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Steroid Hormone Receptors: Links With Cell Cycle Machinery and Breast Cancer Progression

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Progression of cells through cell cycle consists of a series of events orchestrated in a regulated fashion. Such processes are influenced by cell cycle regulated expression of various proteins where multiple families of transcription factors take integral parts. Among these, the steroid hormone receptors (SHRs) represent a connection between the external hormone milieu and genes that control cellular proliferation. Therefore, understanding the molecular connection between the transcriptional role of steroid hormone receptors and cell cycle deserves importance in dissecting cellular proliferation in normal as well as malignant conditions. Deregulation of cell cycle promotes malignancies of various origins, including breast cancer. Indeed, SHR members play crucial role in breast cancer progression as well as management. This review focuses on SHR-driven cell cycle regulation and moving forward, attempts to discuss the role of SHR-driven crosstalk between cell cycle anomalies and breast cancer.

Keywords: steroid hormone receptors (SHRs), cell cycle, breast cancer, estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR)

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INTRODUCTION

Cell cycle progression is a finely regulated process with several checks and balances ensuring that division and proliferation is a favored outcome. The fidelity of cell cycle is regulated by three vital checkpoints, governing the boundaries at G1/S, G2/M, and metaphase/anaphase transition (1). Execution of cell cycle is aided by timely functioning of these checkpoints and associated proteins, deregulation of which influences the occurrence of malignancy (2). For instance, checkpoint error during mitotic progression might result in generation of daughter cells with altered ploidy level (aneuploidy), a hallmark prevalent in almost 70% of solid human tumors (3). Thus, the cell cycle and cancer are found intertwined: cell cycle regulates cell proliferation, and cancer is a disease of unchecked cell proliferation.

As a measure of regulation, there are families of transcription factors which take charge in the timely expression of the cell cycle specific protein molecules. Among them, "steroid hormone receptors (SHRs)" represent an important group of transcription factors, having roles in cellular growth and proliferation (4). Members of this family act upon binding to a steroid hormone ligand. Importantly, evidences accumulated the involvement of SHRs in various cancers and showed their immense potential in targeted therapy (5). Indeed, two members of this family (Estrogen receptor/ER, and Progesterone receptor/PR) are well documented in breast tumor biology as well as they act as clinically established targets in breast cancer (6). Additionally, another member of the SHR

family, androgen receptor (AR), is also gaining importance, specifically in the field of triple negative breast cancer (7). ER and PR (and also, AR) have verified functions in different phases of cell cycle. These cell cycle specific functions and mostly their alterations ought to be instrumental in bringing breast malignancy. With a brief section covering the structure and general functions of SHR, this review focuses on finding the SHR-driven cross-talk between cell cycle and breast cancer.

BIOLOGY OF STEROID HORMONE RECEPTORS (SHRS): STRUCTURE AND FUNCTION

As per the present literatures, the members comprising the SHR family are ER, PR, AR, mineralocorticoid receptor (MR), and glucocorticoid receptor (GR) (8). Though this review centers around ER, PR, and AR, all the SHR members structurally comprise of five domains, as discussed below (**Figure 1**).

A/B: N-terminal domain (NTD): This is a highly variable domain and harbors a little similarity in terms of sequence and size among the SHR members. It also possesses at least one activator function-1 region (AF-1), that acts in a ligand-independent manner (9, 10). The NTD is the target for varied post-translational modifications with variable effects in driving or repressing transcription (11).

C: DNA binding domain (DBD): Among the domains of SHR, the centrally located and mostly preserved DBD contains two zinc finger motifs with each having four cysteine residues. An associated amphipathic helix and an amide loop trail each of these two motifs. Of them, the primary one comprises of the DNA-reading helix that communicates with the principal depression to establish base-explicit associations with DNA. The next motif that has the peptide loop to promote the dimerization of receptors by means of the distal or D box, attaches vaguely with DNA (11, 12).

D: Hinge Region: This region is a small, versatile, and the least conserved amino acid sequence connecting the DBD and LBD. Although the precise function of this domain is unclear, it acts as a site of post translational modifications (PTMs), associated with increased transcriptional activation. Moreover, a nuclear localization signal is also present within this domain (11, 12).

E: Ligand-binding domain (LBD): This domain exhibits a ligand-dependent activation function motif (AF-2), comprising of three helices (3, 4, and 12) present at the C-terminal end. It recruits several coregulators by means of changing conformation of helix 12, upon interacting with ligand. LBD is structurally preserved to a lesser extent and has 11–13 α-helices and four β-strands, organized into three layers in parallel, to create a hydrophobic pocket for ligand binding (LBP). The LBD of SHR shows a higher degree of identity at the upper part, however, the lower part containing LBP confers considerable variation across SHR members to facilitate the interaction of various cadres of ligands (11, 12).

Classical Mode of SHR functions

In absence of a steroid hormone ligand, SHR remains sequestered by a chaperone, heat shock protein 90 (Hsp90) (4, 8). In the classical mode of SHR activation, after ligand docking, a ligand-specific conformational modification of the receptor happens, permitting its dissociation from restrictive Hsp90 complex, followed by auto-phosphorylation (4, 8, 13). These modifications trigger homo- or heterodimerization of SHR, followed by its nuclear translocation and subsequent binding to hormone response element (HRE) in a target gene. This, in turn, initiates the SHR-driven transcriptional regulation of a target gene (4, 8). The ligand-induced conformational modification of a SHR also facilitates its association/dissociation with coregulator complexes, during the course of transcription (4, 8, 12, 14).

