

# Estrogen receptor-positive breast cancer molecular signatures and therapeutic potentials (Review)

MEI HONG ZHANG, HONG TAO MAN, XIAO DAN ZHAO, NI DONG and SHI LIANG MA

College of Biological Science and Biotechnology, Shenyang Agricultural University, Shenyang, Liaoning 110866, P.R. China

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**Abstract.** In this review, the advances in the study of breast cancer molecular classifications and the molecular signatures of the luminal subtypes A and B of breast cancer were summarized. Effective clinical outcomes depend mainly on successful preclinical diagnosis and therapeutic decisions. Over the last few years, the ever-expanding investigations focusing on breast cancer diagnosis and the clinical trials have provided accumulating information on the molecular characteristics of breast cancer. Specifically, among the estrogen receptor (ER)-positive types of breast cancer, the luminal subtype A breast cancer has been shown to exhibit good clinical outcomes with endocrine therapy, whereas the luminal subtype B breast cancer represents the more complicated type, diagnostically as well as therapeutically. Furthermore, even in luminal subtype A breast cancer, the resistance to treatment has become the major limitation for endocrine-based therapy. Accumulating molecular data and further clinical trials may enable more accurate diagnostic and therapeutic decisions. The molecular signatures have emerged as a powerful tool for future diagnosis and therapeutic decisions, although currently available data are limited.

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*Correspondence to:* Professor Shi Liang Ma, College of Biological Science and Biotechnology, Shenyang Agricultural University, 120 Dongling Road, Shenyang, Liaoning 110866, P.R. China  
E-mail: msl@syau.edu.cn

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## 1. Introduction

Breast cancer is one of the major causes of cancer-related morbidity and mortality among women worldwide (1). Breast cancers originate from the epithelial cells of the normal mammary gland. The ducts are lined with luminal epithelial cells, which give rise to the majority of breast cancers (2). As a heterogeneous disease, breast cancer encompasses a wide variety of pathological entities and this heterogeneity is reflected by the differences in cell type composition and proportions, the differences in the proliferation ability between glandular and myoepithelial cells, the proliferation of progenitor cells, the therapeutic responses and patient outcomes (3,4). Breast cancer patients with the same clinical diagnostic and prognostic profiles may exhibit markedly different clinical overall outcomes and treatment responses (5), which may be due to the current breast cancer taxonomies based on the morphological groups, dividing the disease into clinical classes (6). Therefore, the clinical behavior of cancer is not solely dependent on morphology and a molecular taxonomy based on 'signature' profiles may facilitate a more accurate prediction of response to therapy and prognosis (7).

The current molecular classifications of breast cancer molecular subtypes are generally based on the gene expression profiles according to i) luminal cell-related markers, such as cytokeratins (CKs); ii) hormone receptors, such as estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR); iii) growth factor receptors, such as human epidermal growth factor receptor (HER); iv) anti-apoptosis markers, such as Bcl-2 and p53; v) cell proliferation indicators, such as Ki-67 and survivin; vi) cell invasion-related factors, such as matrix metalloproteinases (MMPs) and integrins; vii) signal transduction pathway members, such as the PI3K/AKT pathway members phosphatidylinositol-3-kinase (PI3K) and AKT; viii) cell cycle control members, such as cyclins and cyclin-dependent kinases (CDKs); ix) epithelial-to-mesenchymal transition-indicating factors and regulating factors, such as cadherins and zinc-finger transcription factors Snail, Slug, Zeb1 and Twist; x) metastatic control factors; and xi) blood vessel-forming control factors (8-10). This spectrum also includes stem cell markers, tumor cell and microenvironment interacting factors and other small regulatory molecules, such as microRNAs or other non-coding RNAs. The currently established molecular classification of breast cancers distinguishes breast cancer molecular subtypes into five intrinsic subtypes: i) luminal subtype A (ER<sup>+</sup>

and/or PR<sup>+</sup>, HER2<sup>-</sup> and CK8/18<sup>+</sup>); ii) luminal subtype B (ER<sup>+</sup> and/or PR<sup>+</sup>, HER2<sup>+</sup> and CK8/18<sup>+</sup>); iii) HER2-enriched subtype (ER<sup>-</sup> and/or PR<sup>-</sup> and HER2<sup>+</sup>); iv) basal-like subtype [ER<sup>-</sup> and/or PR<sup>-</sup>, HER2<sup>-</sup>, CK5/6<sup>+</sup>, CK14<sup>+</sup>, CK17<sup>+</sup> and epithelial growth factor receptor (EGFR)<sup>+</sup>]; and v) normal breast-like type (ER<sup>-</sup> and/or PR<sup>-</sup>, HER2<sup>-</sup>, CK5/6<sup>-</sup>, CK14<sup>-</sup>, CK17<sup>-</sup>, EGFR<sup>-</sup>) (11-14) (Table I). Another subtype, referred to as the claudin-low subtype, was later described (15,16). Furthermore, a subpopulation of the luminal A subtype with a Ki-67 proliferation index of >14% was designated as the luminal B subtype (17). As such, the breast cancer molecular subtypes were redefined as follows: luminal A (ER<sup>+</sup> and/or PR<sup>+</sup>, HER2<sup>-</sup> and Ki-67 ≤14%); luminal B (ER<sup>+</sup> and/or PR<sup>+</sup>, HER2<sup>-</sup> and Ki-67 >14%); luminal B HER2/neu<sup>+</sup> (ER<sup>+</sup> and/or PR<sup>+</sup>, HER2<sup>+</sup> and any Ki-67); HER2/neu subtype (ER<sup>-</sup> and PR<sup>-</sup>, HER2/neu<sup>+</sup> and any Ki-67); and triple-negative subtype (ER<sup>-</sup>, PR<sup>-</sup>, HER2<sup>-</sup> and any Ki-67) (18-20).

