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Regulation of steroid hormone receptors and coregulators during the cell cycle highlights potential novel function in addition to roles as transcription factors

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Abbreviations: AF1, activation function 1; AIB1, amplified in breast cancer 1; AR, androgen receptor; ARA, androgen receptor associated; Bub 1, budding uninhibited by benomyl 1; BubR1, bub1-related; CDC, cell division cycle; CDK, cyclin-dependent kinase; CDKi, cyclin-dependent kinase inhibitors; CK2, casein kinase 2; ER, estrogen receptor; ERK, extracellular-signal-regulated kinases; G1, gap 1; G2, gap 2; GR, glucocorticoid receptor; GST, glutathione S-transferase; MAD2, mitosis arrest-deficient 2; MAPK, mitogen-activated protein kinase; M phase, mitosis; Mps1, monopolar spindle 1; mRNA, messenger ribonucleic acid; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; NCOA4, nuclear receptor coactivator 4; NLS, nuclear localization signal; NR, nuclear receptor; PIAS, protein inhibitor of activated signal transducer and activator of transcription protein; PI3K, phosphatidylinositol 3 kinase; PKCζ, protein kinase C ζ; PLK, polo-like kinase; PR, progesterone receptor; PXR, pregnane X receptor; S, serine; SAC, spindle assembly checkpoint; S phase, DNA synthesis; SRC, steroid receptor coactivator; SR, steroid receptor; TR, thyroid receptors

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Cell cycle progression is tightly controlled by several kinase families including Cyclin-Dependent Kinases, Polo-Like Kinases, and Aurora Kinases. A large amount of data show that steroid hormone receptors and various components of the cell cycle, including cell cycle regulated kinases, interact, and this often results in altered transcriptional activity of the receptor. Furthermore, steroid hormones, through their receptors, can also regulate the transcriptional expression of genes that are required for cell cycle regulation. However, emerging data suggest that steroid hormone receptors may have roles in cell cycle progression independent of their transcriptional activity. The following is a review of how steroid receptors and their coregulators can regulate or be regulated by the cell cycle machinery, with a particular focus on roles independent of transcription in G2/M.

Introduction

Cell cycle, its regulation by kinases and association with transcription

The cell cycle consists of four main phases: DNA synthesis (S phase), mitosis (M phase) and two gap phases, G1 and G2. M phase itself is a complex phase, however, and contains five steps (prophase, prometaphase, metaphase, anaphase and telophase), followed by cytokinesis, in order to achieve an equal distribution of two sister chromatids into daughter cells, which later enter early G1 phase. Depending on the cell type and external environment/stimuli, cells can also enter a G0 phase, or quiescent state. A typical mammalian cell usually takes 24 hours to complete a cell cycle (~12 hours for G1, 6 hours for S phase, 6 hours for G2 and 30 minutes for M phase). To ensure faithful DNA synthesis and accurate cell division, cells have three important cell cycle checkpoints: G1/S checkpoint and G2/M checkpoint, and the spindle checkpoint in M phase. The whole cell cycle progression is timely and tightly regulated by various kinases. The sequential activation of complexes of cyclin-dependent kinases (CDKs) and their regulatory cyclins drives cell cycle progression. More specifically, cyclin D and cyclin E are increased at G1, while cyclin A and cyclin E are increased in S phase and cyclin B is an M phase cyclin. Meanwhile, CDK inhibitors (CKIs) negatively regulate CDK activities by binding and inactivating CDK–cyclin complexes. Furthermore, various mitotic

kinases control the cell cycle through regulating centrosome function, spindle assembly, chromosome segregation, and cytokinesis [Fu, 2010]. The spatiotemporal phosphorylation/dephosphorylation of these kinases plays a key role in switching on and off signaling pathways to drive cell cycle progression and protect cells from cell cycle aberrations. For example, mitotic kinases such as Polo-like kinases (PLKs), Aurora kinases and Nek kinases regulate the centrosome cycle and mitotic spindle formation. Other kinases such as budding uninhibited by benomyl 1 (Bub 1) kinase and BubR1 (Bub1-related kinase), Aurora B and the kinetochore kinase Monopolar spindle 1 (Mps1) are involved in the spindle assembly checkpoint (SAC) pathway to ensure all chromosomes are correctly aligned at the metaphase plate before the onset of anaphase [Foley and Kapoor, 2013]. Therefore, cycling of CDK-cyclin complexes/CKIs and phosphorylation/dephosphorylation by mitotic kinases coordinately regulate progression of the cell cycle.

