Expression Pattern of \textit{phb2} and Its Potential Function in Spermatogenesis of Scallop (\textit{Chlamys farreri})

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Abstract Prohibitin (PHB) participates in several biological processes including apoptosis, transcription regulation and suppression of cell proliferation in mammals. In this study, we cloned the full-length cDNA of prohibitin 2 (Cf-phb2) from the testis of scallop (\textit{Chlamys farreri}). The deduced amino acid sequence presented a characteristic of PHB family with the PHB domain, and clustered with PHB2 of other species. Temporal and spatial expression of Cf-phb2 in testis during the reproductive cycle was detected by quantitative real-time PCR (qRT-PCR) and \textit{in situ} hybridization. The expression of Cf-phb2 in the testis increased when testis developed from the resting stage to mature stage. The mRNA abundance of Cf-phb2 was the highest at mature stage, which was about 15-fold higher than that at proliferative stage. The expression of Cf-phb2 could be detected by \textit{in situ} hybridization in all types of germ cells in testis, including spermatogonia, spermatocytes, spermatids and spermatozoa. The intensity of the signal increased with the spermatogenesis and was the highest in spermatids, which suggested that CF-PHB2 might affect the spermatogenesis of \textit{C. farreri}.

Key words \textit{phb2}; spermatogenesis; testis; \textit{Chlamys farreri}

1 Introduction

Prohibitin (PHB) belongs to Band-7 super family and is characterized by the PHB domain or the SPFH domain (Chowdhury et al., 2012; Merkwirth and Langer, 2009; Mishra et al., 2006). It consists of two highly homologous members termed prohibitin 1 (PHB1) and prohibitin 2 (PHB2) (Mishra et al., 2006). McClung et al. (1989) cloned the first \textit{phb1} cDNA from rat \textit{Rattus norvegicus} liver cells, and found \textit{phb1} mRNA blocked DNA synthesis in human fibroblasts \textit{in vitro}. The similar phenomenon was observed in HeLa cells (Nuell et al., 1991) and chronic lymphocytic leukemia B cells (Woodlock et al., 2001). In addition, mutation of PHB1 was detected in human breast tumor cells (Sato et al., 1992), and deletion of PHB1 was detected in human ovarian tumor cells (Foulkes et al., 1993), suggesting that PHB1 plays a role in suppressing tumors. Dixit et al. (2003) reported that the majority of the proliferating thymocytes in rat thymus failed to express PHB1 protein, but nonproliferating thymocytes expressed it at a high level. Aurilide, a potent cytotoxic marine natural product that induces apoptosis in cultured human cells, can selectively bind to PHB1 in mitochondria, and activate the proteolytic processing of OPA1, resulting in mitochondria-induced apoptosis (Sato et al., 2011). Many researches showed that PHB1 inhibits the transcriptional activity of E2F by binding to E2F (Fusaro et al., 2003; Joshi et al., 2003; Wang et al., 1999a; Wang et al., 1999b). Choi et al. (2008) further proved that PHB1 combines directly with E2F1, a kind of multichain transcription factor, to repress the transcription activity of E2F1.

The \textit{phb2} cDNA was first cloned from mouse (\textit{Mus musculus}) B cells (Terashima et al., 1994). PHB2 is named as a repressor of estrogen receptor activity (ERA) because it represses the transcription activity of estrogen receptor (ER) by competing for activator binding sites on ER in the nucleus of human breast cancer cells (Montano et al., 1999). Park et al. (2005) deleted the ERA gene (\textit{phb2}) in the mouse and revealed that the ERA restrains estrogen actions by moderating ER stimulation and enhancing ER repression of E2-regulated genes. In yeast and mammalian cells, PHB2 can inhibit muscle differentiation by repressing the transcription activity of both MyoD and MEF2, and specifically recruit HDAC1 which is important for its repressive activity (Sun et al., 2004).

Data of \textit{phb} gene in spermatogenesis are limited. Recently, Mao et al. (2012) suggested that \textit{phb} may fulfill critical functions in spermatid differentiation of Chinese mitten crab (\textit{Eriocheir sinensis}) by \textit{in situ} hybridization. Fang et al. (2013) found that PHB which was finally clustered into PHB1 subfamily plays a role in spermatoto-

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genesis in crayfish (*Cherax quadricarinatus*). In this study, we isolated the full-length cDNA of *phb2* (Cf-*phb2*) in scallop (*C. farreri*), and revealed its expression characteristic in testis in the reproductive cycle with real-time PCR and *in situ* hybridization techniques. Our aims were to establish an expression pattern of Cf-*phb2* in testis and preliminarily explore its potential role in the spermato-genesis of scallop.

2 Materials and Methods

2.1 Animals and Sampling

Healthy scallops (*C. farreri*) with a mean shell height of 6.38 ± 0.33 cm were purchased from NanShan Aquatic Product Market (Qingdao, China). Six scallops at each developmental stage of testis were employed in this study. The testes were dissected and weighed rapidly. Part of testes was immersed in liquid nitrogen immediately, and then stored at −80°C for total RNA extraction. Part of testes was fixed with 4% paraformadehyde in 0.1 mol L⁻¹ phosphate buffer (pH7.4) at 4°C for 16 h, and then dehydrated with 25, 50, 75 and 100% methanol. Finally they were stored in 100% methanol at −20°C for *in situ* hybridization. Some middle parts of the testes were fixed in Bouin’s solution for 24 h, and then dehydrated with an ascending gradient of ethanol (30%, 50%, 70%, 80%, 95%, 100%). Finally they were embedded in paraffin wax, sliced in a thickness of 6μm, and stained by haematoxylin-eosin (H & E). The histology of testis at different developmental stages was observed and photographed under a Nikon E80i microscope.

Following the morphologic features described by Liao et al. (1983), the testis development of the scallop was divided into four stages according to the histological structure (Fig. 1). Gonadosomatic index (GSI = gonad weight/soft tissue body weight × 100) was 2.46 ± 0.009 at resting stage, 3.89 ± 0.008 at proliferative stage, 3.93 ± 0.012 at growing stage, and 4.53 ± 0.009 at mature stage, respectively.

2.2 RNA Isolation, Reverse Transcription, 3’ and 5’ RACE

Total RNA was extracted from the stored testes (about 200 mg) at each stage using acid phenol-guandine thiocyanate-trichloromethane extraction method according to the described in Molecular Cloning: A Laboratory Manual (Sambrook and Russell, 2001). The RNA quality was estimated by agarose gel electrophoresis, and its concentration and purity was assayed by spectrophotometry at 260 nm and 280 nm. First-strand cDNA was synthesized using PrimeScript™ RT reagent Kit (Takara, Dalian, China).

The cDNAs were amplified with 3’ and 5’ RACE (rapid amplification of cDNA ends) strategy by using

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Fig. 1 The histology of *C. farreri* testis during the reproductive cycle. A, resting stage; B, proliferative stage; C, growing stage; D, mature stage. a–d indicate partial magnifications in the same picture. Fc, follicle cell; Sg, spermatogonium; pSc, primary spermatocyte; sSc, secondary spermatocyte; St, spermatid; Sz, spermatozoon. Scale bar = 30 μm.