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Review

Sex steroid hormone receptors, their ligands, and nuclear and non-nuclear pathways

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Abstract: The ability of a cell to respond to a particular hormone depends on the presence of specific receptors for those hormones. Once the hormone has bound to its receptor, and following structural and biochemical modifications to the receptor, it separates from cytoplasmic chaperone proteins, thereby exposing the nuclear localization sequences that result in the activation of the receptor and initiation of the biological actions of the hormone on the target cell. In addition, recent work has demonstrated new pathways of steroid signaling through orphan and cell surface receptors that contribute to more rapid, "non-nuclear" or non-transcriptional effects of steroid hormones, often involving G-protein-mediated pathways. This review will summarize some of these studies for estrogens, androgens and progestins.

Keywords: sex steroid hormones; receptors; estrogens; androgens; progesterone; genomic pathway; non-genomic pathway; orphan receptor

1. Introduction

There are two types of binding proteins in the superfamily of intracellular receptors: one consists of the unbound receptor of the thyroid hormones, which is fixed on DNA and represses transcription, only to be activated when the thyroid hormone enters the cell and binds, and the second type of receptor protein, that for steroid hormones, which is unable to bind DNA in the absence of its ligand. Only the formation of the hormone-receptor complex produces conformational changes that

allow transfer into the nucleus and binding to the specific DNA sequences that regulate gene transcription. Once in the nucleus, these steroid-receptor complexes regulate transcription by binding, usually as dimers, to the response elements normally localized in the regions of the responsive target genes.

The receptors for androgens and estrogens are members of the superfamily of nuclear receptors that also include those for progesterone, glucocorticoids, mineralocorticoids, vitamin D, thyroid hormones and retinoic acid; all of these hormones can freely diffuse across the plasma membrane and bind with their receptors that then interact with specific binding sites on DNA, thus acting as "transcription factors regulated by hormones". Furthermore, emerging evidence has revealed new pathways of steroid signaling through receptor subfractions localized at the cell membrane that have been implicated as responsible for the non-nuclear effects of steroid hormones [1,2]. These non-conventional signaling mechanisms are often wrongly named "non-genomic" or "non-nuclear"; however they may be more properly labeled "non-transcriptional" so as to emphasize that DNA binding of the receptors and RNA synthesis are not required.

2. Cell membrane sex steroid receptors

The sex steroid receptors (SSRs) are transcription factors mainly localized in the cytoplasm but also to the cell membrane, that regulate the expression of target genes by binding to specific sequences present at the level of DNA called "sex steroid response elements" (SSRE). These are nucleotide sequences specifically recognized by the hormone-receptor complex [3]. This leads to nuclear translocation of ligand and homo or heterodimeric receptors to the SSRE on the promoter of target genes, thereby regulating gene expression [4]. In particular, SSR activation is mediated by two transcriptional activating domains, respectively called AF-1 and AF-2. AF-1 is localized in the N-terminal region and is characterized by a ligand-independent transcriptional activity, while AF-2 is localized within the ligand-binding domain (LBD) and shows ligand-dependent transcriptional activity [5]. This activated complex coordinates the formation of protein complexes composed of several coactivators or corepressors localized on chromatin of their target genes.

The dimerization of the receptors occurs in response to ligand binding; this causes a conformational change that exposes a surface of the receptor with which the transcriptional regulators can interact. The dimer binds SSRE elements that are found in the promoters of genes that respond to sex steroids [6]. For example, some of the coactivator family members of the steroid receptors (SRC) p160 (SRC-1, SRC-2 and SRC-3) bind SSRE through one of three LXXLL motifs, forming amphipathic alpha-helices [7]. The coactivators that are involved belong to the p160 SRC family, which consists of SRC-1 (NCOA-1), SRC-2 (GRIP1/TIF2/NCOA-2), and SRC-3 (AIB1/TRAM-1/ACTR/RAC3/PCIP), proteins more specific for estrogen and progesterone receptors [8], whilst ARA70 and TBLR1 are specific for androgen receptors. The hormone-receptor pairs form a homodimeric complex on specific regulatory elements of the DNA that activate or repress steroid-regulated gene transcription by RNA polymerase, leading to altered levels of specific mRNA. The translation of the RNA messages on cytoplasmic ribosomes influences the appropriate proteins that alter cell function, growth or differentiation. In the absence of hormone, sex steroid receptors (which includes androgen, progesterone and estrogen receptors) exist as inactive oligomeric complexes that are sequestered in the cytoplasm by the heat-shock protein (Hsp) 90, which behaves as a molecular chaperone, as well as heat shock proteins 70, cyclophilin 40, FKBP51, and FKBP52 [9,10]. Another

chaperone, called p23, stabilizes the aporeceptor complex by blocking Hsp90 in the ATP-bound substrate conformation. Co-chaperones utilizing tetratricopeptide repeat motifs are necessary for binding of Hsp90. Furthermore, other chaperones called Hsp40 and Hsp70 and an organizing protein called heat-shock organizing protein (Hop) are important in the assembly of the steroid receptor-Hsp90 complex [11].

