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11β-Hydroxysteroid dehydrogenase-type 2 evolved from an ancestral 17β-Hydroxysteroid dehydrogenase-type 2

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ABSTRACT

11β-Hydroxysteroid dehydrogenase-type 2 (11β-HSD2) regulates the local concentration of cortisol that can activate the glucocorticoid receptor and mineralocorticoid receptor, as well as the concentration of 11-keto-testosterone, the active androgen in fish. Similarly, 17β-HSD2 regulates the levels of testosterone and estradiol that activate the androgen receptor and estrogen receptor, respectively. Interestingly, although human 11β-HSD2 and 17β-HSD2 act at different positions on different steroids, these enzymes are paralogs. Despite the physiological importance of 11β-HSD2 and 17β-HSD2, details of their origins and divergence from a common ancestor are not known. An opportunity to understand their evolution is presented by the recent sequencing of genomes from sea urchin, a basal deuterostome, and amphioxus, a basal chordate, and the availability of substantial sequence for acorn worm and elephant shark, which together provide a more complete dataset for analysis of the origins of 11β-HSD2 and 17β-HSD2. BLAST searches find an ancestral sequence of 17β-HSD2 in sea urchin, acorn worm and amphioxus, while an ancestral sequence of 11β-HSD2 first appears in sharks. Sequence analyses indicate that 17β-HSD2 in sea urchin may have a non-enzymatic activity. Evolutionary analyses indicate that if acorn worm 17β-HSD2 is catalytically active, then it metabolizes novel substrate(s).

1. Introduction

11β-Hydroxysteroid dehydrogenase-type 2 (11β-HSD2) and 17β-HSD2 catalyze the conversion of C11 and C17 alcohols, respectively, to ketones on glucocorticoids, androgens and estrogens (Fig. 1), which make these enzymes important partners with steroid receptors in the regulation of steroid hormone action [1–4]. Tissue-specific expression of 11β-HSD2 and 17β-HSD2 allows these enzymes to act as gatekeepers in regulating access of active glucocorticoids, androgens and estrogens to their receptors. For example, by metabolizing cortisol to cortisone, an inactive steroid, 11β-HSD2 regulates the concentration of cortisol that can activate the glucocorticoid receptor [GR] and mineralocorticoid receptor [MR] [4–7]. Interestingly, in fish, 11β-HSD2 catalyzes the conversion of 11β-hydroxy-testosterone to 11-keto-testosterone [11-keto-T], which is the active androgen in fish [8–10].

Similarly, tissue-specific expression of 17β-HSD2 regulates access of active androgens and estrogens to the androgen receptor [AR] and estrogen receptor [ER] [1–3]. Thus, along with the AR and ER, 17β-HSD2 has a key role in the physiological actions of androgens and estrogens.

Sequence analyses of 17β-HSD2 and 17β-HSD2 reveal that they belong to the short chain dehydrogenases/reductases [SDR], which is a large and diverse family of enzymes that are found in bacteria, plants and animals [11–14]. Previous evolutionary analyses reveal that 11β-HSD2 and 17β-HSD2 are found in fish and land vertebrates [2,3,15–20]. However, several questions remain regarding more ancient events in the evolution of 11β-HSD2 and 17β-HSD2. When did these enzymes evolve; which enzyme evolved first, and when did the second enzyme first appear in animals? Also, what was the substrate(s) of the ancestral enzyme? That is, did the common ancestor of 11β-HSD2 and 17β-HSD2 metabolize glucocorticoids or androgens or estrogens or a combination of these steroids or did it metabolize a different hormone?

