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Integrated actions of PR and cell cycle machinery regulate breast cancer cell proliferation

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Abstract

Multiple laboratories have investigated PR involvement in breast cancer cell cycle progression. There is now a growing body of evidence demonstrating complex interactions between PR and cell cycle regulatory proteins. Here we review the current literature linking PR to cell cycle control and discuss gaps in the current knowledge. A more complete understanding of the relationships between PR and cell cycle regulatory molecules may reveal additional avenues for prevention and treatment of steroid receptor positive breast cancers.

Keywords

progesterone receptor; cell cycle; cyclin D; p21; p27; cyclin dependent kinase 2

The importance of progesterone and the nuclear progesterone receptor (PR) in the progression of steroid hormone receptor-positive breast cancer has recently been a subject of intense study. Exposure of cultured breast cancer cells to progestin initiates cell cycle progression (Figure 1). However, progesterone subsequently induces growth arrest and insensitivity to further progesterone treatment followed by upregulation of tyrosine kinase (erb B) growth factor receptors, in effect “priming” cells for alternate mitogenic stimuli such as EGF [1–5]. While the mechanisms guiding progestin/PR induced cell cycle progression are currently under investigation, several lines of evidence indicate that PR signaling is interwoven with cell cycle regulation during normal breast development and in breast cancer models, suggesting that this linkage may be therapeutically targeted in the clinic.

PR direct and indirect interaction with cyclins

Over-expression of cyclins in breast tumors has been positively linked to estrogen receptor (ER) and PR status [6–9]. Notably, similarities exist between cyclin D and PR knockout mice; both models exhibit identical phenotypes characterized by delayed lobuloalveolar development during pregnancy [10, 11]. In addition, several members of the steroid hormone

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receptor superfamily of ligand-activated transcription factors have been shown to interact with cyclins including ER [12, 13], androgen receptors [14–20] and thyroid receptors [21]. These studies have led researchers to more closely examine progesterone and PR interaction with cell cycle regulatory proteins including cyclins, cyclin dependent kinases (CDKs) and members of the p21^{Cip1}/p27^{Kip1} family of cell cycle regulators.

Work dating back twenty years up to the present shows that acute exposure of growth arrested immortalized breast cancer cells to progestin results in an increase in cyclin D1 mRNA and S phase entry [1, 5, 22] (Figure 1). The ability of anti-progestins to block increases in cyclin D1 [5] and the failure of cells expressing PR-B with a mutation in the DNA binding region to increase cyclin D levels or enter S phase [22, 23] suggested PR involvement. While multiple labs have shown that progestin/PRs are able to induce cyclin D1 gene expression, the PR-dependent mechanisms (i.e. signaling vs. transcriptional activities) are not as clear. Progestin-induced cyclin D1 protein, but not mRNA expression, was dependent upon the sustained activity of c-Src, EGFR and MAPKs, which presumably act to stabilize the short half-life protein through phosphorylation events [24] and/or increase the translation of cyclin D1 mRNAs [25]. Cells expressing PR-B mutants which can bind DNA but cannot rapidly and transiently activate c-Src, showed increased cyclin D and cell cycle progression upon progestin exposure [22, 23]. In similar studies, Faivre, et al. (2007) showed that breast cancer cell proliferation in soft agar required PR DNA binding but was attenuated upon loss of PR rapid signaling events, suggesting that PR-induced protein kinase signaling may promote cell survival. Together, these results demonstrate that the ability of progestin to regulate cyclin D mediated cell cycle progression clearly requires the transcriptional activity of PR-B, yet the precise role and kinetics of extra-nuclear signaling pathways like c-Src and MAPK remain to be defined.

Reciprocal regulation of PR and CDK2

While progestins cause acute upregulation of cyclin D and S phase entry via PR transcriptional activity, chronic exposure of cultured breast cancer cells to progestin results in cell cycle arrest [1, 26] (Figure 1). Long term exposure of breast cancer cell lines to progestin resulted in the inhibition of cyclin D1/CDK4, cyclin D3/CDK4 and cyclin E/CDK2 complex kinase activity as well as decreased overall cyclin D1, cyclin D3 and cyclin E expression [27] (Figure 1). Continuous exposure of T47D cells to progestin also resulted in increased CDK inhibitors p21^{Cip1} and p27^{Kip1} proteins [1] and increased association of cyclin E/CDK2 complexes with kinase inhibiting p27^{Kip1} [27]. The addition of exogenous cyclin D1 to progestin inhibited cells resulted in the resumption of cell cycle progression and the return of CDK2 activity [27] suggesting that one function of cyclin D1 over-expression is to provide a “sink” for p27^{Kip1}, thereby removing it from cyclin E/CDK2 complexes. Interestingly, cells which continually over-express cyclin D1 were not responsive to long term progestin inhibition of cell proliferation, yet showed increases in the proportion of cyclin E/CDK2 complexes associated with p27^{Kip1}. These data suggest that cyclin D plays an additional role in progestin regulated cell cycle progression other than simply acting as a p27^{Kip1} sink [28]. Notably, cyclin E/CDK2 complex kinase activity is hindered by increased p27^{Kip1}, in contrast to cyclin D/CDK4 complexes which are sensitive to the CDK4/6 inhibitor p18^{INK4}. T47D cell exposure to progestin increased p18^{INK4} leading to the

upstream inhibition of cyclin E/CDK2 activity and inhibition of cell cycle progression [26]. Thus, chronic exposure of epithelial derived breast cancer cells to progestin results in the upregulation of multiple CDK inhibitors which may act to initially nucleate cyclin/CDK complexes [29], but ultimately decrease the activity of cyclin E/CDK2 and block cell cycle progression (Figure 1). Overexpression of cyclin D, E, or A molecules or loss of p21^{Cip1} or p27^{Kip1} in PR positive breast cancer is predicted to bypass these controls [27].

