. J Mammary Gland Biol Neoplasia 5:271–281
6. **Ali S, Coombes RC** 2002 Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev Cancer 2:101–112
7. **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
8. **Barnes CJ, Vadlamudi RK, Kumar R** 2004 Novel estrogen receptor coregulators and signaling molecules in human diseases. Cell Mol Life Sci 61:281–291
9. **Russo J, Russo IH** 1987 Development of the human mammary gland. In: Neville M, Daniel CW, eds. The mammary gland. Development, regulation and function. New York: Plenum; 67–93
10. **Hennighausen L, Robinson GW** 1998 Think globally, act locally: the making of a mouse mammary gland. Genes Dev 12:449–455
11. **Nandi S** 1958 Endocrine control of mammary gland development and function in the C3H/He Crgl mouse. J Natl Cancer Inst 21:1039–1063
12. **Couse JF, Korach KS** 1999 Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev 20:358–417
13. **Daniel CW, Silberstein GB** 1987 In: Neville MC, Daniel CW, eds. The mammary gland. New York: Plenum 3–31
14. **Howard BA, Gusterson BA** 2000 Human breast development. J Mammary Gland Biol Neoplasia 5:119–137
15. **Bocchinfuso WP, Lindzey JK, Hewitt SC, Clark JA, Myers PH, Cooper R, Korach KS** 2000 Induction of mammary gland development in estrogen receptor-α knockout mice. Endocrinology 141:2982–2994
16. **Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M** 2000 Effect of single and compound knockouts of estrogen receptors α (ERα) and β (ERβ) on mouse reproductive phenotypes. Development 127:4277–4291

17. **Mallepell S, Krust A, Chambon P, Brisken C** 2006 Paracrine signaling through the epithelial estrogen receptor α is required for proliferation and morphogenesis in the mammary gland. Proc Natl Acad Sci USA 103:2196–2201
18. **Feng Y, Manka D, Wagner KU, Khan SA** 2007 Estrogen receptor expression in the mammary epithelium is required for ductal and alveolar morphogenesis in mice. Proc Natl Acad Sci USA 104:14718–14723
19. **Bocchinfuso WP, Korach KS** 1997 Mammary gland development and tumorigenesis in estrogen receptor knockout mice. J Mammary Gland Biol Neoplasia 2:323–334
20. **Pettersson K, Delaunay F, Gustafsson JA** 2000 Estrogen receptor β acts as a dominant regulator of estrogen signaling. Oncogene 19:4970–4978
21. **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
22. **Beatson GT** 1896 On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. Lancet 2:104–107
23. **O'Malley BW, McGuire WL, Middleton PA** 1968 Altered gene expression during differentiation: population changes in hybridizable RNA after stimulation of the chick oviduct with oestrogen. Nature 218:1249–1251
24. **Jensen EV, Block GE, Smith S, Kyser K, DeSombre ER** 1971 Estrogen receptors and breast cancer response to adrenalectomy. Natl Cancer Inst Monogr 34:55–70
25. **Bundred NJ** 2001 Prognostic and predictive factors in breast cancer. Cancer Treat Rev 27:137–142
26. **Cleator S, Ashworth A** 2004 Molecular profiling of breast cancer: clinical implications. Brit J Cancer 90:1120–1124
27. **Stein RA, McDonnell DP** 2006 Estrogen-related receptor α as a therapeutic target in cancer. Endocr Relat Cancer 13:S25–S32
28. **Kumar V, Green S, Stack G, Berry M, Jin JR, Chambon P** 1987 Functional domains of the human estrogen receptor. Cell 51:941–951
29. **Klein-Hitpass L, Schorpp M, Wagner U, Ryffel GU** 1986 An estrogen-responsive element derived from the 5′ flanking region of the *Xenopus* vitellogenin A2 gene functions in transfected human cells. Cell 46:1053–1061
30. **O'Lone R, Frith MC, Karlsson EK, Hansen U** 2004 Genomic targets of nuclear estrogen receptors. Mol Endocrinol 18:1859–1875
31. **Welboren WJ, Sweep FC, Span PN, Stunnenberg HG** 2009 Genomic actions of estrogen receptor-α: what are the targets and how are they regulated? Endocr Relat Cancer 16:1073–1089
32. **Hayward MA, Brock ML, Shapiro DJ** 1982 Activation of vitellogenin gene transcription is a direct response to estrogen in *Xenopus laevis* liver. Nucleic Acids Res 10:8273–8284
33. **Jost JP, Seldran M, Geiser M** 1984 Preferential binding of estrogen-receptor complex to a region containing the estrogen-dependent hypomethylation site preceding the chicken vitellogenin II gene. Proc Natl Acad Sci USA 81:429–433
34. **Brown AM, Jeltsch JM, Roberts M, Chambon P** 1984 Activation of pS2 gene transcription is a primary response to estrogen in the human breast cancer cell line MCF-7. Proc Natl Acad Sci USA 81:6344–6348
35. **Dubik D, Dembinski TC, Shiu RP** 1987 Stimulation of c-myc oncogene expression associated with estrogen-induced proliferation of human breast cancer cells. Cancer Res 47:6517–6521
36. **Sabbah M, Courilleau D, Mester J, Redeuilh G** 1999 Estrogen induction of the cyclin D1 promoter: involvement of a cAMP response-like element. Proc Natl Acad Sci USA 96:11217–11222
37. **Amiry N, Kong X, Muniraj N, Kannan N, Grandison PM, Lin J, Yang Y, Vouyovitch CM, Borges S, Perry JK, Mertani HC, Zhu T, Liu D, Lobie PE** 2009 Trefoil factor-1 (TFF1) enhances oncogenicity of mammary carcinoma cells. Endocrinology 150:4473–4483
38. **Soubeyran I, Wafflart J, Bonichon F, de Mascarel I, Trojani M, Durand M, Avril A, Coindre JM** 1995 Immunohistochemical determination of pS2 in invasive breast carcinomas: a study on 942 cases. Breast Cancer Res Treat 34:119–128
39. **Gillesby BE, Zacharewski TR** 1999 pS2 (TFF1) levels in human breast cancer tumor samples: correlation with clinical and histological prognostic markers. Breast Cancer Res Treat 56:253–265
40. **Jansen RL, Hupperets PS, Arends JW, Joosten-Achjanie SR, Volovics A, Hillen HF, Schouten HC** 1998 pS2 is an independent prognostic factor for post-relapse survival in primary breast cancer. Anticancer Res 18:577–582
41. **Millour J, Constantinidou D, Stavropoulou AV, Wilson MS, Myatt SS, Kwok JM, Sivanandan K, Coombes RC, Medema RH, Hartman J, Lykkesfeldt AE, Lam EW** 2010 FOXM1 is a transcriptional target of ER α and has a critical role in breast cancer endocrine sensitivity and resistance. Oncogene 29:2983–2995
42. **Ikeda K, Orimo A, Higashi Y, Muramatsu M, Inoue S** 2000 Efp as a primary estrogen-responsive gene in human breast cancer. FEBS Lett 472:9–13
43. **Urano T, Saito T, Tsukui T, Fujita M, Hosoi T, Muramatsu M, Ouchi Y, Inoue S** 2002 Efp targets 14-3-3 σ for proteolysis and promotes breast tumour growth. Nature 417:871–875
44. **Suzuki T, Urano T, Tsukui T, Horie-Inoue K, Moriya T, Ishida T, Muramatsu M, Ouchi Y, Sasano H, Inoue S** 2005 Estrogen-responsive finger protein as a new potential biomarker for breast cancer. Clin Cancer Res 11:6148–6154
45. **Vadlamudi RK, Wang RA, Mazumdar A, Kim Y, Shin J, Sahin A, Kumar R** 2001 Molecular cloning and characterization of PELP1, a novel human coregulator of estrogen receptor α. J Biol Chem 276:38272–38279
46. **Vadlamudi RK, Manavathi B, Balasenthil S, Nair SS, Yang Z, Sahin AA, Kumar R** 2005 Functional implications of altered subcellular localization of PELP1 in breast cancer cells. Cancer Res 65:7724–7732
47. **Nair BC, Nair SS, Chakravarty D, Challa R, Manavathi B, Yew PR, Kumar R, Tekmal RR, Vadlamudi RK** 2010 Cyclin-dependent kinase-mediated phosphorylation plays a critical role in the oncogenic functions of PELP1. Cancer Res 70:7166–7175
48. **den Hollander P, Rayala SK, Coverley D, Kumar R** 2006

Ciz1, a novel DNA-binding coactivator of the estrogen receptor α, confers hypersensitivity to estrogen action. Cancer Res 66:11021–11029

