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Research paper

Functional responses of estrogen receptors in the male and female auditory system

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ABSTRACT

Recently significant progress was made in understanding the mechanisms by which the two estrogen receptors (alpha and beta) are involved in different pathways of estrogen action in a wide variance of tissues. Divergent responses of cells and tissues to estrogens or their ligands have been attributed to various isoforms and signaling pathways of estrogen receptors. Both subtypes of estrogen receptors have been identified in the cochlea and there are indications that they have neuroprotective effects but there is still limited information on the role and specific mechanisms of these two receptors in the auditory system. This review will examine the molecular and functional actions of the two estrogen receptor subtypes, the pivotal role they play in the auditory system and their therapeutic strategies for protecting against hearing loss.

1. Introduction

Estrogen is an important mediator in reproductive and non-reproductive functions in both females and males. Estrogens exert a wide range of biological effects throughout the mammary gland, the immune, cardiovascular, skeletal, and central nervous systems by interacting either directly or indirectly with the estrogen receptors alpha (ERα) and beta (ERβ). Both ERα and ERβ are present in cochlear tissues and their functional role in hearing physiology and pathophysiology are beginning to be elucidated. In the auditory system, there is limited information concerning downstream signaling pathways and co-activation processes for each receptor type. Given the therapeutic potential for these receptors to protect and modulate auditory sensitivity (Meltser et al., 2008), it is important to understand the mechanisms that govern each receptor type.

2. Estrogen receptors

Estrogen receptors (ERs) are members of the nuclear receptor family and act as ligand-activated transcription factors. The two ERs are products of discrete genes found on separate chromosomes and are thought to have opposing biological actions. The human ERα cDNA was cloned for the first time in 1985 (Walter et al., 1985) and sequenced the following year (Greene et al., 1986) and approximately a decade later the ERβ gene was found in the rat (Kuiper et al., 1996) and human (Mosselman et al., 1996). Since then the ER genes have been isolated from a variety of species, including the mouse (Tremblay et al., 1997; White et al., 1987), which is widely used as an experimental model in hearing research.

The human gene for ERα was shown to be more than 140 kb long (Ponglikitmongkol et al., 1988) split into 8 exons and localized on chromosome 6q25.1 (Menasce et al., 1993). The product of the gene was identified to be a 595 amino acids protein with a molecular weight of circa 66 kDa, whereas in mouse the ERα gene was mapped on chromosome 10 (Justice et al., 1990) encoding a 599 amino acids protein with an overall homology of 88% in the sequence between the two species. ERβ differs in length as well as in sequence from ERα. In human, the gene encoding ERβ was mapped on chromosome 14q and its product is a protein of approximately 60 kDa consisting of 530 amino acids, whereas in the mouse it has a molecular weight of 63 kDa and 549 amino acids.

3. Functional domains of estrogen receptors

The sequence of ERα was divided into five functional units (A/B, C, D, E, F) based on the homology between human and chicken ER: domains C and E were found to have a high degree of sequence

Abbreviations: ER, estrogen receptor; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; ERE, estrogen response element; AF-1 or -2, activation function-1 or -2; MAPKs, mitogen-activated protein kinases; ERKO, estrogen receptor alpha knockout mice; BERKO, estrogen receptor beta knockout mice; ARKO, aromatase knockout mice

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homology (100% and 94%, respectively) and were attributed to represent the DNA-binding domain and hormone-binding domain of the receptor (Krust et al., 1986). Apart from the high degree of conservation of C and E domains of the ERα sequence, an extremely well conserved interspecies molecular mechanism of activation of the gene encoding ERα was as shown when human ER was expressed in yeast and apparently acted as an estrogen-dependent transcription factor (Metzger et al., 1988). The greatest differences between the two estrogen receptors are seen in the regulatory domains whereas the highest homology is found in the C domain or DNA-binding domain (96% homology). The ligand binding domain is 58% conserved (Mosselman et al., 1996). The five distinct domains that assemble the estrogen receptors are (Fig. 1):