The steroid receptor-Hsp90 complex seems to be necessary for the receptor to stabilize in a conformation for binding to the ligand with high affinity and also to maintain its solubility in the cell. In addition, although the receptor is held in this complex, it is inactive as a transcription factor, explaining why the Hsp90 complex works as a repressor of transcriptional activity: it prevents nuclear localization, dimerization, DNA binding and interaction of the complex with transcriptional coactivators [12]. Once in the nucleus, SSR binds to the SSREs on promoter/enhancer regions, recruits coregulators, and forms the transcriptional machinery for SSR-regulated gene expression. This SSR-signaling pathway is known as the "genomic pathway" and relies on SSR nuclear translocation and SSR-DNA binding for cell proliferation. This pathway is characterized by increased expression of specific SSR-regulated genes. There are interacting cofactors that improve (i.e.: coactivators) or reduce (corepressors) steroid receptor activation without having any significant effect on the level of basal transcription.

3. Non-genomic signaling mechanisms of sex steroid hormones

There is a much more rapid and reversible pathway that has been shown to be involved in the regulation of cell proliferation particularly in prostate cancer [13,14]. This signaling pathway is known as the "non-genomic pathway", and it requires neither SSRs nuclear translocation nor SSR-DNA binding.

Activated SSRs in the cytoplasm, but especially in the plasma membrane, can stimulate second messenger cascades, interacting with several signaling molecules like phosphatidyl-inositol 3-kinase (PI3K)/Akt, the non-receptor tyrosine kinase c-Src (Src), Ras-Raf-1, protein kinase A (PKA) and protein kinase C (PKC), which in turn converge on mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) activation, leading to cell proliferation [15]. The SSRs can be localized in the plasma membrane or be in specialized micro domains of the lipid bilayer that are enriched with sphingolipids, caveolins, Src family kinases, and G proteins, called "lipid rafts" [16]. In this regard there is a great deal of evidence for the interaction between AR (androgen receptor), ER (estrogen receptor), PR (progesterone receptor) and the tyrosine kinase domain of Src, leading to its activation. The latter is well known to be localized in the inner side of the plasma membrane through post-traslational modifications like myristylation or palmitoylation in the N-terminus that determine a physical interaction with caveolin-1 [17]. Src homology 2 (SH2) and Src homology 3 (SH3) cause an autoinhibition of Src that is relieved when SSR bind SH3 activating an adaptor protein (Shc) that regulates MAPK/ERK-1/2 signaling pathway [18]. Most sex steroids bind sex hormone-binding globulin (SHBG) when circulating in the bloodstream, a protein produced in liver under the influence of these same steroids. The affinity by which hormones bind SHBG is different; particularly, androgens have high affinity for SHBG compared to estrogens. Nowadays it is well established that there is a SHBG receptor (SHBGR), a G-protein coupled receptor, that activates a signaling cascade mediates by cAMP. This leads to the activation of PKA pathways that induce a phosphorylation status change of steroid receptors, and their coregulators that modulate SR transcriptional activity. To induce the cAMP pathway SHBG must first bind to the SHBGR, which is

followed by steroid binding. If steroid binding comes first, interaction with the SHBG receptor cannot happen, though both of these mechanisms may potentially influence the transcriptional activation of the nuclear SR. In addition, the SHBGR may directly bind steroids or indirectly influence the activity of a membrane steroid-binding protein. One of the effects mediated by this putative receptor is to increase intracellular calcium levels. The elevation of intracellular calcium activates signal transduction cascades including PKA, PKC and MAPK, and can modulate the activity of transcription factors [19].

4. Sex steroid hormone actions

4.1. Estrogen receptors

Despite estrogens belonging to the family of sex steroid hormones they are involved in the regulation of many physiological and pathological processes. Estrogen and its receptors mainly exert their effect on the reproductive system but also modulate differentiation, metabolism, and cell proliferation, as well as pathological processes including breast and endometrial cancer and osteoporosis [20].