49. **Sun J, Nawaz Z, Slingerland JM** 2007 Long-range activation of GREB1 by estrogen receptor via three distal consensus estrogen-responsive elements in breast cancer cells. Mol Endocrinol 21:2651–2662
50. **Mishra SK, Talukder AH, Gururaj AE, Yang Z, Singh RR, Mahoney MG, Francí C, Vadlamudi RK, Kumar R** 2004 Upstream determinants of estrogen receptor-α regulation of metastatic tumor antigen 3 pathway. J Biol Chem 279: 32709–32715
51. **Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS, Wade PA** 2003 MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. Cell 113:207–219
52. **Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoute J, Shao W, Hestermann EV, Geistlinger TR, Fox EA, Silver PA, Brown M** 2005 Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell 122: 33–43
53. **Deschênes J, Bourdeau V, White JH, Mader S** 2007 Regulation of GREB1 transcription by estrogen receptor α through a multipartite enhancer spread over 20 kb of upstream flanking sequences. J Biol Chem 282:17335–17339
54. **Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, Chiariotti L, Malorni A, Abbondanza C, Avvedimento EV** 2008 DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science 319:202–206
55. **Pan YF, Wansa KD, Liu MH, Zhao B, Hong SZ, Tan PY, Lim KS, Bourque G, Liu ET, Cheung E** 2008 Regulation of estrogen receptor-mediated long range transcription via evolutionarily conserved distal response elements. J Biol Chem 283:32977–32988
56. **Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY, Wansa KD, Zhao B, Lim KS, Leow SC, Yow JS, Joseph R, Li H, Desai KV, Thomsen JS, Lee YK, Karuturi RK, Herve T, Bourque G, Stunnenberg HG, Ruan X, Cacheux-Rataboul V, Sung WK, Liu ET, Wei CL, Cheung E, Ruan Y** 2009 An oestrogen-receptor-α-bound human chromatin interactome. Nature 462:58–64
57. **Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, Brown GD, Gojis O, Ellis IO, Green AR, Ali S, Chin SF, Palmieri C, Caldas C, Carroll JS** 2012 Differential estrogen receptor binding is associated with clinical outcome in breast cancer. Nature 481:389–393
58. **Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M** 2000 Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843–852
59. **McKenna NJ, O'Malley BW** 2002 Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 108:465–474
60. **Green KA, Carroll JS** 2007 Oestrogen-receptor-mediated transcription and the influence of co-factors and chromatin state. Nat Rev Cancer 7:713–722
61. **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
62. **Lonard DM, O'Malley BW** 2006 The expanding cosmos of nuclear receptor coactivators. Cell 125:411–414
63. **Belandia B, Orford RL, Hurst HC, Parker MG** 2002 Targeting of SWI/SNF chromatin remodeling complexes to estrogen-responsive genes. EMBO J 21:4094–4103
64. **Kurebayashi J, Otsuki T, Kunisue H, Tanaka K, Yamamoto S, Sonoo H** 2000 Expression levels of estrogen receptor-α, estrogen receptor-β, coactivators, and corepressors in breast cancer. Clin Cancer Res 6:512–518
65. **Girault I, Lerebours F, Amarir S, Tozlu S, Tubiana-Hulin M, Lidereau R, Bièche I** 2003 Expression analysis of estrogen receptor α coregulators in breast carcinoma: evidence that NCOR1 expression is predictive of the response to tamoxifen. Clin Cancer Res 9:1259–1266
66. **Mann M, Krishnan S, Vadlamudi RK** 2012 Emerging significance of estrogen cancer coregulator signaling in breast cancer. Minerva Ginecol 64:75–88
67. **Lanz RB, Londard DM, O'Malley BW** 2008 Nuclear receptor coregulators in human diseases. In: Kumar R, O'Malley BW, eds. Nuclear receptor coregulators and human diseases. Singapore: World Scientific Publishing Co.; 1–133
68. **O'Malley BW, Kumar R** 2009 Nuclear receptor coregulators in cancer biology. Cancer Res 69:8217–8222
69. **Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS** 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965–968
70. **Gururaj AE, Peng S, Vadlamudi RK, Kumar R** 2007 E2 induces expression of BCAS3, a novel E2 receptor-α coactivator, through proline-, glutamic acid-, and leucine-rich protein-1 (PELP1). Mol Endocrinol 21:1847–1860
71. **Bultman SJ, Herschkowitz JI, Godfrey V, Gebuhr TC, Yaniv M, Perou CM, Magnuson T** 2008 Characterization of mammary tumors from Brg1 heterozygous mice. Oncogene 27:460–468
72. **Zhang Q, Kao C, Zhang J, Vieth E, Gao H, Cai A, Kim BO, Cheng L, Juliar BE, Li L, Goulet RJ, Miller KD, Sledge GW, Stallcup MR, Jeng MH,** Overexpression of CARM1 in human breast carcinoma stimulated breast cancer cell proliferation. Proc 96th Annual Meeting of the American Association for Cancer Research, Anaheim, CA, 2005 (Abstract 1280)
73. **McBryan J, Howlin J, Kenny PA, Shioda T, Martin F** 2007 ERα-CITED1 co-regulated genes expressed during pubertal mammary gland development: implications for breast cancer prognosis. Oncogene 26:6406–6419
74. **Roy PG, Pratt N, Purdie CA, Baker L, Ashfield A, Quinlan P, Thompson AM** 2010 High CCND1 amplification identifies a group of poor prognosis women with estrogen receptor positive breast cancer. Int J Cancer 127:355–360
75. **Lee H, Kim KR, Noh SJ, Park HS, Kwon KS, Park BH, Jung SH, Youn HJ, Lee BK, Chung MJ, Koh DH, Moon WS, Jang KY** 2011 Expression of DBC1 and SIRT1 is associated with poor prognosis for breast carcinoma. Hum Pathol 42:204–213
76. **Gao X, Mohsin SK, Gatalica Z, Fu G, Sharma P, Nawaz Z** 2005 Decreased expression of e6-associated protein in

breast and prostate carcinomas. Endocrinology 146:1707–1712
77. **Lundy J, Thor A, Maenza R, Schlom J, Forouhar F, Testa M, Kufe D** 1985 Monoclonal antibody DF3 correlates with tumor differentiation and hormone receptor status in breast cancer patients. Breast Cancer Res Treat 5:269–276
78. **Xiao XS, Cai MY, Chen JW, Guan XY, Kung HF, Zeng YX, Xie D** 2011 High expression of p300 in human breast cancer correlates with tumor recurrence and predicts adverse prognosis. Chin J Can Res 23:201–207
79. **Rajhans R, Nair S, Holden AH, Kumar R, Tekmal RR, Vadlamudi RK** 2007 Oncogenic potential of the nuclear receptor coregulator proline-, glutamic acid-, leucine-rich protein 1/modulator of the nongenomic actions of the estrogen receptor. Cancer Res 67:5505–5512
80. **Chooniedass-Kothari S, Hamedani MK, Troup S, Hubé F, Leygue E** 2006 The steroid receptor RNA activator protein is expressed in breast tumor tissues. Int J Cancer 118:1054–1059
81. **Berns EM, van Staveren IL, Klijn JG, Foekens JA** 1998 Predictive value of SRC-1 for tamoxifen response of recurrent breast cancer. Breast Cancer Res Treat 48:87–92
82. **Redmond AM, Bane FT, Stafford AT, McIlroy M, Dillon MF, Crotty TB, Hill AD, Young LS** 2009 Coassociation of estrogen receptor and p160 proteins predicts resistance to endocrine treatment; SRC-1 is an independent predictor of breast cancer recurrence. Clin Cancer Res 15:2098–2106
83. **Spears M, Oesterreich S, Migliaccio I, Guiterrez C, Hilsenbeck S, Quintayo MA, Pedraza J, Munro AF, Thomas JS, Kerr GR, Jack WJ, Kunkler IH, Cameron DA, Chetty U, Bartlett JM** 2012 The p160 ER co-regulators predict outcome in ER negative breast cancer. Breast Cancer Res Treat 131:463–472
84. **Dong XY, Sun X, Guo P, Li Q, Sasahara M, Ishii Y, Dong JT** 2010 ATBF1 inhibits estrogen receptor (ER) function by selectively competing with AIB1 for binding to the ER in ER-positive breast cancer cells. J Biol Chem 285:32801–32809
85. **Ma YX, Tomita Y, Fan S, Wu K, Tong Y, Zhao Z, Song LN, Goldberg ID, Rosen EM** 2005 Structural determinants of the BRCA1: estrogen receptor interaction. Oncogene 24:1831–1846
86. **Xu J, Wang B, Zhang Y, Li R, Wang Y, Zhang S** 2012 Clinical implications for BRCA gene mutation in breast cancer. Mol Biol Rep 39:3097–3102
87. **Tong D, Heinze G, Schremmer M, Schuster E, Czerwenka K, Leodolter S, Zeillinger R** 2007 Expression of the human MTA1 gene in breast cell lines and in breast cancer tissues. Oncol Res 16:465–470
88. **Kumar R, Wang RA, Mazumdar A, Talukder AH, Mandal M, Yang Z, Bagheri-Yarmand R, Sahin A, Hortobagyi G, Adam L, Barnes CJ, Vadlamudi RK** 2002 A naturally occurring MTA1 variant sequesters oestrogen receptor-α in the cytoplasm. Nature 418:654–657
89. **Zhang H, Stephens LC, Kumar R** 2006 Metastasis tumor antigen family proteins during breast cancer progression and metastasis in a reliable mouse model for human breast cancer. Clin Cancer Res 12:1479–1486
90. **Mendes-Pereira AM, Sims D, Dexter T, Fenwick K, Assiotis I, Kozarewa I, Mitsopoulos C, Hakas J, Zvelebil M, Lord CJ, Ashworth A** 2012 Genome-wide functional screen identifies a compendium of genes affecting sensitivity to tamoxifen. Proc Natl Acad Sci USA 109:2730–2735
91. **Simon SL, Parkes A, Leygue E, Dotzlaw H, Snell L, Troup S, Adeyinka A, Watson PH, Murphy LC** 2000 Expression of a repressor of estrogen receptor activity in human breast tumors: relationship to some known prognostic markers. Cancer Res 60:2796–2799
92. **Chan CM, Lykkesfeldt AE, Parker MG, Dowsett M** 1999 Expression of nuclear receptor interacting proteins TIF-1, SUG-1, receptor interacting protein 140, and corepressor SMRT in tamoxifen-resistant breast cancer. Clin Cancer Res 5:3460–3467
93. **Hammerich-Hille S, Bardout VJ, Hilsenbeck SG, Osborne CK, Oesterreich S** 2010 Low SAFB levels are associated with worse outcome in breast cancer patients. Breast Cancer Res Treat 121:503–509
94. **Wu L, Wu Y, Gathings B, Wan M, Li X, Grizzle W, Liu Z, Lu C, Mao Z, Cao X** 2003 Smad4 as a transcription corepressor for estrogen receptor α. J Biol Chem 278:15192–15200
95. **Stuelten CH, Buck MB, Dippon J, Roberts AB, Fritz P, Knabbe C** 2006 Smad4-expression is decreased in breast cancer tissues: a retrospective study. BMC Cancer 6:25
96. **Liu XF, Bagchi MK** 2004 Recruitment of distinct chromatin-modifying complexes by tamoxifen-complexed estrogen receptor at natural target gene promoters in vivo. J Biol Chem 279:15050–15058
97. **Popov VM, Zhou J, Shirley LA, Quong J, Yeow WS, Wright JA, Wu K, Rui H, Vadlamudi RK, Jiang J, Kumar R, Wang C, Pestell RG** 2009 The cell fate determination factor DACH1 is expressed in estrogen receptor-α-positive breast cancer and represses estrogen receptor-α signaling. Cancer Res 69:5752–5760
98. **Lonard DM, Lanz RB, O'Malley BW** 2007 Nuclear receptor coregulators and human disease. Endocr Rev 28:575–587
99. **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
100. **Gururaj AE, Holm C, Landberg G, Kumar R** 2006 Breast cancer-amplified sequence 3, a target of metastasis-associated protein 1, contributes to tamoxifen resistance in premenopausal patients with breast cancer. Cell Cycle 5:1407–1410
101. **Qin L, Liao L, Redmond A, Young L, Yuan Y, Chen H, O'Malley BW, Xu J** 2008 The AIB1 oncogene promotes breast cancer metastasis by activation of PEA3-mediated matrix metalloproteinase 2 (MMP2) and MMP9 expression. Mol Cell Biol 28:5937–5950
102. **Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM** 1997 Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90:569–580
103. **Yi P, Feng Q, Amazit L, Lonard DM, Tsai SY, Tsai MJ, O'Malley BW** 2008 Atypical protein kinase C regulates dual pathways for degradation of the oncogenic coactivator SRC-3/AIB1. Mol Cell 29:465–476
104. **Giamas G, Castellano L, Feng Q, Knippschild U, Jacob J,**