The luminal type of breast cancer tends to be morphologically well differentiated and exhibits a relatively good prognosis, whereas the ER<sup>-</sup> tumors are poorly differentiated and exhibit a poor prognosis. The designation of the luminal type of breast cancer was derived from the finding that this type of breast cancer exhibits mRNA and protein expression of CKs 8/18 (14), which is typically associated with luminal epithelial cells, as opposed to basal cells, which express CKs 5/6. The luminal type of breast cancer was further subdivided into A and B subtypes, with the luminal B subtype exhibiting significant expression differences and worse outcomes (11). Thus, the luminal A and B subtypes are collectively referred to as the luminal type, which accounts for 65-70% of breast cancers, whereas the HER2-enriched subtype accounts for ~10% of breast cancers and the basal-like subtype accounts for 10-15% (14) or, according to other sources, 19% of breast cancers (2,5,6). Those molecular classification studies significantly contributed to the better understanding of the complex properties of different breast cancer types, their response to systemic treatment and their clinical outcomes, including those that respond better to endocrine treatment.

Among the different molecular subtypes of breast cancer, ER<sup>+</sup> breast cancer comprises ~75% of all breast cancers (14). Thus, the ER status has become the most important discriminator of breast cancer molecular subtypes (6), resulting in primary treatment options through targeting the estrogen synthesis (aromatase inhibitors) or the ER functions (tamoxifen). The treatment effectiveness has been proven by the observation that ER<sup>+</sup> tumors were associated with hormonal responsiveness, longer relapse-free survival and overall survival compared to the ER<sup>-</sup> subtypes (21). However, resistance to hormonal therapy was reported in 30% of ER<sup>+</sup> breast cancers, whereas hormonal responsiveness was observed in 5-15% of ER<sup>-</sup> breast cancers (22). Thus, in addition to the ER status, more detailed information associated with the molecular signature profiles and molecular networks is required for designing optimal therapeutic strategies.

## 2. Luminal A subtype breast cancer molecular signatures and therapeutic potentials

*Luminal A subtype breast cancer molecular signatures.* The luminal A subtype of breast cancer is characterized by the luminal-type conventional molecular signatures (ER, PR, Bcl-2

and CK8/18) and the luminal A subtype-specific signatures of ER<sup>+</sup> and/or PR<sup>+</sup>, HER2<sup>-</sup> and Ki-67 ≤14%, which distinguishes luminal A from luminal B subtype. The recognized luminal A subtype breast cancer molecular signatures include GATA binding protein 3 (GATA-3), X-box binding protein 1 (XBP-1), forkhead box A1 (FOXA1) and ADH1B (23-26). The 5-year survival rate of luminal type A breast cancer is 95%, which is the highest among the five types, with a p53 mutation rate of 13% (11).

Studies of the crosstalk between estrogen receptor  $\alpha$  (ER $\alpha$ ), FOXA1 and GATA-3 revealed that, in addition to the ER and PR status, FOXA1 and GATA-3 are also correlated with the luminal A subtype (11,14,25,27). The interaction of FOXA1 with the *cis*-regulatory regions of heterochromatin enhances the binding of ER $\alpha$  to DNA (28) and is involved in controlling almost 50% of the estrogen receptor target genes (29,30). The expression of FOXA1 is significantly positively correlated with the markers of good prognosis or ER-positivity (31) and FOXA1 was recently shown to be required for almost all the ER-binding events in breast cancer cells (32). The transcription factor GATA-3 was recently identified as a key factor involved in luminal cell differentiation in the mammary gland (33). The majority of breast cancers arise from the luminal epithelial cells; therefore, GATA-3 appears to regulate a set of genes involved in the differentiation and proliferation of breast cancer cells (33). Low GATA-3 expression is significantly associated with a higher histological grade, poor differentiation, positive lymph nodes, ER<sup>-</sup> and PR<sup>-</sup> status and HER2/neu overexpression, which are all indicators of poor prognosis (34). The expression of GATA-3 is strongly associated with the expression of ER $\alpha$  in breast cancer and there is increasing evidence that GATA-3 may be used as a clinical molecular signature to determine the response to hormonal therapy and to refine the prognosis of breast cancer patients (33,35,36). The prognosis of the luminal A subtype breast cancer is more favorable compared to that of other subtypes (2,6), which may be due to the fact that the luminal A subtype expresses high levels of GATA-3 that confer a favorable prognosis. It was previously reported that GATA-3 functions as a critical regulator of commitment and maturation of cancer cells in the luminal A epithelial lineage, i.e., the expression of GATA-3 regulates luminal differentiation (37). The anti-apoptotic marker Bcl-2 has been proven to be an independent molecular signature, alone or in combination with Ki-67 as a marker pair, in the luminal type of breast cancer (38-42).

The 70-gene signature (MammaPrint<sup>®</sup>) assay provides a powerful prognostic gene expression signature profile for the prediction of distant recurrence and survival of primary breast cancer, including luminal subtype A breast cancer (43). Other molecular profilings of clinically applicable gene expression-based prognostic panels, such as the Oncotype DX platform, may add more characteristics regarding the prediction of recurrence and survival and the therapeutic options of luminal A type breast cancer (44,45). It was recently demonstrated that microRNA expression profiles may be a promising molecular signature for the classification of breast cancer subtypes (46). The prediction of ER<sup>+</sup> cancer clinical relapse and the sensitivity to endocrine therapy with genomic signatures, such as the PAM50 intrinsic subtyping and risk of relapse (PAM50-ROR) score, the 21-gene assay

Table I. Molecular subtype signatures of breast cancer.

