Hormone Receptors in Cervicovaginal Cells

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Some parts of the female reproductive system such as vagina and ectocervix is covered with stratified squamous epithelium (SSE). SSE consists of many layers and steroid hormones are essential for its normal growth and development. The steroid hormones mediate their effects via intracellular reseptors (3P). These are estrogen receptors, progesterone receptors and androgen receptors. These are found in cytoplasm or nucleus, or both. The steroid hormone receptors are made up of three functional domains: N-terminal domain (NTD), DNA binding domain (DBD) and ligand-binding domain (LBD). Initially, the steroid hormones diffuse into the cell and bind to its spesific receptor protein. Afterwards, hormone-bound receptor dimerizes with another receptor and then the dimer binds to specific DNA sequence through the DBD. Consequently, hormones regulate gene transcription via a direct interaction with DNA. In this review, we will give detailed information about SSE cells, hormone receptors, structure of the receptors and mechanism of their action.

Key Words: Steroid hormone receptors, Estrogen receptors, Progesterone receptors, Androgen receptors

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Introduction

Steroid hormones such as estrogen, progesterone and androgen play key roles in development and maintenance of the female reproductive systems. They affect cells and tissues via their receptors. These receptors are estrogen receptors, progesterone receptors and androgen receptors.

1. Estrogen Receptors

Estrogens are sex steroid hormones. These hormones regulate development, differentiation and function of several tissues and organs such as vagina, uterus, ovary, mammary gland and brain. The three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). The estradiol is the most potent estrogen produced in the body and has a high-affinity to estrogen receptor (ER).[1,2,3]

Estrogens affect their target cells through receptors. SSE which cover vagina and ectocervix have two estrogen receptors. These are estrogen receptor-α (ER α) and estrogen receptor-β (ERβ). These receptors are ligand-inducible transcription factors 4. ERα which is 595 amino acids long and 66kDa molecular weight localizes primarily in the nucleus. It was the first estrogen receptor isolated from human breast cancer cells in the late 1980s.[1,2,5] Ten years after the discovery of ERα, ERβ was discovered using degenerate PCR primers which was taken from rat prostate.[2] This receptor is a protein which is made up of 530 amino acids and 60 kDA molecular weight.1,5 It has been shown that ER α mRNA is abundantly expressed in the rat vagina and ectocervical epithelial cells particularly basal and parabasal cells.[6] It has recently been suggested that ERs are localized at the plasma membrane of cells and responsible for rapid signalling.[7,8]

ERs are comprised of several functional domains.(Figure 1). These are N-terminal or A/B region, C region, D region and COOH-terminal or E/F region. A/B region is a highly variable part of the ERs. This region exhibits only a 17% amino acid homology between ER α and ER β.[2,9,10] ERs have a "Activation function-1" (AF-1) domains in their N-terminal region.[2] This domain is responsible for hormone-independent transcriptional activity.[4] It is suggested that AF-1 is also activated with phosphorylation on serine residues.[2,4] C region is the most conserved domain of ERs. It is also called as Dna-Binding Domain (DBD).[11] ER α and ER β DBDs bind specific DNA sequences which are known as Estrogen Response Elements (EREs). In addition, DBD consists of two zinc fingers. These fingers take part in binding EREs. Each of them is comprised of four cysteine residues and one Zn^{+2} ions. The first one has the specifity for DNA and the second one contributes to DNA binding affinity.[12] Research shows that ER α and ER β share 96% homology of their DBD. Similarity in the DBD of these receptors is one possible explanation for the bind to the same target sites.[2,4] D or hinge region is another site of the receptor. This region contains the nuclear localiza-

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tion signal and contributes to receptor dimerization which requires for binding EREs. It also involves in binding to heat shock proteins. D region of the ER α and ER β exhibits a sequence identity of 36%.[13] The D region is followed by C terminal "E/F" domain which is also called ligand binding doman (LBD). The LBD comprises hormone binding region and another activation function, "Activation Function -2" (AF-2). AF-2 is the major binding site for the ligand. Moreover, AF-2 activity requires the presence of hormone. The E/F domains of ER α and ER β have 53% homology.[4,14]

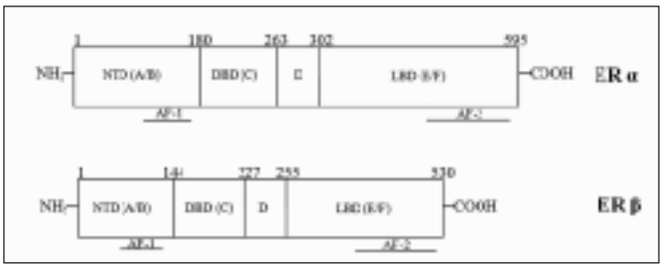

Figure 1: Comparison of structural and functional domains of ER α and ER β. The numbers above each box indicate amino acid residues.

ERs regulatory actions are carried out distinct molecular pathways: genomic or classical mechanism and non-genomic or rapid mechanism.

i. Genomic (Classic) Mechanism: In the absence of ligands such as estrogens, ERs are attached to heat shock protein complex, so they are sequestered away from the cell nucleus[15] (Figure 2).

