

## Targeting rapid action of sex-steroid receptors in breast and prostate cancers

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## 1. ABSTRACT

Human breast and prostate cancers are complex diseases caused by the progressive accumulation of gene mutations combined with epigenetic deregulation of critical genes and derangement of signaling pathways. Compelling evidence indicates that steroid hormones elicit non-genomic responses in cytoplasm of target cells. In this cellular location, steroid-coupled receptors recruit signaling effectors or scaffold proteins, thereafter activating multiple pathways leading to proliferation, survival, migration and invasiveness. Thus, the immediate challenge is the dissection of key upstream events regulating steroid response in target tissues to prevent progression and improve treatment of breast and prostate cancers. Progress in our understanding of the molecular mechanisms that play a master role in these cancers has strongly stimulated the search for specific inhibitors of key signaling molecules. This review aims to give an up-to-date report of the complex network regulating non-genomic action of steroid hormones in target cells. The final section highlights recent advances from our laboratory and future directions in alternative approaches for the treatment of breast and prostate cancers.

## 2. INTRODUCTION

Molecular and clinical evidence has implicated steroid receptors in breast and prostate cancer growth and progression. About 70% of human breast cancers are estradiol receptor alpha (ER alpha)-positive and ER alpha status predicts a favorable disease outcome. Most patients with ER alpha-positive breast cancer in fact receive tamoxifen as adjuvant endocrine therapy (reviewed in 1). Similarly, androgen receptor (AR) is a hallmark of prostate tumors and androgen deprivation treatment is commonly used as a first-line therapy (reviewed in 2). Although hormonal treatment has improved the outcome of breast and prostate cancers, many patients become resistant to this therapy and develop metastatic tumors.

Emerging data indicate that breast and prostate epithelial cells express both ER and AR and are targeted by both estradiol and androgens, thus implying that steroid receptors can substitute each other in mediating breast or prostate cancer cell growth. Sex steroid hormones are master regulators of breast and prostate cancer growth and this effect has so far been attributed to the activation of transcriptional machinery (3). Steroids, however, also exert

very rapid actions by triggering signaling circuits initiated outside the nucleus. These actions were described as early as 1967 by Szego and Davis, who reported an increase in uterine cAMP within 15 seconds after iv treatment with physiological doses of estradiol (4). Interestingly, this effect was insensitive to transcriptional inhibitors. Progress in this field was however slow until almost a decade ago. We now appreciate that rapid steroid actions control development, growth, survival, and motility in very different cell types, such as breast and prostate cancer cells, osteoblasts, neurons, cardiomyocytes, endothelial cells and fibroblasts (reviewed in 5).

Mechanistically, rapid steroid action usually occurs through classical intracellular receptors. These findings have been observed in breast and prostate cancer cells (6, 7, 8, 9, 10), endothelial cells (11) and osteoblasts (12) as well as in cells expressing classical steroid receptors incompetent in transcriptional activation, such as fibroblasts (13 and 14) and stromal uterine cells (15), and in steroid receptor-negative cells ectopically expressing classical steroid receptors (6, 7, 8, 16, 17, 18, 19, 20). In these cells, rapid steroid actions take place through steroid receptor interaction with a number of signaling or scaffold molecules, such as Src, PI3-K, integrins, filamin A and p130Cas (7, 8, 17, 13, 14, 21), or involve the anchorage of conventional receptors to the plasma membrane through palmitoylation/myristoylation (reviewed in 22). The emerging picture indicates that the upstream interaction of classical steroid receptors with signaling or scaffold proteins controls cell cycle (16, 17, 18), proliferation (23 and 24), chromatin remodeling (20), migration (13, 14, 25, 26), and even nuclear exclusion of steroid receptors (27, 28, 29).

It is, however, quite clear that the convergence of transcriptional and non-transcriptional signals results in the integrative effects of sex steroid hormones in breast and prostate cancers. In this light, identification of signaling components and molecules that selectively trigger specific steroid action may offer new hints to achieve a more tailored therapy of human breast and prostate cancers. This report aims to provide a comprehensive review on current opportunities in this field.

