学校编码：10384 密级_____
学号：22420110153615

厦门大学

博 士 学 位 论 文

两种雌雄同体鱼类 Gsdf 基因的克隆和表达

Cloning and expression patterns of gonadal soma-derived factor (Gsdf) in two hermaphrodite fishes

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论文提交日期：2014 年 10 月
论文答辩时间：2014 年 11 月

2014年11月

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摘要

性腺体细胞衍生因子（Gsdf）是转化生长因子β（TGF-β）超家族的新成员。作为一种硬骨鱼特有并仅在性腺表达的生长因子，Gsdf 在早期生殖细胞发育中发挥重要作用。雌雄同体鱼类为研究性腺分化和发育机制提供了理想的材料，然而 Gsdf 在雌雄同体鱼类中的表达模式及功能目前仍不明确。本研究运用组织学、生物化学和分子生物学等技术，对 Gsdf 在雌性先熟的赤点石斑鱼和雄性先熟的黄鳍鲷两种不同类型雌雄同体鱼类中的表达模式进行了初步研究，主要研究成果如下：

1. 观察了赤点石斑鱼和黄鳍鲷性腺发育组织学，根据各级生殖细胞的数量和发育状态以及卵巢和精巢的成熟先后，并结合 GSI 数据，将赤点石斑鱼性腺发育分为雌性阶段（FI—FIV）、性转变阶段（ET 和 LT）和雄性阶段（M），将黄鳍鲷性腺发育分为雄性生殖阶段（MI—MVI）和雌性生殖阶段（F）。血清性类固醇激素水平测定结果表明，类固醇激素 E_2、11-KT 和 DHP 含量均与性腺发育密切相关，赤点石斑鱼和黄鳍鲷血清中 E_2 水平在雌性阶段较高，11-KT 水平在雄性阶段较高，血清 DHP 水平在赤点石斑鱼雌性和雄性成熟阶段较高。

2. 克隆获得了赤点石斑鱼 *gsdf* 基因，序列全长 2307bp；蛋白质编码区可编码 210 个氨基酸，其中 5’端有一个 121bp 的非编码区，3’端有一个 1553bp 的非编码区。克隆获得了黄鳍鲷 *gsdf* 基因，序列全长 1700bp；蛋白质编码区可编码 212 个氨基酸，其中 5’端有一个 149bp 的非编码区，3’端有一个 912bp 的非编码区。赤点石斑鱼和黄鳍鲷的 Gsdf 氨基酸序列有保守的 TGF-β 结构域，并存在一些保守的半胱氨酸残基。序列比对的结果显示，赤点石斑鱼和黄鳍鲷的 Gsdf 与其他硬骨鱼类的 Gsdf 聚为一支。组织表达分析表明，*gsdf* 仅在赤点石斑鱼和黄鳍鲷的性腺中表达，黄鳍鲷精巢 *gsdf* 的表达量显著高于卵巢。

3. 采用 real-time qPCR 方法分析了赤点石斑鱼和黄鳍鲷性转变过程中 *gsdf* 表达模式，结果显示，赤点石斑鱼雌性阶段和性转变的早期阶段 *gsdf* 表达量低，随着性腺由雌性阶段向雄性阶段转变，*gsdf* 表达量逐渐升高，至雄性阶段达到最高值。在赤点石斑鱼性转变后期阶段，*gsdf* 表达量与血清雄性激素 11-KT 水平的

趋势一致。在雄性生殖阶段，黄鳍鲷精巢部分 *gsdf* 在精细胞发育早期表达量最高，随着精细胞的发育表达量逐渐降低；卵巢部分 *gsdf* 的表达模式与精巢部分不同，在雄性生殖阶段早期 *gsdf* 表达量最高，随后显著降低，中后期 *gsdf* 表达量又逐渐增加，但到雌性生殖阶段，*gsdf* 仅有微弱表达。此外黄鳍鲷雄性生殖阶段性腺中未成熟卵巢部分 *gsdf* 表达量与血清 E_2 水平有相似的趋势。

4. 为查明 *gsdf* 的表达位点，通过原位杂交方法研究发现，赤点石斑鱼 *gsdf* 强信号存在于精原细胞边缘的 Sertoli 细胞中；黄鳍鲷 *gsdf* 在精巢的 Sertoli 细胞中有较强表达，在卵巢的性腺体细胞有弱表达。

5. 克隆了赤点石斑鱼脑垂体和性腺的 GtHs 及其受体。分析表明，赤点石斑鱼 FSHβ 和 LHβ 有糖蛋白激素家族成员保守的结构特征，Fshr 和 Lhcgr 有糖蛋白激素受体亚家族保守的结构特征。FSHβ 和 LHβ 仅在脑垂体表达，而 Fshr 和 Lhcgr 除性腺外，在一些非性腺组织也可检测到。采用 real-time qPCR 方法分析赤点石斑鱼性转变过程中 GtHs 及其受体的表达模式发现，FSHβ 与 LHβ 的表达模式不同，脑垂体中 FSHβ 亚基在雌性 FI 阶段表达量最高，随后显著下降，性转变后期阶段表达量迅速上升，雄性阶段表达量再次下降；脑垂体中 LHβ 亚基的高表达量出现在雌性成熟阶段（雌性 FIV 阶段）、性转变后期阶段和雄性阶段。Fshr 和 Lhcgr 在性腺中的表达模式相似，雌性阶段至性转变早期阶段，表达量都很低，性转变后期阶段表达量上升，雄性阶段表达量显著升高并达到最高水平。此外，研究中还发现赤点石斑鱼 GtHRs 的表达模式与 Gsdf 的表达模式相似。

