European Journal of Cell Biology 94 (2015) 611–625

Contents lists available at ScienceDirect

European Journal of Cell Biology

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

Review

ERα36 – Another piece of the estrogen puzzle

Kamil Sołtysik[a], Piotr Czekaj[b,*]

[a] Students Scientific Society, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland
[b] Department of Cytophysiology, Chair of Histology and Embryology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

ARTICLE INFO

Article history:
Received 28 June 2015
Received in revised form 3 September 2015
Accepted 9 October 2015

Keywords:
ERα36
Estrogens
Xenoestrogens
Estrogen receptor alpha
Membrane estrogen receptors
Signal transduction

ABSTRACT

Although the nuclear action of estrogen receptors (ER) is a well-known fact, evidence supporting membrane estrogen receptors is steadily accumulating. New ER variants of unrecognized function have been discovered. ERα is a product of the ESR1 gene. It serves not only as a template for the full-length 66 kDa protein, but also for smaller isoforms which exist as independent receptors. The recently discovered ERα36 (36 kDa), consisting of 310 amino acids of total 595 ERα66 protein residues, is an example of that group. The transcription initiation site is identified in the first intron of the ESR1 gene. C-Terminal 27 amino acids are encoded by previously unknown exon 9. The presence of this unique C-terminal sequence creates an opportunity for the production of selective antibodies. ERα36 has been shown to have a high affinity to the cell membrane and as much as 90% of the protein can be bound with it. Post-translational palmitoylation is suspected to play a crucial role in ERα36 anchoring to the cell membrane. In silico analysis suggests the existence of a potential transmembrane domain in ERα36. ERα36 was found in most cells of animals at various ages, but its exact physiological function remains to be fully elucidated. It seems that cells traditionally considered as being deprived of ER are able to respond to hormonal stimulation via the ERα36 receptor. Moreover, ERα36 displays unique pharmacological properties and its action may be behind antiestrogen resistance. The use of ERα36 in cancer diagnosis gives rise to great expectations.

Contents

* Corresponding author at: Medyków 18 str., 40-752 Katowice, Poland.
E-mail address: pcz@sum.edu.pl (P. Czekaj).

http://dx.doi.org/10.1016/j.ejcb.2015.10.001
0171-9335/

1. Introduction

Preservation of the male and female reproductive function depends only partially on estrogen activity. Individuals with estrogen receptor (ER) gene mutations display specific phenotypic features, i.e. increased body weight, metabolic disorders, and osteoarticular abnormalities. These mutations are not lethal in mammalian species, regardless of gender (Goulding et al., 2010; Hewitt et al., 2010; Quaynor et al., 2013; Smith et al., 1994).

Research on ERs began in the 1960s, when the pioneering works of Jensen and Gorski, with the use of tritium-labeled estradiol, were published (Jensen and Jordan, 2003) Afterwards, in 1985–1986, the sequence of the human ER gene was cloned and determined (Green et al., 1986; Greene et al., 1986; Walter et al., 1985), and rat ER gene was recognized a year later (Koike et al., 1987). Human ER gene was described as the *ESR1* gene (6q25.1), being the matrix for ERα protein with molecular mass of 66 kDa.

However, estrogen signaling is more complicated than originally thought. In the 1990s, Gustafsson and colleagues reported the discovery of yet another ER, primarily isolated from rat prostate cDNA library (Kuiper et al., 1996), but soon detected also in human and mice tissues (Enmark et al., 1997; Mosselman et al., 1996; Ogawa et al., 1998; Tremblay et al., 1997). It was named estrogen receptor-β (ERβ). ERβ is encoded by the *ESR2* gene (14q23.2), and shares 47% amino acid sequence homology with ERα. This homology is even more evident in DNA- and ligand-binding domain (97% and 60%, respectively) (Enmark et al., 1997; Tremblay et al., 1997). As a consequence, both receptors were classified as nuclear receptor superfamily (NRS) members. Similar structure of the domain and phylogenetic relationship do not correlate with functional similarity. Both, tissue sensitivity and gene regulation are differently regulated by ERα and ERβ (Faulds et al., 2012; Harris, 2007; Zhao et al., 2010). As other nuclear hormone receptors (47 in mouse, 46 in human (Germain et al., 2006)), ERα and ERβ are transcription factors. Most of their actions result from the interaction with canonical palindromic ERE (Estrogen Response Elements) sequences GGTCAnnnTGACC (Heldring et al., 2007; Puzianowska-Kuznicka et al., 2013; Zhao et al., 2010). This is the so-called 'classic' or 'genomic' mechanism, which requires a significant amount of time before the final effect occurs. Currently, the non-genomic (non-classic) mechanism, initiated at the level of cell membrane and characterized by much shorter latency period, is the subject of extensive research (Soltysik and Czekaj, 2013). Rapid estrogen action was described relatively early by Szego (Pietras and Szego, 1979; Szego and Davis, 1967), but as far as science is concerned, it has long been playing a secondary role in relation to the nuclear mechanism. Studies indicate that this mechanism comprises an activation of membrane ERα and ERβ (Razandi et al., 1999), and then receptor-dependent signal pathways, including G proteins (Kumar et al., 2007; Razandi et al., 1999), ERK, PI-3K, and PKC kinases (Gutiérrez et al., 2012; Pedram et al., 2006; Wu et al., 2011a), cyclic nucleosides (Pedram et al., 2006; Razandi et al., 1999) and ion influx (Wu et al., 2011b).