Steroid receptors and transcriptional activity overview: structures and main functions

To date, at least 48 steroid hormone and nuclear receptors (NRs) in humans have been found [Klinge, 2008]. Some well-known steroid hormone receptors include estrogen receptor (ERα and ERβ), glucocorticoid receptor (GR), mineralocorticoid receptor, progesterone receptor (PR) and androgen receptor (AR), and these are closely related to some other NRs such as thyroid hormone receptors (TR) and retinoic acid receptors, as well as vitamin D receptors. All steroid hormones originate from the same precursor - cholesterol, and many are initially secreted by the adrenal cortex and/or gonads (ovaries and testes) and diffuse into the bloodstream. Due to their lipid solubility, steroid hormones can freely diffuse through cellular membranes and bind to steroid hormone receptors in their target tissues and organs, where they exert a wide range of biological functions including cell homeostasis, differentiation and regulation of proliferation, survival and cell death. Steroid receptors have distinct cellular distributions. PR and ER are mainly located in the nucleus of target cells, while the majority of GR and AR reside in the cytoplasm of target cells [Ward and Weigel, 2009].

As part of the NR superfamily, steroid receptors share similarity of structure and mode of action as transcriptional factors. These steroid hormone receptors generally contain four structural/functional domains: a variable N-terminal domain, a DNA binding domain, a hinge region and a hormone/ligand binding domain. Nuclear localization signals (NLS) within the hinge region mediate nuclear/cytoplasmic shuttling of receptors. The classical ligand-receptor pathway or so-called genomic pathway can be summarized in four steps: 1) hormone diffuses through cellular membranes and binds to receptor; 2) receptor dimerization and activation; 3) ligand-receptor complexes translocate into nucleus; 4) liganded receptor directly binds to “hormone response elements” on the DNA or indirectly through other transcriptional factors to regulate gene expression. In addition, steroid hormones can also bind membrane associated receptors to activate a variety of rapid intracellular signaling cascades, known as “non-genomic” actions. In addition, steroid receptors can be activated to promote transcription of target genes through ligand-independent pathways, in which activated protein kinases phosphorylate the receptors and/or their coregulators. Unlike kinases, where phosphorylation/dephosphorylation serves as on/off switches within cell signaling pathways, phosphorylation of hormone receptors regulates expression and/or functions of receptors by affecting protein stability, nuclear localization, hormone sensitivity, DNA binding, protein-protein interactions and transcriptional activity [Weigel and Moore, 2007b].

Phosphorylation of receptors can occur in all structural/functional domains, although it has been reported that the majority of phosphorylation sites are within the N-terminal domain [Ward and Weigel, 2009]. Another level of transcriptional regulation of steroid receptors comes from a large group of proteins named steroid receptor coregulators. These can be coactivators or corepressors of steroid receptors, either enhancing or suppressing transcription. Some of the well-known coregulators include the steroid receptor coactivator (SRC) family, steroid receptor RNA activator (SRA), androgen receptor-associated proteins (ARAs) and the PIAS (protein inhibitor of activated signal transducer and activator of transcription) family. [Gao et al., 2002]. Emerging data suggest that steroid receptors can be regulated in a cell-cycle dependent fashion, in a manner suggesting roles in cell cycle phases where transcriptional activity is generally repressed. Here, we review data supporting a cell cycle-dependent expression and/or activity of steroid receptors and their coregulators, some potential mechanisms of cell cycle-dependent functions and expression, and the functional implications of expression in cell cycle phases where transcriptional activity is significantly repressed and the potential impact of such function in disease development and treatment.

Cell cycle-dependent expression of steroid/nuclear receptors and coregulators

ER expression during the cell cycle

ERα levels have been previously reported to fluctuate throughout the cell cycle [Dong et al., 1991; Jakesz et al., 1984; Rostagno et al., 1996b; Vantaggiato et al., 2014]. A rise during G1 is seen and this is consistent

with controlling DNA synthesis and proliferation, the mechanisms of which include a significant regulation of transcription, either directly as a transcription factor or indirectly via non-genomic activities, which can in turn lead to regulation of other transcriptional events [Doisneau-Sixou et al., 2003]. Interestingly, another rise in ERα levels occurs during the S/G2 transition, at a time when little transcriptional activity would be expected [Sonia, 2011; Vantaggiato et al., 2014]. It has been suggested that ERα in G1 is ligand-dependent and ERα in G2 is not [Jakesz et al., 1984; Rostagno et al., 1996a]. However, not all studies have found differences in ERα expression during the cell cycle [He and Davie, 2006; Ikegami et al., 1994]. Even during different stages of M phase, ERα levels may fluctuate. ERα was detected at minimal level during metaphase and anaphase, in contrast to prophase and telophase in MCF7 cells by immunofluorescence assay [He and Davie, 2006]. The discrepancies in results might arise from the use of different cell cycle arrest agents, different assays (immunoblotting or immunofluorescence or immunohistochemistry) and different cell lines (breast cancer cell line vs osteoblastic osteosarcoma cell line) used in different reports [Doisneau-Sixou et al., 2003; He and Davie, 2006; Ikegami et al., 1994; Sonia, 2011; Vantaggiato et al., 2014].