Thomas RS, Coombes RC, Smith CL, Jiao LR, Stebbing J 2009 CK1δ modulates the transcriptional activity of ERα via AIB1 in an E2-dependent manner and regulates ERα-AIB1 interactions. Nucleic Acids Res 37:3110–3123

105. **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
106. **Yu EJ, Kim SH, Heo K, Ou CY, Stallcup MR, Kim JH** 2011 Reciprocal roles of DBC1 and SIRT1 in regulating estrogen receptor α activity and co-activator synergy. Nucleic Acids Res 39:6932–6943
107. **Khurana S, Chakraborty S, Cheng X, Su YT, Kao HY** 2011 The actin-binding protein, actinin α 4 (ACTN4), is a nuclear receptor coactivator that promotes proliferation of MCF-7 breast cancer cells. J Biol Chem 286:1850–1859
108. **Wortham NC, Ahamed E, Nicol SM, Thomas RS, Periyasamy M, Jiang J, Ochocka AM, Shousha S, Huson L, Bray SE, Coombes RC, Ali S, Fuller-Pace FV** 2009 The DEAD-box protein p72 regulates ERα-/E2-dependent transcription and cell growth, and is associated with improved survival in ERα-positive breast cancer. Oncogene 28:4053–4064
109. **Wei X, Xu H, Kufe D** 2006 MUC1 oncoprotein stabilizes and activates estrogen receptor α. Mol Cell 21:295–305
110. **Feng Q, Zhang Y** 2003 The NuRD complex: linking histone modification to nucleosome remodeling. Curr Top Microbiol Immunol 274:269–290
111. **Dobrzycka KM, Townson SM, Jiang S, Oesterreich S** 2003 Estrogen receptor corepressors—a role in human breast cancer? Endocr Relat Cancer 10:517–536
112. **Mazumdar A, Wang RA, Mishra SK, Adam L, Bagheri-Yarmand R, Mandal M, Vadlamudi RK, Kumar R** 2001 Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. Nat Cell Biol 3:30–37
113. **Saad F, Lipton A** 2010 SRC kinase inhibition: targeting bone metastases and tumor growth in prostate and breast cancer. Cancer Treat Rev 36:177–184
114. **Bagheri-Yarmand R, Talukder AH, Wang RA, Vadlamudi RK, Kumar R** 2004 Metastasis-associated protein 1 deregulation causes inappropriate mammary gland development and tumorigenesis. Development 131:3469–3479
115. **Park S, Zhao Y, Yoon S, Xu J, Liao L, Lydon J, DeMayo F, O'Malley BW, Katzenellenbogen BS** 2011 Repressor of estrogen receptor activity (REA) is essential for mammary gland morphogenesis and functional activities: studies in conditional knockout mice. Endocrinology 152:4336–4349
116. **Kurtev V, Margueron R, Kroboth K, Ogris E, Cavailles V, Seiser C** 2004 Transcriptional regulation by the repressor of estrogen receptor activity via recruitment of histone deacetylases. J Biol Chem 279:24834–24843
117. **Hu X, Lazar MA** 1999 The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. Nature 402:93–96
118. **Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen TM, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG, Rose DW** 1998 Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. Proc Natl Acad Sci USA 95:2920–2925
119. **Jiang S, Meyer R, Kang K, Osborne CK, Wong J, Oesterreich S** 2006 Scaffold attachment factor SAFB1 suppresses estrogen receptor α-mediated transcription in part via interaction with nuclear receptor corepressor. Mol Endocrinol 20:311–320
120. **He B, Feng Q, Mukherjee A, Lonard DM, DeMayo FJ, Katzenellenbogen BS, Lydon JP, O'Malley BW** 2008 A repressive role for prohibitin in estrogen signaling. Mol Endocrinol 22:344–360
121. **Huen MS, Sy SM, Chen J** 2010 BRCA1 and its toolbox for the maintenance of genome integrity. Nat Rev Mol Cell Biol 11:138–148
122. **Wilson CA, Ramos L, Villaseñor MR, Anders KH, Press MF, Clarke K, Karlan B, Chen JJ, Scully R, Livingston D, Zuch RH, Kanter MH, Cohen S, Calzone FJ, Slamon DJ** 1999 Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. Nat Genet 21:236–240
123. **Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SB, Herman JG** 2000 Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 92:564–569
124. **Staff S, Isola J, Tanner M** 2003 Haplo-insufficiency of BRCA1 in sporadic breast cancer. Cancer Res 63:4978–4983
125. **Jones LP, Tilli MT, Assefnia S, Torre K, Halama ED, Parrish A, Rosen EM, Furth PA** 2008 Activation of E2 signaling pathways collaborates with loss of Brca1 to promote development of ER-negative and ER-positive mammary preneoplasia and cancer. Oncogene 27:794–802
126. **Hilakivi-Clarke L** 2000 Estrogens, BRCA1, and breast cancer. Cancer Res 60:4993–5001
127. **Escande A, Pillon A, Servant N, Cravedi JP, Larrea F, Muhn P, Nicolas JC, Cavaillès V, Balaguer P** 2006 Evaluation of ligand selectivity using reporter cell lines stably expressing estrogen receptor α or β. Biochem Pharmacol 71:1459–1469
128. **Marik R, Allu M, Anchoori R, Stearns V, Umbricht CB, Khan S** 2011 Potent genistein derivatives as inhibitors of estrogen receptor α-positive breast cancer. Cancer Biol Ther 11:883–892
129. **Lakhani SR, Van De Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, Easton DF** 2002 The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 20:2310–2318
130. **Rebbeck TR, Lynch HT, Neuhausen SL, Narod SA, Van't Veer L, Garber JE, Evans G, Isaacs C, Daly MB, Matloff E, Olopade OI, Weber BL** 2002 Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 346:1616–1622
131. **Bachelier R, Xu X, Li C, Qiao W, Furth PA, Lubet RA, Deng CX** 2005 Effect of bilateral oophorectomy on mammary tumor formation in BRCA1 mutant mice. Oncol Rep 14:1117–1120