Classification	Signature genes	Signaling pathways	Clinical grade	Therapeutic options	5-year survival rate	p53-mutation	Refs.
Luminal A	Marker genes: ER <sup>+</sup> and/or PR <sup>+</sup> , HER2 <sup>-</sup> , CK8/18 <sup>+</sup> , GATA-3, XBP-1, FOXA1 and ADH1B gene overexpression	Estradiol response	I	Tamoxifen; anastrozole (Arimidex)	95%	13%	(11,14,23,24,26)
Luminal B	Marker genes: ER <sup>+</sup> and/or PR <sup>+</sup> , HER2 <sup>+</sup> , CK8/18 <sup>+</sup> , FGFR1, HER1, Ki-67 and/or cyclin E1, CCNB1 and MYBL2 gene overexpression	IGF-1 FGF PI3K	I (III also observed)	Bevacizumab combined with paclitaxel, tamoxifen combined with small-molecule inhibitors or antibodies against IGF-1R/IR, FGF, FGFR, PI3K and EGFR/HER2	50%	40%	(11,12,14,17,70,84,110,111)
ErbB2/HER2 <sup>+</sup>	Marker genes: ER <sup>-</sup> and/or PR <sup>-</sup> , HER2 <sup>+</sup> and GRB7 overexpression	IGF-1 HER2	More likely III	Trastuzumab (Herceptin), lapatinib (Tykerb). For patients with resistance to trastuzumab, combine with a PI3K/mTOR inhibitor	30%	71%	(11,14,112,113)
Basal-like	Marker genes: ER <sup>-</sup> and/or PR <sup>-</sup> , HER2 <sup>-</sup> , CK5/6 <sup>+</sup> , CK14 <sup>+</sup> , CK17 <sup>+</sup> , EGFR <sup>+</sup> HER1 and/or c-Kit, FOXC1, p63, P-cadherin, vimentin and laminin overexpression	IGF-1 Wnt/ $\beta$ -catenin	More likely III	Chemotherapy; antiangiogenic agents; platinum salts; PARP inhibitors	30%	83%	(11,12,14,23,112,114-117)
Normal breast-like	Marker genes: ER <sup>-</sup> and/or PR <sup>-</sup> , HER2 <sup>-</sup> , CK5/6 <sup>-</sup> , CK14 <sup>-</sup> , CK17 <sup>-</sup> , EGFR <sup>-</sup> and ADH1B overexpression				50%	33%	(11,14,26)

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; GATA-3, GATA binding protein 3; XBP-1, X-box binding protein 1; FOXA1, forkhead box A1; FGFR1, fibroblast growth factor receptor 1; MYBL2, myeloblastosis oncogene-like 2; CK, cytokeratin; ADH, alcohol dehydrogenase; GRB, growth factor receptor-bound protein; IGF, insulin-like growth factor; PI3K, phosphatidylinositol-3-kinase; mTOR, mammalian target of rapamycin; PARP, poly ADP ribose polymerase.