Among the target tissues of estrogens are breast, endometrium, bone, brain, liver and heart. Like other steroid hormones they are synthesized from cholesterol, which is also the starting molecule for the androgenic compounds androstenedione and testosterone. Estrogens are first converted to progestins, androgens, and finally, through a series of enzymatic reactions, into estrogens. They are mainly synthesized in the ovaries in premenopausal women; in older women and in men the main site for the synthesis of estrogen is the peripheral tissue, especially adipose tissue [21]. In addition to this, the aromatization of testosterone to estradiol makes available a source of estrogen that can bind estrogen nuclear receptors and promote estrogenic actions on the hypothalamus, pituitary, bone, lipids and cardiovascular system.

The most important forms of estrogens include 17β-estradiol, estrone and estriol. The first is the most potent and it is produced and secreted from ovaries and moves throughout the body; the others are produced in the liver and adrenal glands, and have less effect than 17β-estradiol [22]. Recently a new isoform of estrogen named estrone sulfate has been identified. It is the most abundant form of circulating estrogen during the postmenopausal period, as well as in non-pregnant women and men. This isoform can be converted to estrone by sulfatase enzyme activity and then into estradiol. It is noteworthy that estradiol is the most potent estrogen in premenopausal women, estrone in postmenopausal women, and estriol in pregnant women [23,24].

Estrogens, in addition to acting on the reproductive system, perform their function in the skin and mucous membranes [25]; at the sebaceous-follicle level, they reduce the secretion of sebum and facilitate hair growth [26]; at the level of the bone they play a positive role in metabolism [27], while in adipose tissue estrogens work on the deposition of fat, characteristic for women; they also promote lipogenesis and insulin secretion [28].

Estrogen activities are exerted through intracellular receptors that regulate gene expression after their movement into the nucleus. Indeed, these effects can be rapid or take several days, so a difference needs to be established between transcription-independent (nongenomic) and transcription-dependent (genomic) actions [29]. Some effects of estrogen cannot be justified by purely genomic mechanisms due to the rapidity of their effects. Estrogen receptors are found in cell

membrane invaginations called caveolae. Human studies on osteoblasts, endothelial cells, neurons, and breast cancer cells show a relationship between estrogen interaction with its receptor on the cell membrane and the mitogen-activated protein kinase cascade [30].

The classical pathway of gene activation by estrogen takes from 30 minutes to several hours; non-genomic pathways, unlike the classical, are characterized by a more rapid response time, from a few seconds to about ten minutes. The latter also mediates responses such as immediate vasodilation of the coronary arteries and the effect of estrogen on the rapid insulinotropic cells of the pancreas [31].

Estrogen action in different target tissues is carried out through binding to the nuclear estrogen receptors α and β; the first was previously cloned from rat uterus in 1987 [32] while the second was cloned from rat prostate and is highly expressed in the secretory epithelial cells of the prostate and also in the granulosa cells of the ovary [33]. The two receptors are located on different chromosomes: in humans (which is different from other organisms) chromosome 6 contains the alpha isoform and chromosome 14 the beta isoform. These receptors are present in the target cells and are ligand-dependent transcription factors; that is they are inactive until they are bound by their ligand. Some evidence suggests the presence of ERs also at the level of mitochondria and endosomes of some cells in breast cancer and in endothelial cells. In this capacity, ERs could inhibit the production of mitochondrial reactive oxygen species (ROS) essential to induce cell death in cancer cells. This would lead to the survival of tumor cells [34].

With regards the estrogen receptor (ER), both nuclear and membrane/cytoplasmic pools of ER α and ER β have affinity for steroid ligand. Estrogens activate or repress gene transcription by binding target nuclear receptors, forming a steroid-receptor complex that binds to DNA response elements in the promoter of the estrogen-target genes. Another mechanism involves the interaction of nuclear estradiol (E2)/ER with transcription factors such as activator protein 1 (AP-1) or Sp-1.

Recent studies have suggested the idea that both ER-α and the nuclear membrane form are derived from the same gene and are the same protein in a variety of cells, including breast cancer. Membrane ER rapidly activates signaling through G-protein coupled receptors to generate calcium flux by stimulating cAMP and cGMP production, and activating PI3K and extracellular signal-regulated kinase (ERK) pathway activation. Rapid estrogen signaling from the pool of cell membrane ER has an effect on gene transcription and non-genomic functions. In all of these ways, ER membrane signaling contributes to many actions of this sex steroid in breast cancer cells [35].

A dynamic post-translational modification, palmitoylation, facilitates interaction with caveolin-1 (Cav-1), which carries a limited group of these steroid receptors to plasma membrane caveolae rafts (CR). At the membrane level, ER is bound by estrogen, activating rapid signals including G-proteins and Src.