132. **Andrieu N, Goldgar DE, Easton DF, Rookus M, Brohet R, Antoniou AC, Peock S, Evans G, Eccles D, Douglas F, Noguès C, Gauthier-Villars M, Chompret A, Van Leeuwen FE, Kluijt I, Benitez J, Arver B, Olah E, Chang-Claude J, EMBRACE, GENEPSO, GEO-HEBON, IBCCS Collaborators Group** 2006 Pregnancies, breast-feeding, and breast cancer risk in the International BRCA1/2 Carrier Cohort Study (IBCCS). J Natl Cancer Inst 98:535–544
133. **Spillman MA, Bowcock AM** 1996 BRCA1 and BRCA2 mRNA levels are coordinately elevated in human breast cancer cells in response to estrogen. Oncogene 13:1639–1645
134. **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
135. **Eakin CM, Maccoss MJ, Finney GL, Klevit RE** 2007 E2 receptor is a putative substrate for the BRCA1 ubiquitin ligase. Proc Natl Acad Sci USA 104:5794–5799
136. **Fan S, Ma YX, Wang C, Yuan RQ, Meng Q, Wang JA, Erdos M, Goldberg ID, Webb P, Kushner PJ, Pestell RG, Rosen EM** 2002 p300 modulates the BRCA1 inhibition of E2 receptor activity. Cancer Res 62:141–151
137. **Kim MY, Woo EM, Chong YT, Homenko DR, Kraus WL** 2006 Acetylation of E2 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
138. **Wen J, Li R, Lu Y, Shupnik MA** 2009 Decreased BRCA1 confers tamoxifen resistance in breast cancer cells by altering estrogen receptor-coregulator interactions. Oncogene 28:575–586
139. **Hosey AM, Gorski JJ, Murray MM, Quinn JE, Chung WY, Stewart GE, James CR, Farragher SM, Mulligan JM, Scott AN, Dervan PA, Johnston PG, Couch FJ, Daly PA, Kay E, McCann A, Mullan PB, Harkin DP** 2007 Molecular basis for E2 receptor α deficiency in BRCA1-linked breast cancer. J Natl Cancer Inst 99:1683–1694
140. **Katiyar P, Ma Y, Fan S, Pestell RG, Furth PA, Rosen EM** 2006 Regulation of progesterone receptor signaling by BRCA1 in mammary cancer. Nucl Recept Signal 4:e006
141. **Sutherland RL, Musgrove EA** 2004 Cyclins and breast cancer. J Mammary Gland Biol Neoplasia 9:95–104
142. **Eeckhoute J, Carroll JS, Geistlinger TR, Torres-Arzayus MI, Brown M** 2006 A cell-type-specific transcriptional network required for estrogen regulation of cyclin D1 and cell cycle progression in breast cancer. Genes Dev 20:2513–2526
143. **Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV** 1994 Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature 369:669–671
144. **Ormandy CJ, Musgrove EA, Hui R, Daly RJ, Sutherland RL** 2003 Cyclin D1, EMS1 and 11q13 amplification in breast cancer. Breast Cancer Res Treat 78:323–335
145. **Herber B, Truss M, Beato M, Müller R** 1994 Inducible regulatory elements in the human cyclin D1 promoter. Oncogene 9:1295–1304
146. **Ogba N, Chaplin LJ, Doughman YQ, Fujinaga K, Montano MM** 2008 HEXIM1 regulates 17β-estradiol/estrogen receptor-α-mediated expression of cyclin D1 in mammary cells via modulation of P-TEFb. Cancer Res 68:7015–7024
147. **Dyson N** 1998 The regulation of E2F by pRB-family proteins. Genes Dev 12:2245–2262
148. **Musgrove EA, Hamilton JA, Lee CS, Sweeney KJ, Watts CK, Sutherland RL** 1993 Growth factor, steroid and steroid antagonist regulation of cyclin gene expression associated with changes in T-47D human breast cancer cell cycle progression. Mol Cell Biol 13:3577–3587
149. **Planas-Silva MD, Weinberg RA** 1997 Estrogen-dependent cyclin E-cdk2 activation through p21 redistribution. Mol Cell Biol 17:4059–4069
150. **Prall OW, Sarcevic B, Musgrove EA, Watts CK, Sutherland RL** 1997 Estrogen-induced activation of Cdk4 and Cdk2 during G1-S phase progression is accompanied by increased cyclin D1 expression and decreased cyclin-dependent kinase inhibitor association with cyclin E-Cdk2. J Biol Chem 272:10882–10894
151. **Foster JS, Henley DC, Bukovsky A, Seth P, Wimalasena J** 2001 Multifaceted regulation of cell cycle progression by estrogen: regulation of Cdk inhibitors and Cdc25A independent of cyclin D1-Cdk4 function. Mol Cell Biol 21:794–810
152. **Lamb J, Ladha MH, McMahon C, Sutherland RL, Ewen ME** 2000 Regulation of the functional interaction between cyclin D1 and the estrogen receptor. Mol Cell Biol 20:8667–8675
153. **Nielsen NH, Emdin SO, Cajander J, Landberg G** 1997 Deregulation of cyclin E and D1 in breast cancer is associated with inactivation of the retinoblastoma protein. Oncogene 14:295–304
154. **Kenny FS, Hui R, Musgrove EA, Gee JM, Blamey RW, Nicholson RI, Sutherland RL, Robertson JF** 1999 Overexpression of cyclin D1 messenger RNA predicts for poor prognosis in estrogen receptor-positive breast cancer. Clin Cancer Res 5:2069–2076
155. **Foster JS, Wimalasena J** 1996 Estrogen regulates activity of cyclin-dependent kinases and retinoblastoma protein phosphorylation in breast cancer cells. Mol Endocrinol 10:488–498
156. **Stendahl M, Nilsson S, Wigerup C, Jirström K, Jönsson PE, Stål O, Landberg G** 2010 $p27^{Kip1}$ is a predictive factor for tamoxifen treatment response but not a prognostic marker in premenopausal breast cancer patients. Int J Cancer 127:2851–2858
157. **Levin ER** 2009 Membrane oestrogen receptor α signalling to cell functions. J Physiol 587:5019–5023
158. **Manavathi B, Kumar R** 2006 Steering estrogen signals from the plasma membrane to the nucleus: two sides of the coin. J Cell Physiol 207:594–604
159. **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
160. **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
161. **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 recep-

tor-α to the plasma membrane. Proc Natl Acad Sci USA 101:2076–2081
162. **Becker MA, Ibrahim YH, Cui X, Lee AV, Yee D** 2011 The IGF pathway regulates ERα through a S6K1-dependent mechanism in breast cancer cells. Mol Endocrinol 25:516–528
163. **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
164. **Conzen SD** 2008 Nuclear receptors and breast cancer. Mol Endocrinol 22:2215–2228
165. **Kim R, Kaneko M, Arihiro K, Emi M, Tanabe K, Murakami S, Osaki A, Inai K** 2006 Extranuclear expression of hormone receptors in primary breast cancer. Ann Oncol 17:1213–1220
166. **Mann M, Krishnan S, Vadlamudi RK** 2012 Emerging significance of estrogen cancer coregulator signaling in breast cancer. Minerva Ginecol 64:75–88
167. **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
168. **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
169. **Fernando RI, Wimalasena J** 2004 Estradiol abrogates apoptosis in breast cancer cells through inactivation of BAD: Ras-dependent extranuclear pathways requiring signaling through ERK and Akt. Mol Biol Cell 15:3266–3284
170. **Björnström L, Sjöberg M** 2005 Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 19:833–842
171. **Joung I, Strominger JL, Shin J** 1996 Molecular cloning of a phospho tyrosine-independent ligand of the p56lck SH2 domain. Proc Natl Acad Sci USA 93:5991–5995
172. **Kumar R, Zhang H, Holm C, Vadlamudi RK, Landberg G, Rayala SK** 2009 Extranuclear coactivator signaling confers insensitivity to tamoxifen. Clin Cancer Res 15:4123–4130
173. **Vadlamudi RK, Rajhans R, Chakravarty D, Nair BC, Nair SS, Evans DB, Chen S, Tekmal RR** 2010 Regulation of aromatase induction by nuclear receptor coregulator PELP. J Steroid Biochem Mol Biol 118:211–218
174. **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
175. **Manavathi B, Acconcia F, Rayala SK, Kumar R** 2006 An inherent role of microtubule network in the action of nuclear receptor. Proc Natl Acad Sci USA 103:15981–15986
176. **Wang X, Yang Z, Zhang H, Ding L, Li X, Zhu C, Zheng Y, Ye Q** 2008 The estrogen receptor-interacting protein HPIP increases estrogen-responsive gene expression through activation of MAPK and AKT. Biochim Biophys Acta 1783:1220–1228
177. **Mishra SK, Yang Z, Mazumdar A, Talukder AH, Larose L, Kumar R** 2004 Metastatic tumor antigen 1 short form (MTA1s) associates with casein kinase I-γ2, an estrogen-responsive kinase. Oncogene 23:4422–4429
178. **Giancotti FG, Tarone G** 2003 Positional control of cell fate through joint integrin/receptor protein kinase signaling. Annu Rev Cell Dev Biol 19:173–206
179. **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 E2 receptor α and modulates non-genomic E2 signaling in breast cancer cells. J Cell Sci 117:1603–1611
180. **Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S** 2004 Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 73:39–85
181. **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
182. **Tommiska J, Bartkova J, Heinonen M, Hautala L, Kilpivaara O, Eerola H, Aittomäki K, Hofstetter B, Lukas J, von Smitten K, Blomqvist C, Ristimäki A, Heikkilä P, Bartek J, Nevanlinna H** 2008 The DNA damage signaling kinase ATM is aberrantly reduced or lost in BRCA1/BRCA2-deficient and ER/PR/ERBB2-triple-negative breast cancer. Oncogene 27:2501–2506
183. **Le Romancer M, Poulard C, Cohen P, Sentis S, Renoir JM, Corbo L** 2011 Cracking the estrogen receptor's posttranslational code in breast tumors. Endocr Rev 32:597–622
184. **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
185. **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
186. **Duplessis TT, Williams CC, Hill SM, Rowan BG** 2011 Phosphorylation of estrogen receptor α at serine 118 directs recruitment of promoter complexes and gene-specific transcription. Endocrinology 152:2517–2526
187. **Murphy L, Cherlet T, Adeyinka A, Niu Y, Snell L, Watson P** 2004 Phospho-serine-118 estrogen receptor-α detection in human breast tumors *in vivo*. Clin Cancer Res 10:1354–1359
188. **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
189. **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
190. **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
191. **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