In case of estrogen receptor (ER), there exists two isoforms, ER-α and ER-β, each of them translated from completely different genes with similar however not identical structure (15, 16). E2 (17β-estradiol) is the steroid hormone ligand that binds to ER, changing its conformation and activating ER-mediated transcription. E2-activated ERs homodimerize and function as transcription factors, upon interacting with estrogen response element (ERE) on a target gene (17). Likewise, there

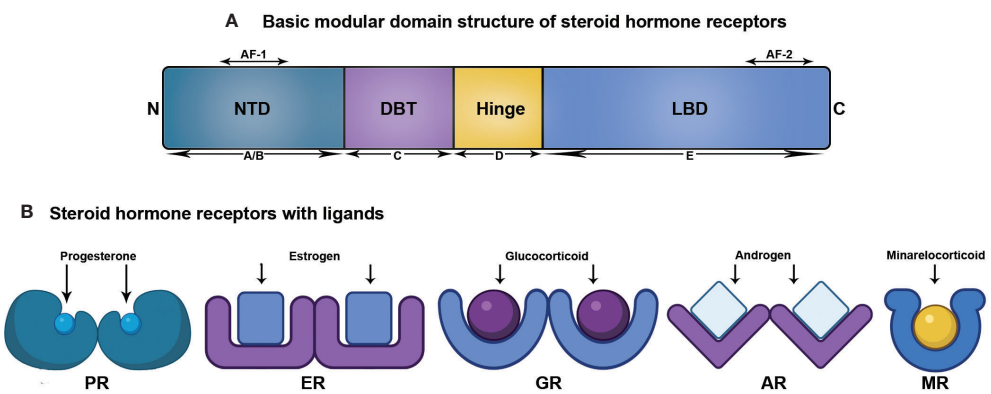

FIGURE 1 | Structural organization of SHRs associated with ligand. **(A)** Linear representation of domains of SHRs. **(B)** SHRs and their corresponding ligand.

exists two isoforms of progesterone receptor (PR): PR-A is deleted for 164 amino acids at the N-terminal end, while PR-B refers to the entirely intact receptor. An additional isoform, PR-C is represented with a truncation in the DBD and is efficient in impeding the function of PR-B. Progesterone acts through PR-A and PR-B; however, the functional roles of PR-C remain to be characterized (18).

The classical or genomic androgen receptor (AR) signaling pathway involves the dissociation of chaperones from the androgen-activated AR, followed by its translocation to the nucleus. Dimerized AR attaches to androgen response element (ARE) within the genome, leading to AR-mediated transcription of the target genes. This is assisted by recruitment of several coregulators, resulting in the expression of androgen-regulated genes (19).

Non-Classical Mode of SHR Function

Though the members of SHR family are mostly localized in nucleus (ER and PR) or cytoplasm (AR, MR, and GR), a distinct pool of them, approximately 5%, are located at the plasma membrane, including ER, PR, and AR (20, 21). This pool is responsible of extra-nuclear signaling cascades mediated by SHR and this defines the non-classical mode of SHR function. The membrane trafficking of these receptors is initiated by palmitoylation of an internal cysteine residue, which is driven by two palmitoylacyltransferase proteins, DHHC7 and DHHC21 (22, 23). Cav-1 transports the palmitoylated receptors to the caveolae rafts in the membrane. Indeed, Cav-1 also serves as a scaffold protein for different signaling molecule assembly, including G-protein-coupled receptors, protein kinase C, components of the MAPK pathway, and endothelial nitric oxide synthase (eNOS), to SHR. Although membrane-associated signaling pathways are extensively diverse for different steroid hormones, some of the common pathways include: ion fluxes (mostly calcium), secondary messengers such as inositol trisphosphate (IP_3) and cyclic AMP (cAMP), activation of PKC and the extracellular signal regulated kinase–MAPK (ERK-MAPK), and PI3K-Akt pathways (20, 21, 24, 25). Membrane steroid receptors (mSRs) are also reported to mediate the quick non-genomic effects of steroid hormones through inhibition of adenylate cyclase (AC) and cyclic nucleotide (cAMP) production and activation of MAPK (26).

Membrane-coupled ER, upon ligand binding, dimerizes which in turn, makes it interacting to G protein alpha and beta/gamma subunits. This results in various rapid signal transduction events including early kinase (e.g. SRC) activation (21). In endothelial cells, membrane localized caveole-bound ER can activate endothelial nitric oxide synthase (eNOS) through protein kinase-mediated phosphorylation (27). Binding of E2 to scaffolded ER-α in the caveole results in interaction with $G_{\alpha i}$ proteins and activation of the PI3k-Akt pathway that involves subsequent eNOs activity and rise in nitric oxide levels (28). Additionally, the presence of ER-β at the cell membrane suggests that it can mediate extranuclear actions through its association with steroid receptor coactivator (Src) (29). There is another ER variant (ER-α-36), acting at non-classical level, and is primarily localized in plasma membrane and cytosol. It expresses through alternative splicing, lacking both of the transactivation domains, as present in the classical ER-α. Upon binding to various ligands, it involves in rapid membrane-initiated mitogenic signaling pathways, such as MAPK/ERK, PI3K/Akt, and PKC-δ to regulate biological functions of cells (30).

