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Follicle-stimulating hormone receptor (FSHR) in Chinese alligator, *Alligator sinensis*: Molecular characterization, tissue distribution and mRNA expression changes during the female reproductive cycle

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ABSTRACT

The follicle-stimulating hormone (FSH) plays a central role in vertebrate reproduction, with the actions of FSH mediated by FSH receptors (FSHRs) on the granulosa cells of the ovary. The present study reports the cloning and characterization of FSHR in Chinese alligator, *Alligator sinensis* (caFSHR), and its tissue distribution and mRNA expression changes during the reproductive cycle. The mature protein of caFSHR displays typical features of the glycoprotein hormone receptor family, but also contains some remarkable differences when compared with other vertebrate FSHRs. The deduced amino acid sequence of the caFSHR shares identity of 85% with Chinese softshell turtle, 84–87% with birds, 77–78% with mammals, 67–73% with amphibians and 51–58% with fishes. Phylogenetic tree analysis of the FSHR amino acid sequence indicated that alligators cluster into the bird branch. Tissue expression analysis showed that caFSHR was not only expressed in the ovary, but also in the stomach, intestine, pancreas liver and oviduct at similar levels, while it was not detectable in heart, thymus or thyroid. Expression of caFSHR in the ovary is high in May (breeding prophase) and peaks in July during the breeding period, where it is maintained at high levels through September (breeding anaphase). Expression decreases significantly in November (hibernating period) and then remains relatively low from January to March (hibernating period). These temporal changes in FSHR expression suggest that it plays an important role in promoting ovarian development during the female reproductive cycle of Chinese alligator.

1. Introduction

The Chinese alligator, *Alligator sinensis*, belonging to the Crocodilia Alligatoridae family, is an endangered species indigenous to China and was listed by the Chinese government as a first-level state-protected species in 1972 (Yan et al., 2005). In response, nature reserves and artificial farms of the Chinese alligator were set up in Anhui and Zhejiang Provinces. Though the issues with artificial incubation and breeding of the Chinese alligators have been successfully resolved, the productivity of the Chinese alligator is considerably affected by inherent problems such as lower and highly annual fluctuating egg-laying rate (Cheng et al.,

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2003). The cause of these problems remains unclear. Application of assisted reproductive technologies (e.g. hormonal treatment) has the potential to improve reproductivity.

The reproduction of vertebrates is mainly under the endocrine control of hypothalamus–pituitary–gonad axis. The pituitary gonadotropins, follicle-stimulating hormone (FSH), a glycoprotein hormone, is the central hormone regulating vertebrate reproduction, it functions together with luteinizing hormone(LH) to promote the growth and development of gonads, to control gametogenesis and regulate gonadal endocrine functions (Chauvigné et al., 2012). In females, FSH stimulates ovarian follicular development and egg production (Zhao et al., 2010). The follicles receiving insufficient FSH support are doomed to atretic degeneration during the critical stage of follicle development (Zhao et al., 2010). FSH could increase serum oestradiol-17 beta concentrations, number of growing follicles and yolk deposition in aging hens (*Gallus gallus domesticus*) with decreased egg production (Palmer and Bahr, 1992). Expression of FSHβ in the Chinese alligator ovary increases significantly during the breeding prophase and reaches a peak in the breeding period, then decreases significantly during the breeding anaphase and hibernating periods (Zhang et al., 2015). *In vivo* FSH challenge in five-month-old female American alligators (*Alligator mississippiensis*) could increase circulating estradiol concentrations, ovarian steroidogenic enzyme mRNA expression levels, and oviducal mRNA expression levels of sex steroid hormone receptors, activin-binding proteins, and mitotic factors (Moore et al., 2012).