### 3. SIGNALING BY SEX-STEROID RECEPTORS IN BREAST CANCER

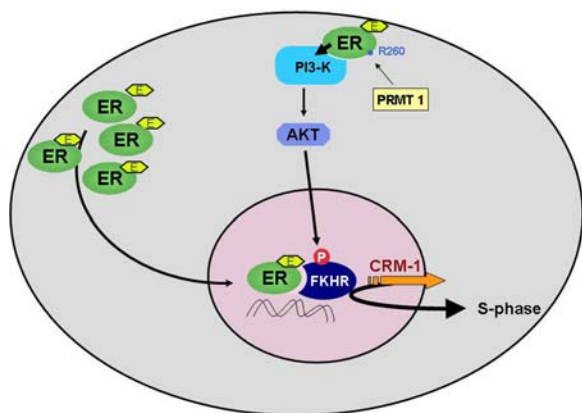
Sex steroids, ER alpha, progesterone receptor (PgR), and growth factors are required for the development of normal mammary gland. Estrogens stimulate ductal elongation, progestins induce ductal side-branching and alveologenesis, while epidermal growth factor (EGF) promotes proliferation of terminal end buds and enhances both estrogen-induced ductal outgrowth and progesterone-induced side-branching (30 and 31). While only a few, non-dividing epithelial cells in the lumen of the mature mammary gland express ER alpha and PgR, the majority (about 70%) of newly diagnosed breast cancers express ER alpha. Over-expression of ER alpha is a prognostic and predictive factor in breast cancer patients and a large subset of breast cancers shows a single-gene amplification of the

ER alpha gene, thus suggesting that this amplification may be a common mechanism in proliferative breast disease and a very early genetic alteration in breast cancer progression (32 and 33). Expression of PgR in breast cancer serves as a functional assay because it indicates that the ER transcriptional pathway is intact. Thus, the existence of steroid receptor (SR)-positive proliferating cells in breast cancer implies that signaling pathways involved in normal mammary gland growth and development are likely reactivated in breast cancer. This mechanism is poorly understood.

Estradiol challenging of breast cancer MCF-7 cells rapidly and transiently triggers activation of the Src/Ras/Raf/MAPK pathway (34 and 6). This activation ensures DNA duplication, as small molecule inhibitors, dominant negative versions or neutralizing antibodies of signaling effectors impair estradiol-induced S-phase entry in these cells (16). Mechanistically, estradiol triggers association of classical ER alpha with Src in MCF-7 cells. This occurs through direct interaction between the single tyrosine-phosphorylated ER alpha at position 537 and the SH2 domain of Src (8). A similar mechanism also takes place upon EGF stimulation of breast cancer MCF-7 cells (9). EGF induces ER/Src association, thereby activating the Src-dependent pathway. This activation increases DNA synthesis and induces cytoskeleton changes in breast cancer MCF-7 cells (9). These findings indicate that ER mediates signaling events even in the absence of estradiol. Thus, ER/Src interaction represents a novel and promising target in breast cancer therapy, since these tumors respond to both steroid hormones and growth factors.

Numerous findings support the concept that protein-protein interactions integrate signaling activation by estradiol and related functions in breast cancer cells. Association of the adapter protein, p130Cas, with ER alpha and Src in breast cancer T47D cells (21) indicates that complexation of these proteins modulates estradiol-induced cytoskeleton changes and invasiveness in breast cancer cells, as p130Cas interacts with focal adhesion kinase (FAK). Again, estradiol activation of the Src-dependent pathway occurs alongside PI3-K (17). Hormone stimulation of MCF-7 cells triggers the assembly of a complex made up of ER alpha, Src, p85 alpha, the regulatory subunit of PI3-K, and the atypical protein kinase C, PKC-zeta. This complex simultaneously activates Src- and PI3-K-dependent pathways. Estradiol-activated PI3-K targets Akt and PKC-zeta. Once activated, Akt increases cyclin D1 transcription, whereas PKC-zeta facilitates Ras recruitment to the ER/Src/PI3-K complex. In such a way, Erk-2 translocates into the nuclei, where it induces cytoplasmic release of p27. The interplay between estradiol-activated signaling effectors and cell cycle markers positively affects DNA synthesis in MCF-7 cells (17 and 18).

