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N-terminal truncations in sex steroid receptors and rapid steroid actions

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ABSTRACT

Sex steroid receptors act as ligand activated nuclear transcription factors throughout the body, including the brain. However, post-translational modification of these receptors can direct them to extranuclear sites, including the plasma membrane, where they are able to initiate rapid signaling. Because of the conserved domain structure of these receptors, alternative exon splicing can result in proteins with altered nuclear and extranuclear actions. Although much attention has focused on internal and C-terminal splice variants, both estrogen and androgen receptors undergo N-terminal truncations, as well. These truncated proteins not only influence the transcriptional activity of the full-length receptors, but also associate with caveolin and initiate signaling at the plasma membrane. Such actions may have important physiological consequences in neuronal, endothelial, and cancer signaling and cell survival.

1. Introduction

Receptors for androgens, estrogens, and progestins (ARs, ERs, PRs) belong to the steroid receptor superfamily of nuclear transcription factors that share a common structural organization (for reviews see: [1–3]). The defining feature of these receptors is a three domain structure centered around two zinc finger motifs in the DNA binding domain (DBD) that allow interaction with specific promoter elements termed hormone responsive elements (HREs). The carboxy-terminal domain (CTD) contains the ligand binding domain (LBD) and ligand dependent activation function 2 (AF2), the region responsible for recruiting coactivators and interacting with the basal transcriptional machinery to initiate activation of regulated genes. The amino-terminal domain (NTD) is the most variable region, and is primarily associated with the ligand-independent activation function 1 (AF1), that also interacts with AF2 in the ligand-activated state. In addition to direct DNA binding, these receptors can regulate transcription through protein-protein interactions with other transcription factors to modulate their activity.

As nuclear transcription factors, sex steroid receptors (SRs) are primarily localized to the nucleus, but nuclear-cytoplasmic shuttling and post-translational modifications act to localize a portion of SRs to the plasma membrane. The best-described examples of these actions are from cancer and endothelial cells that demonstrate a small proportion of SRs are trafficked to the plasma membrane through palmitoylation of the CTD [4] and interactions with caveolin-1 [5–7]. A role for rapid steroid actions in neurons has been recognized since the 1970's, but the nature of the receptors underlying the various effects of steroids in the brain continues to evolve [8].

In addition to trafficking of full-length receptors to the membrane and the discovery of structurally unrelated membrane SRs, evidence from studies of ERs and PRs supports a role for N-terminal truncated receptors as mediators of extra-nuclear and membrane actions. Recent evidence from our [9,10] lab supports the role of membrane-associated NTD truncated androgen receptor in membrane lipid rafts of neurons (Fig. 1). In this mini-review, we examine the potential role of SR NTD splice variants in rapid neuronal signaling (Fig. 2).

2. Steroid receptor splice variants

In addition to steroid receptor isoforms encoded by different genes (i.e. ERα, ERβ) steroid receptors undergo extensive alternative splicing [11]. Alternative 5′ untranslated regions (UTRs)/exons appear to be a common characteristic of SRs including glucocorticoid receptors [12,13], mineralocorticoid receptors [14,15], ERs [16–18], and ARs [19]. In most cases, these alternative exon sequences do not affect the coding sequence of the SR, but rather tissue specific distribution or expression levels. However, as discussed below, in some cases, alternative splicing of the NTD yields SRs with altered expression, localization, and function. With respect to the functional properties of SR splice variants, those that result in changes to the coding region have been the subject of more study. Numerous splice variants in the coding

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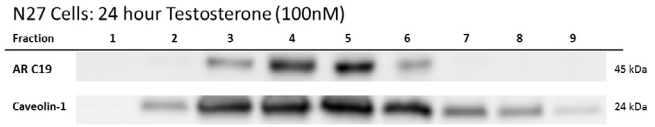

Fig. 1. N27 dopaminergic cells derived from fetal rat mesencephalic tissue express AR45 protein in membrane lipid rafts. N27 cells were treated with 100 nM testosterone for 24 h to stabilized the androgen receptor. N27 cells were homogenized and separated into membrane, cytosol, and nuclear fractions. The membrane portion of the cells were further separated into 9 fractions using a sucrose gradient and ultracentrifugation in order to examine lipid rafts. Primary antibodies targeting AR45 (Santa Cruz sc-815/AR-C19 androgen receptor antibody) and lipid raft markers (Cell Signaling 3267 caveolin-1 antibody) were used. AR45 was only observed in caveolin positive lipid raft fractions. N = 3 per treatment group.

sequence of SRs have been detected in peripheral tissues, and are abundant in cancer cells. For example, the sensitivity of RT-PCR allows the detection of 18 different ERα splice variants and 17 different PR splice variants in the human endometrium [20,21] and brain [22]. Depending on the exon(s) spliced out of the coding sequence, these splice variants can result in transcripts that produce either functional or non-functional proteins. The domain structure of SRs and the close overlap of exons with functional domains allow functional splice variants to act as constitutively active, dominant negative, or modulatory factors for full-length receptors [11]. Some of these variants result in internal start codons that produce functional proteins. However, beyond their transcriptional actions, SR variants may also mediate various non-genomic actions depending on their ability to interact with cytoplasmic and membrane proteins through their remaining domains.