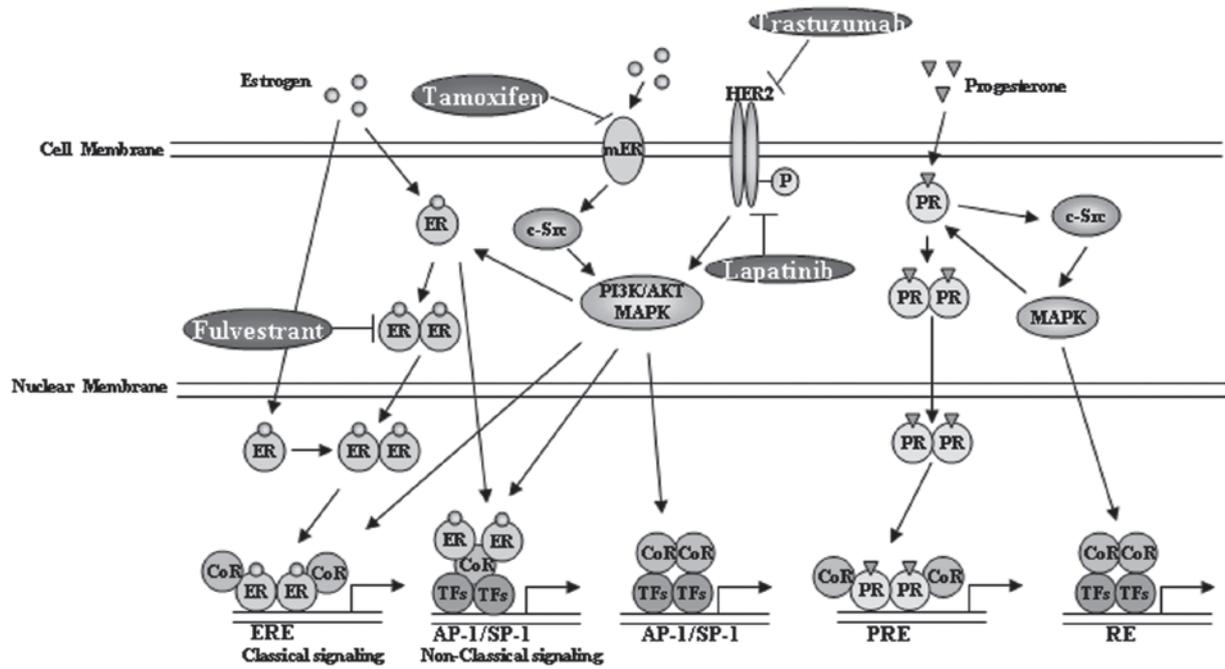


Figure 1. The estrogen receptor (ER) pathway includes the canonical and the non-canonical ER pathways. In the canonical pathway, the cytoplasmic estrogens bind directly to the nuclear membrane estrogen receptor and activate signaling. In the non-canonical pathway, the extracellular estrogens bind to the plasma membrane receptors and activate the phosphatidylinositol-3-kinase (PI3K) or Ras signaling pathway. Alternatively, the estrogens first penetrate through the cell membrane and then bind to the estrogen receptor monomer, which is then dimerized and transported into the nucleus to activate signaling. Treatment with tamoxifen blocks the binding of estrogen and, thus, inhibits the estrogen-activated signaling. Treatment with fulvestrant inhibits the cytoplasmic dimerization of estrogen. Treatment with aromatase inhibitors, such as letrozole, anastrozole and exemestane blocks estrogen production. Another characteristic correlated to the ER pathway is the progesterone receptor (PR) signaling, which is also involved in the regulation of luminal type breast cancer development. CoR, coregulators; RE, response element; TFs, transcription factors; mER, membrane ER; HER, human epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; ERE, estrogen response element; PRE, progesterone response element.

(Oncotype DX) recurrence score, the MammaPrint assay score and the Rotterdam 76-gene assay score, demonstrated that low-risk tumors (>90% distal recurrence-free survival at 8.5 years) were identified mostly as luminal A tumors (47).

#### *Luminal A subtype breast cancer therapeutic potentials.*

Estrogen is a steroid hormone that is crucial for growth, development and reproduction (48). Estrogens have been shown to play an important role in human breast cancer development and ~1/3 of breast cancers are stimulated by estradiol (49). Estrogens exert their effects through the action of the estrogen receptors  $\alpha$  and  $\beta$  (ER $\alpha$  and ER $\beta$ ), which belong to the steroid hormone superfamily of nuclear receptors (NRs) (50,51). ERs are members of the large NR family of transcription factors that are typically activated upon binding to small lipophilic molecules (52). The activities of steroid receptors, particularly ERs, have been associated with the regulation of breast epithelial cell cycle transition from the G0 into the S phase (53,54). Since ~75% of breast cancers express ER $\alpha$ , the significance of ER $\alpha$  in breast cancer is well-established (55,56).

Since luminal subtype A and B breast cancer cells are ER $^{+}$  and/or PR $^{+}$ , patients with these two types of breast cancer are always subjected to endocrine therapy with tamoxifen, to inhibit the functions of ER (57), and aromatase inhibitors or inactivators (58), to block estrogen production or to inactivate ERs, or even to inhibit the dimerization and downregulate ERs (59-61). The therapeutic strategies that may interrupt the estrogen signaling pathway have been proven effective for

the ER $^{+}$  subtype of breast cancer (62) and are currently the first-line clinical treatment option (63,64) (Fig. 1).

However, in ER $^{+}$  patients, the clinical therapeutic practices with endocrine therapy to antagonize ER signaling were proven to be 30% effective (65). Hormonal therapy with tamoxifen may only be beneficial for tumors exhibiting nuclear ER expression, since membrane ERs may activate EGFR and/or HER2 signaling, which may then stimulate tumor growth (66) (Fig. 2). Other signaling pathways may also be involved, such as the insulin-like growth factor 1 (IGF-1) or the vascular endothelial growth factor (VEGF) signaling pathways (67,68) (Fig. 2). Thus, for ER $^{+}$  breast cancers, different molecular subtypes have been further described, such as the five-biomarker panel (69) signatored by ER, PR, HER2, CK 5/6 and EGFR. The primary considerations regarding treatment options are the gene signature profiles, including the Ki-67 status (17) that distinguishes luminal A from luminal B subtype; the nuclear ER signaling pathway components that distinguish luminal A from other subtypes; the HER2 signaling pathway components that distinguish the ER $^{+}$ /PR $^{+}$  with HER2 $^{+}$  from the HER2 $^{-}$  subtype; and the EGFR, or IGF-1, VEGF and PI3K/AKT signaling pathway components. To overcome the ineffectiveness of tamoxifen treatment, luminal type breast cancers are also subjected to other therapeutic strategies, such as treatment with the anti-VEGF antibody bevacizumab combined with paclitaxel, which has also been proven effective (70). Treatment with motesanib (which is a novel, highly selective inhibitor of