It has been shown that estradiol-induced arginine methylation of ER alpha is required for association of this receptor with Src and PI3-K in extra-nuclear compartment of breast cancer MCF-7 cells (29). Thus, ER might be trapped in cytoplasm through its interaction with signaling effectors. Consistently, we have shown that estradiol



**Figure 1.** Model of ER alpha nuclear export in breast cancer cells. A model of ER alpha nuclear export in breast cancer MCF-7 cells based on experimental evidence from our laboratory is shown. Estradiol-stimulated PI3-K/Akt pathway leads to FKHR phosphorylation at Ser 256, thus triggering the associated export of FKHR/ER. This export removes the transcriptional repressor activity of FKHR and triggers DNA synthesis (ref. 28 in the text). The model here depicted is compatible with findings showing that methylation of ER alpha by PRMT1 tethers the receptor in cytoplasm of MCF-7 cells, where it recruits and activates PI3-K, Src and FAK. Hypermethylated ER alpha is a hallmark of aggressive human breast cancers (ref. 29 in the text).

activation of the PI3-K/Akt pathway controls the nuclear export of ER alpha in MCF-7 cells. We have also found initial evidence that COOH-terminus of ER alpha contains the nuclear export sequence (NES) that interacts with exportin/Crm1. A NES-ER alpha mutant is unable to interact in vitro with exportin/Crm1, does not exit nuclei, and fails to mediate S-phase entry upon hormonal stimulation of MCF-7 cells (28). Thus, estradiol activation of PI3-K signaling pathway links ER alpha nuclear export with cell cycle progression in breast cancer cells. Our findings pointed to the master role of forkhead (FKHR) in this process. Estradiol activation of PI3-K/Akt pathway triggers FKHR phosphorylation, thus increasing its association with ER. In this way, the nuclear export of FKHR/ER alpha complex is facilitated and the inhibitory action of FKHR on cell cycle is removed. This results in cell cycle progression of breast cancer cells (28 and Figure 1). The model in Figure 1 suggests that trapping of ER in nuclear compartment blocks DNA synthesis in breast cancer cells by inhibiting cytoplasmic release of FKHR.

Relevant to these findings is the observation that a shortened form of the metastatic tumor antigen 1 (MTA1s) sequesters ER alpha in the cytoplasm and leads to malignant phenotypes by enhancing non-genomic functions of ER in MCF-7 cells (35). Further, a subset of human breast cancer specimens exhibits hypermethylated ER alpha that is exclusively localized in the cytoplasm, where it recruits and activates both Src and PI3-K (29). It is clear from these findings that ER alpha location regulates the estradiol signaling output, such as DNA synthesis,

anchorage-dependent growth and transformation of target cells. In turn, signaling pathways control ER location in breast cancer cells.

Increasing evidence implicates PgR in development and progression of human breast cancers (reviewed in 36). Progesterone activation of the Src/MAPK pathway has been described in T47D breast cancer cells (7). In these cells, which express PgR under basal conditions, progestin-bound PgR-B triggers association of ER alpha with Src and consequent activation of the Src/Ras/MAPK pathway (7). This activation controls progestin-induced DNA synthesis (16). Remarkably, experiments in Cos-7 cells ectopically expressing PgR-B and ER alpha suggested that PgR-B/ER-alpha cross talk either induces or amplifies the progestin signal. The mechanism underlying progesterone activation of the Src-dependent pathway by cross talk between PgR-B and ER alpha was subsequently clarified (19). This activation depends on the association of unliganded ER alpha with PgR-B via two domains of PgR (19). Progestin activation of MAPK cascade triggers phosphorylation of histone H3, thereby inducing chromatin remodeling and expression of target genes in T47D cells (reviewed in 37). The observation that this effect is also prevented by pure anti-estrogens again implicates the regulatory role of PgR-B/ER cross talk in progestin signaling (20).