Another integral membranous receptor is GPR30, which is coupled to Gαs in its inactive state and its activation causes heterotrimeric G proteins to induce adenylate cyclase, Src, and EGFR signaling (31, 32). Through mPR, progesterone alters signal transduction pathways by activating mitogen-activated protein kinases (MAPKs) and inhibiting adenylyl cyclase and cAMP formation. Upon progesterone binding, mPR involves in various signaling cascades, including stimulation of extracellular signal-regulated kinases 1/2 (ERK1/2orp42/44) or p38MAPKs, and stimulation of intracellular Ca2+ mobilization (33). E2-activated GPR30 can activate downstream adenylate cyclase, AKT, and MAPK signaling (34). Further, reduction of GPR30 expression is reported to stop growth stimulation of TNBC cells (that lack ER-α) by E2 (35, 36); conversely, different reports counsel that GPR30/GPER expression correlates with higher prognosis in ER-α positive carcinoma patient (36, 37).

The extranuclear activity of progesterone is channeled by membrane progesterone receptor (mPR) and also the membrane-bound progesterone receptor membrane component one (PGRMC1). In most of the cell models examined, progestin-bound mPR-α involves in alteration of second messenger pathways, by activating pertussis toxin-sensitive inhibitory G-proteins (38). The ligand-activated extranuclear PR can interact with c-Src, by means of a polyproline (PPD) domain within its N-terminal domain. This interaction triggers rapid activation of Src/Ras/Raf/MAPK and other downstream targets (39). In human myometrial cells, mPR-α can activate p38, a MAPK, that phosphorylates myosin light chain protein in these cells (40). Another signaling pathway, which is executed in Atlantic croaker oocytes through mPR-α, is the Akt/PI3 kinase pathway (41). Progestin induction of mPR-α can either increase (42) or decrease (40) the cAMP production through the involvement of two different G proteins. A number of studies suggest both mPR-α and PGRMC1 act in concert to initiate calcium mobilization from intracellular compartments, upon progestin binding (43, 44). PGRMC1 also contains binding sites for Src homology 2 (SH2) and Src homology 3(SH3) domain-containing proteins (45).

Studies on membrane-associated androgen receptor depicted that it can interact with AKT to activate the downstream signaling, upon androgen stimulation (46). A splice variant lacking NTD, AR45 is localized in plasma membrane that might involve in intracellular calcium signaling upon interacting with the membrane-associated Gαo and Gαq proteins (47).

Integration of Non-Classical and Classical Mode of SHR Functions

Importantly, these non-classical effects could integrate with classical mode of SHR action, in which the extranuclear

signal pathways converge on SHR-driven transcriptional regulation. Apart from kinase activation and calcium signaling at the extranuclear compartment, rapid membrane-associated pathways could result in downstream genomic effects through regulating transcription factors and cofactors (**Figure 2**).

Several studies on knockout mice enhance the current understandings of the highly coupled genomic and nongenomic action of SHRs. As E2-dependent lipid biogenesis was decreased in a nuclear receptor knockout mice, it indicates extensive membrane ER signaling, resulting in phosphorylation and sequestration of the transcription factor SREBP1c at cytoplasm. This renders the critical lipid synthetic genes to remain inactive in the nucleus (48). Contrariwise, the mice expressing only nuclear ER that exhibited the membrane-initiated ERK-MAPK cascade by membranous ER-α, was extremely necessary in driving the recruitment of nuclear ER to the promoter and subsequent transactivation of pS2 gene (49). Another study discussed the role of AR in cell motility and invasiveness in TNBC, where the authors documented AR/Src assembly upon androgen induction (50). This complex triggers downstream non-classical signaling, that involves the recruitment of p85α, the regulatory subunit of PI3K and, FAK (Focal Adhesion Kinase). The activated PI3K phosphorylates Akt at Ser 473 residue, while autophosphorylation of FAK (on Tyr 397) results in Tyr 118 phosphorylation of paxillin. These two signaling cascades act in concert to promote migration and invasiveness of TNBC cells (50, 51). On a similar note, a membrane-localized splice variant of AR, AR8, is reported to promote the interaction of Src and AR with EGFR, upon EGF stimulation. Membrane-associated AR8 brings AR in close proximity of the plasma membrane to facilitate the formation of a dynamic signaling complex, with AR, AR8, Src, and EGFR as components. Src thereafter phosphorylates AR at Tyr-534 residue to promote the nuclear translocation of AR, resulting in AR-driven transcription. Thus, this pathway acts in concert with classical mode of transcriptional activation of AR (52).

ROLE OF SHRS IN CELL CYCLE REGULATION

The unanimous role of SHRs as transcription factors is widely worked upon in various research findings (4, 8, 53). Additionally, its non-genomic role is also established through a number of elegant works (20, 21). Among these numerous findings, we focused on those directly relevant to the regulation of cell cycle.