In the meantime, reports claiming that membrane estrogen signaling includes more receptors have appeared in the literature (Doolan and Harvey, 2003; Nadal et al., 2000). In 2005, an orphan receptor known as CEPR, DRY12 or Gpr30, later called GPER (G protein-coupled estrogen receptor), was discovered to bind estradiol (E2). The binding triggered the inflow of Ca^{2+}, and the activation of PI-3K and G_s proteins (Revankar et al., 2005; Thomas et al., 2005). The unique feature of GPER (GPER1 7p22.3), as compared to other ERs, is that it belongs to the seven-transmembrane receptor family (7TM receptors) (Davenport et al., 2013). The receptor is usually found in cell and endoplasmic reticulum membranes, and the effects of its action should be classified as non-classic.

The *ESR1* gene serves not only as a template for a full-length 66 kDa protein, described as ERα66, but also for at least two alternative isoforms functioning as independent receptors. In early studies on human *ESR1*, immunoblotting assays revealed the existence of 46 kDa band. This band was described as the product of ERα66 degradation rather than as the receptor isoform itself (Walter et al., 1985). Moreover, during the analysis of proteins isolated from endothelial cells and osteoblasts, bands of higher electrophoretic mobility (35–39 kDa), as compared to intact protein, were observed (Denger et al., 2001; Li et al., 2003). Existence of shorter protein sequences became more evident when first *ESR1* knockout mice were introduced (Lubahn et al., 1993). In order to disrupt gene continuity, thus preventing the synthesis of the functional receptor, Lubahn and colleagues inserted the so-called 'Neo cassette' (Neomycin resistance gene cassette) into exon 1 of the *ESR1* gene. Surprisingly, cells of these animals retained partial ability to bind to E2 (Couse et al., 1995; Lubahn et al., 1993). The explanation of this phenomenon could be that there exist variants of the receptor that naturally bypass exon 1 during transcription, and thus are not a target for knockout. Nevertheless, the expression of ER variants appeared to be small since E2 binding capacity remained at the level of 5% (Chaudhri et al., 2012; Lubahn et al., 1993).

The first newly recognized and confirmed isoform was ERα46 (46 kDa). Synthesis of ERα46 comprises two possible mechanisms: (1) use of the promoter F and direct transcription of exon 2, omitting exon 1 (Flouriot et al., 2000), and/or (2) use of the initiation site of translation induced by an internal ribosome entry (Barraille et al., 1999). ERα46 is deprived of 173 N-terminal amino acids. ERα46 was localized in the cell nucleus and cytosol. It is known for its high membrane affinity and ability to induce eNOS in the endothelial cells (Li et al., 2003, 2007).

The second recently discovered ER isoform is ERα36 (36 kDa). It differs from ERα66, both at the N′- and C′-end. Currently, the use of ERα36 in breast cancer diagnostics has given rise to great expectations (Fowler et al., 2009).

Multiple receptors for E2 offer a chance to regulate independent processes in several organs. Moreover, one receptor protein might be functionally replaced by others, what could explain the fact that mutations in the estrogen-receptor genes are not lethal.

2. Synthesis and structure of ERα isoforms

Human *ESR1* gene encoding the ERα66 protein sequence is located on the long arm of chromosome 6 and includes over 140 kbp. It consists of eight (1–8) exons separated by seven introns (Ponglikitmongkol et al., 1988). There are nine non-coding exons and at least seven promoters, starting from the initiation site toward 5′-end (Fig. 1) (Flouriot et al., 1998; Kos et al., 2001). Promoters give rise to several mRNAs with different non-coding 5′-ends. However, if translated, the protein product is the same, i.e. ERα66 (Kos et al., 2001). Promoter F seems to be an exception, since it initiates ERα46 mRNA, in which exon 1 is omitted. Hence, ERα46 originates from exons 2 to 8 (Flouriot et al., 2000; Kos et al., 2001). Usage of different promoters explains how *ESR1* can be transcribed in tissue-specific manner (Brand et al., 2002; Goulding et al., 2010).

ERα66 represents evolutionarily conserved five-domain structure specific for members of NRS. In humans, its 595 amino acids are organized in the A/B, C, D, E and F domains (Krust et al., 1986). The C domain (181–263 aa), the so-called 'DNA-binding domain' (DBD), is structurally comparable between various receptors, even in evolutionarily distant species. It contains two zinc fingers and recognizes EREs. The A/B domain (1–180 aa) activates transcription without ligand binding through the activation function domain 1 (AF-1). As in the case of other NRS members, its crystal structure is not yet known. It seems to be the main target for ERα66 phosphorylation. The E domain (303–552 aa), which is known as the