Even if they have a high homology with the ERs, estrogen-related receptors (ERRs) are a family of nuclear receptors that are constitutively active transcription factors regulating many homeostatic processes [36]. These do not bind estradiol and instead activate transcription in a ligand-independent way, hence their classification as orphan nuclear receptors [37]. The ERRs were previously used to discover estrogen-receptor beta by comparing the sequence with that of the receptor α [38]. There is a strong conservation among ERRs and ERs: 36% in the ligand-binding domain and 68% in the DNA-binding domain. The ERRs are able to bind both EREs and ERREs (ERR response elements) and modulate the transcription of target genes [39].

The ligand-independent pathway is a phenomenon that most likely is joined to the classical pathway, as it allows the activation of the ER even in the presence of low concentrations of estradiol,

amplifies the effects of growth factors, and stimulates mitogenesis within ER-positive tissues [40]. Until now there have been identified three different receptors that can regulate these homeostatic processes: estrogen-receptor alpha, beta and gamma. ERRα plays a key role in the regulation of metabolic processes and is mostly expressed in tissues with high-energy demand, such as heart, kidney, skeletal muscle and brown adipose tissue [41]. Evidence shows the potential involvement of ERRα in cancer progression and development. Previous studies have shown that suppression of ERRα causes tumor cell death through the production of ROS [42]. Moreover, ERRα is involved in phosphoenolpyruvate carboxykinase enzyme (PEPCK) regulation, which plays a key role in hepatic gluconeogenesis, through action on the transcriptional coactivator PGC-1α. ERRα also increases expression of mitochondrial genes such as ATP synthase subunit β and cytochrome c-1, but decreases gluconeogenic genes, including PEPCK and glycerol kinase [43].

The ER family of receptors binds to specific DNA recognition sequences present in conserved responsive genes, often found in the neighborhood of the promoters, about 100 kb from the gene promoter responsive. The canonical estrogen ERE is the sequence 5'-AGGTCA-3' followed by three bases with undetermined sequence, followed by the reverse sequence 5'-TGACCT-3' [44]. The nature of the palindromic ERE sequence makes possible the interaction of the two DNA binding domains on the receptor.

It is noteworthy that the ERRα binds particularly well if the canonical sequence is preceded by 5'-TAA-3' or 5'-TCA-3'. This suggests that the response element to ERRs are formed by 5'-TA/AGGTCA-3'CA [45]. Regarding the other two receptors ERRβ and ERRγ, it is now known that their expression is important for the control of energy balance and food intake. Just as ERRα and ERRγ, the expression of ERRβ is abundant in the retina, liver, adipose tissue, and skeletal and cardiac muscle [46].

ERRγ is mainly expressed in the heart, brain, kidneys, pancreas and liver; it is involved in important cellular processes including metabolism of alcohol, the hepatic metabolism of glucose as well as the stress response in the endoplasmic reticulum. Studies carried out indicate that ERRs, including ERRγ are constitutively active without a natural ligand; there are different ligands that stimulate or repress the activity of ERRγ promoting or disrupting its interaction with coactivators [47].

Another orphan receptor discovered in 1997 in breast cancer is GPR30. It recently was renamed as a G protein-coupled estrogen receptor (GPER) and defined as a functional receptor of estrogen. It is mainly localized intracellularly and is not restricted to the cell membrane. This should have an important affect on estrogen signaling in many tissues; GPER is coupled with a G protein act to activate adenylate cyclase and epidermal growth factor receptor (EGFR) [48]. The activation of GPER by binding estrogen causes a mobilization of intracellular calcium and synthesis of phosphatidylinositol 3,4,5-triphosphate in the nucleus [49]. Studies in genetic variants of GPER were started to better understand its role in the onset of some tumors, such as human seminoma [50]. All of these estrogen related receptors are extranuclear mediators for the non-genomic actions of steroids.

4.2. Androgen receptors

Androgens simultaneously function as sex steroid hormones and anabolic hormones. Their anabolic effects on various tissues such as bone, muscle and red blood cells have long been known [51]. The side effects are not serious in women even if they have many virilizing effects (e.g.

hirsutism, voice change) but could be dramatic in men by increasing the risk of prostate cancer or benign prostatic hyperplasia [52].