192. **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
193. **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
194. **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
195. **Kirkegaard T, Witton CJ, McGlynn LM, Tovey SM, Dunne B, Lyon A, Bartlett JM** 2005 AKT activation predicts outcome in breast cancer patients treated with tamoxifen. J Pathol 207:139–146
196. **Jordan NJ, Gee JM, Barrow D, Wakeling AE, Nicholson RI** 2004 Increased constitutive activity of PKB/Akt in tamoxifen resistant breast cancer MCF-7 cells. Breast Cancer Res Treat 87:167–180
197. **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
198. **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
199. **Medunjanin S, Weinert S, Schmeisser A, Mayer D, Braun-Dullaeus RC** 2010 Interaction of the double-strand break repair kinase DNA-PK and estrogen receptor-α. Mol Biol Cell 21:1620–1628
200. **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
201. **Patrone C, Gianazza E, Santagati S, Agrati P, Maggi A** 1998 Divergent pathways regulate ligand-independent activation of ERα in SK-N-BE neuroblastoma and COS-1 renal carcinoma cells. Mol Endocrinol 12:835–841
202. **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
203. **Arnold SF, Obourn JD, Jaffe H, Notides AC** 1995 Phosphorylation of the human estrogen receptor on tyrosine 537 *in vivo* and by Src family tyrosine kinases *in vitro*. Mol Endocrinol 9:24–33
204. **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
205. **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
206. **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
207. **Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS, Katzenellenbogen JA** 2001 Estrogen receptor-β potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. J Med Chem 44:4230–4251
208. **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
209. **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
210. **Le Corre L, Chalabi N, Delort L, Bignon YJ, Bernard-Gallon DJ** 2005 Resveratrol and breast cancer chemoprevention: molecular mechanisms. Mol Nutr Food Res 49:462–471
211. **Heine GF, Parvin JD** 2007 BRCA1 control of steroid receptor ubiquitination. Sci STKE 2007:pe34
212. **La Rosa P, Pesiri V, Marino M, Acconcia F** 2011 17β-Estradiol-induced cell proliferation requires estrogen receptor (ER) α monoubiquitination. Cell Signal 23:1128–1135
213. **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
214. **Karamouzis MV, Konstantinopoulos PA, Badra FA, Papavassiliou AG** 2008 SUMO and estrogen receptors in breast cancer. Breast Cancer Res Treat 107:195–210
215. **Le Romancer M, Treilleux I, Bouchekioua-Bouzaghou K, Sentis S, Corbo L** 2010 Methylation, a key step for extranuclear estrogen signaling in breast tumors. Steroids 75:560–564
216. **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
217. **Bartel DP** 2004 MicroRNAs: genomics, biogenesis, mechanism and function. Cell 116:281–297
218. **Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM** 2004 Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 101:2999–3004
219. **Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM** 2005 miR-15 and miR-16 induce apoptosis by

targeting BCL2. Proc Natl Acad Sci USA 102:13944–13949
220. **Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY** 2007 MicroRNA-34b and microRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res 67:8433–8438
221. **Ma L, Teruya-Feldstein J, Weinberg RA** 2007 Tumour invasion and metastasis initiated by micro-RNA-10b in breast cancer. Nature 449:682–688
222. **Ucar A, Vafaizadeh V, Jarry H, Fiedler J, Klemmt PA, Thum T, Groner B, Chowdhury K** 2010 miR-212 and miR-132 are required for epithelial stromal interactions necessary for mouse mammary gland development. Nat Genet 42:1101–1108
223. **Klinge CM** 2009 Estrogen regulation of microRNA expression. Curr Genomics 10:169–183
224. **Cicatiello L, Mutarelli M, Grober OM, Paris O, Ferraro L, Ravo M, Tarallo R, Luo S, Schroth GP, Seifert M, Zinser C, Chiusano ML, Traini A, De Bortoli M, Weisz A** 2010 Estrogen receptor α controls a gene network in luminal-like breast cancer cells comprising multiple transcription factors and microRNAs. Am J Pathol 176:2113–2130
225. **Ferraro L, Ravo M, Nassa G, Tarallo R, De Filippo MR, Giurato G, Cirillo F, Stellato C, Silvestro S, Cantarella C, Rizzo F, Cimino D, Friard O, Biglia N, De Bortoli M, Cicatiello L, Nola E, Weisz A** 2012 Effects of oestrogen on microRNA expression in hormone-responsive breast cancer cells. Horm Cancer 3:65–78
226. **Fujiyama-Nakamura S, Yamagata K, Kato S** 2010 Hormonal repression of miRNA biosynthesis through a nuclear steroid hormone receptor. Adv Exp Med Biol 700:43–55
227. **Wickramasinghe NS, Manavalan TT, Dougherty SM, Riggs KA, Li Y, Klinge CM** 2009 Estradiol downregulates miR-21 expression and increases miR-21 target gene expression in MCF-7 breast cancer cells. Nucleic Acids Res 37:2584–2595
228. **Bhat-Nakshatri P, Wang G, Collins NR, Thomson MJ, Geistlinger TR, Carroll JS, Brown M, Hammond S, Srour EF, Liu Y, Nakshatri H** 2009 Estradiol-regulated microRNAs control estradiol response in breast cancer cells. Nucleic Acids Res 37:4850–4861
229. **Han M, Liu M, Wang Y, Mo Z, Bi X, Liu Z, Fan Y, Chen X, Wu C** 2012 Re-expression of miR-21 contributes to migration and invasion by inducing epithelial-mesenchymal transition consistent with cancer stem cell characteristics in MCF-7 cells. Mol Cell Biochem 363:427–436
230. **Maillot G, Lacroix-Triki M, Pierredon S, Gratadou L, Schmidt S, Bénès V, Roché H, Dalenc F, Auboeuf D, Millevoi S, Vagner S** 2009 Widespread estrogen-dependent repression of microRNAs involved in breast tumor cell growth. Cancer Res 69:8332–8340
231. **Yu X, Zhang X, Dhakal IB, Beggs M, Kadlubar S, Luo D** 2012 Induction of cell proliferation and survival genes by estradiol-repressed microRNAs in breast cancer cells. BMC Cancer 12:29
232. **Castellano L, Giamas G, Jacob J, Coombes RC, Lucchesi W, Thiruchelvam P, Barton G, Jiao LR, Wait R, Waxman J, Hannon GJ, Stebbing J** 2009 The estrogen receptor-α-induced microRNA signature regulates itself and its transcriptional response. Proc Natl Acad Sci USA 106:15732–15737
233. **Lee YM, Lee JY, Ho CC, Hong QS, Yu SL, Tzeng CR, Yang PC, Chen HW** 2011 MicroRNA 34b as a tumor suppressor in estrogen-dependent growth of breast cancer cells. Breast Cancer Res 13:R116
234. **Cheng C, Fu X, Alves P, Gerstein M** 2009 mRNA expression profiles show differential regulatory effects of microRNAs between estrogen receptor-positive and estrogen receptor-negative breast cancer. Genome Biol 10:R90
235. **Yamagata K, Fujiyama S, Ito S, Ueda T, Murata T, Naitou M, Takeyama K, Minami Y, O'Malley BW, Kato S** 2009 Maturation of microRNA is hormonally regulated by a nuclear receptor. Mol Cell 36:340–347
236. **Adams BD, Furneaux H, White BA** 2007 The micro-ribonucleic acid (miRNA) mir-206 targets the human estrogen receptor-α and represses ER-α messenger RNA and protein expression in breast cancer cell lines. Mol Endocrinol 21:1132–1147
237. **Kondo N, Toyama T, Sugiura H, Fujii Y, Yamashita H** 2008 Mir-206 expression is down-regulated in estrogen receptor α-positive human breast cancer. Cancer Res 68:5004–5008
238. **Pandey DP, Picard D** 2009 miR-22 inhibits estrogen signaling by directly targeting the estrogen receptor α mRNA. Mol Cell Biol 29:3783–3790
239. **Xiong J, Yu D, Wei N, Fu H, Cai T, Huang Y, Wu C, Zheng X, Du Q, Lin D, Liang Z** 2010 An estrogen receptor α suppressor, microRNA-22, is downregulated in estrogen receptor α-positive human breast cancer cell lines and clinical samples. FEBS J 277:1684–1694
240. **Zhao Y, Deng C, Wang J, Xiao J, Gatalica Z, Recker RR, Xiao GG** 2011 Let-7 family miRNAs regulate estrogen receptor α signaling in estrogen receptor positive breast cancer. Breast Cancer Res Treat 127:69–80
241. **Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E** 2007 let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell 131:1109–1123
242. **Qian P, Zuo Z, Wu Z, Meng X, Li G, Wu Z, Zhang W, Tan S, Pandey V, Yao Y, Wang P, Zhao L, Wang J, Wu Q, Song E, Lobie PE, Yin Z, Zhu T** 2011 Pivotal role of reduced let-7g expression in breast cancer invasion and metastasis. Cancer Res 71:6463–6474
243. **Cochrane DR, Cittelly DM, Howe EN, Spoelstra NS, McKinsey EL, LaPara K, Elias A, Yee D, Richer JK** 2010 MicroRNAs link estrogen receptor α status and Dicer levels in breast cancer. Horm Cancer 1:306–319
244. **Zhao JJ, Lin J, Yang H, Kong W, He L, Ma X, Coppola D, Cheng JQ** 2008 MicroRNA-221/222 negatively regulates estrogen receptor α and is associated with tamoxifen resistance in breast cancer. J Biol Chem 283:31079–31086
245. **Rao X, Di Leva G, Li M, Fang F, Devlin C, Hartman-Frey C, Burow ME, Ivan M, Croce CM, Nephew KP** 2011 MicroRNA-221/222 confers breast cancer fulvestrant resistance by regulating multiple signaling pathways. Oncogene 30:1082–1097
246. **Guttilla IK, Phoenix KN, Hong X, Tirnauer JS, Claffey KP, White BA** 2012 Prolonged mammosphere culture of