PR, like other steroid receptors, is phosphorylated at multiple sites under varying conditions [30, 31]. Of the fourteen confirmed PR-B phosphorylation sites, 8 are cyclin/CDK sites [32]. Differential phosphorylation of PR, and closely related glucocorticoid receptor altered transcriptional activity and is a mechanism for promoter selectivity [33–35]. Given that PRs are CDK2 substrates, it is not surprising that PR has been shown to interact with cyclin E/CDK2 and cyclin A/CDK2 complexes [36, 37]. Progestins increase CDK2 kinase activity and PR phosphorylation at serine 400, a CDK2 site that is also basally phosphorylated, and sensitive to mitogens [36]. Forced expression of a constitutively active kinase mutant of CDK2 in breast cancer cells increased both liganded and unliganded PR transcriptional activity on luciferase reporter genes [36]; ser400 phosphorylation via CDK2 kinase activity was required for rapid PR nuclear localization and ligand –independent PR activity [36]. Nonetheless, the functional significance of PR/cyclin/CDK interaction is not fully understood. PR co-immunoprecipitated with cyclin A in the presence and absence of ligand [37]. Overexpression of cyclin A increased ligand dependent PR activity on a MMTV-luc reporter construct and this required CDK2 activity. However, CDK2 did not appear to directly target known PR phosphorylation sites. While cyclin A physically associated with PR in the absence of CDK2, CDK2 activity was required for transcriptional co-activation of PR. Further investigation showed that the association of PR with the cyclin A/CDK2 complex resulted in the phosphorylation of SRC1 and its increased association with PR, thus enhancing PR transcriptional activity during S phase [37].

PR regulation of cell cycle inhibitors

In addition to positive regulators of cell cycle progression like the cyclins and CDKs, progestins also directly regulate p21^{Cip1} and p27^{Kip1} inhibitors of cell cycle progression. Prolonged progestin exposure resulted first in increased expression of p21^{Cip1} followed by increased p27^{Kip1} [1]. PR induced upregulation of cell cycle inhibitors is presumed to contribute to the progestin induced cell growth inhibition of cultured breast cancer cells (Figure 1). While increases in p21^{Cip1} mRNA and protein levels have been detected there are no hormone response elements in the proximal promoters of this gene. However, recent studies demonstrated that PR, when phosphorylated at Ser³⁴⁵, strongly associates with specificity protein 1 (SP1) transcription factors [38] to upregulate transcription of SP1-regulated genes, including p21^{Cip1} [39] and EGFR [38]. The mechanism of progestin regulation of p27^{Kip1} has not yet been resolved.

PR strongly associates with S phase cyclins and CDK2, and these associations promote PR transcriptional activity. Thus, PR activity levels change with cell cycle progression and appear highest during S phase [40] when whole cell transcriptional activity is also high. Interestingly, both liganded and unliganded PR activity on MMTV-luciferase reporter

constructs are increased during S phase of synchronized cells, and the patterns of PR phosphorylation and SRC-1 recruitment to PRE change during cell cycle progression. Taken together, these studies indicate that CDK2 dependent phosphorylation of PR (Ser⁴⁰⁰) and differential recruitment of coactivators (SRC-1) may preferentially regulate PR nuclear localization [36] as well as receptor-coactivator interactions at selected promoters during S phase (i.e. when CDK2 activity is highest) relative to G₂/M or G₁ [40].

Conclusions

Coordination of PR transcriptional activity, phosphorylation state and association with specific cyclin/CDK complexes suggests that PR regulates genes in a cell cycle phase-dependent manner. As most of the current studies looking at PR target genes have used unsynchronized cells, it is possible that key PR target genes have been missed. In particular, a subset of S phase genes may be regulated by phosphorylated PR in the absence of progesterone. The discovery of these genes may provide clues to how progesterin/PR may contribute to development and/or progression of breast cancer. A more complete understanding of the coupling of PR target gene regulation to cell cycle control may reveal additional avenues for prevention and treatment of steroid receptor positive breast cancers.

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Figure 1.

Summary of PR-B Expressing T47D Breast Cancer Cell Proliferation and Cell Cycle Regulatory Protein Expression Following Progestin Exposure. Unsynchronized PR-B expressing T47D breast cancer cells, which are sensitive to progesterone but are not strongly dependent on estrogen, undergo a burst of proliferation upon exposure to progestin (initial 24 hours) followed by growth arrest at the G₁/S boundary (dashed line) [1, 3, 26]. Breast cell exposure to continuous progestin results in a transient increase in cyclin D RNA and protein followed by inhibition [1, 5, 22, 23]. Cyclin E levels initially decrease (within 24 hours) upon exposure to progestin followed by a transient cyclin E upregulation (36–60 hours post progestin exposure) [1, 5]. Breast cancer cyclin A levels decrease after 18 hours progestin treatment and remain undetectable through 60 hours [1]. Cyclin B levels also decrease upon progestin treatment of breast cancer cells 36 hours post treatment [1]. Progestin treatment causes a transient increase in CDK2 activity between 12–42 hours post treatment [1, 36]. The cell cycle inhibitor p21^{Cip1} protein levels transiently increase over the course of 72 hours progestin treatment [1, 39] while p27^{Kip1} protein levels increase following 40 hours progestin treatment and remain high while progestin is present [1].