Little understanding exists regarding the mechanisms associated with this fluctuation or the functional implications, especially of the G2/M-associated ER. Interestingly, a recent publication [Vantaggiato et al., 2014] has suggested that the cell cycle fluctuation of ERα may, at least in part, be due to a transcriptional elongational RNA Polymerase II block in intron 1 of the ERα gene, such that ERα mRNA is low in S phase but elevated in G2/M and G1. The data show that full-length ERα mRNA transcripts are only found during a 3–6 h interval during late S/G2 phase and are likely responsible, at least in part, for the ER protein rise in G2/M and the following increases in G1. In addition, phosphorylation of ERα may be involved, at least in part, in the elevated expression of ERα protein in the G2/M phases of the cell cycle [Vazquez-Martin et al., 2013; Zheng et al., 2015]. Other studies have suggested that phosphorylation may be linked to the mitotic stability of some transcription factors [Chuang et al., 2008]. It is possible that multiple mechanisms, including transcriptional regulation and post-translational modification (phosphorylation, ubiquitination, etc.), may be associated with fluctuations of ERα during the cell cycle. Irrespective of mechanisms by which ERα fluctuates during the cell cycle, little if any understanding of the functional role of ERα during G2/M exists.

Expression of other steroid receptors during the cell cycle

The expression and/or activity of other members of the steroid hormone/NR superfamily have also been reported to fluctuate during the cell cycle. The glucocorticoid receptor (GR) undergoes both ligand-dependent and -independent nuclear import in interphase, and is rapidly excluded from the nucleus at the onset of mitosis and into early G1[Matthews et al., 2011]. The G2/M nuclear exclusion of GR was accompanied by a prolonged glucocorticoid-induced activation of extracellular signal-regulated kinases (ERK), suggesting an increase in the non-genomic actions of the receptor. In addition, a marked loss of general transcriptional activity was detected, although a relative increase in ligand-independent activity was observed associated with its G2/M expression. The latter was thought to be due to a marked increase in phosphorylation in the activation-function (AF1) region of the GR, a situation similar to that observed with ERα [Vazquez-Martin et al., 2013; Zheng et al., 2015]. Interestingly, the phosphorylation of one of the GR serine residues (S211) was found to be important in mediating the cell cycle-dependent, ligand-independent transcriptional activity, as measured by a reporter plasmid. Although the latter suggests some endogenous transcriptional activity may be involved, the question that arises, due to generally decreased endogenous transcription at G2/M, is whether the reporter assay is merely a surrogate marker of another function, unrelated to the transcription factor activity of the GR during G2/M. Indeed, GR has recently been shown to regulate chromosome segregation, a role independent of transcriptional activity [Matthews et al., 2015]. These results are consistent with some other publications [Hu et al., 1994], but not all studies agree [Abel et al., 2002]. However, all such studies have measured receptor activity using transcriptional read-outs, and since other transcription factors have been found potentially associated with the mitotic machinery [Astrinidis et al., 2010], it is tempting to suggest that transcription-independent cell cycle regulatory functions might also exist.

The PR has also been shown to exhibit both expression and activity changes during the cell cycle [Narayanan et al., 2005]. Both phosphorylation and transcriptional activity have been documented to change during the cell cycle. In contrast to GR, PR activity was highest in S phase, followed by a marked reduction in G2/M. A significant component of the high S phase activity of PR seems to be dependent on casein kinase 2 (CK2) phosphorylation of S81, which is only found in PR-B [Hagan et al., 2011]. This phosphorylation is ligand-independent, and furthermore, CK2 nuclear localization is cell cycle-dependent and is nuclear-localized in S phase [Hagan et al., 2013; Hagan et al., 2011]. Interestingly,

phosphorylation of PR at S162 and S294 is abolished in G2/M and phosphorylation at S294 has been associated with transcriptional activation previously [Shen et al., 2001]. Therefore, low phosphorylation levels and transcriptional activity of PR at G2/M suggests that phosphorylation of PR regulates its transcriptional activation in a cell cycle-dependent manner, predominantly in G1/S. Indeed, phosphorylation at multiple sites on hormone receptors is generally thought to positively or negatively modify transcriptional activities of receptors.

