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Structural and evolutionary analysis of the co-activator binding domain in vertebrate progesterone receptors

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ABSTRACT

Biochemical studies show that binding of co-activators to the progesterone receptor [PR] is an important mechanism for regulating of PR-mediated gene transcription. Unfortunately, unlike other steroid receptors, the PR has not been crystalized with a co-activator. Fortunately, the PR has strong structural similarity to the mineralocorticoid receptor [MR] and glucocorticoid receptor [GR], which have been crystalized with co-activators. This similarity allowed us to construct 3D models of the PR with steroid co-activator 1-Box 4 [SRC1-4] and transcriptional intermediary factor 2-Box 3 [TIF2-3], which were extracted from the crystal structures of human MR and GR, respectively. Comparisons of 3D models of human PR with SRC1-4 and TIF2-3 and human MR with SRC1-4 and GR with TIF2-3 identified some unique interactions between the PR and SRC1-4 and TIF2-3. An evolutionary analysis of the sequence of the co-activator binding groove in human PR found strong conservation in terrestrial vertebrates. However, there are some differences between human PR and the PRs in lamprey, shark and fishes. These differences among the PRs and between the PR, MR and GR may have contributed to the evolution of specificity for progestins, mineralocorticoids and glucocorticoids in vertebrates.

1. Introduction

The progesterone receptor [PR] mediates the actions of progesterone, which regulates many aspects of female reproductive physiology, including fertilization, maintenance of pregnancy and preparation of the endometrium for implantation and parturition [1–6]. Although progesterone usually is considered a reproductive steroid in females, progesterone also has important physiological actions in males [7,8]. The PR belongs to the nuclear receptor [NR] family of transcription factors, which also includes receptors for adrenal steroids, aldosterone and cortisol, and the sex steroids, estradiol and testosterone [9–14].

Binding of progesterone to the PR induces a conformational change that increases the affinity of the PR for co-activators, which is an important mechanism for achieving specificity in the response to progesterone and to other steroids by their cognate receptors [15–22]. The p160 family of co-activators, so called because they are approximately 160 kDa in size, are transcriptional activators of the PR and other steroid receptors. These co-activators are multifunctional proteins containing domains called NR boxes, which interact with nuclear receptors. NR boxes have a characteristic LXXLL sequence motif, where L is leucine and X is any amino acid. Some co-activators have several names because they were cloned by different laboratories at about the same time. Steroid receptor co-activator 1 [SRC1], also called NCoA1, which has four NR boxes, was first found to activate the PR [15,17]. Transcriptional intermediary factor 2 [TIF2], also called NCoA2/GRIP-1/SRC2, which also activates the PR, contains three NR boxes [16,18,20]. Hu and Funder [23] found that in a mammalian two-hybrid assay, SRC1-4 activated human PR, GR and MR, and TIF2 activated human PR and GR, but not human MR. SRC1-4 was a little stronger than TIF2 in activating transcription by human PR.

Crystal structures indicate that co-activators bind to a groove consisting of helices 3, 4, 5 and 12 on the glucocorticoid receptor [GR] [24], mineralocorticoid receptor [MR] [25], androgen receptor [AR] [26] and estrogen receptor [ER] [27,28].

Our interest in the evolution of the PR [29,30] and other steroid receptors [31–34] stimulated us to perform a comparative analysis of the structure of the co-activator binding groove on the PR with the MR and GR, as well as an evolutionary analysis of sequence of this site in vertebrate PRs. Unfortunately, in contrast to other steroid receptors, crystal structures of the PR containing a co-activator have not been reported. Fortunately, superposition of the crystal structure of the PR onto the GR [24] and MR [25,35] indicates that their overall 3D structures are conserved. Thus, we used the mineralocorticoid receptor [MR] complexed with SRC1-Box 4

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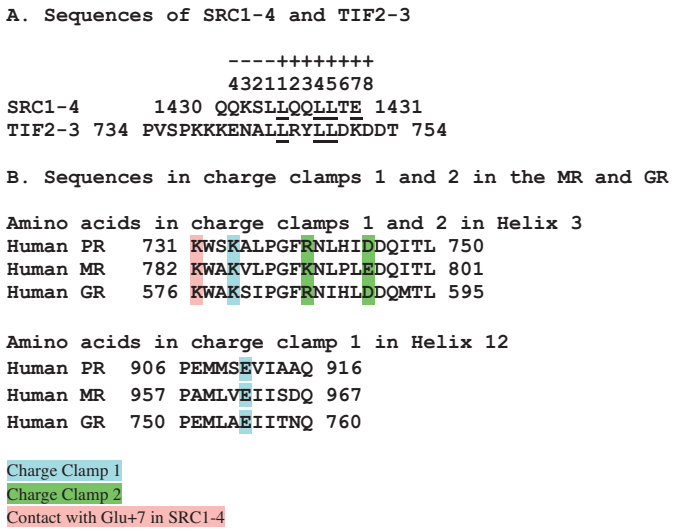

Fig. 1. Alignment of LXXLL motifs in SRC1-4 and TIF2-3 and charge clamps in human PR, MR and GR. (A) LXXLL motifs. The LXXLL motif in SRC1-4 and TIF2-3 is a hydrophobic peptide that contacts the co-activator binding groove on the PR, MR, GR and other steroid receptors. Sequences of SRC1-4 and TIF2-3 are taken from the crystal structure of the GR [PDB:1M2Z] and MR [PDB:2A3I], respectively. (B) Charge clamps. In human PR, K-734 and E-911 align with K-579 and E-755 on the GR and K-785 and E-962 on MR, which comprise charge clamp 1. In human PR, D-745 and R-740 align with D-590 and R-585, respectively, in human GR, which comprise charge clamp 2. D-590 and R-585 in charge clamp 2 contact, R+2 and D+6, respectively, in TIF-2-3. In human PR, K-731 aligns with K-782 in human MR. K-782 contacts E+7 in SRC1-4, which does not have a second charge clamp.