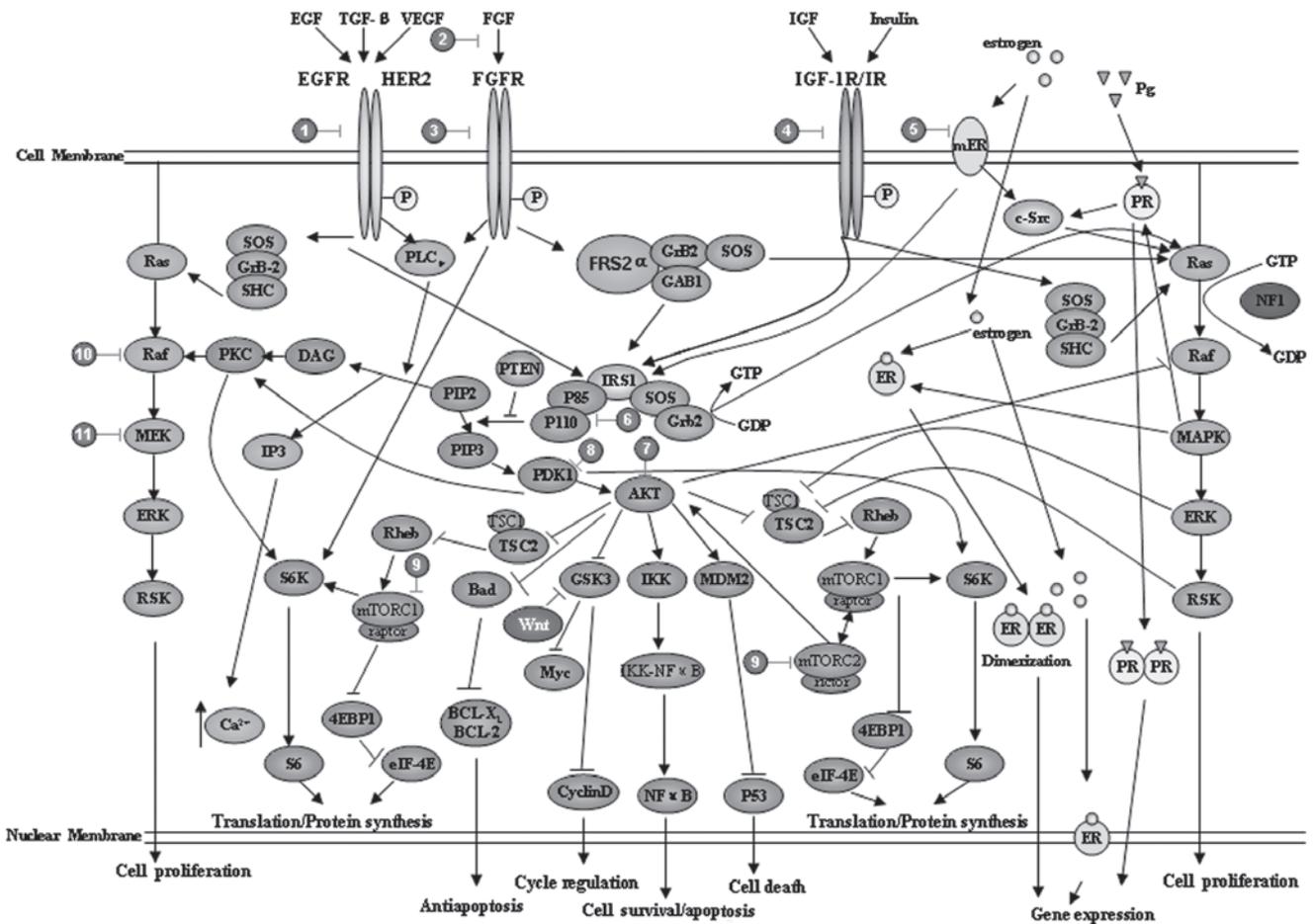


Figure 2. Crosstalk of signaling pathways in breast cancer and the potential clinical therapeutic targets. The receptors of extracellular small molecules shown here include: epidermal growth factor (EGF), transforming growth factor (TGF)- $\beta$ , vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF)-1, insulin and estrogen, which activate their corresponding receptors and further transduce the signals mainly through the phosphatidylinositol-3-kinase (PI3K)/AKT pathway or the Ras/MEK pathway. A number of PI3K/AKT and Ras/MEK signaling pathway inhibitors have been developed, some of which may be used in combination (details also summarized in Table II). Pg, progesterone; ER, estrogen receptor; PR, progesterone receptor; ERK, extracellular signal-regulated kinase; RSK, ribosomal S6 kinase; IP, inositol phosphate; PKC, protein kinase C; PTEN, phosphatase and tensin homolog; PDK, phosphoinositide-dependent kinase; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase.

VEGFR1, VEGFR2, VEGFR3, KIT and platelet-derived growth factor receptor) in combination with the ER modulator tamoxifen also resulted in a significant reduction in tumor volume (71). Of note, inhibition of the PI3K pathway signaling in endocrine treatment-resistant breast cancer cell lines was shown to reduce cell survival and improve the treatment response to endocrine agents, providing further scientific rationale to target both ERs and the PI3K pathway in order to improve the outcome of ER<sup>+</sup> breast cancer (72).

Based on the molecular signatures of luminal A subtype breast cancer, the luminal A subtype signature genes, such as GATA-3 and FOXA1, also appear to be promising therapeutic targets. Among these, the expression of FOXA1 was positively correlated with ER-positivity, particularly luminal A type ER-positivity, and negatively correlated with tumor size, tumor grade, nodal status, the expression of Ki-67 and HER2 and basal-like subtype of breast cancer (31). A previous study reported that the expression of FOXA1 was positively correlated with ER<sup>+</sup> and PR<sup>+</sup> status, but inversely correlated with nuclear grade and the Ki-67 index, suggesting the therapeutic potentials of FOXA1 targeting (73). Moreover, the forkhead

box O3a (FOXO3a) transcription factor was identified as an intracellular mediator of ER $\alpha$  expression and an important downstream target of the PI3K/AKT pathway, thus representing a potential therapeutic target in ER<sup>+</sup> breast cancer (74).

An alternative way for the therapeutic considerations in luminal A subtype of breast cancer is targeting other members that are coexpressed with ER in the superfamily of steroid receptors, including estrogen-related receptors, PRs (Fig. 1), ARs, glucocorticoid and mineralocorticoid receptors. The PR A and B isoforms were demonstrated to play different roles in breast cancer cell growth and have thus been considered as therapeutic targets of antiprogestin (75). PR has been clinically used for evaluating ER activity (64) and the loss of PR in ER<sup>+</sup> tumors is considered to be predictive of the lack of response to hormone therapy (76). However, the estrogen response element (ERE) transcriptional activity remains a better readout of ER function, as PR is just one of the numerous ER target genes and is regulated by several other transcription factors, such as Sp1 or AP-1 (77,78). High ERE-activity is correlated with the luminal A type of breast cancer and low ERE-activity is correlated with the malignancy biomarker Ki-67 (79).