Other NRs such as the pregnane X receptor (PXR) have been suggested to actually associate with condensed chromatin during mitosis [Saradhi et al., 2005]. The AR has also been reported to be associated with mitotic chromatin in some circumstances [Singh and Kumar, 2005] and its function there suggested to provide a form of "transcription memory" or "book marking" [Sarge and Park-Sarge, 2009; Zaidi et al., 2010]. Other data suggest that AR levels decline at the G1/S transition and increase as prostate cancer cells progress through S phase, with markedly lower AR levels again being detected as cells exit mitosis [Chen et al., 2006]. Protein degradation has been implicated in this decrease in AR levels, and an involvement of Cdk1 in the process suggested. This mitotic kinase is associated with and can phosphorylate and stabilize AR. As cells exit mitosis, however, the activity of cdk1 is markedly decreased [Chen et al., 2006]. These data, it can be argued, support a potentially non-transcription factor role for at least some NRs, especially in the M phase of the cell cycle.

Steroid receptor coactivator expression/activity during the cell cycle

Interestingly, some NR coactivators have been suggested to have distinct roles at different phases of the cell cycle. Nuclear receptor coactivator 4 (NCOA4), also known as ARA70, is a coactivator of several nuclear factors [Kollara and Brown, 2012]. However, several lines of evidence now suggest a potential role in mitosis, due to its frequent association with the mitotic spindle and midbody at cytokinesis [Kollara and Brown, 2012].
Another coactivator, steroid receptor coactivator-3/amplified in breast cancer 1 (SRC3/AIB1), has also been suggested to have roles in both S phase and mitosis [Ferrero et al., 2011]. Expression levels of AIB1 are highest during G1, and decrease in S and G2/M phases. However, in mitosis, specifically in prometaphase, a slower migrating form of AIB1 is seen. The slower migrating form of AIB1 is due mostly to phosphorylation. During mitosis, AIB1 interacts with and is phosphorylated by cdk1, and it has been speculated that this is a mechanism to negatively regulate the transcriptional coactivator activity of AIB1 to prevent abnormal transcription during mitosis [Ferrero et al., 2011].

Differential expression, regulation and potential activity during the cell cycle of several NRs and/or their coactivators support the need for a more detailed investigation of differential functions.
Mechanisms of fluctuations in expression and activity of steroid receptors throughout the cell cycle
Studies at genome-wide transcription levels have found that about 10% of genes are related to cell-cycle functions, while the majority of genes are not [Cho et al., 1998; Spellman et al., 1998]. These phase-specific genes are closely related to cell cycle progression or cell cycle-dependent events. For instance, genes transcribed during the M interval are proteins that are required for late cell cycle events, such as spindle formation, cytokinesis and separation of two daughter cells [McInerny, 2004]; those transcripts that are upregulated in late G1 are involved in DNA replication; genes responsible for DNA damage response peak in S phase [Cho et al., 2001]. In the appropriate target cells, steroid hormone receptors regulate the transcription of genes that directly regulate the cell cycle. In addition, steroid hormone receptors can directly affect cell cycle regulatory protein complexes via protein-protein interactions or by effecting protein translation and/or degradation. In turn, several members of the cell cycle machinery can regulate steroid hormone receptor activity, often by phosphorylation/dephosphorylation, and in some cases via a transcriptional coregulatory function. Therefore, multiple mechanisms are associated with steroid hormone receptor regulation throughout the cell cycle.

Steroid receptors can regulate expression and/or activity of cell cycle machinery

The association between steroid or steroid receptor pathways and the G1/S cell cycle machinery, including both cyclins and CDKs, is well known to activate gene transcription, promote cell proliferation and to drive the G1/S transition and enhance cell cycle progression through S phase. Extensive study has shown that steroids and steroid receptors promote G1/S cell cycle progression by activation of several cell cycle-dependent genes such as cyclin D1, cyclin E and modulation of CDK inhibitors p21 and p27 [Shupnik, 2004]. During G1/S, CDK4/6/2 and their associated cyclins D/E/A are known to be activated and play important roles in G1 entry and G1/S transition [Vermeulen et al., 2003]. For example, estrogen regulates the transcription and function of c-Myc and cyclin D1, which further activates the cyclin E-CDK2 complex. This latter protein complex phosphorylates various substrates such as the retinoblastoma susceptibility gene pRB, which allows DNA synthesis initiation [Weinberg, 1995]. Similarly, another recent study showed that estrogen (ERα)-

