Journal of Steroid Biochemistry & Molecular Biology 117 (2009) 87–92

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Mini-review

Specific involvement of glycogen synthase kinase-3 in the function and activity of sex steroid hormone receptors reveals the complexity of their regulation

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ARTICLE INFO

Article history:
Received 10 July 2009
Received in revised form 12 August 2009
Accepted 13 August 2009

Keywords:
Estrogen receptor
Androgen receptor
Phosphorylation
Stabilization
Glycogen synthase kinase-3

ABSTRACT

Protein kinases represent key nodes for the integration of multiple intracellular signalling pathways, resulting in modulation of both ligand-dependent and ligand-independent mechanisms of sex steroid receptor (sSR) signalling cascades. The proline-directed Ser/Thr kinases including mitogen-activated protein kinases and cyclin dependent kinases were especially reported to contribute to the function and activity of sSRs. The relevant effects of these kinases are well-documented but the impact of glycogen synthase kinase-3 (GSK-3), another member of this kinase family, has been underestimated. Indeed, the specific role of GSK-3 regarding the different sSRs will help to understand further the complexity of sSR signalling. So far, AR and ERα were identified as GSK-3 substrates. Additionally, the docking properties of GSK-3 were demonstrated to play a crucial role in sSR signal transduction. Reciprocally, GSK-3 was described as a potential target of non-genomic effects of sSRs. Therefore, GSK-3 regulates and is regulated by sSRs. This review focuses on the emerging and promising involvements of GSK-3 regarding the signalling cascade of the respective sSRs. This review represents a necessary complement of information to highlight the importance of GSK-3 regarding sSR function and activity.

Contents

1. Introduction

Sex steroid hormone receptors (sSRs) including the androgen receptor (AR), estrogen receptor-alpha (ERα) and ERβ, and progesterone receptors (PRs) A and B are members of the nuclear receptor class I superfamily. Mammalian sSRs show a modular structure [1]: an N-terminal A/B domain containing the ligand-independent activation function-1 (AF-1), a C-domain required for DNA binding (DBD), a D-domain or hinge region with a nuclear localisation signal, an E-domain containing the ligand binding domain (LBD) and the ligand-dependent activation function-2 (AF-2), and a C-terminal F-domain with so far unclear function. AF-1 and AF-2 act independently or synergistically to regulate the transcriptional activity of the respective receptor [2]. Importantly, AF-2 requires the binding of a ligand in order to be activated, whereas AF-1 activity is regulated by phosphorylation mainly in a ligand-independent manner [3,4]. Ligand-independent activation of sSR is triggered by growth factor receptors such as epidermal growth factor receptor (EGFR) and type I insulin-like growth factor receptor (IGF-IR) [5,6]. The AF-1 domain may also be activated/phosphorylated by

Abbreviations: AR, androgen receptor; Cdk, cyclin-dependent kinase; DBD, DNA binding domain; ER, estrogen receptor; EGFR, epidermal growth factor receptor; GSK-3, glycogen synthase kinase-3; GST, glutathione S-transferase; IGF, insulin-like growth factor; IGF-IR, type I IGF receptor; LBD, ligand binding domain; MAPK, mitogen-activated protein kinase; MNAR, modulator of non-genomic action of estrogen receptor; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKB/Akt, protein kinase B; PKC, protein kinase C; PR, progesterone receptor; Shc, Src homologous and collagen; sSR, sex steroid receptor; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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doi:10.1016/j.jsbmb.2009.08.002

rapid non-genomic actions of steroids [7]. Thus, posttranslational phosphorylation of sSRs is related to both ligand-dependent and ligand-independent transcriptional activity of sSRs and regulates various mechanisms of transcriptional activation such as subcellular localisation, receptor dimerisation, and coactivator recruitment.