### 3. Luminal B subtype breast cancer molecular signatures and therapeutic potentials

*Luminal B subtype breast cancer molecular signatures.* The major molecular distinctions between luminal type A and B tumors are that luminal type A tumors exhibit a higher expression of ER-related genes and luminal type B tumors exhibit a higher expression of proliferation-related genes, such as CCNB1, MKI67 and myeloblastosis oncogene-like 2 (MYBL2) (2,11,23,57,80). In contrast to the luminal A subtype, the 5-year survival rate of luminal B breast cancer is 50%, with a p53 mutation rate of 40% (11), indicating the similarities between luminal B subtype and p53-mutated tumors. Badve *et al* (24) suggested that the better prognosis of luminal A compared to that of luminal B breast cancer may be due to the different function of ER in luminal A and B cancers and the effect of additional factors, such as coactivators, corepressors and transcription factors that modulate ER $\alpha$  activity.

In addition to sharing similar signatures with luminal subtype A, such as ER and Bcl-2, luminal subtype B tumors also share similar signatures with the basal-like subtype tumors, including the proliferation markers Ki-67, survivin and CCNB1, as well as similar signatures with the HER2 subtype, such as the overexpression of HER2 (4). The Ki-67 proliferation index was previously used as a potential unidimensional proliferation marker to distinguish luminal B from luminal A tumors (17). Thus, the Ki-67 index is the most useful signature that distinguishes high-risk luminal B from low-risk luminal A tumors (81). In addition to the differences in ER-related and proliferation-related gene expression, emerging evidence demonstrated the amplification of growth receptor signaling genes in luminal type B tumors, such as the overexpression of fibroblast growth factor receptor 1 (FGFR1) in luminal type B cancer patients (82), which may contribute to the poor prognosis of luminal B compared to luminal A cancer patients (2,11), despite their clinical ER $^+$  status (83). Thus, the typical signature genes in luminal subtype B tumors include FGFR1, HER1, cyclin E1 and Ki-67 (2,11,12,17,82). Using the luminal A subtype as a reference, according to the multivariate analysis of untreated early-stage breast cancer, the relapse-free survival of luminal B breast cancer exhibited a hazard ratio of 2.43 ( $P < 0.0001$ ), similar to ErbB2/HER2 amplified tumors with a hazard ratio of 2.53 ( $P = 0.00012$ ) (23,84). The immunohistochemical analysis further demonstrated that ~20% of luminal B cancers were HER2 $^+$  and ~30% of HER2-expressing tumors were of the luminal B subtype (85). Clinically, the luminal (A and B) type breast cancers are often grade I; however, the luminal B breast cancers are often of the HER2 $^+$  genotype and are more likely to be high-grade compared to luminal A cancers (6,11,57). More comprehensive gene signature profiles of the luminal type breast cancers have been provided by assays based on the platforms of the 21-gene signature assay Oncotype DX (Genomic Health, Redwood City, CA, USA) and the 70-gene signature-based MammaPrint assay (Agendia, Amsterdam, The Netherlands), as well as others (8,86).

Although luminal B cancers are ER $^+$ , they do not appear to exhibit a corresponding expression of estrogen-regulated genes and may therefore depend on alternative pathways for growth. Candidate pathways that may be targeted in luminal B

cancer cells include those involving growth factor receptors, such as HER2 and EGFR, as well as the PI3K/AKT/mTOR pathway. The standards for distinguishing luminal A from luminal B cancers are the Ki-67 index (cut-off value, 14%), clinicopathological factors, such as age at diagnosis, intensity score of ER and PR, histological grade, Bcl-2 (cut-off value, 33%) and disease-free survival (DFS).

*Luminal B subtype breast cancer therapeutic potentials.* Similar to luminal A, luminal B breast cancer is currently treated as an ER $^+$ , hormone-sensitive disease (20,86). However, luminal B breast cancer is often considered as a more aggressive form of the luminal (ER $^+$ ) type of breast cancer. The PAM50 assay results demonstrated that, although luminal subtype B breast cancers share some gene expression patterns with luminal subtype A breast cancers (e.g., ER genes ESR1, FOXA1 and Bcl-2), they also share some gene expression patterns with basal-like breast cancers (e.g., Ki-67 gene MKI67, survivin gene BIRC5 and cyclin B1 gene CCNB1) (87).

A major characteristic of luminal type B breast cancer cells is the expression of the HER2 gene (4,88). The immunohistochemical results demonstrated that 30% of HER2 $^+$  breast cancer cells are luminal type B and in this type of cell the PI3K/AKT, Ras/mitogen-activated protein kinase (MAPK) and phospholipase C $\gamma$  (PLC $\gamma$ )/protein kinase C (PKC) signaling pathways are also involved (84) (Fig. 2 and Table II). Thus, recent clinical trials considered targeting of alternative pathways in luminal B cancer, such as using the drug gefitinib to target EGFR (89) and everolimus to target PI3K/AKT/mTOR (90). Numerous small-molecule inhibitors or antibodies targeting these signaling pathway elements have been designed and investigated, such as BMS-754807, cixutumumab, MK-0646, dalotuzumab, OSI-906 and CP-758171. Other examples include targeting the IGF-1R pathway with TKI-258 and AZD-4547 and targeting the PI3K/AKT pathway with MK-2206, XL-147 and XL-765 (84).

Another characteristic of luminal B tumors is the high expression levels of Ki-67 combined with HER2 expression, exhibiting high scores in the Oncotype DX gene expression profile. Thus, for patients with HER2 $^+$  and ER $^+$  tumors, combination treatment with endocrine and anti-HER2 therapy may achieve therapeutic benefits (91). In addition, the Ki-67 and p53 signature subtypes mainly belong to the luminal B subtype (92,93), preconditioning for endocrine resistance (94). Moreover, previous studies demonstrated that, following neoadjuvant endocrine treatment, changes in the expression of Ki-67 may predict long-term outcome (95,96). Among the prognostic factors, ER  $< 10\%$ , Ki-67  $> 14\%$  and HER2 overexpression are considered as risk factors (97).