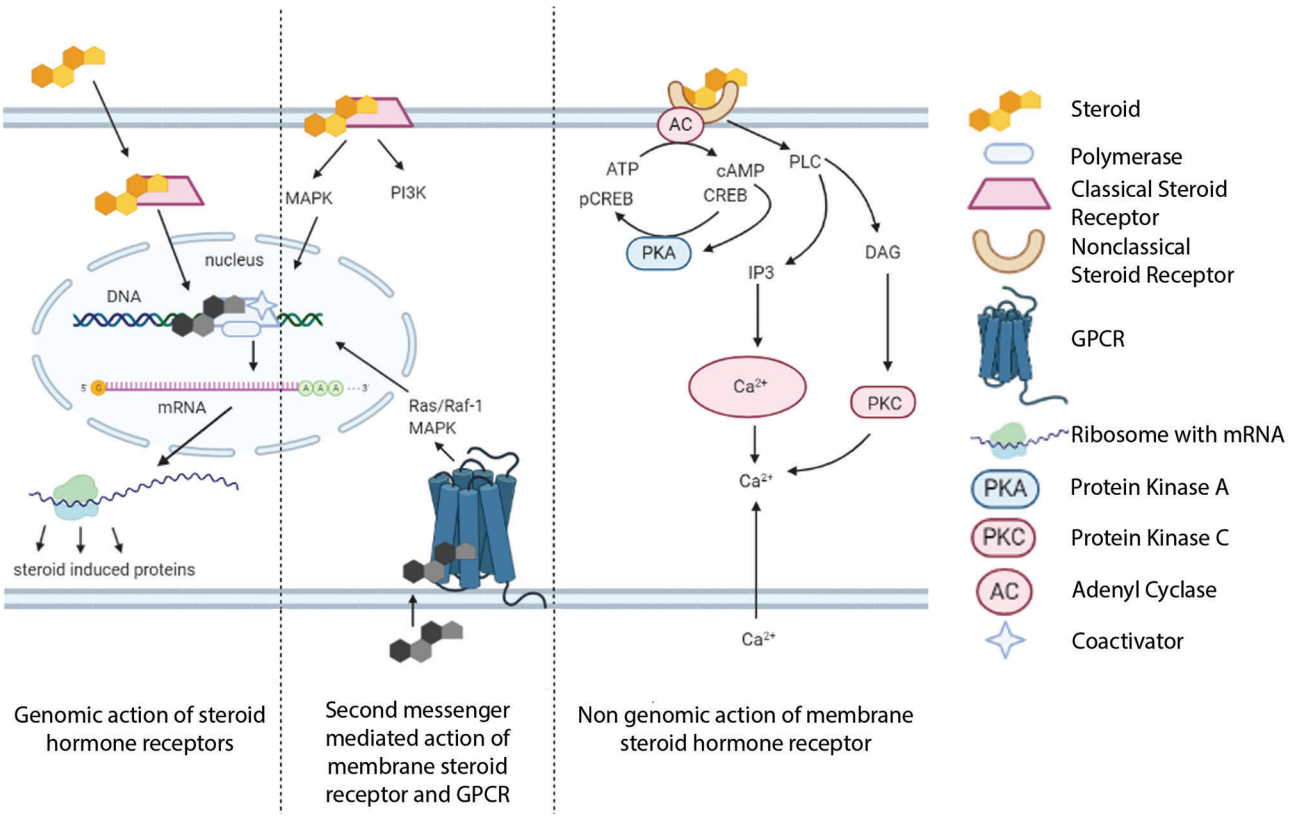

FIGURE 2 | Convergence of genomic and nongenomic mechanisms of steroid hormone receptor action. Both of these pathways together can alter biological processes.

G1 Phase

This phase regulates the rapid growth of eukaryotic cells, and is governed by a series of restrictive events. Cyclin D1 is an ER-responsive gene and this activation is of utmost importance in cellular progression through G1 phase. Ligand-bound-ER binds to an ERE site on the *CCND1* (Cyclin D1 coding) promoter to induce its expression. Cyclin D1 associates with CDK4/6, which in turn hyperphosphorylates and inactivates the retinoblastoma (Rb) tumor suppressor protein, thereby promoting G1/S transition of the cells (54, 55). Another ER-responsive gene is *c-MYC* that is involved in G1/S transition, acting in parallel to pRb/E2F pathway (56). ER, along with AP-1 transcription factors, can activate an upstream enhancer element of *c-MYC* promoter (57). Upon nuclear translocation, Estrogen-activated dimeric ER weakly seats on a half ERE present in the enhancer. This complex is stabilized by the interaction of ER and AP-1 factors (JunD and FosB), which further bind to the AP1 element. The resulting complex transactivates *MYC* gene expression (57). Similarly, ER-α interacts with p21-bound-Cyclin E/Cdk2 to cause the dissociation of p21 and the active Cyclin E/Cdk2 phosphorylates Rb, allowing the progression of cells from the G1 to S phase (58). Moreover, two reports highlighted an ERE element independent (non-genomic) mode of *CCND1* trans-regulation by ER. One such finding documented that the ER-mediated activation of CCND1 occurs through Sp1 transcription factor binding at GC-rich promoter sequences (59), where the other report informed the recruitment of cAMP response element (CRE) on *CCND1* by the combination of c-Jun and ER (60). Moreover, several groups documented a direct crosstalk between ER and Cyclin D1. This interaction was shown to lead the ER-driven transcription which is independent of Cyclin D1 association with Cdk4 and unrelated to the presence of hormone ligand (61–64).