Human PgR directly interacts with the Src-SH3 domain through a proline-rich (PXXP) motif and this interaction is stimulated by progestin (38). The role of hormone-induced PgR-B association with Src has been mostly investigated in different PgR-B- as well as ER-negative cells ectopically expressing PgR-B. Depending on the cell type and experimental conditions, growth arrest (38) or stimulation of cell proliferation (39 and 40) occur upon progestin stimulation. These findings ultimately converge in supporting the regulatory role of Src in progestin-induced proliferation. In addition to the rapid activation of MAPK, the progestin-bound PgR-B induces sustained activation of MAPK in T47D cells. This activation results from PgR-mediated transcriptional up-regulation of the secreted glycoprotein Wnt1, which binds to the seven-transmembrane receptor Frizzled and stimulates the MMP-dependent cleavage of EGFR ligands. In this way, EGFR transactivation occurs and sustained activation of the downstream Src and MAPK effectors follows (41). Again, progestin activation of MAPK leads to phosphorylation of Ser 294 of PgR. This phosphorylation mediates PgR nuclear export and degradation. Leptomycin B inhibits this event, which is involved in regulation of PgR transcriptional activity (27). These latter findings suggest that a feedback loop between transcriptional and non-transcriptional action regulates location as well as the coupled functions of PgR.

AR has only recently been considered as a potential biomarker in breast cancer. It is commonly co-expressed with ER and PgR and androgen signaling has been identified in benign and malignant breast tissue (42). AR expression, however, has been observed in a subset of ER/PgR-negative breast cancers (43), thus raising the

possibility that AR might serve as a therapeutic target in these tumors. Hormonal manipulations of androgen signaling (ie androgen blockade with the anti-androgen bicalutamide) are currently being investigated in a clinical trial of ER/PgR-negative/AR-positive breast cancer patients and selective androgen receptor modulators (SARMs) are in development stage (reviewed in 44).

In breast cancer MCF-7 cells, we discovered that a small fraction of ER alpha and AR are associated under basal conditions. Challenging of these cells with androgens or EGF causes simultaneous interaction of both receptors with Src (8 and 9). Although association of either ER or AR with Src activates this kinase, the presence of both receptors induces stronger Src activation, possibly because each of the receptors interrupts only one of the two intramolecular inhibitory interactions that keep Src in an inactive conformation under basal conditions (8). The assembly of ER/AR complex strongly activates the Src-dependent pathway leading to cell proliferation and cytoskeleton changes in breast cancer cells (8 and 9). Findings obtained with EGF also underline the role of ER/AR complex in signaling elicited by this growth factor in breast cancer.

Evidence collected in recent years has revealed a complex network of proteins recruited to SR outside cell nuclei or near cell membranes in breast cancer cells. This network integrates rapid action of sex steroids with later events involving gene transcription (reviewed in 45 and 37). The balance between these two effects mediates the net outcome of breast cancer cells. Breast tumors frequently develop resistance to endocrine treatments as they progress. The majority of these resistant cancers, however, retain high levels of ER alpha or PgR. In these resistant tumors, the rapid action of steroid receptors could be activated by sub-optimal hormonal concentrations and steroid receptor signaling might inappropriately trigger gene transcription independently of receptor nuclear location. Further analysis may reveal the way in which the assembly of signaling platforms outside cell nuclei modulates the functions of intermediate signaling effectors or final target genes in breast cancer.

#### 4. SIGNALING BY SEX-STEROID RECEPTORS IN PROSTATE CANCER

Prostate cancer is very common in Western society and much evidence points to a critical role of androgens and estrogens in prostate growth and cancerogenesis. In the early stages, prostate cancer requires androgens for its proliferation. As the tumor progresses, cancer cells invade neighboring tissues and escape from the prostate gland. Metastatic lesions then appear in bone and lymph nodes. The standard therapy is treatment with anti-androgenic agents to reduce the efficacy of circulating levels of testosterone. In spite of its initial efficacy, prostate carcinoma cells become insensitive to this therapy. The mechanism responsible for prostate cancer progression and androgen independence is still debated and includes overexpression or mutation of AR, deregulation of

interactions between androgen receptor and transcriptional machinery, and derangements of growth factor-, Src tyrosine kinase- and integrin-dependent signaling (reviewed in 2 and 44). Thus, analysis of molecular mechanisms responsible for prostate cancer progression and hormone resistance is fundamental for the development of effective therapies.