Testosterone is the principal androgen circulating in the blood and in many tissues it is metabolized by 5-alpha-reductase to form 5-alpha-dihydrotestosterone (DHT), which binds to and activates the androgen receptor (AR) [53]. Only 0.5–4% of circulating steroid hormones are free in the serum. Approximately 60% is bound to sex hormone-binding globulin (SHBG) with the remainder bound to albumin, which has an affinity 1,000 times less than that SHGB. This fraction of steroids, being linked with lower affinity and therefore more rapidly dissociable, eventually ends up in a pool of free testosterone steroid that is readily usable, so-called bioavailable testosterone, which constitutes about 50% of total testosterone [54].

The receptor for SHBG has been identified in many tissues including the prostate, testes, breast, and liver, but not in lymphocytes and muscle. SHBG, in addition to acting as a regulator of concentration, plays a central role in allowing some steroid hormones to act without entering the cell [55]. SHBG is a glycoprotein of 373 amino acids, known as a carrier protein, produced by the liver and secreted into the circulation, which binds with high affinity to testosterone and with lower affinity to estradiol (E2) and modulates their bioavailability [56]. Insulin is an important regulator of SHBG production [57]. In addition to the liver, SHBG is also produced in other organs, including the brain [58], uterus [59] and testis [60].

The biological activity of testosterone and DHT occurs predominantly through binding to the AR, a member of the nuclear superfamily that function as a ligand-activated transcription factor. The appropriate regulation of androgen activity is necessary for a range of developmental and physiological processes, particularly male sexual development and maturation, as well as the maintenance of male reproductive organs and spermatogenesis [61]. Hypertrophy of muscle fibers is a process under the complex control of several myogenic pathways. Inoue and colleagues elucidated the role played by ARs as potential mediators of exercise-induced muscle fiber hypertrophy and demonstrated substantial increases in the concentration of AR in response to exercise [62,63,64]. In addition, satellite cells, the major source for the addition of new myonuclei into the hypertrophying muscle fiber, express AR and they are direct targets for testosterone action, with the increase in androgen-binding sites important for the regulation of pathways involved in the control of the satellite cell mitotic activity [65,66]. For these hypertrophic effects, for many years the anabolic androgenic steroids (AAS) have been used by many athletes and bodybuilders; however, it has been shown that supraphysiological doses can exert toxic effects on the neuron-like differentiated pheocromocytoma cell line PC12 [67].

With regards to pathways activated by hormone binding, AR associate with other effectors that may regulate the fate of target cells by mediating biological effects such as cell survival, profilation and migration. For example Filamin A (FlnA), an actin-binding protein that crosslinks actin filaments into the cytoplasm and participates in the anchoring ARs to the membrane, cooperates with ARs in the induction of non-genomic pathway, working as a scaffold. Together with its proteolytic products, FlnA intercepts the action of steroids at different levels and cellular compartments by hooking directly to the steroid receptors (e.g. AR) or effectors (e.g. GTPases) that mediate the rapid effects of steroids [68].

Association of cSrc with AR is responsible for the rapid activation of kinase-signaling cascades observed in the non-genomic effects of AR, leading to enhanced kinase activity. The biological effects are evident through activation of the MAPK/ERK pathway, following the translocation of activated ERK-1/2 into the nucleus and phosphorylation of transcription factors such as Elk1, the

latter which regulates transcription of early genes such as c-fos that in turn acts on the expression of several genes controlling cell proliferation. After AR/Src activated complex formation there should be an association with p85; the androgen-induced ternary AR/Src/p85 complex activates downstream Erk-2 and Akt effectors [69]. Sometimes following cellular stimulus, Src forms a complex with autophosphorylated FAK, a truly multi-functional signaling protein that rapidly phosphorylates the p85 subunit of phosphoinositol-3-Kinase (PI-3K). PI-3K activation is accompanied by the downstream upregulation of the Rho small GTPases Cdc42, Rac1, RhoA and RhoB; FAK activity is particularly required for Rac activation. Rapid activation of these GTPases resultes in actin cytoskeleton reorganization affecting motility and apoptosis [70,71].

The human androgen receptor gene is made up of 8 exons encompassing about 90 kilobases (kb) of DNA in the q11–12 region of the X chromosome. Gene expression is regulated by a single promoter that contains two initial sites of transcription localized within a region of about 13 bases. Two isoforms of AR were identified: A, of 87 kDa, and B, of 110 kDa, encoding a receptor protein that contains a variable number of amino acids in relation to the presence of two polymorphic sites in the N-terminal sequence (CAGn, GGNn, SNPs) (it's "GAG repetitions") [72]. The AR has several functional domains: the transactivation domain (exon 1), the DNA binding domain (exons 2 and 3), the nuclear localization sequence and the hinge region (junction of exons 3 and 4), and the steroid binding domain (exons 4 to 8) [73].