Contrary to the role of ER in Cyclin D1 upregulation and G1 progression, the ligand-activated androgen receptor (AR) decreases the transcriptional activity of Cyclin D1. This reduction in Cyclin D1 levels is mediated by binding of AR in combination with an orphan nuclear receptor, DAX1, to an androgen response element (ARE) located on the *CCND1* proximal promoter (65). Interestingly, AR and ER-α actively compete to interact with a steroid receptor coactivator, A1B1. This coactivator molecule acts as the functional determinant of ER-α, facilitating its useful coupling with the *CCND1* promoter (66). The transcriptional activity of AR is markedly reduced at G1/S transition. This is due to downregulation of receptor levels and involvement of histone deacetylase that brings about chromatin remodeling to repress transcription (67). Hence, it could be assumed that in absence of AR activity, ER continues to trans-activate Cyclin D1 and thus aids in the cellular progression through G1 phase. On the separate note, the activity of the progesterone receptor is markedly reduced in G1 part, owing to the influence of histone deacetylases (68).

S Phase

Cyclin A, in association with Cdk2 or Cdk1, regulates cellular progression through S phase *via* its phosphorylation activities. Among the substrates of this S-phase kinase, ER-α is also reported. ER-α is positively regulated by Cyclin A/Cdk2-mediated phosphorylation at its AF-1 domain (55, 69) and this indicates for cell cycle specific activity of ER-α. Overexpression of Cyclin A2 also increases AR activity (70). Reportedly, PR activity was also highest in S part (68). Research findings showed a significant increase of nuclear PR as a result of hormone treatment, exclusively at S phase (68). The ligand-independent phosphorylation of PR-B at S81 residue is catalyzed by Casein Kinase 2 (CK2), resulting in pronounced PR function during S phase (71). The transcriptional activity of PR is regulated by Cyclin A/Cdk2 (72). However, the ablation of phosphorylation sites found in the Ser/Thr-Pro motif of PR didn't significantly lower the Cyclin A/Cdk2-mediated kinase activity, suggesting an indirect mode of phosphorylation of PR. Cyclin A/Cdk2 can act as PR coactivator and the interaction of Cyclin A and PR was reported. The binding of Cyclin A/Cdk2 to the promoters of PR-responsive genes facilitates phosphorylation of associated proteins (72). An *in vitro* study reported the reduced binding of SRC-1 to PR upon phosphatase treatment (72); however, the interaction was restored by rephosphorylation with Cyclin A/Cdk2. The LXXLL motifs in SRC-1 mediate its interaction with PR (73). Therefore, PR-dependent recruitment of Cyclin A/Cdk2 is able to elicit a rise in kinase concentrations, required for phosphorylating SRC-1 on those motifs to trigger its PR-affinity (70, 72).

G2 Phase

ER-α can modulate G2-M transition by repressing a cell cycle inhibitor, Reprimo (RPRM). ER-α, histone deacetylase 7 (HDAC7), and FoxA1 together form a complex to inhibit RPRM, to drive the cell cycle progression (55). In G2 phase, the transcriptional activity of PR is significantly reduced, and site-specific phosphorylation of PR at Ser162 and Ser294 is abolished (68). HSPB8, a heat-shock protein at the G2/M phase requires cyclin D1 for its expression (74). The expression and transcriptional activity of AR largely depends on the CDK1-mediated phosphorylation, which stabilizes AR (75); however, androgen-stimulated transactivation is absent in G2/M (76). The transcriptional activities of steroid receptors can be regulated by the involvement of G2/M kinases. Such a kinase, named PLK1 (polo-like kinase 1) communicates with ER-α and modulates the transcription of ER-α target genes in breast cancer cells (77). Similarly, Aurora A, a serine/threonine kinase, is involved in phosphorylation of ER-α at S167 and S305, a modification involved in transcriptional activity of ER-α (78).

M Phase

The accessibility of chromatin is largely reduced at M-phase. As the genome remains transcriptionally suppressed, no such role of SHR in M-phase is documented (**Figure 3**).

SHR-DRIVEN CROSS-LINK BETWEEN CELL CYCLE AND BREAST CANCER

Breast cancer is the commonest malignancy in women across the world and predominantly related to cancer-related deaths in