MCF-7 cells induces an EMT and repression of the estrogen receptor by microRNAs. Breast Cancer Res Treat 132: 75–85

247. **de Souza Rocha Simonini P, Breiling A, Gupta N, Malekpour M, Youns M, Omranipour R, Malekpour F, Volinia S, Croce CM, Najmabadi H, Diederichs S, Sahin O, Mayer D, Lyko F, Hoheisel JD, Riazalhosseini Y** 2010 Epigenetically deregulated microRNA-375 is involved in a positive feedback loop with estrogen receptor α in breast cancer cells. Cancer Res 70:9175–9184
248. **Spizzo R, Nicoloso MS, Lupini L, Lu Y, Fogarty J, Rossi S, Zagatti B, Fabbri M, Veronese A, Liu X, Davuluri R, Croce CM, Mills G, Negrini M, Calin GA** 2010 miR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor-α in human breast cancer cells. Cell Death Differ 17:246–254
249. **Li X, Mertens-Talcott SU, Zhang S, Kim K, Ball J, Safe S** 2010 MicroRNA-27a indirectly regulates estrogen receptor α expression and hormone responsiveness in MCF-7 breast cancer cells. Endocrinology 151:2462–2473
250. **Leivonen SK, Mäkelä R, Ostling P, Kohonen P, Haapa-Paananen S, Kleivi K, Enerly E, Aakula A, Hellström K, Sahlberg N, Kristensen VN, Børresen-Dale AL, Saviranta P, Perälä M, Kallioniemi O** 2009 Protein lysate microarray analysis to identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines. Oncogene 28: 3926–3936
251. **Hossain A, Kuo MT, Saunders GF** 2006 Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Mol Cell Biol 26:8191–8201
252. **Pinzone JJ, Stevenson H, Strobl JS, Berg PE** 2004 Molecular and cellular determinants of estrogen receptor α expression. Mol Cell Biol 24:4605–4612
253. **Thomas C, Gustafsson JÅ** 2011 The different roles of ER subtype in cancer biology and therapy. Nat Rev Cancer 11:597–608
254. **Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE** 1994 Methylation of the E2 receptor gene CpG island marks loss of E2 receptor expression in human breast cancer cells. Cancer Res 54:2552–2555
255. **Lapidus RG, Nass SJ, Butash KA, Parl FF, Weitzman SA, Graff JG, Herman JG, Davidson NE** 1998 Mapping of ER gene CpG island methylation by methylation-specific polymerase chain reaction. Cancer Res 58:2515–2519
256. **Champagne FA, Curley JP** 2008 Maternal regulation of E2 receptor α methylation. Curr Opin Pharmacol 8:735–739
257. **Yang X, Ferguson AT, Nass SJ, Phillips DL, Butash KA, Wang SM, Herman JG, Davidson NE** 2000 Transcriptional activation of estrogen receptor α in human breast cancer cells by histone deacetylases inhibition. Cancer Res 60:6890–6894
258. **Bovenzi V, Momparler RL** 2001 Antineoplastic action of 5-aza-2-deoxycytidine and histone deacetylase inhibitor and their effect on the expression of retinoic acid receptor and estrogen receptor genes in breast carcinoma cells. Cancer Chemother Pharmacol 48:71–76
259. **Stoica A, Pentecost E, Martin MB** 2000 Effects of arsenite on estrogen receptor α expression and activity in MCF7 breast cancer cells. Endocrinology 141:3595–3602
260. **Sundar SN, Marconett CN, Doan VB, Willoughby Sr JA, Firestone GL** 2008 Artemisinin selectively decreases functional levels of estrogen receptor-α and ablates estrogen-induced proliferation in human breast cancer cells. Carcinogenesis 29:2252–2258
261. **Garcia-Morales P, Saceda M, Kenney N, Kim N, Salomon DS, Gottardis MM, Solomon HB, Sholler PF, Jordan VC, Martin MB** 1994 Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. J Biol Chem 269:16896–16901
262. **Jang SY, Jang SW, Ko J** 2011 Celastrol inhibits the growth of estrogen positive human breast cancer cells through modulation of estrogen receptor α. Cancer Lett 300:57–65
263. **Huang Y, Keen JC, Pledgie A, Marton LJ, Zhu T, Sukumar S, Park BH, Blair B, Brenner K, Casero Jr RA, Davidson NE** 2006 Polyamine analogues down-regulate estrogen receptor α expression in human breast cancer cells. J Biol Chem 281:19055–19063
264. **Yao Y, Zhou Q** 2010 A novel antiestrogen agent Shikonin inhibits estrogen-dependent gene transcription in human breast cancer cells. Breast Cancer Res Treat 121:233–240
265. **Martin MB, Angeloni SV, Garcia-Morales P, Sholler PF, Castro-Galache MD, Ferragut JA, Saceda M** 2004 Regulation of estrogen receptor-α expression in MCF-7 cells by taxol. J Endocrinol 180:487–496
266. **Guilbaud N, Pichon MF, Faye JC, Bayard F, Valette A** 1988 Modulation of estrogen receptors by phorbol diesters in human breast MCF7 cell line. Mol Cell Endocrinol 56: 157–163
267. **Fortunati N, Bertino S, Costantino L, De Bortoli M, Compagnone A, Bandino A, Catalano MG, Boccuzzi G** 2010 Valproic acid restores estrogen receptor α and antiestrogen sensitivity to ER α-negative breast cancer cells. Mol Cell Endocrinol 314:17–22
268. **Helguero LA, Faulds MH, Gustafsson JA, Haldosén LA** 2005 Estrogen receptors α (ERα) and β (ERβ) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11. Oncogene 24: 6605–6616
269. **Zhou Q, Atadja P, Davidson NE** 2007 Histone deacetylase inhibitor LBH589 reactivates silenced estrogen receptor α gene expression without loss of DNA hypermethylation. Cancer Biol Ther 6:64–69
270. **Shen J, Tan C, Zhang Y, Li X, Li W, Huang J, Shen X, Tang Y** 2010 Discovery of potent ligands for estrogen receptor β by structure-based virtual screening. J Med Chem 53: 5361–5365
271. **Stoica A, Saceda M, Doraiswamy VL, Coleman C, Martin MB** 2000 Regulation of estrogen receptor-gene expression by epidermal growth factor. J Endocrinol 165:371–378
272. **Stoica A, Saceda M, Fakhro A, Joyner M, Martin MB** 2000 Role of insulin-like growth factor-I in regulating estrogen receptor-gene expression. J Cell Biochem 76: 605–614
273. **Dhasarathy A, Kajita M, Wade PA** 2007 The transcription factor snail mediates epithelial to mesenchymal transitions by repression of estrogen receptor α. Mol Endocrinol 21: 2907–2918
274. **Lewis-Wambi JS, Kim H, Curpan R, Grigg R, Sarker MA, Jordan VC** 2011 The selective estrogen receptor modulator bazedoxifene inhibits hormone-independent breast cancer cell growth and down-regulates estrogen receptor α and cyclin D1. Mol Pharmacol 80:610–620