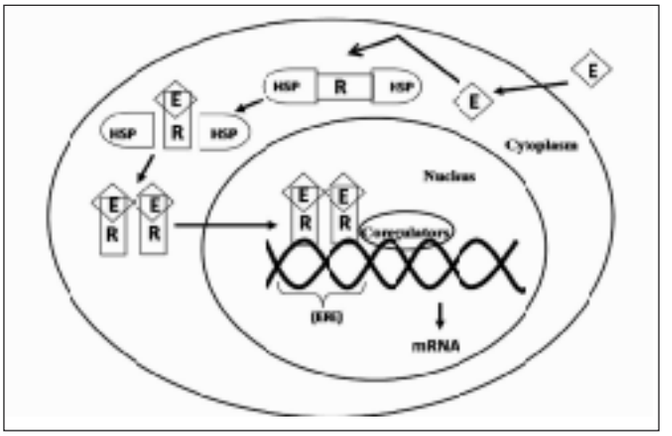

Figure 1: Comparison of structural and functional domains of ER α and ER β. The numbers above each box indicate amino acid residues.

Initially, estrogens diffuse into the target cells and bind to ERs. After binding hormone, ERs are activated by phosphorilation and then heat shock proteins dissociate ERs. Hormone bound (activated) ERs alter their self-conformation. Conformational changing of ERs facilitate receptors-ERE interactions.[13,15,16] Afterwards, activated ERs dimerize with another ER. Hormone-ER-dimer complex passes through the nucleus and directly bind DNA promoter site via the ERE sequence.[13]

Coregulators are proteins that regulate transcription activity of DNA. They have an important function as receptors do. There are two kinds of coregulators: coactivator and corepressor. Whereas coactivator proteins enhance transcription, corepressors inhibit its action. Coregulators recruit at the target gen promoter and interact with hormone-ER-dimer complex. Hence, transcription begin.[2,16,17]

ii. Non-genomic (Rapid) Mechanism: In this mechanism estrogens regulate transcription of genes within seconds via membrane receptors.[17,18] Recent studies have reported that ER α and ER β localize in many cells on the plasma membrane and cytoplasmic organelle membrane such as mitochondria. Further studies also suggested that membrane ER binds similar antibodies to classical ER α.7 On the other hand, it has been thought that this signalling events include transmembrane growth factor receptors and G-protein coupled receptors (GPCR) which assosicate with plasma membrane ERs.[7,18] When estrogen binds plasma membrane ERs, ERs activate GPRC subunits. Subsequently, Ca^{+2} or NO levels increase and kinases like phospholipase C (PLC), mitogen activated protein kinase (MAPK) and cyclic AMP (cAMP) are activated in the cytoplasm. Consequently, cellular responses such as proliferation or apaptosis occure more quickly than genomic mechanism.[13,18]

2. Progesterone Receptors

Progesterone is one of the steroid hormones. It regulates function of reproductive tract such as uterus, ovary and mammary gland. It also plays an important role in preparing the uterus to receive a blastocyst.[19]

The effects of progesterones are mediated through the progesterone receptors (PRs). PRs contain several domains like ERs. These are N-terminal or A/B domain, DBD domain and C-terminal or LBD (Figure 3). N-terminal region is the most variable domain in the receptors. In contrast to estrogens, PRs have an extra activation domain, "Activation Function -3" (AF-3). Its function is recruiting coactivator proteins to the promoter site of DNA. N-terminal side also includes an "Inhibition domain" (ID) which is responsible for interaction with corepressor proteins. The most conserved region of PRs is DBD. It comprises roughly of 66-68 amino acids and consist of two zinc finger like ERs DBD. LBD is located C-terminal side of the receptor protein and includes AF-2. AF-2 is necessary for hormone-dependent activation, receptor dimerization and interacting with heat shock proteins like ERs.[20,21,22]

Studies showed that PR is localized predominantly in the nucleus of epithelial cells.[23] PRs have three isoforms: Progesteron receptor A (PR-A), Progesteron receptor B (PR-

B) and Progesteron receptor C (PR-C). These are expressed from a single PR gene which consist of eight exons.[24] PR-A and PR-B are independently transcribed from this gene.[22] PR-A and PR-B are two main nuclear isoforms of PRs. PR-B contains 960 amino acids and its molecular weight is 114kDA. But, PR-A which is truncated form of PR-B lacks 164 amino acids from PR-B and its molecular mass is 94 kDA. Adverse PR-A and PR-B, PR-C is primarily cytoplasmic isoform of PRs. PR-C does not include N-terminus, a full DBD and two activation domains (AF-1 and AF-3). Though PR-C can bind hormone, dimerize and localize in the nucleus, it would not directly bind Progesterone Response Elements (PREs). Research suggests that, PR-C is not expressed in vivo, thus, it does not take part in progesterone signalling.[22,25,26]

Progesterone effects are mediated by genomic mechanism like estrogens.