induced activation of cyclin E-CDK2 results in the complex binding to and phosphorylating ERα at S341, which enhances ERα interaction with a coactivator S-phase kinase-associated protein 2 (SKP2). This in turn enhances E2F-1 gene transcription, which further induces expression of SKP2, cyclin E, cyclin A and other E2F-1 target genes that drive S and G2/M progression[Zhou et al., 2014]. Cdc25, which was also identified to be a coactivator of ER, was shown to bind ER and increase its transcriptional activity [Ma et al., 2001]. Interestingly, Cdc25A is a phosphatase that activates cyclin E- and cyclin A-dependent kinases and promotes G1/S transition [Blomberg and Hoffmann, 1999; Hoffmann et al., 1994]. It is still unclear whether the interaction between Cdc25 and ER directly contributes to G1/S cell cycle progression. Moreover, many different cell signaling pathways are involved in the regulation of ERα transcription and G1/S progression. For example, the phosphatidylinositol 3-kinase (PI3K)-protein kinase C ζ (PKCζ)-ras signaling pathway acts on ERα, increasing cyclin D expression, which helps to drive G1/S transition in MCF7, a breast cancer cell line [Castoria et al., 2004]. It is also important to note that c-Myc and Cyclin D1 are both ERα and PR target genes in breast cancer, and in many cases these two genes are likely regulated by novel ERα/PR complexes [Giulianelli et al., 2012].

AR can also regulate G1/S cell cycle progression [Balk and Knudsen, 2008] through several mechanisms that affect cyclin expression and CDK activities in prostate cancer cells. Androgen and AR can also drive cell cycle progression by increasing cyclin D levels through a mammalian target of rapamycin (mTOR) complex 1 (mTORC1)-dependent enhancement of translation [Xu et al., 2006], through activation of mTORC2/AKT pathways and decreased activity of the cyclin-dependent kinase inhibitor p27 [Fang et al., 2012]. Such activity promotes assembly of active CDK/cyclin complexes. Moreover, several components of the G1/S cell cycle machinery have in turn been shown to regulate AR transcriptional activity [Balk and Knudsen, 2008]. It has been shown that overexpression of cyclin E upregulated AR activity, while cyclin D1 overexpression inhibited AR function [Knudsen et al., 1999; Reutens et al., 2001; Yamamoto et al., 2000]. Indeed, some of the cyclins have effects on AR which are independent of their ability to regulate CDK activity. In addition, while some G1/S CDKs can phosphorylate the AR and hence regulate its activity, some CDKs can also coactivate the receptor independently of their kinase activity [Koryakina et al., 2014; Lim et al., 2005]. There is also a significant amount of literature showing the association of PR with cell cycle mediators. The data show that progestins, through PR, can increase the expression of cyclin D1 RNA and protein levels [McGowan et al., 2007]. In addition, progestins, through PR, can increase cyclin E activity [Rivas et al., 2012]. The accumulated data not only show that PR signaling can regulate cell cycle progression [Groshong et al., 1997; McGowan et al., 2007; Sutherland et al., 1998], but also suggest that PR expression, phosphorylation and activity can be regulated in a cell cycle-dependent fashion [Dressing et al., 2014; Moore and Weigel, 2011; Weigel and Moore, 2007a] .

Steroid hormone receptor activity in G2/M and interactions with G2/M kinases

While most studies have focused on the function and regulation of steroid hormone receptors in G1 and G1/S progression, several receptors have been shown to interact with and be regulated by G2/M kinases that are known primarily for their G2/M functions. For example, the G2 phase activated kinase, CDK1, can phosphorylate AR and modulation of CDK1 activity can alter AR expression and transcriptional activity [Chen et al., 2012; Chen et al., 2006].

During G2/M phase, while cells prepare for mitosis and cell division and continue to divide into two cells, ligand-independent phosphorylation of steroid receptors and repressed gene transcription were frequently observed within the target cells. For instance, significant ligand-independent phosphorylation of the PR can occur in G2/M synchronized cells [Dressing et al., 2014] and there seems to be a subset of PR target genes that can be selectively transcribed/expressed during the G2/M phase of the cell cycle. Others have shown that PR transcriptional activity is highest in S phase, lowest in G0/G1 and impaired in G2/M [Narayanan et al., 2005], using reporter gene assays in stably transfected T47D breast cancer cells. Similarly, GR-mediated transcription was also shown to be impaired in G2/M phases [Burnstein, 2002]. These transcriptional activities of receptors are closely related to the phosphorylation status of receptors, accessibility of the chromosomes and nuclear localization of receptors. The impaired transactivation of some receptors in mitosis has largely been thought to be regulated by their subcellular localization. It was shown using live cell imaging analysis that GR stays in the nucleus throughout interphase, while it is rapidly excluded from DNA in M phase and early G1[Matthews et al., 2011]. In addition, phosphorylation of receptors might also play a role in the cell cycle phase-specific regulation of NRs. Phosphorylation of GR increased three-fold in G2/M compared to hormone-induced phosphorylation of GR during S phase [Hu et al., 1994]. Furthermore, despite the hyperphosphorylation in G2/M, the phosphorylation of the N-terminal domain S203 and S211 phosphosites was not required for GRs chromosomal segregation regulatory function [Matthews et al., 2015]. Presumably, the hormone-

independent phosphorylation in G2/M functions differently than the hormone-induced hyperphosphorylation occurring in S phase in its transactivation of target genes [Hu et al., 1994].