In luminal B-type tumors, the high expression levels of Ki-67 combined with HER2 expression exhibit high scores in the Oncotype DX gene expression profile; thus, for patients with HER2 $^+$  and ER $^+$  tumors, the combination of endocrine and anti-HER2 therapy may achieve therapeutic benefits (91). However, in ER $^+$ /PR $^-$  luminal breast tumors, aggressive behavior and tamoxifen resistance are characteristic, despite the ER $^+$  status (98). This subtype of luminal type breast cancers was classified as luminal B tumors, with greater genomic instability and a higher proliferation rate, as well as elevated growth factor signaling and membranous ER

Table II. Inhibitors or antibodies for signaling pathway elements in breast cancer.

No.	Target	Target agents	Refs.
1	HER2	Trastuzumab (Herceptin); pertuzumab (Omnitarg); CP-724,714; TAK165; CI-1033; 2C4; AG1478; ARRY-380	(113,118-120)
	EGFR/HER2	Lapatinib (Tykerb); AEE-788; BMS-599626; ARRY-334543; BIBW2992; HKI-272; MP-412; CI-1033 (canertinib); neratinib (HKI-272); CUDC-101; AZD8931; BMS-599626; ARRY-334543; dacomitinib (PF-00299804); TAK-285	(113,121-128)
	EGFR	Gefitinib (ZD-1839, Iressa); CPI-358,774; cetuximab; OSI-774; PD153035; erlotinib	(118, 129, 130)
	VEGFRs	Regorafenib (BAY 73-4506) <sup>a</sup> ; BAY 43-9006; OSI-930; SU11248 <sup>b</sup> ; axitinib (AG-013736); cediranib (AZD2171); vandetanib (Zactima, ZD6474); motesanib diphosphate (AMG-706); E7080 (lenvatinib); ENMD-2076; telatinib (BAY 57-9352); linifanib (ABT-869); apatinib (YN968D1) <sup>c</sup> ; cabozantinib (XL184); RAF265 (CHIR-265)	(71,131-144)
	EGFR/VEGFRs	AEE-788; EXEL-7647 (XL647)	(113,145)
2	FGF	1A6; FP-1039; palifermin (Kepivance)	(146)
3	FGFRs	SU5402; PD173074; TKI-258; BIBF 1120; BMS-582,664 (brivanib); E7080; TSU-68; IMC-A1; PRO-001; R3Mab; AZD-4547; ENMD-2076; AZD4547	(84,139,146,147)
4	IGF-1R	BMS-754807; NVP-AEW541	(84,148)
	IGF-1R/IR	Cixutumumab; MK-0646; dalotuzumab; CP-758171; BMS-554417; BMS-536924; GSK1904529A; OSI-906 (linsitinib); AG-1024 (Tyrphostin); GSK1838705A	(84,125,149-152)
5	ER	ICI 182,780; tamoxifen; Casodex; fulvestrant; letrozole; anastrozole; exemestane	(60,153,154)
6	PI3K	Wortmannin; BKM120; LY294002; XL-147; GDC-0941; PX-866; ZSTK474; SF1126; IC486068; fused heteroaryl and imidazopyridine-based inhibitors; WAY-266176; WAY-266175; PIK75	(60,84,111,129,153, 155-157)
	PI3K/mTOR	BEZ235; XL-765; GSK2126458; PKI-402; GDC-0980; PF-05212384 (PKI-587)	(60,84,158-161)
7	AKT	MK-2206; PX-316; perifosine (KYX-0401); UCN-01; GSK690693; AT7867; PHT-427; triciribine	(84,129,162-165)
8	PDK1	OSU-03012; PHT-427; BX-795; BX-912; BX-320;	(129,164,166)
9	mTORC1	Everolimus; rapamycin; RAD-001; temsirolimus (CCI-779); ridaforolimus (AP23573; MK-8669); PF-04691502;	(60,167-170)
	mTORC1/2	OXA-01; OSI-027; AZD8055; WYE-125132 (WYE-132); GSK2126458; GDC-0980; WAY-600; WYE-687; WYE-354; AZD2104; IKK-128; XL388	(133,158,171-176)
10	Raf	Regorafenib (BAY 73-4506) <sup>b</sup> ; sorafenib (BAY 43-9006, Nexavar); RAF265	(131,132,144)
11	MEK1/2(MAPK)	UO126; PD98059; CI-1040 (PD184352); UCN-01; PD318088	(119,153,156,177,178)

<sup>a</sup>Regorafenib (BAY 73-4506) is an oral multikinase inhibitor of VEGFR1/2/3, PDGFR $\beta$ , KIT, RET and Raf-1 (131). <sup>b</sup>SU11248, an oral multitargeted TKI with antiangiogenic and antitumor activity, inhibits VEGF, PDGF, KIT and FLT3 receptor TKs (70). <sup>c</sup>Apatinib (YN968D1) is a small-molecule TKI that inhibits VEGFR2 (Flk-1/KDR), RET (rearranged during transfection), c-Kit (stem cell factor receptor) and c-Src tyrosine kinases (142). HER, human epidermal growth factor receptor; ER, estrogen receptor; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; FGF, fibroblast growth factor receptor; IGF, insulin-like growth factor; PI3K, phosphatidylinositol-3-kinase; mTOR, mammalian target of rapamycin; PDK, phosphoinositide-dependent kinase; MAPK, mitogen-activated protein kinase; PDGFR, platelet-derived growth factor receptor; FLT, FMS-like tyrosine kinase; TKI, tyrosine kinase inhibitor.

activity (98). Luminal subtype B cancer patients also exhibit a higher expression of HER1 and HER2 and active growth factor signaling mediated by the PI3K/AKT/mTOR pathway (98) (Fig. 2 and Table II). Thus, the optimal treatment approach

for this subset of patients may be the combination of aromatase inhibitors, fulvestrant and chemotherapy. It was also demonstrated that PR<sup>+</sup> luminal B tumors that were treated with neoadjuvant chemotherapy (adriamycin/cyclophosphamide)

exhibited a significantly improved response compared to other types of tumors (99).