It was initially observed that stimulation of prostate cancer-derived LNCaP cells with androgen rapidly activates the Erk-2 pathway (10). Subsequent studies showed that androgen or estradiol treatment of LNCaP cells promotes Erk-2 activation and proliferative effects by inducing the rapid assembly of a signaling complex containing classical AR, ER beta and Src (8). This ternary complex follows direct interaction of a proline-rich motif of AR with the SH3-Src domain, and a phosphorylated tyrosine of ER beta, most likely the Tyr 443 residue, with the SH2-Src domain (8). As discussed in the previous section, this complex fully activates Src and its dependent network in prostate and breast cancer cells (8). Consistently, Src family kinases are frequently deregulated in prostate cancers (reviewed in 2) and estrogens play an important role in prostate carcinogenesis (46 and 44). Beneficial effects of the selective estrogen receptor modulator (SERM), toremifene, have been shown in phase II trials (47).

Increasing evidence from our and other laboratories also points to the role of cross talk between sex steroid receptors and growth factor signaling. EGF challenging of LNCaP cells also induces the assembly of ER beta/AR/Src complex. This results in stimulation of DNA synthesis and filopodia formation (9). Thus, the ternary complex conveys the mitogenic and migratory signals of EGF even in the absence of steroids. On the other hand, both androgens and estrogens up-regulate IGF-1R expression in prostate cancer cells through non-genomic pathways (48, 49, 50). These findings are consistent with the observed deregulation of IGF-1 axis in human prostate cancers (51).

The scaffold protein MNAR contributes to the Src/AR complex assembly and its constitutive up-regulation is correlated with androgen-independent transition of LNCaP cells (52). Interaction of AR with scaffolds/adapters is expected. AR directly interacts with the scaffold protein filamin A (FlnA) or its fragments and these interactions have been correlated to nuclear translocation (53) and transcriptional action (54) of AR as well as to androgen dependence in prostate cancer LNCaP cells (55). Cytoplasmic localization of FlnA has been correlated with metastatic and hormone-refractory phenotype in human prostate cancer (56), thus suggesting that intracellular localization of FlnA orchestrates invasiveness and even hormone responsiveness of prostate cancers. Most likely, the AR/FlnA complex increases motility and invasiveness when assembled in cytoplasm, while it helps the transcriptional machinery in nuclei. This idea is supported by our recent findings showing that cytoplasmic AR/FlnA complex triggers the migratory phenotype in androgen-stimulated fibroblasts (14).

**Table 1.** Antiproliferative peptides targeting steroid receptor/protein interactions

PEPTIDE	PROPERTIES
pY-peptide (ref. 24 in the text) Ac-Leu-pTyr-Asp-Leu- Leu-Leu-NH <sub>2</sub>	- inhibits estradiol-induced ER alpha/Src association and DNA synthesis in MCF-7 cells at 1 nM - does not affect ERE-dependent transcriptional activity - suppresses growth of MCF-7 cell xenografts.
Tat-conjugated ER-NES peptide (ref. 28 in the text) Ac-EFVCLKSIILLNS- AAA-RKKRRQRRR-NH <sub>2</sub>	- competes with Crm1/ER alpha interaction and sequesters ER alpha in nuclei of MCF-7 cells - inhibits DNA synthesis induced by estradiol in MCF-7 cells - does not affect ERE-dependent transcriptional activity.
S1 peptide (ref. 23 in the text) Ac-PPPHPHARIK-NH <sub>2</sub>	-inhibits androgen-induced AR/Src interaction and DNA synthesis in LNCaP cells at 1-10 nM - does not affect ARE-dependent transcriptional activity - suppresses growth of LNCaP xenografts.

In LNCaP cells that lack PTEN, androgen activation of Akt depends on AR/Akt-1 interaction in lipid rafts. This interaction is disrupted by the anti-androgen bicalutamide, but is independent of PI3-K inhibitors (57). Thus, a particular location of AR might control Akt signaling independently of PI3-K.