[SRC1-4] [25] and the glucocorticoid receptor [GR] complexed with TIF2-Box 3 [TIF2-3] [24] as templates for transferring SRC1-4 and TIF2-3 to the human PR.

Analysis of the 3D models of human PR with SRC1-4 and TIF2-3 reveals that many contacts between these co-activators and the PR, MR and GR are conserved and also that the PR has some unique interactions with SRC1-4 and TIF2-3. An evolutionary analysis of the sequence of co-activator binding groove in vertebrate PRs [Fig. 1] finds excellent conservation in terrestrial vertebrates, which have some differences with PRs in lamprey, shark and fish. Differences in the co-activator binding groove among the PRs and between the PR, MR and GR may have contributed to the evolution of specificity for progestins, mineralocorticoids and glucocorticoids in vertebrates [Fig. 2].

2. Methods

2.1. Construction of 3D models of human PR with SRC1-4 and TIF2-3

To construct 3D models of the steroid-binding domain on human PR with SRC1-4 and TIF2-3, we superimposed the crystal structure of human PR with progesterone [PDB:1A28] [36] onto the crystal structures of the MR complexed with corticosterone and SRC1-4 [PDB:2A3I] [25] and GR complexed with dexamethasone and TIF2-3 [PDB:1M2Z] [24]. Accurate superposition of the PR on the MR and GR depends on strong conservation of their 3D structures [37]. Structural similarity of the steroid-binding domain on human PR to human MR [25,35] and GR [24] has been demonstrated, which is consistent with the strong amino acid sequence similarity between the steroid binding domains in human PR and MR [56% sequence identity] and GR [54% sequence identity]. This is encouraging because one can construct a useful 3D model of a protein if it has over 40% sequence identity with a crystalized protein

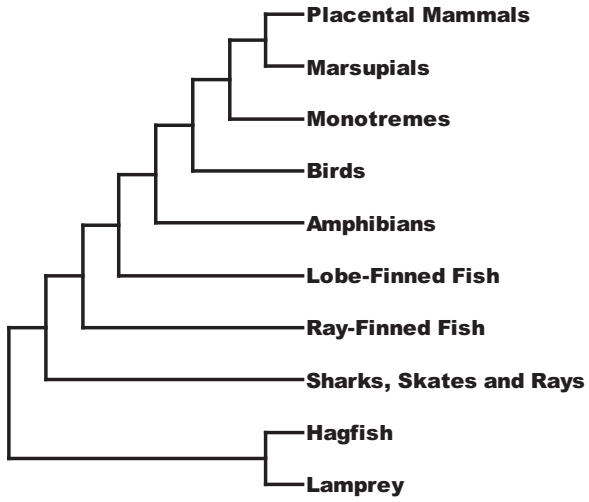

Fig. 2. Evolutionary relationships among vertebrate groups. Lampreys and hagfish are jawless fish, belonging to the cyclostome taxon. Sharks, skates, sharks and rays are cartilaginous fishes, belongs to the elasmobranch subclass. Land vertebrates are descended from lobe-finned fishes (coelacanths and lungfishes).

[37]. Sequence similarity between the steroid-binding domain on human PR with MR and GR exceeds this threshold.

We find that superposition of the entire the ligand binding domain of the PR on the MR and GR yields a root mean square deviation [RMSD] between the PR and the MR and GR of 1.0 Å and 1.1 Å, respectively. Because we are interested in the interaction of co-activators with the PR, we superimposed the segments on the PR, MR and GR corresponding to the co-activator binding groove, which consists of helices 3–5 and 12 and then extracted TIF2-3 from the GR and SRC1-4 from the MR. The RMSDs for superposition of the co-activator groove on the PR with the MR and GR are 0.7 Å and 0.8 Å, respectively.

The 3D models of the PR with SRC1-4 or TIF2-3 were refined using Discover 3 from the Insight II software package (Accelrys) with the CVFF force field and a distant dependent dielectric constant of 2 for 1000 iterations.

2.2. Evolutionary analysis of the co-activator groove in the PR

A 59 residue segment from the human PR corresponding to helix 3 through helix 5 and a 32 residue segment corresponding to helix 12 and the preceding loop [29,30,36,38] were used as probes for BLAST [39] to search GenBank and Ensembl for the corresponding segments in other animal PRs. The accessions for each gene are presented in Table 1 in the Supplement.

3. Results

3.1. Alignment of TIF2-3 and SRC1-4 and their binding sites on human PR, MR and GR

Fig. 1A shows the alignment of the LXXLL motif of TIF2-3 and SRC1-4, in which the first leucine in this motif is numbered +1 and the preceding amino acid numbered -1, according to convention.

Fig. 1B shows the alignment of segments containing charge clamps 1 and 2 in human PR, GR and MR. Human GR contacts TIF2-3 with two charge clamps [24]. SRC1-4 does not contain the two amino acids corresponding to the second charge clamp on TIF2-3. However, there is a unique contact between Glu+7 on SRC1-4 and Lys-782 on human MR [25].

The PR conserves all of the residues forming the charge clamps in the MR and GR. Lys-734 and Glu-911 on the PR, which correspond