275. **Swami S, Krishnan AV, Feldman D** 2000 1,25-dihydroxyvitamin D3 down regulates estrogen receptor abundance and suppresses estrogen actions in MCF7 human breast cancer cells. Clin Cancer Res 6:3371–3379
276. **Bentrem D, Dardes R, Liu H, MacGregor-Schafer J, Zapf J, Jordan V** 2001 Molecular mechanism of action at estrogen receptor α of new clinically relevant antiestrogen (GW7604) related to tamoxifen. Endocrinology 142:838–846
277. **Savoldi G, Ferrari F, Ruggeri G, Sobek L, Albertini A, Di Lorenzo D** 1995 Progesterone agonists and antagonists induce down and upregulation of estrogen receptors and estrogen inducible genes in human breast cancer cell lines. Int J Biol Markers 10:47–54
278. **Wijayaratne AL, Nagel SC, Paige LA, Christensen DJ, Norris JD, Fowlkes DM, McDonnell DP** 1999 Comparative analyses of mechanistic differences among antiestrogens. Endocrinology 140:5828–5840
279. **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
280. **Chiang CH, Cheng KW, Igarashi S, Nathwani PS, Leung PCK** 2000 Hormonal regulation of estrogen receptor and gene expression in human granulosa-luteal cells *in vitro*. J Clin Endocrinol Metab 85:3828–3839
281. **Andò S, Panno ML, Salerno M, Sisci D, Mauro L, Lanzino M, Surmacz E** 1998 Role of IRS-1 signaling in insulin-induced modulation of estrogen receptors in breast cancer cells. Biochem Biophys Res Commun 253:315–319
282. **Cho J, Kim D, Lee S, Lee Y** 2005 Cobalt chloride-induced estrogen receptor α down-regulation involves hypoxia-inducible factor-1α in MCF-7 human breast cancer cells. Mol Endocrinol 19:1191–1199
283. **Ford CE, Ekström EJ, Andersson T.** 2009 Wnt-5a signaling restores tamoxifen sensitivity in estrogen receptor-negative breast cancer cells. Proc Natl Acad Sci USA 106:3919–3924
284. **Guo S, Sonenshein GE** 2004 Forkhead box transcription factor FOXO3a regulates estrogen receptor α expression and is repressed by the Her-2/neu/phosphatidylinositol 3-kinase/Akt signaling pathway. Mol Cell Biol 24:8681–8690
285. **Madureira PA, Varshochi R, Constantinidou D, Francis RE, Coombes RC, Yao KM, Lam EW** 2006 The forkhead box M1 protein regulates the transcription of the estrogen receptor α in breast cancer cells. J Biol Chem 281:25167–25176
286. **Eeckhoute J, Keeton EK, Lupien M, Krum SA, Carroll JS, Brown M** 2007 Positive cross-regulatory loop ties GATA-3 to estrogen receptor α expression in breast cancer. Cancer Res 67:6477–6483
287. **Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li HI, Eaves CJ** 2006 Purification and unique properties of mammary epithelial stem cells. Nature 439:993–997
288. **Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE** 2006 Generation of a functional mammary gland from a single stem cell. Nature 439:84–88
289. **Stingl J** 2011 E2 and progesterone in normal mammary gland development and in cancer. Horm Cancer 2:85–90
290. **Asselin-Labat ML, Shackleton M, Stingl J, Vaillant F, Forrest NC, Eaves CJ, Visvader JE, Lindeman GJ** 2006 Steroid hormone receptor status of mouse mammary stem cells. J Natl Cancer Inst 98:1011–1014
291. **Asselin-Labat ML, Vaillant F, Sheridan JM, Pal B, Wu D, Simpson ER, Yasuda H, Smyth GK, Martin TJ, Lindeman GJ, Visvader JE** 2010 Control of mammary stem cell function by steroid hormone signalling. Nature 465:798–802
292. **Dick JE** 2003 Breast cancer stem cells revealed. Proc Natl Acad Sci USA 100:3547–3549
293. **Reya T, Morrison SJ, Clarke MF, Weissman IL** 2001 Stem cells, cancer, and cancer stem cells. Nature 414:105–111
294. **Dontu G, El-Ashry D, Wicha MS** 2004 Breast cancer, stem/progenitor cells and the estrogen receptor. Trends Endocrinol Metab 15:193–197
295. **Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA** 2008 The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133:704–715
296. **Visvader JE** 2011 Cells of origin in cancer. Nature 469:314–322
297. **Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF** 2003 Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988
298. **Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G** 2007 ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 1:555–567
299. **Visvader JE, Lindeman GJ** 2008 Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 8:755–768
300. **Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur MH, Diebel ME, Monville F, Dutcher J, Brown M, Viens P, Xerri L, Bertucci F, Stassi G, Dontu G, Birnbaum D, Wicha MS** 2009 Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res 69:1302–1313
301. **Croker AK, Goodale D, Chu J, Postenka C, Hedley BD, Hess DA, Allan AL** 2009 High aldehyde dehydrogenase and expression of cancer stem cell markers selects for breast cancer cells with enhanced malignant and metastatic ability. J Cell Mol Med 13:2236–2252
302. **Simões BM, Piva M, Iriondo O, Comaills V, López-Ruiz JA, Zabalza I, Mieza JA, Acinas O, Vivanco MD** 2011 Effects of estrogen on the proportion of stem cells in the breast. Breast Cancer Res Treat 129:23–35
303. **Fillmore CM, Gupta PB, Rudnick JA, Caballero S, Keller PJ, Lander ES, Kuperwasser C** 2010 Estrogen expands breast cancer stem-like cells through paracrine FGF/Tbx3 signaling. Proc Natl Acad Sci USA 107:21737–21742
304. **Kok M, Koornstra RH, Margarido TC, Fles R, Armstrong NJ, Linn SC, Van't Veer LJ, Weigelt B** 2009 Mammosphere-derived gene set predicts outcome in patients with ER-positive breast cancer. J Pathol 218:316–326
305. **Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryis-**

kaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K 2007 Molecular definition of breast tumor heterogeneity. Cancer Cell 11:259–273

306. **Morimoto K, Kim SJ, Tanei T, Shimazu K, Tanji Y, Taguchi T, Tamaki Y, Terada N, Noguchi S** 2009 Stem cell marker aldehyde dehydrogenase 1-positive breast cancers are characterized by negative estrogen receptor, positive human epidermal growth factor receptor type 2, and high Ki67 expression. Cancer Sci 100:1062–1068
307. **Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, Pilotti S, Pierotti MA, Daidone MG** 2005 Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 65:5506–5511
308. **Clarke RB, Spence K, Anderson E, Howell A, Okano H, Potten CS** 2005 A putative human breast stem cell population is enriched for steroid receptor-positive cells. Dev Biol 277:443–456
309. **Xu WR, Lin HS, Chen XY, Zhang Y** 2011 Estrogen receptor of breast cancer stem cells depending on the original breast cancers. Med Hypotheses 77:71–73
310. **O'Brien CS, Howell SJ, Farnie G, Clarke RB** 2009 Resistance to endocrine therapy: are breast cancer stem cells the culprits? J Mammary Gland Biol Neoplasia 14:45–54
311. **Zhao C, Dahlman-Wright K, Gustafsson JA** 2008 Estrogen receptor β: an overview and update. Nucl Recept Signal 6:e003
312. **Roger P, Sahla ME, Mäkelä S, Gustafsson JA, Baldet P, Rochefort H** 2001 Decreased expression of receptor β protein in proliferative preinvasive mammary tumors. Cancer Res 61:2537–2541
313. **Loveday RL, Greenman J, Simcox DL, Speirs V, Drew PJ, Monson JR, Kerin MJ** 2000 Genetic changes in breast cancer detected by comparative genomic hybridisation. Int J Cancer 86:494–500
314. **Bürki NG, Caduff R, Walt H, Moll C, Pejovic T, Haller U, Ward DC** 2000 Comparative genomic hybridisation of fine needle aspirates from breast carcinomas. Int J Cancer 88:607–613
315. **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
316. **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
317. **Treeck O, Juhasz-Boess I, Lattrich C, Horn F, Goerse R, Ortmann O** 2008 Effects of exon-deleted estrogen receptor β transcript variants on growth, apoptosis and gene expression of human breast cancer cell lines. Breast Cancer Res Treat 110:507–520
318. **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
319. **Zhao C, Matthews J, Tujague M, Wan J, Ström A, Toresson G, Lam EW, Cheng G, Gustafsson JA, Dahlman-Wright K** 2007 Estrogen receptor β2 negatively regulates the transactivation of estrogen receptor α in human breast cancer cells. Cancer Res 67:3955–3962
320. **Ogawa S, Inoue S, Watanabe T, Orimo A, Hosoi T, Ouchi Y, Muramatsu M** 1998 Molecular cloning and characterization of human estrogen receptor βcx: a potential inhibitor of estrogen action in human. Nucleic Acids Res 26:3505–3512
321. **Das SK, Tan J, Raja S, Halder J, Paria BC, Dey SK** 2000 Estrogen targets genes involved in protein processing, calcium homeostasis, and Wnt signaling in the mouse uterus independent of estrogen receptor-α and -β. J Biol Chem 275:28834–28842
322. **Wada-Hiraike O, Hiraike H, Okinaga H, Imamov O, Barros RP, Morani A, Omoto Y, Warner M, Gustafsson JA** 2006 Role of estrogen receptor β in uterine stroma and epithelium: insights from estrogen receptor $\beta-/-$ mice. Proc Natl Acad Sci USA 103:18350–18355
323. **Filardo EJ, Quinn JA, Bland KI, Frackelton Jr AR** 2000 Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. Mol Endocrinol 14:1649–1660
324. **Thomas P, Pang Y, Filardo EJ, Dong J** 2005 Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology 146:624–632
325. **Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER** 2005 A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307:1625–1630
326. **Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ** 1997 Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. Genomics 45:607–617
327. **Albanito L, Madeo A, Lappano R, Vivacqua A, Rago V, Carpino A, Oprea TI, Prossnitz ER, Musti AM, Andò S, Maggiolini M** 2007 G protein-coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17β-estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. Cancer Res 67:1859–1866
328. **Prossnitz ER, Oprea TI, Sklar LA, Arterburn JB** 2008 The ins and outs of GPR30: a transmembrane estrogen receptor. J Steroid Biochem Mol Biol 109:350–353
329. **Albanito L, Sisci D, Aquila S, Brunelli E, Vivacqua A, Madeo A, Lappano R, Pandey DP, Picard D, Mauro L, Andò S, Maggiolini M** 2008 Epidermal growth factor induces G protein-coupled receptor 30 expression in estrogen receptor-negative breast cancer cells. Endocrinology 149:3799–3808
330. **Pandey DP, Lappano R, Albanito L, Madeo A, Maggiolini M, Picard D** 2009 Estrogenic GPR30 signalling induces proliferation and migration of breast cancer cells through CTGF. EMBO J 28:523–532
331. **Filardo EJ, Graeber CT, Quinn JA, Resnick MB, Giri D, DeLellis RA, Steinhoff MM, Sabo E** 2006 Distribution of GPR30, a seven membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. Clin Cancer Res 12:6359–6366