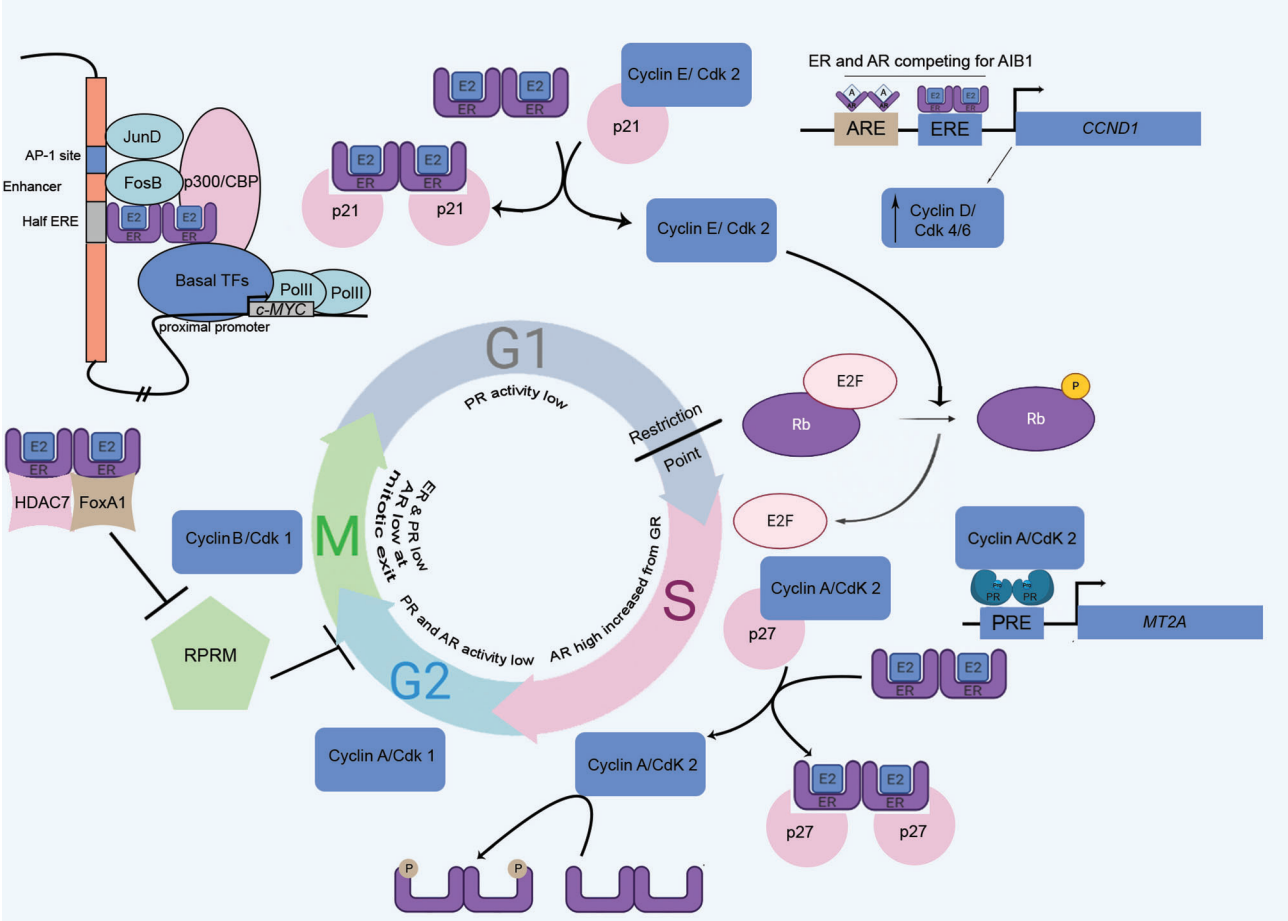

FIGURE 3 | The cell cycle, driven by the master regulators: steroid hormone receptors and kinases. SHRs involve in various cell cycle phase-specific functions, engaging the retinoblastoma protein (Rb), cyclins, and, cyclin-dependent kinases (cdk). MT2A, Metallothionein IIA, a PR target gene; A, Androgen; Pro, Progesterone; ERE, Estrogen Response Element; PRE, Progesterone Response Element; ARE, Androgen Response Element.

women (79). Breast cancer can be subdivided as either luminal (ER and/or PR+ve), human epidermal growth factor receptor 2 (HER2)-enriched or triple negative (TNBC) subtype (ER, PR, and HER2 –ve) (80). Over the previous decades, the insights into the molecular heterogeneity of this dreaded disease have been extensively divulged and the integral role of cell cycle machinery in oncogenesis as well as hormone therapy resistance has picked up expanding consideration. In the following section, this review will elaborate the current knowledge on the role of breast cancer associated SHR members (specially emphasizing ER, PR, and AR) in oncogenesis of breast through deregulation of cell cycle.

Cell Cycle Anomalies in Breast Cancer Development and Progression

The safeguards and checkpoints of the cell cycle are often overridden in cancer cells. A number of molecular alterations in several checkpoints of the cell cycle are noticeable in the different subtypes of breast cancer (81).

Luminal tumors (ER and/or PR+ve) are generally characterized by an enhanced expression of Cyclin D1, *via* estrogen receptor activation that binds directly to the *CCND1* promoter, enhancing its expression (82, 83). This cellular phenomenon is surely a prerequisite step for cell cycle progression, while ER upregulation might bring cellular hyper-proliferation upon influencing Cyclin D1 overexpression. This might be the case in breast cancers where *CCND1* gene is overexpressed in at least 50% of incidences (84). Indeed, E2-stimulated Cyclin D1 expression in ER+ve breast cancer cells largely relies on an enhancer element downstream of *CCND1* coding sequence, corresponding to the predominant ER-α recruiting site. FoxA1 mediates chromatin remodeling to activate this enhancer, and drives ER-α-mediated *CCND1* expression and concomitant cell proliferation, upon recruiting additional transcription factors, such as Sp1. Simultaneously, a tight regulation of Cyclin D1-mediated cell