3. Estrogen receptor NTD splice variants

Using RT-PCR, numerous ERα splice variants have been detected in the rat and human brain. In the rat brain, these include deletions of exon 3 [23], exon 4 [24,25], exons 3/4 [25], and exons 5/6 [25]. Alternative promoter expression can also result in an NTD truncation of exon 1 in ERα [26]. This deletion produces a 46 kDa protein (ERα46) from a start codon early in exon 2, and is also found in mice and humans [26]. In transfected cells overexpressing ERα46, this variant appears predominantly nuclear and can stimulate transcription in response to estrogen, but inhibits the activity of the full-length ERα66 [26]. However, in human endothelial cells ERα46 localizes to the plasma membrane, where it can mediate the estrogen-induced stimulation of endothelial nitric oxide synthase (eNOS) through interactions with PI3 kinase [27,28]. Similarly, ERα46 interacts with PI3K p85a subunit in rat cerebral cortex and this interaction declines with age [29]. ERα46 and additional isoforms are also localized to rat cerebral endothelial cells [30], but it is unclear whether it is localized to neurons.

A second major NTD truncated ERα, first noted in endometrium [27] and cloned from human breast tumors [31], is ERα36. Like ERα46, ERα36 lacks exon 1 [27,31–33]. However, it also skips exons 6 and 7 of the full-length ERα and has a unique 27 amino acid CTD [33]. Thus, the resulting protein lacks both the AF1 and much of the AF2/LBD. In the breast, ERα36 is predominantly localized to the plasma membrane where it mediates estrogen activation of MAPK and PI3/Akt signaling [34,35]. Further evidence from transfection experiments supports a role for ERα36 in the mobilization of calcium in breast cancer cells [36]. In addition to its role at the membrane, there is also evidence that ERα36 can inhibit transcriptional activation by the full-length ERα66 [34]. Although initially found in peripheral tissues, ERα36 has also been localized to neurons in the cortex and hippocampus of rats where it is mostly extra-nuclear [37]. In both the rat and human brain ERα36 is associated with caveolin-1 in cortical and hippocampal neurons, confirming membrane localization [37]. Ovariectomy and cerebral

A.

Steroid Receptor NTD Splice Variants Localized to the Plasma Membrane

Name	Location	Function
ERa46	Endothelial cells, Brain (cerebral cortex)	PI3 kinase activity
ERa36	Breast, Brain (cortical and hippocampal neurons)	MAPK and PI3 kinase activity
AR8	Prostate	Unknown
AR45	Brain (Entorhinal cortex, hippocampus, substantia nigra)	G protein (Gaq & Gao) signaling

B.

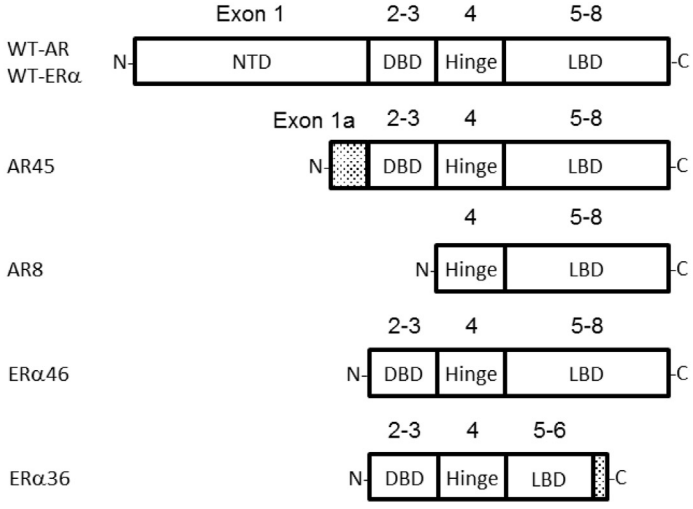

Fig. 2. Characterized N-terminal deletions of AR and ER. A: Location and signaling function of N-terminal deleted variants. B: Structure of variants ARs and ERs relative to the wild-type (WT) full-length receptors. Exons are noted above domain schematics. Shaded areas represent unique sequences not present in the WT receptors.