This is an opportune time to seek answers to these questions about the origins and evolution of 11β-HSD2 and 17β-HSD2 because the genomes from sea urchin (*Strongylocentrotus purpuratus*), a basal deuterostome and amphioxus (*Branchiostoma floridae*), a basal chordate, have been sequenced. In addition, recently, many sequences from the acorn worm (*Saccoglossus kowaleski*), another basal deuterostome, were deposited in GenBank, and a draft of the elephant shark genome [21] and lamprey genome also are available (Fig. 2). As a result, a more complete genomic dataset can be used for analysis of the origins of 11β-HSD2 and 17β-HSD2. With this in mind, we searched GenBank

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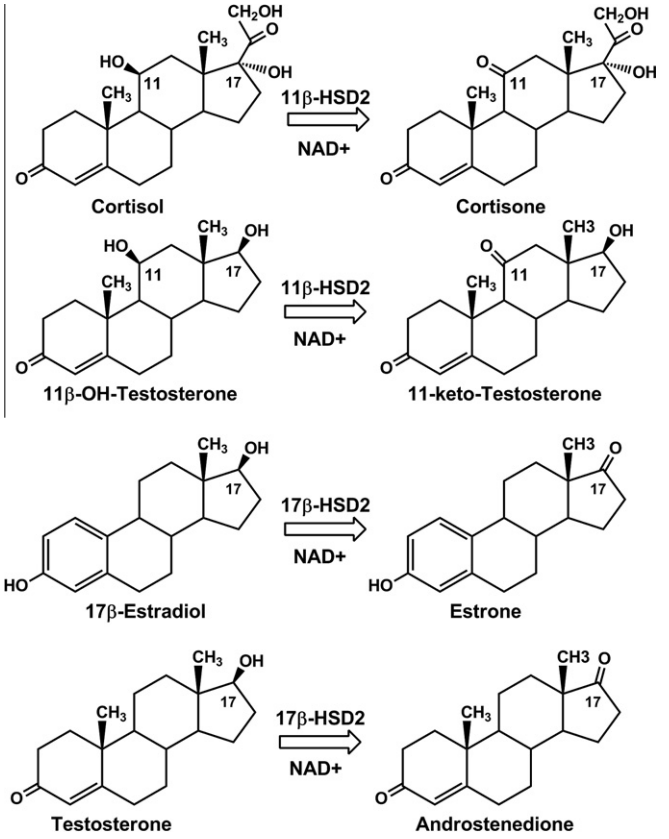

Fig. 1. Reactions catalyzed by 11β-HSD2 and 17β-HSD2. 11β-HSD2 and 17β-HSD2 are NAD^+-dependent enzymes that metabolize the C11-alcohol and C17-alcohol, respectively, on steroids.

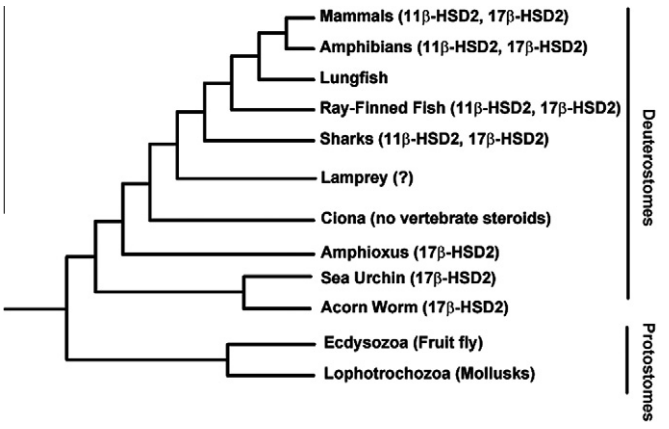

Fig. 2. Phylogeny of deuterostomes and protostomes [22]. Sea urchin and acorn worm, basal deuterostomes, contain an ortholog of 17β-HSD2. There is no evidence for steroid receptors in sea urchin [29,54]. 11β-HSD2 is present in elephant shark, but not in amphioxus or Ciona. It is not known if either 11β-HSD2 or 17β-HSD2 is present in lamprey.

and other databases with 11β-HSD2 and 17β-HSD2 for orthologs. These searches found an ortholog of 17β-HSD2 in sea urchin, acorn worm and amphioxus, while 11β-HSD2 first appears in the elephant shark. This indicates that 17β-HSD2 is the ancestor of 11β-HSD2 and suggests that 17β-HSD2 and 11β-HSD2, respectively, may have had a role in evolution of basal deuterostomes [22] and sharks.