proliferation is also observed at cellular level. NFIC, a tumor suppressor, directly represses. the transcriptional activity of Cyclin D1, upon an E2 stimulus (85). It could be well assumed that the loss of tumor suppressor, as frequent in breast cancer cells, might contribute to the ER-CyclinD1 crosstalk and bring forward cellular malignancy. Furthermore, in ER+ve breast cancer models, dihydrotestosterone (DHT)-mediated activation of AR has been shown to inhibit ER-α signaling and cell cycle progression through a reduction in *CCND1* transcription (66). There involves a competition in transcriptional activity of AR and ER-α that is more precisely dependent on the bio-availability of AIB1, a steroid receptor coactivator. It was reported that the rise in AR intracellular concentration dictated a marked decrease in E2-induced AIB1 recruitment at the AP1 site of *CCND1* promoter (66), resulting in reduced ER-driven transcription of *CCND1* and concomitant inhibition of estrogen-induced cell proliferation (86). On the other hand, in AR+ TNBC, DHT has been shown to increase levels of Cyclin D1, while decreasing p73 and p21 expression (7). p21 is a Cyclin-dependent kinase that involves in tumor suppression by cell cycle arrest and its downregulation was reported to cause breast cancer (87). AR blocks the promoter regions of p73 and p21 for their co-activators, leading to the downregulation of these two tumor suppressors. These studies experimentally validated the influence of AR on TNBC cell proliferation, exploiting the above mechanism (7). Additionally, the luminal androgen receptor (LAR) subgroup, a subtype of TNBC, might be sensitive to inhibition of CDK4/6 pathway due to the association of AR expression and RB1 expression (81, 88). The Cyclin E overexpression in TNBC exhibits a poor prognostic significance and is associated with the absence of ER and PR (89). This finding is concordant with a number of studies (90, 91), one of which depicted the correlation of highly expressed Cyclin E in steroid receptor negative tumors with the failure of endocrine therapy (91).

Targeting the Cell Cycle in Breast Cancer

CDK4/6 inhibitors in SHR-positive breast cancer: Due to the central role of Cyclin D/CDK4/6 complex in the control of the breast cancer proliferation and in the estrogen receptor signaling pathway, several CDK4/6 inhibitors have been investigated as breast cancer therapeutics in the last decades, in particular in luminal breast cancer (**Figure 4**).

Selective ATP-competitive inhibitors of CDK4/6, including palbociclib, ribociclib, and abemaciclib have recently been evaluated in clinical trials in combination with standard endocrine therapies in metastatic breast cancer, and have demonstrated significant improvements in tumor response rate and progression-free survival compared to endocrine therapy alone, which has led to their FDA approvals in combination with aromatase inhibitors or fulvestrant (92, 93). The steroid hormone receptor-positive breast cancer cell lines show the most sensitivity towards palbociclib (94). In estrogen-responsive cells, the effects of estrogen on the cell cycle progression depend highly on Cyclin D1 expression (95). In ER+ve breast tumors, there is an increased expression of Cyclin D1 or CCND1 gene amplification, as well as, p16 or Rb losses (81, 96). These molecular signatures predicted the sensitivity of ER+ve breast cancer towards palbociclib (94). This drug effectively arrests the cells at G1-S boundary and inhibits cell proliferation, in Rb-positive cells of different tumor types, with concomitant Rb dephosphorylation at specific serine residues (Ser780/Ser795) (97).

CDK4/6 inhibitors in triple negative breast cancer: A major challenge in developing targeted therapies in TNBC patients dwells on the variable level of response to treatments which reflects underlying heterogeneity within this subtype. Advances in the knowledge of gene expression profiling of TNBC have led to complementary classification systems that may be associated with response to therapy agents (98). For example, new insights in TNBC classification profiling isolated a subset of TNBC that is enriched for Androgen Receptor (AR) expression. AR positive TNBC are particularly sensitive to CDK 4/6 inhibition. The molecular mechanism underpinning this observation depends on cells bearing two different subpopulations of CDK2, CDK2-high and CDK2-low, dictating a biphasic mitotic exit of cells into proliferative and quiescent state, respectively (99, 100). Palbociclib-sensitive LAR (Luminal androgen receptor positive; a subtype of TNBC) cancer cells typically exit into a quiescent $CDK2^{low}$ state post-mitosis, from which CDK4/6 is required to phosphorylate RB1 and pass the restriction point (101–103). The requirement of CDK4/6 sensitizes these LAR cells towards CDK4/6 inhibitors. This led several ongoing trials to clinically evaluate the role of the antiandrogen drug bicalutamide in combination with CDK inhibitors in AR-positive metastatic breast cancer (104).

Complex relations exist between Rb protein, TNBC and AR expression. In fact, although Rb is commonly lost in TNBC, studies showed an association between Rb and AR expression in those tumors and sensitivity to palbociclib was seen in luminal androgen receptor (LAR) cell lines (105–107). A prior study reported that AR antagonist enzalutamide enhanced the palbociclib-induced G1 arrest in AR-positive/RB-proficient (MDA-MB-453) cells rather than AR-positive/RB-negative (BT-549), AR-negative/RB-proficient (MDA-MB-231), and AR-negative/RB-negative (MDA-MB-468) cells (108). It was previously documented that the interaction of RB with AR is androgen-independent and the protein functions as an AR-coactivator (109). Moreover, DNA replication is indirectly stimulated by AR through hyperphosphorylated RB (110). Palbociclib decreases the phosphorylation of RB, and enzalutamide might decrease the RB coactivator recruitment leading to RB-mediated cell cycle arrest.