Numerous phosphorylation sites of sSRs have been described to be potentially relevant for ligand-dependent and ligand-independent activation of these receptors. Several protein kinases including proline-directed Ser/Thr kinases have been described as key players in the regulation of sSRs but their specific role must still be clarified. These kinases mainly belong to the GMCC group of kinases comprising glycogen synthase kinases (GSKs), mitogen-activated protein kinases (MAPKs) and cyclin-dependent kinases (Cdks). Among these kinases, MAPKs and Cdks have been extensively studied and their implication in the phosphorylation of sSR has been largely reviewed. Indeed, well-detailed reviews describe the importance of MAPKs [8] and Cdks [9] for phosphorylation of AR, ER and PR.

Recently, the serine/threonine protein kinase GSK-3 has been identified as a key regulator of sSRs. So far, GSK-3 has been identified to phosphorylate AR and ERα. However, the emerging role of mammalian GSK-3 regarding sSR function involves not only phosphorylation of the receptors but additionally, the docking properties of GSK-3 play a crucial role in sSR regulation. Reciprocally, GSK-3 was described as a potential target of non-genomic effects of sSRs. These data suggest a central role of GSK-3 in the sSR signalling pathways. The specific aim of this article is to provide a comprehensive review of the particular role of GSK-3 in the modulation of AR and ER function and activity and to discuss the points of convergences and divergences of the different studies performed in this field. GSK-3 has not been attributed a role in PR phosphorylation so far. Therefore PR phosphorylation will not be further discussed in this review.

2. Multiple involvements of GSK-3 in the regulation of androgen receptor and estrogen receptor function and activity

2.1. General aspects of GSK-3 function

GSK-3 has numerous functions. Best known is its role as key component in insulin and Wnt signalling and as regulator of the activity and intracellular localisation of numerous transcription factors. It also plays a role in neurological disorders like Alzheimer's disease and schizophrenia [10,11]. The properties and multiple functions of GSK-3 have been summarized in several excellent reviews [10–14]. Therefore, in this review we will only summarize those properties and focus on those functions of GSK-3 that play a role in the regulation of sSR function.

Two highly homologous forms of mammalian GSK-3, GSK-3α and GSK-3β, have been described [15] which show 97% sequence similarity in their protein kinase domain [14]. The isoforms show similar substrate specificity but the isoform-specific functions are still unclear. The disruption of the GSK-3β gene in mice results in embryonic lethality, indicating that GSK-3α cannot compensate the loss of GSK-3β [16]. On the other hand, GSK-3β can partly compensate the loss of GSK-3α. GSK-3α knockout animals are viable but display enhanced glucose and insulin sensitivity [17]. Therefore, GSK-3β seems to be the more essential isoform.

The predominant role of GSK-3β in the control of numerous intracellular pathways demands careful regulation of its activity. GSK-3β is one of the few protein kinases, which is inactivated by phosphorylation. In fact, phosphorylation at Ser-9 inhibits GSK-3β activity [15]. Enzymes involved in Ser-9 phosphorylation include PKB/Akt, MAPKAP kinase-1 (p90Rsk), mammalian target of rapamycin (mTOR), PKA and PKC. These protein kinases belong to growth factor and hormone-stimulated signal transduction pathways and stringently control GSK-3β activity [11]. Inversely, GSK-3β phosphorylation at Tyr-216 may increase its activity but the protein kinases involved have not yet been identified in mammals [11]. Additionally, protein complex formation (as in the Wnt signalling pathway) and distinct intracellular localisation are other ways to regulate the activity of this enzyme.

GSK-3β has unique substrate specificity. Most GSK-3β substrates e.g., glycogen synthase (GS), eukaryotic initiation factor-2B (eIF2B) and β-catenin, require a priming phosphate at $n+4$ (where n is the site of phosphorylation by GSK-3β) to be in turn bound to and phosphorylated by GSK-3β. Primed substrates bind to the respective "priming phosphate site" and are then phosphorylated at the "active site" of GSK-3β [13]. Other proteins such as axin, seem to be unprimed substrates of GSK-3β [10]. These proteins bind to a distinct substrate binding site of GSK-3β and are phosphorylated without previous priming phosphorylation by another kinase [13]. 'Primed' and 'unprimed' GSK-3β substrates include a vast number of metabolic and signalling proteins, structural proteins and transcription factors.