In sum, investigating AR using proteomic approach offers a way of assessing new proteins that contribute to androgen biology. AR interacts with a plethora of signaling molecules or scaffolds or co-regulators (reviewed in 58) that act in a variety of subcellular locations, bridging AR with the basal signaling machinery, modulating AR nuclear translocation, influencing DNA binding and gene transcription, and conferring AR stability.

## 5. INHIBITORS OF STEROID RECEPTOR/SIGNALING MOLECULE INTERACTION IN BREAST AND PROSTATE CANCERS

Evidence collected in the last few years reveals a crucial role of non-genomic steroid action in breast and prostate cancers. It also identifies a new and unexpected role for SR localization in controlling proliferation and motility of target cells (13, 14,28). Moreover, our findings support the notion that specific targeting of the interaction between SR and signaling effectors (eg Src) or nuclear export receptors (eg exportin/Crm1) antagonizes the proliferative rate of breast and prostate cancer cells. In this respect, it was intuitively obvious to design new compounds specifically interfering in SR/Src association or ER alpha/Crm1 interaction.

The observation that ER alpha phosphotyrosine 537 is required for the association of ER alpha with the Src-SH2 domain (8) led us to design and synthesize a small peptide derived from ER alpha sequence surrounding Tyr 537 (24). The peptide was amino-terminal acetylated and carboxyl-terminal amidated to reduce cleavage by exopeptidases. At very low concentration (1 nM) the peptide

rapidly penetrates across cell membranes through a temperature-independent mechanism and inhibits DNA duplication induced by estradiol or EGF in breast cancer MCF-7 cells. Further, the peptide inhibits the survival effect induced by estradiol in MCF-7 cells, while it does not affect EGF-induced S-phase entry in ER-negative breast cancer MDA-MB231 cells. Thus, the peptide specifically targets ER-mediated effects by disrupting the upstream ER alpha/Src association and the consequent events (Src and Erk-2 activation, cyclin D1 up-regulation) in MCF-7 cells (24). Remarkably, the peptide inhibits tumor growth in MCF-7 cell xenografts established in immunodepressed mice in a dose-dependent fashion, leaving unaffected estradiol-induced ERE-dependent transcriptional activity (24). Table 1 summarizes the properties of the peptide.

The 444-456 sequence of ER contains a putative NES. This sequence shows homology with the conserved leucine-rich and REV-like NES of p53 and, remarkably, is conserved in other steroid receptors, such as ER beta, PgR, AR, GR and MR, suggesting that this sequence is responsible for Crm1-dependent nuclear export of most steroid receptors (28 and 59). Mutations in the hydrophobic core of this sequence impair estradiol-induced nuclear export of full-length ER alpha in MCF-7 cells (28). Since the NES-ER alpha mutant we prepared fails to mediate estradiol-induced DNA synthesis, we reasoned that targeting of NES-ER sequence by synthetic peptides likely inhibits estradiol-induced DNA synthesis by trapping ER/FKHR complex in nuclei of target cells. Therefore, using a well-accepted approach to deliver peptides into the cell nuclei (60), we designed and synthesized a Tat-conjugated peptide corresponding to the residues 444-456 of ER alpha (Table 1). The carboxyfluorescein-coupled peptide accumulates in nuclei of MCF-7 cells within 30 min (28). This peptide displaces the estradiol-induced in vitro interaction between recombinant Crm1 and ER alpha, and sequesters the receptor in nuclei of 60 min estradiol-treated breast cancer MCF-7 cells (28).

The Tat-conjugated peptide does not affect the transcriptional activity of ER whereas it leaves unaltered p27, p53 and Rev nuclear export (28 and unpublished data). Thus, it specifically interferes in ER alpha localization. Interestingly, the Tat-conjugated peptide markedly reduces estradiol-induced DNA synthesis, without interfering in serum-induced DNA synthesis in MCF-7 cells. The peptide does not affect the nuclear export of other SR (unpublished data). These findings indicate that the compound we designed specifically interferes in estradiol action and ER localization.