DISCUSSION

In recent years, increasing evidence implicating SHR in cross-talk with cell cycle anomalies in breast cancer pathogenesis has elicited immense interest in understanding the combinatorial treatment options exploiting breast cancer specific SHR members with cell cycle inhibitors. SHR-induced deregulation in cell-cycle

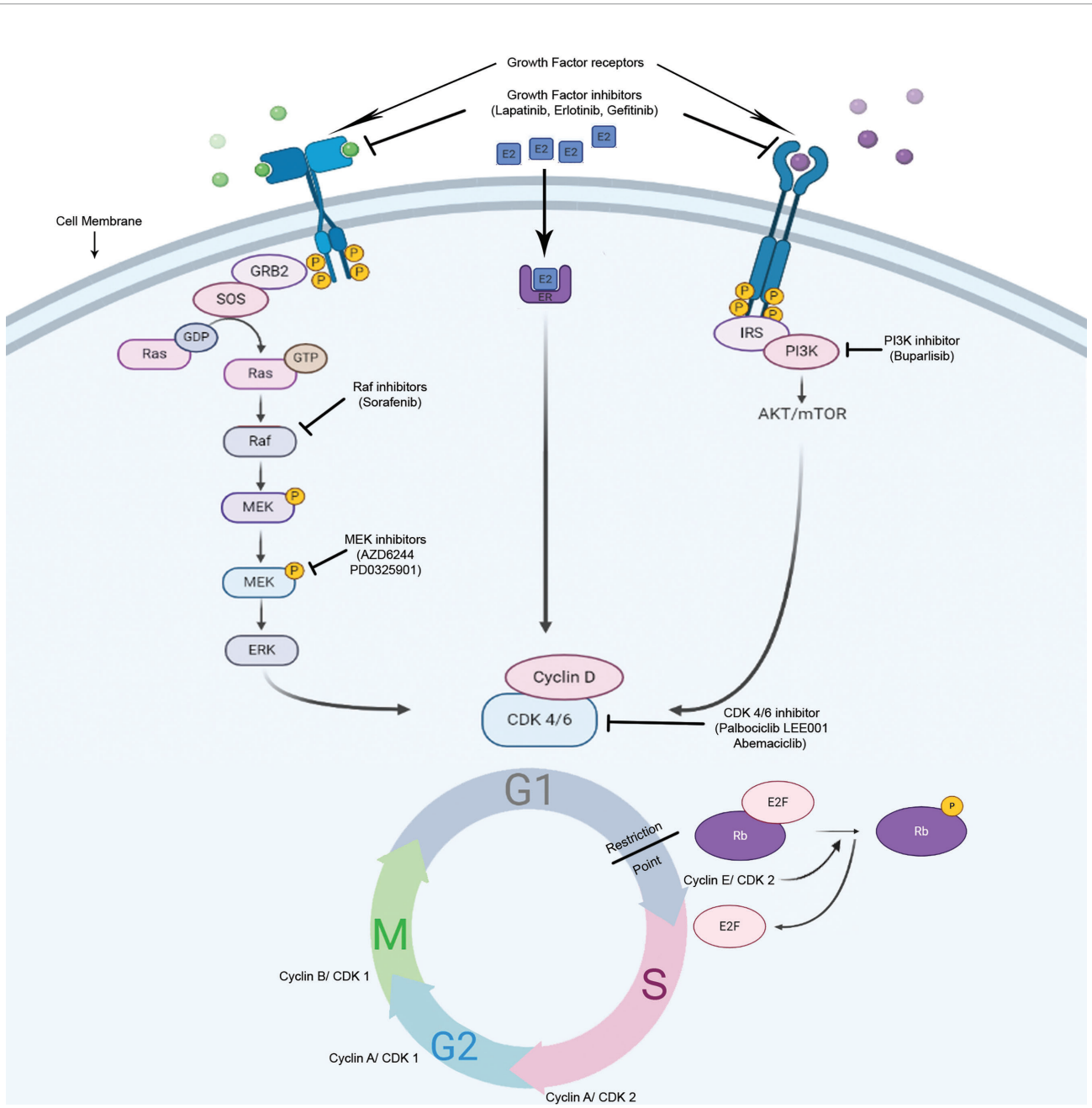

FIGURE 4 | Potential mode of action of cell cycle inhibitors in hormone-responsive breast tumors. There are numerous anti-cancer strategies that specifically target cell proliferation in breast cancer. The dependence of the HR+ve cells on Rb/E2F/CDK4/6 axis for progression through restriction point makes them vulnerable for CDK4/6 inhibitors.

control and the subsequent breast cancer progression unraveled a potential unexplored regimen in breast cancer treatment by introducing several clinical trials with cell-cycle inhibitors in clinical practice (111). In this era of precision medicine, different CDK inhibitors have emerged as novel therapeutics due to their selective abrogation of only the cancer cells. Although targeted monotherapies have resulted in favored outcome in the clinic, the emergence of acquired therapy-resistance is almost unavoidable. To overcome these circumstances, the selective drug combinations (combinatorial drug therapy) will be the only option to control the disease in a more efficacious manner. Until recently, several CDK4/6 inhibitors have been clinically developed and further understandings will be required to administer optimal combination of these agents with the other highly selective SHR-related therapeutics towards a better management of breast cancer.

AUTHOR CONTRIBUTIONS

SS and SN conceptualized and wrote the manuscript. SD wrote the manuscript. All authors contributed to the article and approved the submitted version.

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