The observation of altered and often enhanced phosphorylation of some steroid hormone receptors in G2/M suggests the involvement of G2/M kinases. The interaction of steroid receptors with G2/M phase-specific kinases seems less well understood than that with G1/S kinases. Similar to G1/S kinases, G2/M kinases also can participate in the regulation of transcriptional activities of steroid receptors. For example, PLK1, a serine/threonine kinase, is well-known to regulate mitotic entry, spindle formation and cytokinesis during cell division [van Vugt and Medema, 2005]. PLK1 interacts with ERα and is recruited to ERα target genes, where it can modulate estrogen-dependent gene transcription in breast cancer cells [Wierer et al., 2013]. Some studies have also found that in estrogen-treated MCF7 cells, the upregulation of PLK1 and cyclin B correlated directly with ERα protein levels [Karadedou, 2006]. However, it was not determined if the elevation of G2/M kinases by estrogen treatment was due to a general effect on cell cycle, since expression and activity levels of both PLK1 and cyclin B increase at G2/M and the expression level of ERα itself oscillates during the cell cycle, as discussed above.

Aurora A kinase is another serine/threonine kinase and a proto-oncogenic mitotic kinase. It functions mainly in centrosome separation and spindle formation, but may also have roles in chromosome alignment and chromosome segregation during mitosis [Lens et al., 2010]. It can be activated by mitogen- activated protein kinase (MAPK) signaling, which can induce down-regulation of ERα expression and endocrine resistance, as well as tumor progression in breast cancer cells [Opyrchal et al., 2014]. It has also been shown that Aurora A interacts with and phosphorylates ERα at S167 and S305, which increased DNA-binding and transcriptional activity of ERα [Opyrchal et al., 2014]. Aurora A kinase can be activated by aberrant MAPK signaling that results in ERα down-regulation through phosphorylation and activation of SMAD5 nuclear signaling that can lead to endocrine resistance and tumor progression. Another estrogen receptor, ERβ, which shares significant homology in DNA and hormone binding domains with ERα, was found to directly interact with the spindle assembly checkpoint protein mitosis arrest-deficient 2 (MAD2) in a yeast two-hybrid system and glutathione S-transferase (GST) pulldown assay [Poelzl et al., 2000]. Although the functions of ERβ/MAD2 interaction are still unknown, it suggested a potential function in the regulation of cell cycle apart from the previously established role as a transcriptional factor. However, these results have not been independently replicated as yet. Interestingly, unlike other NRs which predominantly drive G1/S and cell cycle progression, ERβ over-expression in ERα+ breast cancer cell-lines induced ligand-independent G2 arrest by inhibiting CDK1 activities, which determine G2 progression [Paruthiyil et al., 2011] through increased levels of GADD45A and BTG2 and decrease expression level of cyclin B.

Another Aurora kinase family member, Aurora B kinase, is a well-known G2/M kinase; it maintains spindle integrity and regulates cytokinesis during mitosis. Apart from its function in cell cycle regulation, it was shown that Aurora B can directly interact with TR and this activity is essential for TR-dependent growth hormone gene transcription at G0/G1. Meanwhile, liganded TR in turn increases kinase activity of Aurora B [Tardaguila et al., 2011].

As discussed, the association between steroid receptors and some G2/M kinases can result in enhanced target gene transcription in interphase cells. Despite the expression and activity of G2/M proteins, such as PLK1, Aurora A, Aurora B and cyclin B being lower in G1/S, they promote the transcriptional activities of steroid receptors which often peak at G1/S. It is postulated that G2/M kinases could also interact with some steroid receptors during G2/M and this would result in novel alternative activities.

Post-translational modification of steroid receptors in the regulation of transcription and cell cycle

As discussed above, phosphorylation of receptors may play a role in the cell cycle phase-specific regulation of NRs. Further data supporting this idea are as follows. GR phosphorylation at S203 and S211 in the AF1 domain are important for its transactivation [Matthews et al., 2008], however, these two sites are also hyperphosphorylated at G2/M in a ligand-independent manner, but only phosphorylation at S211 in mitotic cells is required for GR activity [Matthews et al., 2011]. In contrast, phosphorylation at both these sites in interphase cells is minimal and in the presence of hormone, phosphorylation at both sites is significantly increased.