In addition to its kinase activity, GSK-3 shows specific docking properties which have been extensively investigated in the canonical Wnt signalling pathway [10]. Typical for this pathway is formation of a protein complex consisting of active GSK-3β, adenomatous polyposis coli (APC), axin and β-catenin, in which GSK-3β phosphorylates β-catenin and targets it for proteolytic degradation. Activation of the Wnt-pathway results in dissociation of the protein complex and translocation of unphosphorylated β-catenin into the nucleus where it binds to members of the T-cell factor (Tcf) family of transcription factors and stimulates the transcription of genes required for embryogenesis during the developmental stage as well as the transcription of genes especially involved in tissue proliferation and differentiation in the adult stage [10].

Grimes and Jope emphasize [11] that one of the most important roles of GSK-3β is the regulation by phosphorylation of numerous transcription factors and thereby the control of the expression of the respective target genes, thus playing a crucial role in cell growth, development, differentiation, proliferation and cell death as well as in immune system regulation. Most of the transcription factors regulated by GSK-3 are bound to the enzyme and kept in an inactive state by phosphorylation, while others are activated by phosphorylation through GSK-3 (Fig. 1). The first steroid hormone receptor reported to be phosphorylated by GSK-3 was rat glucocorticoid receptor (GR). GSK-3 phosphorylates the rat GR at Thr-171 and inhibits the receptor activity. This amino acid residue is lacking in the human GR [18]. More recently, GSK-3 phosphorylation of the human GR at Ser-404 in a glucocorticoid-dependent manner was shown to decrease GR transcriptional activity and function. This phosphorylation was also suggested to increase GR nuclear export and to enhance GR protein degradation [19]. This example suggests that GSK-3 phosphorylation of steroid hormone receptors alters their intracellular location, content and activity. In this review we specifically address the regulation of the sSRs AR and ERα activity by GSK-3 phosphorylation.

2.2. Sex steroid receptors are substrates of GSK-3

AR: Numerous serine phosphorylation sites have been identified in the AR. In LNCaP prostate cancer cells, AR was found constitutively phosphorylated at Ser-94 and in response to stimulation by its ligand, phosphorylation at Ser-16, Ser-81, Ser-256, Ser-308, Ser-424 and Ser-650 was observed [20]. Additionally, AR phosphorylation at Ser-213 and Ser-791 was described and partly characterised *in vitro* and *in vivo* in response to androgens [21] (Fig. 2). Salas et al. [22] showed that GSK-3 phosphorylates the AR, thereby inhibiting AR-driven transcription. The AR hinge and lig-

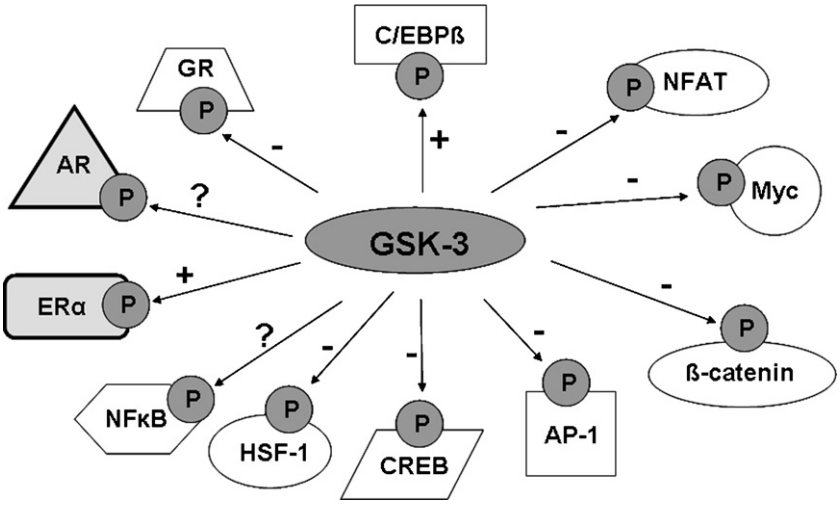

Fig. 1. Selected transcription factors including steroid receptors that are positively (+) or negatively (−) regulated by GSK-3β. Question mark (?) means "under debate".