Deletion of the 371-422 sequence of the hAR, which contains a proline stretch, abolishes the in vitro association of the receptor with the Src-SH3 domain (5). Thus, a peptide corresponding to the amino acids 377-386 of the hAR was designed (Table 1). It was amino-terminal acetylated and carboxyl-terminal amidated. The uptake of carboxyfluorescein-conjugated S1 peptide was followed by confocal microscopy in quiescent, unfixed LNCaP and MCF-7 cells. Within 30 min at 37 °C, the peptide was

delivered into the cells in a temperature-independent fashion (5). In LNCaP and MCF-7 cells the S1 peptide (at 1nM) inhibits DNA synthesis by disrupting the interaction of AR and ER with Src induced by androgens or estradiol. As expected, the S1 peptide also prevents the hormonal activation of both Src and Erk-2 (5). Remarkably, the S1 peptide also abolishes the EGF effect in LNCaP and MCF-7 cells. These findings further reinforce our data on EGF-triggered AR/ER/Src complex assembly in prostate and breast cancer cells (9). The S1 peptide does not affect EGF-induced DNA synthesis in AR-negative PC3 or DU145 cells and does not modify intrinsic Src activity. These data clearly demonstrate that the S1 peptide specifically interferes in AR-dependent Src activation. Interestingly, the S1 peptide does not significantly affect hormonal action on ER- or AR-dependent transcription as shown by gene reporter assay in LNCaP and MCF-7 cells (5). Lastly, treatment with S1 peptide strongly inhibits tumor growth of LNCaP xenografts established in nude mice (5).

Table 1 illustrates the properties of the S1 peptide.

## 6. SUMMARY AND PERSPECTIVES

Breast and prostate cancers are both hormone-dependent cancers, at least in some stages of their progression. Hormonal manipulation represents an important therapeutic approach. Although most breast and prostate cancers initially respond to hormone therapy, the majority of tumors start to grow again. Hormone therapy in relapsed tumors may result in additional short-term response. Finally, hormone-resistant metastatic breast and prostate cancers will frequently develop. Thus, the challenge lies in dissecting the mechanisms by which SR signaling pathways continue to influence cell growth and invasiveness of cancer cells. Recent years have seen the discovery of a significant numbers of master proteins in breast and prostate cancers. Collectively, these findings are providing valuable insights into diagnosis, prognosis, drug development and therapy management of these cancers. Particularly, basic research has generated a wealth of data characterizing the molecular changes that occur in breast and prostate cancers. These findings have advanced our understanding of steroid action and allowed the identification of more discriminating action of SR in these cancers.

Studies in cultured cells and animals have revealed important details regarding SR non-genomic regulatory complexes and their functional role. Many SR interacting proteins have been discovered by yeast two-hybrid assay, GST pull-down assay, and far Western analysis. Although these techniques have improved our understanding of non-genomic steroid action, they are limited by their ability to detect only one interacting partner at a time. Regardless of this limitation, many protein-protein complexes regulating rapid steroid actions have so far been detected. As discussed in this review, this field is one of the most promising and dynamic areas of non-genomic steroid action because of its therapeutic impact.

Rapid development in this field will depend on large-scale (omics) data acquisition as well as analysis of multiple component conformations. Many molecular switches, including G-proteins, can be reversibly shifted between two or more stable states, thus interacting with a different set of signaling effectors (reviewed in 61). These analyses, together with the study of temporal and spatial involvement of single components or rearrangement of complexes in response to cellular milieu and cellular changes, should lead to new advances and create major opportunities in the treatment of breast and prostate cancers.

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**Abbreviations:** AR, androgen receptor; Crml, chromosome region maintenance 1; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estradiol receptor; FAK, focal adhesion kinase; FKHR, forkhead in rhabdomyosarcoma; IGF-1, insulin-like growth factor 1; IGF-1R, insulin-like growth factor 1 receptor; MAPK, mitogen activated protein kinases; MEK-1, mitogen-activated kinase kinase; MNAR, modulator of non-genomic action of steroid receptors; MTA1, metastatic tumor antigen; NES, nuclear export sequence; PgR, progesterone receptor; PI3-K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PTEN, phosphatase and tensin homologue deleted on chromosome; SR, steroid receptors.

**Key Words:** Breast And Prostate Cancers, Steroids, Signaling Activation, Targeted Therapy, Review

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