The hormone-receptor complex binds activated steroid responsive elements on DNA, made up of a recognition core of 6 bases. Generally, these consist of two coupled cores (half-sites) separated by a space of variable length. The nucleotide sequence of the core is specific for subgroups of receptors. The androgen, glucocorticoid and progesterone receptors bind to a hexamer with a common sequence TGTTCT. It is important to consider that the activation of transcription occurs not just due to the migration of the activated hormone-receptor complex into nucleus but also for the action of some modulators that interact with the elements of the core sequence, in the promoter region, to induce basal transcription. RNA-polymerase-II and general transcription factors are assembled into a transcriptional complex that contains associated factors like binding protein, and TATA box binding proteins (TBP) [74]. The coactivators are cellular proteins that interact with the ligand- steroid receptor complex to raise the transactivation of target genes.

AR is the most highly expressed receptor in breast cancer and has historically been considered anti-proliferative and beneficial [75]. A new class of drugs is represented by selective AR modulators (SARMs) that have a high specificity for AR and can control their activity and mediate the response to androgens. These showed an absence of virilizing side effects and this provides the possibility of extending androgen therapy to women [76].

Recently has been discovered an orphan receptor named GPRC6A, which transduces the non-genomic effects of testosterone; compared with the first class of receptors, the bind between ligand and receptor is not specific at all. GPRC6A is a pertussis toxin-sensitive member of the C family of GPCRs that senses calcium, amino acids and osteocalcin, all of which can activate this member of the G-protein family [77]. Previous studies have shown that in a cell model, removal of GPRC6A, rather than classic nuclear AR, abolished the intracellular signaling sensitivity to androgens. GPRC6A can work as a receptor for anabolic steroids and is able to coordinate the responses of different tissues to changes in nutrients and other factors. In this regard, the removal of this orphan G-protein coupled receptor involves testicular feminization in male mice, suggesting that GPRC6A may also modulate sex steroids and organ responses [78].

5. Progesterone receptors

Progestins are important steroids regulating final maturation of reproductive tissues in male and female vertebrates. Natural progestin in humans is progesterone, an essential regulator of normal human female reproductive function in the uterus, ovary, mammary gland and brain, that also plays an important role in non-reproductive tissues such as the cardiovascular system, bone, and the central nervous system, highlighting the widespread role of this hormone in normal physiology [79,80]. Membrane progestin receptors (mPRs), identified in 2003, are key mediators of rapid, non-genomic actions of progestins [81]. In addition to mPRs, there are two receptor candidates for mediating progestin signaling from the cell surface: nuclear progestin receptors (nPRs) and progestin receptor membrane components (Pgrmc). The latter are single-transmembrane proteins that include the family members Pgrmc 1, 2, neudesin, and neuferricin [82]. Each of these molecules is found in the brain, but only the first one seems to bind progesterone. In astrocytes, Pgrmc1 exerts its neuroprotective effetcts by increasing Brain Derived Neurotrophic Factor (BDNF) levels following activation of ERK signaling pathways [83]. There is data suggesting that Pgrmc1 is the same molecule as the sigma-2 (σ2) receptor, which is responsible for motor function and emotional response. It is possible that the σ2 receptor binding site could be located in Pgrmc1 and that progesterone binds both proteins [84].

There are two different subtypes of σ receptors (1 and 2), with progesterone binding acting as an antagonist in σ-receptor-mediated modulation. The mechanisms of these actions remain still unclear. Since their tissue-specific expression coincides, it seems likely that their functions do as well. Thus, the physiological roles of receptors mediating progestin actions might be complicated and difficult to clarify [85,86]. For example, in addition to its function as a nuclear transcription factor, nPR modulates cell signaling pathways by activating c-Src and downstream MAPK pathways outside the nucleus [87].

The membrane progestin receptor was discovered in fish ovaries in 2002 and three related mPRs were subsequently identified in humans and other vertebrates, which were named mPRα, mPRβ and mPRγ [88]. Recently, two additional related proteins, named mPRδ and mPRε, have also been shown to bind progesterone [89]. These receptors have no apparent homologies with known G protein–coupled receptors (GPCRs) or nuclear progesterone receptors, but belong to a highly conserved family of proteins termed “progestin and adiponectin receptors” (PAQRs). All the PAQR family members have a seven-transmembrane region, but the membrane topologies of the mPRs and adipoQRs probably differ. Moreover, the mPRs bind small steroid molecules resulting in G-protein activation, whereas the adipoQRs bind the large adiponectin molecule to activate G-proteins [90]. Studies show that physiological concentrations of progesterone caused a decrease of intracellular cyclic adenosine monophospate (cAMP), which was reversed upon treatment with pertussis toxin [91]. This observation has been confirmed in several cell models, further supporting the coupling of mPR to G protein signaling [92].