and binding domains were important for both the phosphorylation and the inhibition of transcriptional activity of the receptor. Overexpression of constitutively active GSK-3β (GSK-3 missing the first 9 amino acids including Ser-9) in prostate cancer cells increased total AR phosphorylation and more precisely phosphorylation of the ligand binding domain of AR. Additionally, pull-down assays with different GST–AR constructs followed by *in vitro* kinase assay with recombinant GSK-3β demonstrated that GSK-3β phosphorylates AR at the N-terminal domain [23]. However, the amino acids phosphorylated by GSK-3 were not determined. Recently, Chen et al. [24] predicted by computer database analysis Ser-424, Ser-514 (N-terminal domain) and Ser-650 (hinge region) as putative phosphorylation sites for GSK-3 (Fig. 2) but phosphorylation of these sites have not been experimentally demonstrated until now.

ERα: In ERα eight Ser/Thr-phosphorylation sites have been functionally characterised to date: Ser-104, Ser-106, Ser-118, Ser-154 and Ser-167 in the AF-1 domain; Ser-236 in the DNA binding domain; and Ser-305, Thr-311, and Tyr-537 in the AF-2/ligand binding domain [25,26] (Fig. 2). In addition Ser-102, Ser-212, Ser-294, Ser-554, and Ser-559 have been identified as potential phosphorylation sites by mass spectrophotometry [26,27]. Our group established functional links between GSK-3β and ERα and provided evidence that GSK-3β modulates ERα function by phosphorylation of relevant serine residues. *In vitro* kinase assays performed by using wild type and mutant (Ser → Ala) GST-ERα fusion proteins identified the Ser-102, 104, 106 motif and Ser-118 residue in the N-terminal AF-1 domain of ERα as phosphorylation sites of GSK-3β [28] (Fig. 2). In agreement with these results, Ser-118 phosphorylation was also observed by immunodetection after *in vitro* kinase assay using recombinant GSK-3β and ERα proteins [29]. The sequence of the Ser-102, 104, 106 motif and Ser-118 motif suggests that Ser-118 represents an unprimed substrate, while Ser-106 seems to be a primed substrate. Use of the GSK-3β inhibitor LiCl or silencing of GSK-3 by RNAi techniques resulted in decrease of both E2-induced ERα phosphorylation at Ser-118 and E2-induced ERα transcriptional activity in breast cancer cells [28,29]. This suggests a positive regulation of ERα activity by GSK-3β. Unexpectedly, the use of the maleimide inhibitor SB415286 resulted in ERα phosphorylation at Ser-118 and increased transcriptional activity in absence of estrogen which suggests estrogenic properties of this compound (Grisouard and Mayer, unpublished).

Beside the almost ubiquitously expressed ERα subtype, ERβ exhibits a limited expression pattern [30]. The role of ERβ is still under debate and the protein kinases responsible for its phosphorylation have not been clearly identified. The putative ERβ phosphorylation sites include Ser-16, Ser-106, Ser-124 and Ser-255 [8,31]. A potential role of GSK-3β in ERβ phosphorylation and signalling has not been reported so far.

2.3. Docking properties of GSK-3 regarding function of sex steroid receptors

AR: Co-immunoprecipitation of AR with GSK-3 [22,23,32] suggests interaction or complex formation of GSK-3 with AR. However, the effects of GSK-3 docking regarding AR stability and regulation of AR activity are still under debate. Wang et al. [23] reported that androgen-mediated AR transcriptional activation was repressed by wild type GSK-3β or constitutively active S9A-GSK-3β in a dose-dependent manner. In contrast, a catalytically inactive kinase mutant GSK-3β showed little effect on the AR transactivation.