FSH exerts its biological activities through interaction with specific receptors (FSHRs) present on target cell surfaces (Minj et al., 2008). FSHR belongs to the large family of G protein-coupled receptors (GPCRs), which also includes the TSH receptor (TSHR) and the LH receptor (LHR). FSHR, together with TSHR and LHR, constitute the subfamily of glycoprotein hormone receptors (GpHRs) that are a part of a broader family of leucine-rich repeat-containing GPCRs (Szkudlinski et al., 2002). Members of this family are characterized by a large extracellular (EC) domain with multiple imperfect leucine-rich repeats (LRRs), flanked by N- and C-terminal cysteine-rich sub-domains, and a rhodopsin-like domain of seven transmembrane (7TM) helices followed by a C-terminal intracellular tail (Hsu et al., 2000). Residues present in the β-strand motifs of the LRRs provide specificity and affinity for ligand binding, while the TM domain is responsible for receptor activation and signal transduction through G proteins (Smits et al., 2003). Hormone binding to the EC domain of a GpHR induces changes in the 7TM domain that promote cAMP accumulation via activation of heterotrimeric Gs, which then initiate a signaling cascade leading to steroid synthesis (Szkudlinski et al., 2002). The nucleotide and amino acid sequences of FSHRs have been extensively studied in mammals, birds and fish, wherein conservation of the molecular structure of FSHRs was found, while some specific features were also reported in different species (Kumar et al., 2001; Zhao et al., 2010). Relatively less data are available concerning the molecular structure of reptilian FSHRs. In Crocodilia, only two partial FSHR mRNA sequences for the American alligator (DQ010157.1 and JN568480.1) and two predicted FSHR mRNA variants for the Chinese alligator (XM_006014720.1 and XM_006014721.1) are available in GenBank, no Crocodilian FSHRs have yet been characterized.

FSHR was found to be highly expressed in granulosa cells of the ovary and in Sertoli cells of the testis (Rocha et al., 2007; An et al., 2009; Shen and Yu, 2002). However, many studies on bird and fish tissue show that FSHR is also expressed in non-gonadal tissues (Zhao et al., 2010; Mu et al., 2013). For example, in chick, FSHR was also expressed in the pancreas and glandular stomach (Zhao et al., 2010); in Korean rockfish, FSHR was also detected in the spleen, head kidney, caeca, heart, liver, gill, kidney and intestine (Mu et al., 2013); and in channel catfish, FSHR was also expressed in the spleen (Kumar et al., 2001). These results suggest that FSHR expression is not restricted to gonadal tissues and may have various physiological functions in different tissues. To assess its roles in regulating ovarian development, the temporal changes in FSHR expression have been documented in the gonadal tissues of various vertebrate species during sexual maturation, including mammals, birds and fish, such as mouse (O'Shaughnessy et al., 1996), chick (Zhao et al., 2010), Zi geese (Kang et al., 2010), Korean rockfish (Mu et al., 2013), channel catfish (Kumar et al., 2001), whereas no related work has been reported in the Crocodilian order so far.

In the present study, we report the molecular cloning and characterization of FSHR in the Chinese alligator, and its tissue distribution and mRNA expression changes during the female reproductive cycle. The aim of this study was to expand our knowledge of FSHR phylogenetic evolution and to provide basic data for further study on the mechanisms that regulate Chinese alligator reproduction, which could be useful for maximizing the success of artificial breeding.

2. Materials and methods

2.1. Animals and RNA isolation

Sexually mature female Chinese alligators were obtained from the Xuanzhou Alligator Culturing Centre of Anhui Province. Ovary, stomach, intestine, pancreas, liver, heart, thymus, thyroid and oviduct tissues were excised and immediately put into RNA-Be-Locker A (Sangon Biotec, Shanghai) and stored at −80 °C. Tissue (100 mg) was ground in liquid nitrogen, followed by extraction of total RNA using Total RNA Extractor (Sangon Biotec, Shanghai) according to the manufacturer's instructions. To remove genomic DNA contamination, RNase-free DNase I (TaKaRa, Dalian, China) was used. Concentration of total RNA was determined by measuring absorbance at 260 nm, and purity was determined by dividing absorbance at 260 nm by absorbance at 280 nm (A_{260}/A_{280}). Only RNA samples with A_{260}/A_{280} ratios of 1.8–2.0 were used for RT-PCR. Total RNA was stored at −80 °C until further use.

2.2. Cloning of the coding region of caFSHR cDNA

Reverse transcription of mRNA into cDNA was achieved using PrimeScript 1st cDNA Synthesis Kit (TaKaRa, Dalian).