Noteworthy are the effects of progesterone’s metabolites, allopregnanolone and its isomer pregnanolone, as positive allosteric modulators of $GABA_A$ receptors (an ionotropic receptor). Their anesthetic, anticonvulsant, sedative and anxiolytic properties are well established as well as their neuroprotective and neurogenic effects in neurodegenerative diseases like Alzheimer’s disease [93]. Another non-classical pathway by which progesterone and its metabolites elicit their effect, is through sigma 1 receptor binding. Since the latter is involved in neuroprotection, this may be an important component of progesterone’s protective properties [94].

As previously mentioned, the effects of progesterone are also mediated by its nuclear receptor (nPR) which interacts with transcriptional coregulators, moves into nuclear aggregates (due to the structural constraints imposed by nuclear compartmentalization, and to the efficiency gains of co-location of functionally-related molecules) and regulates gene expression. More than 300 coregulators have been described and more nPR-binding coactivators exist than are required to form a functional nPR activation complex. It has been hypothesized that the specific combination of coactivators that associate with PR in a given cell is dependent on their relative abundance, which varies in a tissue-specific manner [95].

nPR is expressed as two isoforms, PRA and PRB, which are virtually identical except that PRA lacks 164 amino acids that are present at the N-terminus of PRB [96]. Although the precise mechanisms underlying the differential activities of the two human PR isoforms is not fully understood, structure-function studies suggest that the AF3 domain located within the PRB upstream sequence region, which is absent in PRA, contributes to PRB transcriptional activity by suppressing the activity of an inhibitory domain (ID) contained within the sequences common to PRA and PRB [97]. Moreover, evidence suggests that the two receptors adopt different conformations within the cell allowing PRA to interact with several coregulators that are different from those that interact with PRB. This is supported by studies demonstrating that PRA has a higher affinity for the corepressor SMRT than PRB in the presence of PR antagonists, and PRA does not interact directly or with as high an affinity as PRB with the coactivators SRC-1 and SRC-2 upon agonist binding, potentially contributing to differences in the transcriptional activities of the two isoforms and further enhancing the complexity of this regulatory system [98,99].

In a cell that co-expresses both PR isoforms, there is the potential for 3 molecular species (PRB homodimer, PRA homodimer and PRA-PRB heterodimer) to exist at the same time and to contribute to the complexity of PR action. In human physiology, however, the majority of PR positive cells express PRA and PRB at equivalent levels, and cells that express only one PR isoform are uncommon. This suggests that in the human, progesterone exerts its effects in cells that co-express both PR isoforms, and that the PRA-PRB heterodimer is the predominant molecular species.

In addition, the PR isoform ratio influences the capacity of PRA to regulate the activity of PRB. Previous studies have shown that PRA is a dominant inhibitor of the transcriptional activity of PRB [100]. mPR-α and -β activation leads the transactivation of PRB, revealing an intersection between classical and membrane associate signaling; the amino terminus binds to the SH3 domain of Src, activating Ras/Raf/ERK1/2 signaling independent of its transcriptional activity. On the other hand activation of the MAPK pathway results in the phosphorylation and activation of transcription factors such as c-Fos, c-Jun and nuclear PRs to control gene transcription.

6. Conclusion

The response to sex steroid hormones can be either genomic or non-genomic. The difference between the pathways depends upon the type of receptors that are involved. Generally, nuclear/cytoplasmic receptors induce a genomic response, while membrane receptors activate non-genomic pathways. More evidence shows that these membrane receptors exhibit characteristics of G-protein coupled receptors. Some examples of receptors identified that are responsible for the genomic response of steroids are GPR30 for estrogen, mPR for progesterone and GPRC6A for androgen.

Sex steroid receptors associated with the non-genomic pathways are associated with the

modulation of intracellular calcium levels, though this seems to be through different mechanisms depending on the cell types.

It is now evident that there is crosstalk between non-genomic and genomic signaling pathways, in which the first can regulate the latter (Figure1). For example it is known that ERK increases the transcriptional activity of steroid receptors directly through phosphorylation of SR and its coregolators. In any case, genomic and non-genomic signaling pathways can work together to coordinate the regulation of gene expression targets.