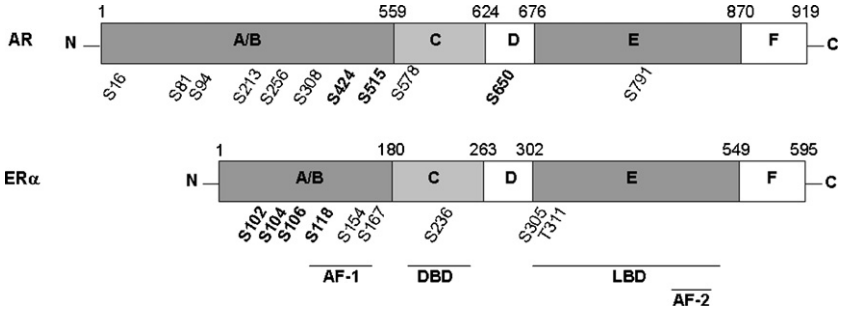

Fig. 2. Known serine (S)/threonine (T) phosphorylation sites of androgen receptor (AR) and estrogen receptor alpha (ERα). GSK-3 phosphorylation sites reported in the literature are highlighted in bold. AF-1 denotes activation function-1; AF-2, activation function-2; DBD, DNA binding domain; LBD, ligand binding domain and letters A–F, the different domains of these receptors with their amino acid sequence numbering.

Salas et al. [22] not only reported that overexpression of wild-type or constitutively active GSK-3β repressed ligand-dependent transcriptional activity of the wild-type AR. They also showed that truncated AR missing the ligand binding domain and hinge region were still responsive to androgen, and androgen-stimulated transcriptional activity of the truncated AR was not inhibited by wild-type GSK-3β overexpression. This suggests that the ligand binding domain of AR is required for the interaction with GSK-3β and for the GSK-3β-related decrease of AR transcriptional activity.

The data reported by Wang et al. and Salas et al. are at variance with those of Liao et al. [33] who showed that LiCl and siRNA targeting GSK-3 reduced androgen-stimulated gene expression and especially expression of the prostate specific antigen (PSA) in LNCaP cells. In agreement with these results, Mazor et al. [32] showed that GSK-3 increased AR transcriptional activity and that GSK-3 inhibitors of the maleimide-type reduced AR protein levels and prostate cancer cell growth. Similarly, Rinnab et al. [34] showed down-modulation of AR protein by the maleimide inhibitor SB216763 and reduction of transcriptional activity due to an increase of proteasomal degradation of AR. These authors also observed a decrease of AR protein and transcriptional activity after depletion of GSK-3 protein by shRNA. This suggests that GSK-3 stabilizes AR triggering its transcriptional activity. However, the decrease of AR stability after GSK-3 inhibition in CWR-R1 cells [32] or GSK-3 silencing or inhibition in 22Rv1 and LNCaP cells [34] was not observed in PC3 cells transfected with an expression plasmid coding for a GFP-AR fusion protein [34] or after silencing of GSK-3 in LAPC-4 prostate cancer cells [33]. The different cell lines used and the different method chosen for reduction of GSK-3 activity (silencing or chemical inhibition) may be responsible for these discrepancies. Furthermore, an important point of concern is the possible androgenic activity of GSK-3 inhibitors. Indeed, AR-014418, a known GSK-3 inhibitor was shown to increase AR transcriptional activity in absence of androgenic stimulus [34].