Similarly, AR S81 phosphorylation peaks at mitosis, which is mediated by CDK1 activation. It has been suggested that AR S81 phosphorylation in mitosis provides a pool which can be rapidly recruited to chromatin to regulate target gene transcription during G0/G1 phase, since AR S81 phosphorylation regulates cellular distribution of AR [Fang et al., 2012]. Interestingly, in experiments in which MCF7 cells were programmed to have a more cancer stem cell-like phenotype and higher mitotic rate by stable over-expression of SOX2, increased ERα phosphorylation on S118 was found during

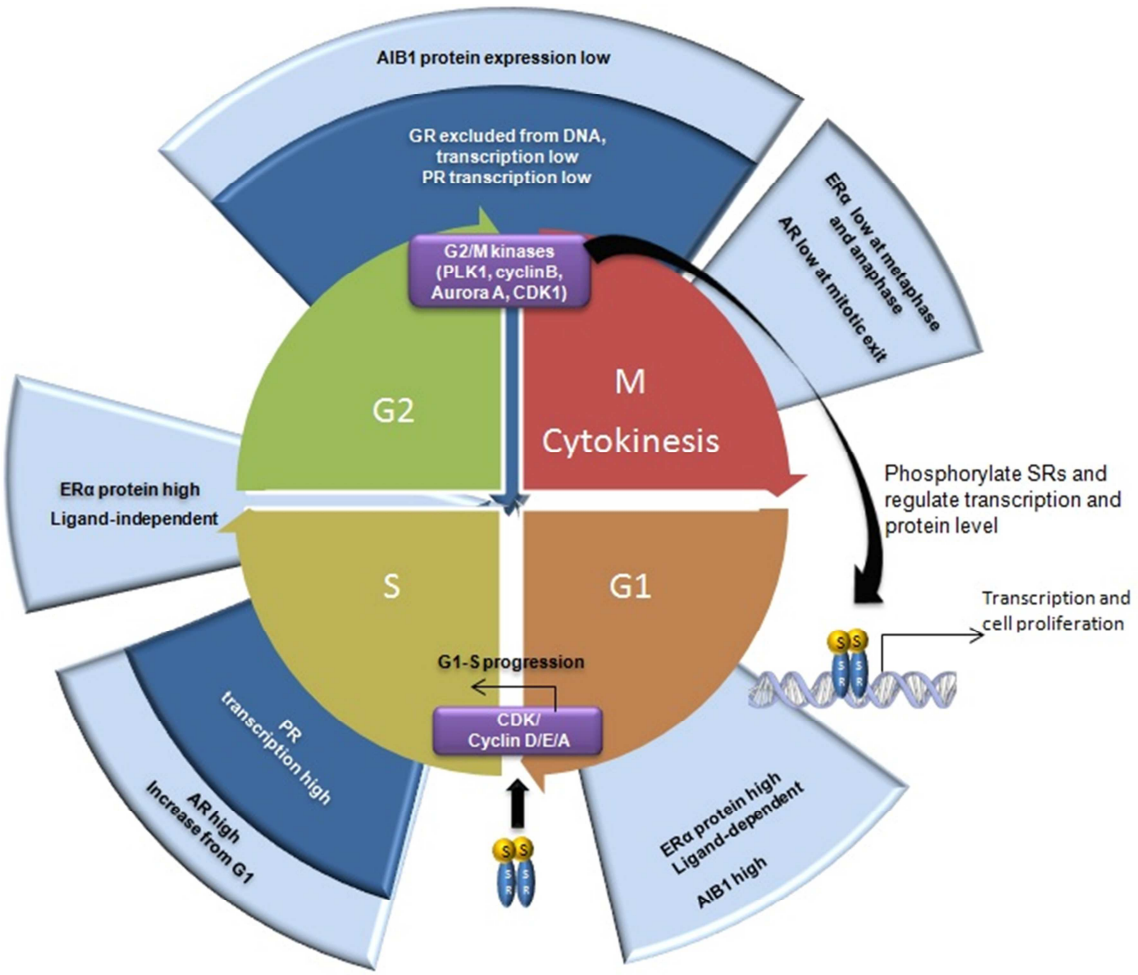

Figure 1: Steroid receptors and cell cycle regulatory kinases during the cell cycle. Protein expression (in light blue) and transcriptional activity (in dark blue) of steroid receptors (SRs) and coactivators (ERα, AR, PR, GR and AIB1) during different phases of the cell cycle are shown. During G1/S or S/G2 transition, SR and coactivators generally have high levels of protein expression and transcriptional activities. Both are decreased as the cells progress through G2/M. SRs directly interact with cell cycle kinases (Cyclin D/E/A) to drive G1-S progression. While maintaining the fidelity of cell division, G2/M kinases (PLK1, Aurora A, cyclin B and CDK1, etc.) fine-tune transcriptional levels and protein stability of SRs (ER, AR).

metaphase [Vazquez-Martin et al., 2013]. These results suggest that phosphorylation of steroid receptors in particular may be intimately involved in regulating the putative novel function(s) of steroid receptors during mitosis.