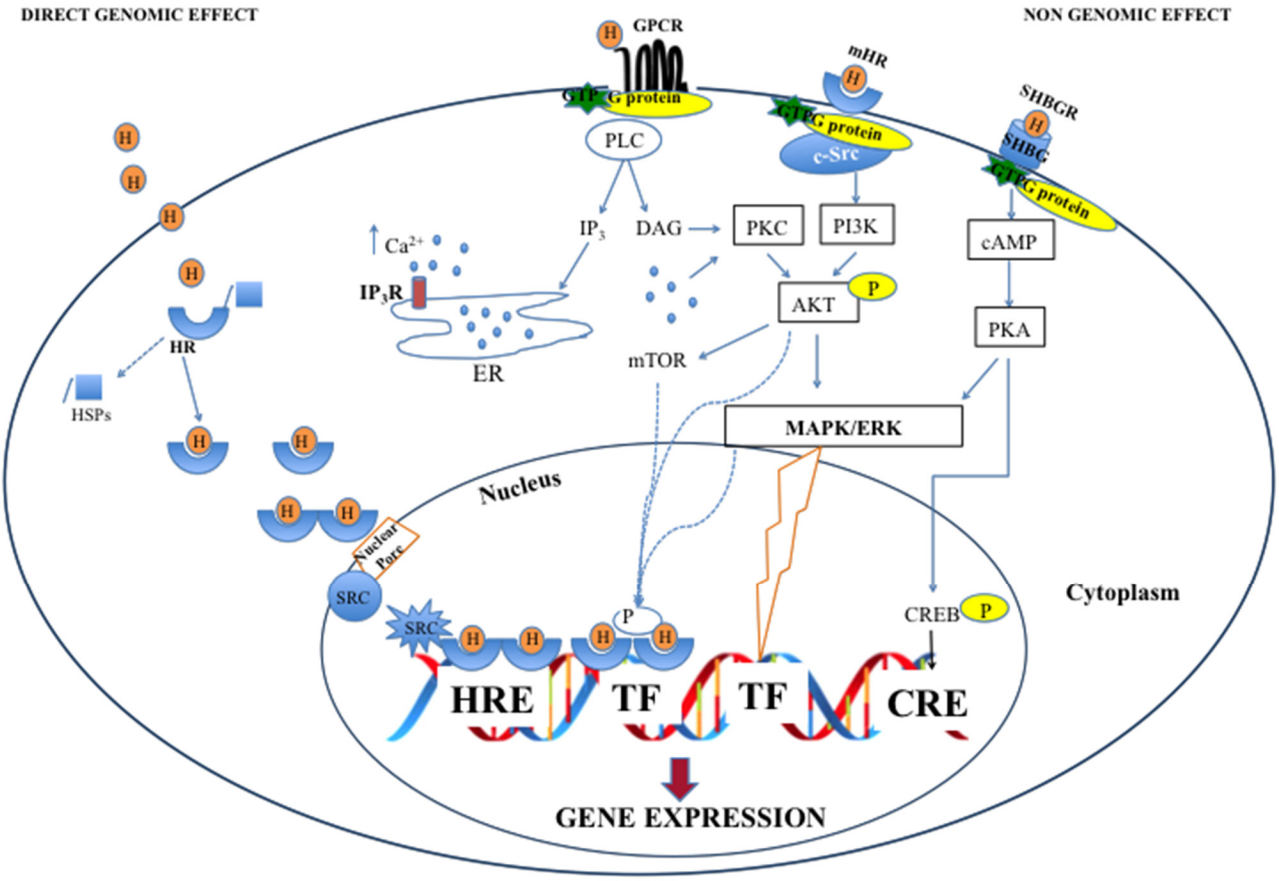

Figure 1. Convergence between genomic and non-genomic sex steroid hormone receptor (HR) pathways. The molecular mechanism for HRs lacking HREs to regulate gene expression involve MAPK/ERKs, both of which have been shown to enhance transcriptional activity through direct phosphorylation of the HRs and their coregulators. The genomic effects of sex steroids occur through their binding to cytoplasmic steroid receptors. These then become active hormone-receptor complexes that can translocate to the nucleus and bind to sex steroid response elements in the promoters of target genes. Even though this is the classic model of mechanism of action, there are also indirect genomic effects by which sex steroid hormones act on gene expression by regulating other transcription factors. These pathways begin upon binding to receptors that mediate the membrane-initiated actions of sex steroids. In addition to classical membrane steroid receptors, there are novel receptors, coupled to G-proteins, that activate a protein kinase cascade or act at the level of secondary messengers such as PI3K/AKT/mTOR or PI3K/AKT/cAMP/Ca^{2+} or ERK-mediated pathways like PI3K/AKT/MAPK-ERK/Elk1.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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