Liao et al. [33] described that AR is mainly localized in the cytoplasm and translocates into the nucleus after androgen stimulation. They also suggested using LiCl that GSK-3 was not involved in androgen-stimulated AR nuclear translocation in LNCaP or LAPC-4 cells. In agreement with this finding Salas et al. [22] using fluorescence microscopy observed that distribution of both AR and GSK-3β did not influence each other. However, Rinnab et al. [34] demonstrated that inhibition of GSK-3 activity by SB216763, GSK inhibitor XIII or GSK-3 silencing reduced the cytoplasmic to nuclear translocation of AR upon androgen stimulation. They further showed that SB216763 induced a nuclear export of AR in the transfected PC3 cells pretreated by androgens [34]. Salas et al. [22] showed that the intracellular distribution of GSK-3β depends on the GSK-3β type or mutant expressed and the state of GSK-3 activity. Indeed, wild type GSK-3β was localized in both the cytoplasm and nucleus of AR negative and positive prostate cancer cells. The constitutively active GSK-3β was predominantly expressed in the nucleus and the GSK-3β mutated at Tyr-216 to Phe was only found in the cytoplasm [22]. This may restrict GSK-3 interaction with AR to subcellular compartments where GSK-3 is located.

The contradictory observations regarding GSK-3 effects reported above demand a special note of caution. Our own work suggests that maleimide-type inhibitors of GSK-3β decrease GSK-3β phosphorylation at Ser-9 while LiCl increases this phosphorylation (Grisouard and Mayer, unpublished). An increase of GSK-3β phosphorylation at Ser-9 was also observed after LiCl treatment in mice [35]. This indicates that LiCl and maleimide inhibitors affect GSK-3 phosphorylation in different ways and that the effects related to GSK-3 inhibition may be different from one inhibitor to the other. Taken together, GSK-3 inhibition requires careful studies using appropriate GSK-3 mutants or inhibitors to specify the effects of GSK-3 and potential different GSK-3 pools and their precise intracellular action. Understanding the fine tune of GSK-3 regulation and intracellular localisation will highlight its particular involvement in AR activation.

Moreover, the multifaceted interaction between the AR and Wnt signalling pathways seems to be implicated in the development, progression and growth of prostate cancer [36]. Indeed, some groups [37,38] suggest that PI3K/Akt stimulates androgen-activated pathway through GSK-3β inhibition and nuclear β-catenin accumulation. However, Mazor et al. [32] showed that depletion of β-catenin using RNA interference increased rather than decreased AR activity suggesting that endogenous β-catenin is not a transcriptional coactivator for AR. They further showed that competitive binding of axin and FRAT [32] to GSK-3 may prevent GSK-3/AR complex formation and thus interfere with AR transcriptional activity. This reinforces the role of GSK-3 in the regulation of AR activity and simultaneously, complicates our understanding of the modalities of such a regulation.

ERα: Concerning ERα, our group recently reported that GSK-3 interacts with this receptor, playing an important role in both ligand-independent [39] and E2-dependent activation of ERα [28] in breast cancer cells. GSK-3 plays a role in E2-independent TPA-induced ERα nuclear translocation and activation [39]. TPA activates PKCδ, leading to phosphorylation and inhibition of GSK-3β at Ser-9 and release of ERα which then translocates into the cell nuclei. In accordance with these results, immunoprecipitation and confocal microscopy, respectively, showed that GSK-3β and ERα co-immunoprecipitate and co-localize in the cytoplasm of unstimulated breast cancer cells [28]. In cells stimulated with E2, GSK-3β and ERα co-localized in the nucleus. Use of RNAi technology permitted to analyse the involvement of GSK-3 in ligand-dependent ERα function. We found that complex formation between ERα and GSK-3β stabilizes the receptor in the cytoplasm in unstimulated cells and protects it from proteasomal degradation. In E2-treated cells GSK-3β modulates ERα transcriptional activity via phosphorylation of the nuclear receptor at Ser-118 [28] and GSK-3 is required for full transcriptional activity of ERα [29]. Taken together, these observations revealed the crucial role of GSK-3 in the regulation of ERα function and activity