Alterations of steroid receptors and cell cycle perturb hormonal control and lead to tumor formation and progression

Long term exposure to hormones plays an integral role in the development of hormone-dependent tumors such as breast, prostate and endometrial cancers. Although exact mechanisms are not well established, it is generally believed that hormones acting through hormone receptors stimulate gene transcription and cell proliferation, and support the growth of cells with genetic mutations. Dysregulation of ER and AR expression, and the subsequent altered signaling pathways, are pivotal events in breast and prostate cancer, respectively. Often, one of the primary events is increased expression of the steroid hormone receptor at an early stage of tumorigenesis [Lanari and Molinolo, 2002; Singh and Kumar, 2005]. Following that, cancer progression and metastasis occur by multiple mechanisms associated with short circuiting the agonist requirement for activation of the steroid hormone receptor, which in a few cases includes gain-of-function mutations in ER and AR [O'Mahony et al., 2008; Pasqualini, 2002], resulting in ligand-independent constitutive activation. Loss of steroid hormone receptors is not frequently seen, but can occur [Sighoko et al., 2014]. Mutation and/or overexpression of many genes encoding kinases and associated regulatory proteins that regulate the cell cycle are also common themes in cancer development, e.g., Aurora A kinase over-expression and constitutive activation leads to aneuploidy,

centrosome anomalies and chromosome instability in prostate, ovarian and breast cancers, as well as in animal models of these cancers [Buschhorn et al., 2005; Das et al., 2010; Gritsko et al., 2003; Hontz et al., 2007]. Patients having both increased steroid receptors and cell cycle kinase activities may therefore benefit at an early stage from combination therapies consisting of anti-hormone therapy and kinase inhibitors. Targeting cell cycle kinases, such as CDK4/6, in combination with antihormonal therapies, such as aromatase inhibitors, as therapy for advanced breast cancer is already in progress, with some success [Finn et al., 2014]. The emerging data reviewed above suggest that there are many other interactions and novel functions to be explored and elucidated that involve steroid receptors and cell cycle kinases.

There are multiple studies from several different laboratories showing steroid hormone and other NRs both regulating expression and/or activity of the cell cycle/mitotic machinery, as well as showing that the activity and/or expression of the receptors themselves can be altered during the cell cycle (Figure 1). Most often, determination of cell cycle effects on receptor function has only focused on measuring transcriptional activity. However, by analogy to the demonstration of alternative functions, independent of the cell cycle mediator roles, of several components of the cell cycle machinery (e.g., kinase independent actions of CDKs, cyclins and CKIs [Yamamoto et al., 2000]; cyclin D1 has roles in steroid hormone receptor transcription that are independent of its cell cycle mediator role [Zwijsen et al., 1997]), it is possible that the steroid and other NRs may also have transcription-independent functions depending on the cell cycle phase. Such alternative activities are often difficult to separate experimentally and the extensive cross-talk, that in part has been reviewed above, suggests a tight linkage of the cell cycle with transcriptional events. For example, during G2/M phase in synchronized cells, PR and cyclin D1 interact in a ligand-independent fashion and both can be detected in the same transcriptional complexes at endogenous target genes that are known to be cyclin D1-dependent [Dressing et al., 2014], and this may be part of the mechanism associated with target genes that only become sensitive to PR regulation during specific phases of the cell cycle [Dressing et al., 2014]. Uncovering the mechanisms underlying particularly the steroid receptor roles in G2/M of the cell cycle may open up completely new approaches for endocrine therapy.

Conclusion

Increasing evidence suggests that cell cycle kinases may have differential roles independent of those traditionally identified with their regulation of the cell cycle (Figure 1). As described in this review, they can assist in the transcriptional activities of steroid hormone receptors. In particular, it is now well established that mitotic kinases can regulate steroid hormone receptor transcriptional activity in interphase. In addition, emerging data hint that the steroid hormone receptors may have functions in mitosis that are likely independent of their transcriptional activity. Therefore, the mitotic kinases may well interact with some steroid hormone receptors in G2/M, resulting in alternative novel activities. Identification of these interactions may well provide novel targets for therapy in various diseases where steroid hormone receptors are known to play important roles.

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