2. Methods

We used the sequences for human 11β-HSD2 (Accession: P80365) and human 17β-HSD2 (Accession: P37059) to search GenBank (http://www.ncbi.nlm.nih.gov/entrez), Ensembl (http://www.ensembl.org/), elephant shark server (http://esharkgenome.imcb.a-star.edu.sg/), Sea Urchin Genome Database (http://www.spbase.org/SpBase), Joint Genome Initiative Database (http://genome.jgi-psf.org/) and Gene Indices (http://compbio.dfci.harvard.edu/tgi/tgipage.html) with BLAST [23]for orthologs. The accessions for each gene are presented in (Supplementary Table 1).

Multiple alignments of 11β-HSD2 and 17β-HSD2 were done with Clustal X using the iteration option for each step in the multiple alignment [24,25]. This alignment was converted to a phylogenetic tree using the neighbor-joining algorithm with a correction of branch lengths for rate heterogeneity between sites [26].

3. Results

3.1. Identification of orthologs of 11β-HSD2 and 17β-HSD2

Analysis of the evolution of 11β-HSD2 and 17β-HSD2 depends on tracing their orthologs in multi-cellular animals. Two genes are orthologs, if they have evolved only by speciation events [27]. Thus, human and mouse 11β-HSD2 are orthologs. In contrast, human 11β-HSD2 and 17β-HSD2 are paralogs, having evolved through a gene duplication and divergence to yield enzymes with two different catalytic activities. The amino acid sequences of human 11β-HSD2 and 11β-HSD2 are about 41% identical over 341 amino acids, with 14% conservative replacements and only one gap. This difference in their sequences is not surprising because glucocorticoids and estrogens have different functional groups at C11 and C17 (Fig. 1). Nevertheless BLAST searches show that 11β-HSD2 and 17β-HSD2 are closest to each other in GenBank. That is, a search with 11β-HSD2 finds a series of 11β-HSD2 orthologs in vertebrates, followed by a series of vertebrate 17β-HSD2 paralogs. A similar BLAST search with 17β-HSD2 finds a series of 17β-HSD2 orthologs, followed by 11β-HSD2 paralogs.

3.2. Phylogenetic analysis of 11β-HSD2 and 17β-HSD2

Proteins identified with BLAST as being possible orthologs of 11β-HSD2 or 17β-HSD2 were collected for the phylogenetic analysis, shown in Fig. 3. This analysis reveals that 17β-HSD2 has orthologs in sea urchin, acorn worm and amphioxus. Both 11β-HSD2 and 17β-HSD2 are found in fish and land vertebrates, as has been previously reported [2,3,15–17,19,20]. BLAST searches of the elephant shark genome found partial sequences of 11β-HSD2 and 17β-HSD2 (Supplementary Table 2), but the sequences were not long enough to be included in the phylogenetic analysis. BLAST searches did not find either 11β-HSD2 or 17β-HSD2 in Ciona, which lacks steroid receptors [28,29], or in sea lamprey. Although there is substantial coverage of the sea lamprey genome, it is incomplete. Thus, the absence of 11β-HSD2 and 17β-HSD2 from lamprey is not yet established. With this caveat, it appears that 11β-HSD2 arose in a shark, after 17β-HSD2 arose in a basal deuterostome [22].

3.3. Unusual amino acids in the catalytic site of amphioxus and sea urchin 17β-HSD2

11β-HSD2 and 17β-HSD2, like other SDRs, contain a triad consisting of tyrosine, lysine and serine at the catalytic site [11,13]. A highly conserved asparagine also is important in catalysis [30]. In Fig. 4 we show a multiple alignment of the amphioxus, acorn