In N2a neuroblastoma cells, Mendez and Garcia-Segura [40] observed GSK-3 and ERα localized in different cellular compartments. Therefore, they were unable to detect an interaction between ERα and GSK-3 in these cells. They suggest an alternative mechanism of control of ERα transcription by GSK-3 mediated by β-catenin. In their model the PI3K/GSK-3 pathway indirectly affects the stability of ERα, inhibition of GSK-3 protecting ERα from proteasomal degradation and activation of GSK-3 by wortmannin accelerating the degradation rate of ERα. A protective role of β-catenin in ERα proteolysis was hypothesized. Other groups described a link between GSK-3/ERα and GSK-3/β-catenin signalling pathways. Cardona-Gomez et al. [41] reported that estradiol regulates interaction of ERα, GSK-3β and β-catenin in rat hippocampus. Kouzmenko et al. [42] suggested a functional interaction between β-catenin and ERα in human colon and breast cancer cells. However, other components involved in the potential cross-talk between Wnt and estrogen signalling pathways must still be identified, and GSK-3β is only a putative candidate.

As outlined above, the docking properties of GSK-3 are of great importance for the regulation of both AR and ERα functions. It will therefore be highly relevant to decipher the key amino acids of GSK-3 and sSRs involved in their physical interaction. Comparison of these amino acid sequences with the binding sites of axin and β-catenin to GSK-3 will contribute further to detect and unravel the potential cross-talk between the sSRs and the Wnt signalling pathways. A recent review describes in more details the interaction of nuclear receptors with Wnt/β-catenin/Tcf signalling [43]. Further investigations in the interactions between Wnt and AR and

Wnt and ERα signalling with emphasis on the role of GSK-3 may highlight the specific function of GSK-3 in the modulation of sSR transcriptional activity.

2.4. Sex steroid receptors' non-genomic signalling induces GSK-3 phosphorylation

Liao et al. [33] described that androgen rapidly (15–120 min after treatment) increased phosphorylation of GSK-3 at Tyr-216 via a PI3K-dependent signalling pathway in prostate cancer cells. A less prominent although significant increase of Ser-9 phosphorylation of GSK-3 was observed in response to longer (24 h) androgen stimulation, which may also be attributed to the PI3K pathway. These androgen-induced phosphorylation reactions have so far not been attributed to non-genomic effects of AR in prostate cancer cells, although a non-genomic AR-dependent activation of PI3K/Akt signalling pathway by androgens was reported in osteoblasts [44].

Rapid biological effects of E2 have been observed in the bone, breast, vasculature, and nervous system [45]. These non-genomic effects of estrogens may be mediated through cell-surface associated ERα and downstream activation of intracellular signal transduction proteins such as protein kinases. ERα localisation at the plasma membrane is still controversial but Song et al. [46] suggested that Shc and IGF-IR serve as key elements in the translocation of ERα to the cell membrane and in the facilitation of ERα-mediated rapid E2 action. In human MCF-7 breast cancer cells, estrogens via binding to the membrane-bound ERα may rapidly activate several protein kinases, i.e., MAPK, PKB/Akt, PKA and PKC. This rapid signal transduction cascades may be relevant for breast cancer cell proliferation in response to E2 [47]. Interestingly, Greger et al. [48] identified a so-called modulator of non-genomic action of estrogen receptor (MNAR) in MCF-7 cells and they suggested that phosphorylation of this novel scaffold protein promotes activation of PI3K upon E2 stimulation, leading to increased phosphorylation of PKB/Akt. They suggest that this mechanism could be responsible for the rapid phosphorylation of the PKB/Akt-substrate GSK-3 at Ser-9 in E2 treated cells. Simultaneous to the E2-induced rapid phosphorylation of GSK-3 at Ser-9, Cardona-Gomez et al. [41] also reported Tyr-216 phosphorylation and a transient activation of GSK-3 in the rat hippocampus. A non-genomic effect of estrogen is hypothesized. In uterine epithelial cells, E2-induced PI3K pathway activation inhibits GSK-3 via Ser-9 phosphorylation and activates cell proliferation [49]. Progesterone was shown to antagonize the effects of E2 via inhibition of GSK-3 phosphorylation at Ser-9 resulting in sustained activation of this enzyme. These data suggest that non-genomic effects of sex steroid hormones may fine tune GSK-3 activity and this may play a crucial role in sex steroid hormones intracellular effects.