332. **Otto C, Fuchs I, Kauselmann G, Kern H, Zevnik B, Andreasen P, Schwarz G, Altmann H, Klewer M, Schoor M, Vonk R, Fritzemeier KH** 2009 GPR30 does not mediate estrogenic responses in reproductive organs in mice. Biol Reprod 80:34–41
333. **Pedram A, Razandi M, Levin ER** 2006 Nature of functional estrogen receptors at the plasma membrane. Mol Endocrinol 20:1996–2009
334. **Filardo EJ, Quinn JA, Sabo E** 2008 Association of the membrane estrogen receptor, GPR30, with breast tumor metastasis and transactivation of the epidermal growth factor receptor. Steroids 73:870–873
335. **Kleuser B, Malek D, Gust R, Pertz HH, Potteck H** 2008 17-β-Estradiol inhibits transforming growth factor-β signaling and function in breast cancer cells via activation of extracellular signal-regulated kinase through the G protein-coupled receptor 30. Mol Pharmacol 74:1533–1543
336. **Wang D, Hu L, Zhang G, Zhang L, Chen C** 2010 G protein-coupled receptor 30 in tumor development. Endocrine 38:29–37
337. **Lapensee EW, Tuttle TR, Fox SR, Ben-Jonathan N** 2009 Bisphenol A at low nanomolar doses confers chemoresistance in estrogen receptor-α-positive and -negative breast cancer cells. Environ Health Perspect 117:175–180
338. **Ruan SQ, Wang ZH, Wang SW, Fu ZX, Xu KL, Li DB, Zhang SZ** 2012 Heregulin-β1-induced GPR30 upregulation promotes the migration and invasion potential of SkBr3 breast cancer cells via ErbB2/ErbB3-MAPK/ERK pathway. Biochem Biophys Res Commun 420:385–390
339. **De Marco P, Bartella V, Vivacqua A, Lappano R, Santolla MF, Morcavallo A, Pezzi V, Belfiore A, Maggiolini M** 19 March 2012 Insulin-like growth factor-I regulates GPER expression and function in cancer cells. Oncogene doi: 10.1038/onc.2012.97
340. **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
341. **Lee LM, Cao J, Deng H, Chen P, Gatalica Z, Wang ZY** 2008 ER-α36, a novel variant of ER-α, is expressed in ER-positive and -negative human breast carcinomas. Anticancer Res 28:479–483
342. **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
343. **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 signalling. Proc Natl Acad Sci USA 103:9063–9068
344. **Lacassagne A** 1936 Hormonal pathogenesis of adenocarcinoma of the breast. Am J Cancer 27:217–225
345. **Jordan VC, Morrow M** 1999 Tamoxifen, raloxifene and the prevention of breast cancer. Endocr Rev 20:253–278
346. **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
347. **Brueggemeier RW, Hackett JC, Diaz-Cruz ES** 2005 Aromatase inhibitors in the treatment of breast cancer. Endocr Rev 26:331–245
348. **Morris C, Wakeling A** 2002 Fulvestrant ('Faslodex')—a new treatment option for patients progressing on prior endocrine therapy. Endocr Relat Cancer 9:267–276
349. **Saxena NK, Sharma D** 2010 Epigenetic reactivation of estrogen receptor: promising tools for restoring response to endocrine therapy. Mol Cell Pharmacol 2:191–202
350. **Sappok A, Mahlknecht U** 2011 Ribavirin restores ESR1 gene expression and tamoxifen sensitivity in ESR1 negative breast cancer cell lines. Clin Epigenetics 3:8
351. **Vansteenkiste J, Van Cutsem E, Dumez H, Chen C, Ricker JL, Randolph SS, Schöffski P** 2008 Early phase II trial of oral vorinostat in relapsed or refractory breast, colorectal, or non-small cell lung cancer. Invest New Drugs 26:483–488
352. **Luu TH, Morgan RJ, Leong L, Lim D, McNamara M, Portnow J, Frankel P, Smith DD, Doroshow JH, Wong C, Aparicio A, Gandara DR, Somlo G** 2008 A phase II trial of vorinostat (suberoylanilide hydroxamic acid) in metastatic breast cancer: a California Cancer Consortium study. Clin Cancer Res 14:7138–7142
353. **Ramaswamy B, Bhalla K, Cohen B, Pellegrino C, Hershman D, Chuang E, Somlo G, Goetz M, Swaby R, Hopkins U, Christos P, Espinoza-Delgado I, Sparano JA** 2009 Phase II study of the histone deacetylase inhibitor (HDACi) vorinostat plus paclitaxel and bevacizumab in metastatic breast cancer (MBC). New York Cancer consortium trial P7703. Proc 100th Annual Meeting of the American Association for Cancer Research, Denver, 2009 (Abstract 09-AB4116)
354. **Munster PN, Thurn KT, Thomas S, Raha P, Lacevic M, Miller A, Melisko M, Ismail-Khan R, Rugo H, Moasser M, Minton SE** 2011 A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. Br J Cancer 104:1828–1835
355. **Pathiraja TN, Stearns V, Oesterreich S** 2010 Epigenetic regulation in estrogen receptor positive breast cancer—role in treatment response. J Mammary Gland Biol Neoplasia 15:35–47
356. **Hwang C, Giri VN, Wilkinson JC, Wright CW, Wilkinson AS, Cooney KA, Duckett CS** 2008 EZH2 regulates the transcription of estrogen-responsive genes through association with REA, an estrogen receptor corepressor. Breast Cancer Res Treat 107:235–242
357. **Reijm EA, Jansen MP, Ruigrok-Ritstier K, van Staveren IL, Look MP, van Gelder ME, Sieuwerts AM, Sleijfer S, Foekens JA, Berns EM** 2011 Decreased expression of EZH2 is associated with upregulation of ER and favorable outcome to tamoxifen in advanced breast cancer. Breast Cancer Res Treat 125:387–394
358. **McDonnell DP, Chang CY, Norris JD** 2000 Development of peptide antagonists that target estrogen receptor-cofactor interactions. J Steroid Biochem Mol Biol 74:327–335
359. **Shao D, Berrodin TJ, Manas E, Hauze D, Powers R, Bapat A, Gonder D, Winneker RC, Frail DE** 2004 Identification

of novel estrogen receptor α antagonists. J Steroid Biochem Mol Biol 88:351–360

360. **Leduc AM, Trent JO, Wittliff JL, Bramlett KS, Briggs SL, Chirgadze NY, Wang Y, Burris TP, Spatola AF** 2003 Helix-stabilized cyclic peptides as selective inhibitors of steroid receptor-coactivator interactions. Proc Natl Acad Sci USA 100:11273–11278
361. **Yudt MR, Koide S** 2001 Preventing estrogen receptor action with dimer-interface peptides. Steroids 66:549–558
362. **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 estradiol stimulated MCF-7 cells. EMBO J 20:6050–6059
363. **Arteaga CL** 2011 The phosphatidylinositol-3 kinase/mTOR pathway: new agents. Breast Cancer Res 13(Suppl 2):O8
364. **Mayer E, Baurain J, Sparano J, Strauss L, Campone M, Fumoleau P, Rugo H, Awada A, Sy O, Llombart-Cussac A** 2009 Dasatinib in advanced HER2/neu amplified and ER/PR-positive breast cancer: phase II study CA180088. J Clin Oncol 27(Suppl S):1011