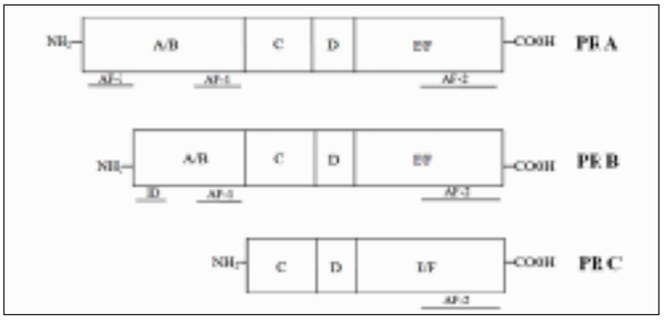

Figure 3: A schematic structural and functional comparison of PR A, PR B and PR C.

3. Androgen Receptors

Androgens are steroid hormones which regulate functions of ovary, uterus, oviduct, clitoris and mammary gland and secondary sex characteristics. They are required for woman reproductivity and precursor molecules for estrogen sythesis. Testosterone and 5α-dihydrotestosterone are two-main androgens that bind to androgen receptors (ARs).[27,28]

Androgen effect is mediated via androgen receptors. AR gene is located on the X chromosome (Xq 11-12) and spans about 90kb of DNA. This gene encodes AR protein which is 918 amino acids in length and has 110 kDa molecular weight. ARs are made of specific regions such as ERs and PRs (**Figure** 4). The least evolutionary conserved region of AR is N terminal domain (NTD) including 555 amino acids. Research suggests that the NTD amino acids sequence of human AR is only 20% identical to rat AR. In the NTD, two transactivation domains are determined, AF-1 and AF-5, which has weaker activation than AF-1.[29,30] It has been thought that these regions contact with transciption factors and coregulators.[28] The DBD is a well conserved region in the AR and consist of 68 amino acids residue. Like ER and PR, AR DBD comprises two zinc fingers which can bind to Androgen Response Element (ARE) in the DNA promoter site.[30] There is 100% amino acids identity between human and rat AR.[29] Examination of mouse mammary tumor virus showed that AR binds to "GGTACA nnnTGTTCT" sequence in DNA promoter.4 The last region of the AR is LBD. This side includes AF-2 and takes part in receptor dimerization and hormone binding which are essential steps for receptor mechanism.[30]

ARs signalling are mediated by genomic mechanism like estrogens.

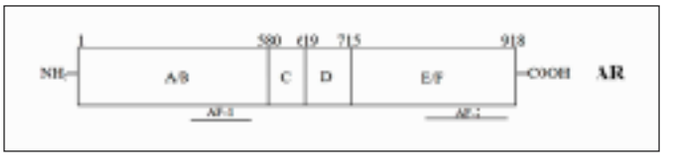

Figure 4: Schematic representation of structural and functional domains of the AR. Amino acid sequence position is indicated for each domain.

Conclusion

Estrogen, progesterone and androgen receptors have been reported in vaginal tissue (SSE cells) through immunohistochemistry method.[23,28] ERs take part in vaginal epithelial cell proliferation and cytodifferentiation.[31] PRs are essential for the regulation of progesterone-regulated genes expression. ARs regulate mucin production and proliferation of vaginal epithelial cells.[28] In summary, these receptors play an important role in development and differentiation in SSE. Furthermore, additional effort is required to fully understand the detailed mechanisms of their action and their effects on cells.

Servikovajinal Hücrelerin Hormon Reseptörleri

Kadın üreme sisteminin vajina ve ektoserviks gibi bazı kısımları çok katlı yassı epitel (ÇKYE) hücreleri ile kaplıdır. ÇKYE hücreleri birçok tabakadan meydana gelirler ve steroid hormonlar bu hücrelerin normal büyümesi ve gelişmesi için gereklidir. Steroid hormonlar hücre içi reseptörler aracılığıyla etkilerini gösterirler. Bunlar östrojen reseptörü, progesteron reseptörü ve androjen reseptörüdür. Bu reseptörler hücre içerisinde sitoplazmada, çekirdekte ya da her ikisinde de bulunurlar. Steroid hormon reseptörleri işlevsel olarak 3 bölgeden meydana gelirler: N-terminal bölge, DNA- bağlayan bölge ve ligand-bağlayan bölge. İlk olarak, steroid hormonlar difüzyonla hücre içerisine girerler ve özgül protein reseptörlerine bağlanırlar. Ardından, hormon bağlı reseptör diğer bir reseptör ile dimerize olur ve sonra DNA bağlayan bölgesi aracılığıyla özgül DNA dizilerine bağlanır. Sonuç olarak, hormonlar gen transkripsiyonunu DNA ile doğrudan temas kurarak düzenlerler. Bu derlemede ÇKYE hücrelerinin hormon reseptörleri, bu reseptörlerin yapısı ve mekanizması hakkında detaylı bilgi verilecektir.

Anahtar Kelimeler: Steroid hormon reseptörleri, Östrojen reseptörü, Progesteron reseptörü, Androjen reseptörü

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