关键词：性腺体细胞衍生因子；雌雄同体；赤点石斑鱼；黄鳍鲷；促性腺激素及其受体

Abstract

Gonadal soma-derived factor (Gsdf) is a new member of the transforming growth factor beta (TGF-β) superfamily. As an unique growth factor that only expresses in gonads of teleost, Gsdf plays an important role in early germ cell development. Hermaphroditic fishes provide a good experimental model for understanding the mechanisms of sex determination and differentiation. However expression patterns and functions of Gsdf in hermaphroditic fishes are still unclear. In the present study, histological, biochemical and molecular biological techniques were used to analyze the expression patterns of Gsdf in the two different types of hermaphrodite fishes, protogynous *Epinephelus akaara* and protandrous *Acanthopagrus latus*. The main results were as followed:

1. The gonadal development of *E. akaara* and *A. latus* was observed histologically during sex change. On the basis of the number and developmental stages of germ cells, mature sequence of ovary and testis and GSI changes, the process of gonadal development of *E. akaara* was divided into female phase (FI, FII, FIII and FIV stages), transition phase (ET and LT stages) and male phase (M stage); while the process of gonadal development of *A. latus* was divided into male phase (MI, MII, MIII, MIV, MV and MVI stages) and female phase (F stage). Analyses of the plasma steriod revealed that E_2, 11-KT and DHP levels were closely related to gonadal development. The plasma E_2 levels were high in female phase, while 11-KT levels were high in male phase in both *E. akaara* and *A. latus*, the plasma DHP levels showed two peaks at mature stages of both female and male phases in *E. akaara*.

2. One single copy of *gsdf* cDNA was cloned in *E. akaara*. The cloned complete sequence was 2307bp, which encoded a protein of 210 amino acids, flanked by 121bp of 5'UTR and 1553bp of 3'UTR. One single copy of *gsdf* cDNA was cloned in *A. latus*. The cloned complete sequence was 1700bp, which encoded a protein of 212 amino acids, flanked by 149bp of 5'UTR and 912bp of 3'UTR. In predicted amino

acid sequences of *E. akaara* and *A. latus*, the conserved TGF-β domains were found. Multiple alignments of *E. akaara* and *A. latus* Gsdf TGF-β domains with other teleost Gsdfs showed that *E. akaara* and *A. latus* also present several conserved cysteine residues. The phylogenetic analyses showed that *E. akaara* and *A. latus* Gsdfs were clearly associated with other teleost Gsdfs. The expression of *gsdf* was restricted to the gonads in both *E. akaara* and *A. latus*, and the expression of *gsdf* in testicular tissue showed significantly higher than in ovarian tissue of *A. latus*.

3. The expression patterns of *gsdf* were analyzed by real-time qPCR during sex change both in *E. akaara* and *A. latus*.The results showed in *E. akaara* the expression levels of *gsdf* were low in female phase and early transition phase, then gradually increased and reached a peak in male phase. In *E. akaara* the expression levels of *gsdf* showed a similar trend to the plasma 11-KT levels at LT stage of transition phase. In the male phase of *A. latus*, the highest expression level of *gsdf* was observed in the testicular tissue at early stage of germ cell development, and decreased continuously with the development of germ cells. Conversely, the expression pattern of *gsdf* in the ovarian tissue was different from that in the testicular tissue in *A. latus*. In the ovarian tissue, the highest expression of *gsdf* appeared in early male phase, and then decreased significantly. Afterwards, the *gsdf* expression levels gradually increased in middle-later male phase. In the female phase weak expression of *gsdf* was detected. Additionally, the expression pattern of *gsdf* in immature ovarian tissue showed a similar trend to the plasma E_2 level in the male phase.

4. Identification of specific zone expression of *gsdf* mRNA was accomplished using in situ hybridization (ISH). Strong signals were observed in Sertoli cells in the periphery of spermatogonia in *E. akaara*. In *A. latus*, stronger signals were observed in Sertoli cells of the testicular zone, and weaker signals in somatic cells in the ovarian zone.

5. cDNAs encoding GtHs and their receptors were cloned from *E. akaara*. The results showed FSHβ and LHβ subunits of *E. akaara* had conserved structural features of the glycoprotein hormones family. Fshr and Lhcgr of *E. akaara* had conserved structural features of the glycoprotein hormone receptors subfamily. FSHβ and LHβ

subunits only expressed in pituitary, while Fshr and Lhcgr were detected in some other tissues besides the gonads. Expression patterns of GtHs and their receptors were analyzed during sex change of *E. akaara* by real-time qPCR. The results showed there were different expression patterns between FSHβ and LHβ subunits. In pituitary, the expression levels of FSHβ subunit exhibited the highest at FI stage, then decreased significantly. Afterwards, the expression levels of FSHβ increased rapidly at LT stage of transition phase, and decreased again in male phase. High expression levels of LHβ subunit were observed in mature female phase (FIV stage), LT stage of transition phase and male phase. In the gonad, the expression patterns of Fshr and Lhcgr were similar. The expression levels of GtHRs were low from female phase to ET stage of transition phase, and increased at LT stage of transition phase. The expression levels of GtHRs significantly increased and reached a peak in male phase. Furthermore, it was found that the expression patterns of *gsdf* and GtHRs showed a similar trend in *E. akaara*.

Keywords: Gonadal soma-derived factor; hermaphrodite; *Epinephelus akaara*; *Acanthopagrus latus*; GtHs and GtHRs