Higher PI3K signature scores have been observed in ER<sup>+</sup> tumors and the cell lines of the more aggressive luminal B compared to those of the less aggressive luminal A molecular subtype, suggesting that targeting PI3K in these tumors may reverse the loss of ER expression and signaling and restore hormonal sensitivity (100). Inhibition of PI3K pathway signaling in endocrine-resistant luminal B subtype breast cancer cells reduces cell survival and improves treatment response to endocrine agents (72), providing further potential therapeutic strategies by targeting both the ER and the PI3K pathway, in order to improve the outcome of ER<sup>+</sup> breast cancer. In the ER<sup>+</sup>/PR<sup>-</sup> luminal B subtype breast cancer cells, the binding of estrogen or tamoxifen to the ER on the plasma membrane activates EGFR and HER2 and their downstream signaling pathways, including MAPK and PI3K/AKT/mTOR pathway, leading to tamoxifen resistance (76,101,102). The corresponding strategies for this type of breast cancer are aromatase inhibitors, fulvestrant and chemotherapy, plus targeted therapies, such as gefitinib, to overcome tamoxifen resistance (103).

Charafe-Jauffret *et al* (104) demonstrated that ErbB2 is mainly overexpressed in luminal breast cancer cell lines, rather than in basal-like breast cancer cell lines. Additional characteristics of the luminal type B breast cancer cells include the expression of CCNB1, MKI67, MYBL2, FGFR1 and ZNF703 genes that are associated with resistance to tamoxifen and poor treatment outcome (25,105,106). Thus, the treatment effectiveness of tamoxifen for luminal subtype B is lower compared to that for luminal type A breast cancers. Other luminal subtype B breast cancer signature genes, such as ZNF703, identified in luminal subtype B breast cancer, were shown to modulate luminal B cancer stem cells (106). The NHERF1 gene, expressed in luminal B subtype breast cancer, was associated with a worse survival in ER<sup>+</sup> breast cancer and the low NHERF1 expression genotype may benefit from endocrine therapy (107). The P-Rex1 gene, a Rac-specific guanine nucleotide exchanging factor, is highly overexpressed in ErbB2<sup>+</sup> and ER<sup>+</sup> double-positive luminal type breast cancers, thus suggesting the possibility of targeting the P-Rex1/Rac signaling pathway for luminal subtype B breast cancer therapy (98). Bergamaschi *et al* (108) demonstrated that the elevated 14-3-3 $\zeta$  expression promoted resistance to endocrine therapy with tamoxifen in luminal B cancer patients and corresponding to the elevated 14-3-3 $\zeta$  expression were genes such as aurora kinase B, polo-like kinase 1, CDC25B, BIRC5/survivin and FOXM1, providing a wider target selection base for luminal B subtype breast cancers. In a previous study of a 96-patient cohort, Glynn *et al* (109) reported that retinoic acid receptor  $\alpha$  levels were significantly higher in HER2/neu<sup>+</sup> and ER<sup>+</sup> patients compared to HER2/neu<sup>-</sup> and ER<sup>-</sup> patients, adding another gene expression signature to the therapeutic options for HER2/neu<sup>+</sup> and ER<sup>+</sup> luminal subtype B breast cancer.

#### 4. Challenges and perspectives

The present molecular classifications assigned ER<sup>+</sup> breast cancer into two categories: the luminal subtype A, characterized by ER<sup>+</sup>/PR<sup>+</sup>/HER2<sup>-</sup>/low Ki-67; and the luminal subtype B,

characterized by ER<sup>+</sup>/PR<sup>+</sup>/HER2<sup>+</sup> or ER<sup>+</sup>/PR<sup>+</sup>/high Ki-67. Based on these molecular signatures, effective diagnostic and therapeutic decisions are enabled, with subsequent improved clinical outcomes. However, these simplified signatures are unable to accurately represent the complex intrinsic processes of tumor cell growth. The details of the molecular processes within the tumor cell have not been elucidated, nor have the mechanisms of treatment resistance, the clear panorama of the molecular networks and the molecular cascade upon treatment. In addition, the treatment interventions in the tumor suppressor genes have not been adequately investigated and the effect of the microenvironment on treatment efficacy has not been determined. The applications of several molecular signature sets, including the Oncotype DX and MammaPrint assays mentioned earlier, have been demonstrated to be powerful tools enabling more accurate predictions and effective therapeutic decisions. The prediction values of these assays have also been confirmed when combined with other approaches. However, due to the heterogeneous properties of the breast cancer subtypes and the complex molecular processes underlying breast cancer development, these molecular signature sets are not considered sufficient for practical clinical application. In addition, accumulating data have demonstrated the individual differences based on genomic sequencing results of clinical samples. Although the individual genomic differences may not reflect the decisive molecular mechanisms, they are a reminder of the heterogeneity and complexity of the decision-making process. The combination of the present molecular signature sets with accumulating data obtained by the new-generation sequencing technology and the high throughput gene expression quantification technology may enable obtaining more reliable molecular signature sets for future diagnosis and prediction of human breast cancer.

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