3. Conclusion

Recent research demonstrates that GSK-3 plays an important and specific role in the regulation of the function and activity of sSRs. Both kinase activity and docking properties of GSK-3 are highly relevant for phosphorylation of AR and ERα and regulation of their function. So far, GSK-3β was the only GSK-3 isoform described leading to sSR phosphorylation.

Regarding ERα, GSK-3 may represent the missing link between the non-genomic and the genomic effects of ERα as it plays a role in both rapid estrogen effects and transcriptional activity of ERα. The interaction between GSK-3 and AR clearly has a role in the regulation of the receptor activity. GSK-3 interacts with and phosphorylates sSRs and thus modulates sSR activity and signalling pathways. In fact, the complex formation between GSK-3 and sSR was demonstrated as a key process regulating sSR protein stability and GSK-3β/sSR complex formation was suggested as a modulator of intracellular sSR localisation. Activation of sSRs with their specific ligands directly triggers GSK-3 phosphorylation at Ser-9 by rapid activation of Akt/PKB. The resulting inhibition of GSK-3 activity was mainly imputed to the non-genomic effects of sSRs and this may constitute a first step in sSR signalling cascade. Fig. 3 summarizes the known effects of GSK-3 on AR and ER as well as the non-genomic effects of these receptors on GSK-3.

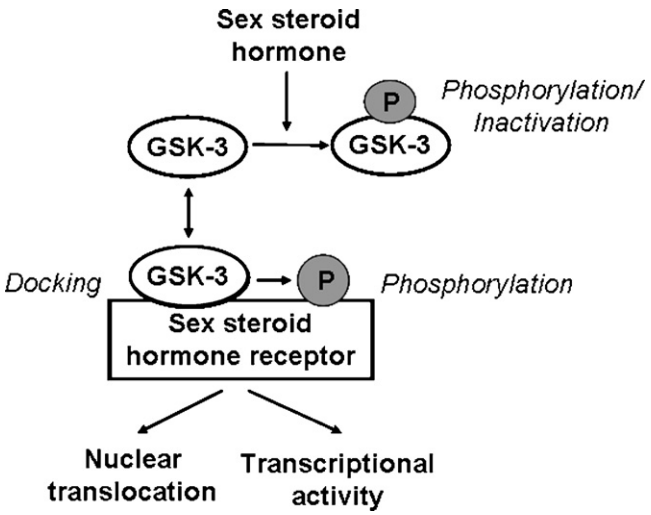

Fig. 3. Summary of the multiple interactions between GSK-3 and sex steroid receptors (sSRs). On the one hand active GSK-3 binds to and phosphorylates sSRs leading either to activation or inhibition of sSR translocation and transcriptional receptor activity. On the other hand sex steroid hormones rapidly activate protein kinases which phosphorylate and thus inactivate GSK-3.

However, the role of GSK-3 in the regulation of sSR activity may be more complex as it first appears. This review highlights the substantial need for further studies regarding the interaction between GSK-3 and sSR signalling pathways. GSK-3 must be studied more systematically while studying the impact of kinases on sSR. Moreover, the non-genomic effects of sSR on GSK-3 phosphorylation need to be investigated further to understand their potential role in the function and activity of sSR. Together, deciphering the role of GSK-3 in sSR phosphorylation will contribute to the understanding of the regulation of the activity of nuclear receptors.

Interestingly sex steroids activate the PI3K/Akt signalling pathway which is also activated by insulin and IGF. Thus, in prostate and breast cancer cells, GSK-3 especially represents a key node for cross-talk between major signalling pathways including the IGF-I receptor, Wnt, and sSR involved in cell proliferation. Targeting GSK-3 in a directed way may therefore help to treat hormone-dependent prostate, uterus and breast cancer progression. The use of small-molecule GSK-3 inhibitors as selective anticancer agents has been suggested only recently by Patel and Woodgett [50]. However, unexpected effects on sSR may limit the application of small molecule inhibitors. Furthermore, the role of GSK-3 regarding ERα has revealed recent and promising interest in neuroendocrinology.

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