- The **A/B domain** or NTD (amino-terminal domain) is the more variable in length and sequence. It contains the transcriptional activation function-1 domain (AF-1) and plays a role in interaction of co-receptor proteins with the receptor. One reason for the different function of the two receptors is attributed to the low homology found in this domain that results in differences in activation of the two receptors.
- The **C domain** or DNA-binding domain is the one that binds with high affinity to the estrogen response elements (EREs), the specific DNA sequences of estrogens' target genes. It consists of two zinc fingers containing the P-box (proximal) responsible for the specific recognition of the EREs and the D-box (distal) involved in the dimerization of the receptor, as well as a C′-terminal extension (CTE) important for the monomers' ERE binding and interaction with co-regulatory proteins like high-mobility-group B proteins (HMGB) (Melvin et al., 2004).
- The **D domain** is also called the hinge region as its' main role is to interconnect the C and E domains keeping a distance between them so that they maintain their function, and it is now thought to be involved not only in the nuclear localization of the receptor but also in the modulation of co-activators' interaction and post-translational modifications regulating the transcriptional potential of the ERs (Sentis et al., 2005).
- The **E domain** or ligand binding domain (LBD) binds specifically to the ligand and activates ligand-dependent transcription via an activation function-1 domain (AF-2) in the C′-terminus. It also contains sites for dimerization, for interaction with heat shock proteins and nuclear localization signals. The LBDs of ERα and ERβ vary quite a lot but still they have similar affinity for estradiol, the main estrogen in the body.
- The **F domain** is found in the carboxyl-terminus of the receptor and is characterized by a great variance in length and sequence. For many years it was thought not to have any significant function but recently it was shown to modify ligand binding, interaction with co-regulatory proteins including steroid receptor co-activator-1 (SRC-1), transcription activation, and accumulation at an endogenous promoter (reviewed in Skafar and Zhao, 2008).

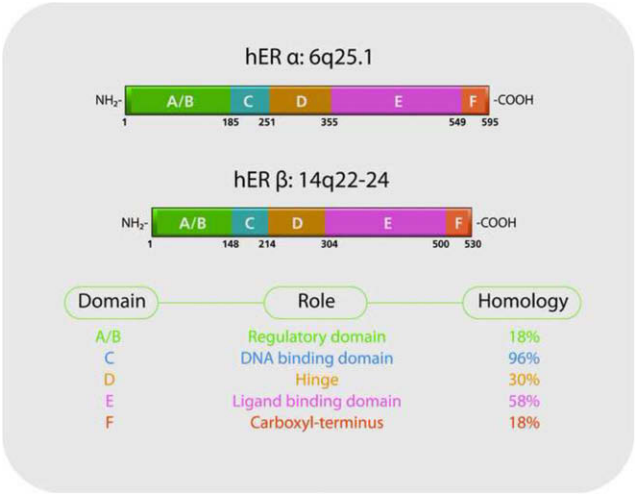

Fig. 1. Modular organization and functional domains of the human estrogen receptors alpha and beta (hERα and hERβ). Estrogen receptors (ERs) belong to the superfamily of nuclear receptors. Two subtypes of ERs have been identified [alpha(α) and beta(β)] existing as many isoform variants that may differ in their cell and tissue specificity and transcriptional capacity. Both human ER (hER) genes are composed of five domains (A/B to F). The main functional role of each domain and the homology between the five domains between hERα and hERβ are shown. The DNA-binding domain is the one with the highest degree of conservation between the two subtypes of hERs (96%) whereas the A/B domain that functions as a region of interaction with co-regulators is only 18% homologous among hERα and hERβ and is thought to be to some extent responsible for the differences in activation between the two receptors.

4. Promoters

Multiple promoters have been identified for ERα and ERβ in humans, rats, mice and other species. The promoter region upstream of the gene encoding the human ERα contains at least seven different promoters, resulting in variant mRNA products differing only in their 5′-untranslated regions (5′-UTRs) (Reid et al., 2002). Since these regions do not get translated, the 5′-end of the produced protein is not variant and the final ERα protein is identical for all different promoters, thus the full-length 140 kb or 66-kDa ERα. There are some cases where divergent splicing takes place to result in the formation of ERα isoforms. A functional 46-kDa isoform has been identified as a product of alternative splicing and divergent promoter utilization (Flouriot et al., 2000). In this case the transcription starts from a promoter quite far upstream from the one first identified and the 5′-UTR splicing with exon 2 skipping exon 1 that contains AF-1 results in the 20-kDa shorter isoform. Interestingly this shorter isoform can form homo- or heterodimers together with the full-length ERα with a different affinity to the EREs. It seems like the existence of various promoters could explain the presence of different mRNA variants and ER isoforms, as well as the differential expression of the receptors in different tissues and cell types.

5. Mechanisms of estrogen action

The innate complexity of sex hormones' action lies not only in the numerous variants and isoforms of the two ER subtypes, but also in the multiple signaling pathways that have been characterized. Sophisticated control systems are necessary to obtain a tight equilibrium in estrogen action and regulation of ER expression in tissues and cells. The different pathways by which estrogen exert their actions will be discussed below (Fig. 2).

5.1. Classic signaling (ligand dependent)

Estrogen receptors are kept inactive in the nucleus and cytoplasm of the cell forming a complex with various heat shock proteins that act as chaperones when the cell is not exposed to estrogens. Such proteins are hsp90, hsp70 and hsp56 and by forming a complex with the ERs they are believed to prevent them from binding to their response elements, but also keep them capable of binding to their ligands (estrogens) with high affinity. When the estrogens diffuse across the cell and nuclear membrane they interact with the inactive form of the ERs and separate them from the hsp-complex. ERs are now activated and can form homodimers and to a lesser extent heterodimers to bind to their estrogen re-