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Molecular and functional characterization of grass carp squint/nodal-related 1: a potential regulator of activin signaling in teleost pituitary cells

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Abstract

Nodal, a member of the transforming growth factor-β superfamily, plays important roles in embryogenesis in vertebrates, including fish. However, the functional characterization of the fish nodal-related gene in nonembryonic cells is still unclear. In teleost, three nodal-related genes, nodal-related (*ndr*)1/squint, *ndr2*/cyclops, and *ndr3*/southpaw have been reported. In this study, a full-length cDNA for grass carp squint (*gcSqt*) was cloned, and its transcript was detected in the selected organs, including pituitary, brain, heart, head kidney, kidney, spleen, and gonad. To further define its functional role, recombinant grass carp squint (rgcSQT) was produced in *Escherichia coli* in a homodimer form. Furthermore, we examined the effects of rgcSQT on activin and its receptor gene expression with the use of grass carp pituitary cell as a model. Results showed that rgcSQT stimulated the mRNA expression of activin βA and βB subunit, as well as activin receptor *ActRIB* and *ActRIIB*. These findings not only contribute to the understanding of nonembryonic functions of nodal gene in fish, but they also provide new insight into the regulation of activin signaling in vertebrates.

Keywords: Grass carp; Squint; Pituitary cell; Functional characterization; Gene expression

1. Introduction

Nodal was originally cloned from a mouse embryo cDNA library. Cumulative evidence has established that nodal is crucial for mesendoderm formation and left–right asymmetry patterning in the vertebrate embryos [1–4]. In addition to its core functions in embryogenesis, the potential role of nodal signaling in a few nonembryonic cells has been implicated. For example, nodal-activin receptor-like kinase 7 (ALK7) pathway induces the apoptosis of ovarian granulosa cells [5] and ovarian epithelial cancer cells [6].

In higher vertebrates, including mouse, human, and chick, a single nodal ligand is identified [4,7,8]. However, further nodal orthologs are isolated in fish species. For example, three nodal-related (*ndr*) genes (called *ndr1*/squint, *ndr2*/cyclops, and *ndr3*/southpaw) are characterized in zebra fish [7,8]. Furthermore, some cDNAs of nodal-related genes have been isolated from other fishes, such as *ndr1* in cyprinid fish [9], *ndr1/2/3* in medaka [10] and *ndr1/2* in bichir [11]. In accordance with their mammalian orthologs, these genes have been

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implicated in mesendoderm induction, embryonic axes formation, and left–right development [1,2,8,12]. However, to date, the functional role of fish nodal-related genes in other tissues remains undefined.

Both nodal and activin are the members of transforming growth factor-β (TGF-β) superfamily and share a common signal transduction pathway, which is initiated by the ligand-induced formation of a heteromeric complex of type I and type II transmembrane serine/threonine kinase receptors. The activated activin/nodal receptors phosphorylate the receptor-regulated similar to mothers against decapentaplegic homolog (Smad), Smad2/3, which subsequently form a complex with Smad4, and translocate to the nucleus to regulate transcription of target genes [13]. Moreover, nodal and activin can bind to or activate the same receptors, including type I receptors ALK4 and ALK7, and activin type II receptors (ActRIIs) ActRIIA and ActRIIB [14], although nodal function needs some additional coreceptors [8]. Therefore, an interesting question is raised whether there is crosstalk between nodal and activin signaling at the ligand and receptor levels.

In the present study, a full-length cDNA of grass carp (*Ctenopharyngodon idellus*) squint (*gcSqt*) was isolated from the pituitary, which implicated its potential function in the teleost pituitary. This notion was further supported by the findings that the key components of the signaling pathway for nodal and activin [15,16], including ActRIIA, ActRIIB, ActRIB, and Smads, have been detected in fish pituitary [17–20]. In addition, the coreceptor, one-eyed pinhead (*oep*), which is essential for nodal [21], was detected in grass carp pituitary. These findings prompted us to investigate whether nodal plays a functional role in the fish pituitary. To test this hypothesis, recombinant grass carp squint (rgcSQT) in its mature form with a homodimer was prepared. With the use of a static incubation method, we determined the effects of rgcSQT on the mRNA expression of activin subunits (βA and βB), *ActRIB* and *ActRIIB* in grass carp pituitary cells. This study contributes to the understanding of nonembryonic functions of squint in fish and provides new insight into the regulation of activin signaling in vertebrates.

2. Materials and methods

All animal experiments complied with the Regulation of Animal Experimentation of Sichuan Province, China, and were approved by the Animal Ethical Committee at the University of Electronic Science and Technology of China.

2.1. Fish

One-year-old grass carp were purchased from Chengdu Tongwei Aquatic Science and Technology Company (Chengdu, China). The fish at this stage were sexually immature, and sexual dimorphism was not apparent. All the fish used in the experiments ranged from 30 to 50 cm in length. The fish were kept at 20 °C in a running water system and fed to satiation daily with commercial carp pellets. The fish were acclimated to this environment for at least 1 wk before use in experiments. During the procedures of cell preparation, the fish were killed by anesthesia in 0.05% MS222 (Sigma-Aldrich, St. Louis, MO, USA), and their pituitaries were collected.

2.2. Cloning of gcSqt cDNA

Total RNA was isolated from grass carp pituitary with the use of TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). About 2 μg of RNA was subjected to reverse transcription with the use of Oligo$(dT)_{18}$ as the primer with SuperScript II Reverse Transcriptase (Invitrogen). The partial sequence of *gcSqt* was amplified by the degenerate primers (sqtF and sqtR; all primer sequences are listed in Supplementary Table 1), which were designed according to the conserved regions of its counterparts in zebra fish (GenBank accession no.: NM130966), medaka (GenBank accession no.: EF206724), and bichir (GenBank accession no.: AB500934). The obtained PCR fragment was cloned into pGM-T vector (Tiangen, Beijing, China) and sequenced. Subsequently, 3′ and 5′ rapid amplification of cDNA ends (RACE) were performed to obtain the full-length cDNA sequence of *gcSqt*. For 5′-RACE, first-strand cDNA was synthesized with Oligo$(dT)_{18}$ primer and SuperScript II Reverse Transcriptase (Invitrogen), and then a homopolymeric tail was added to the 5′-end of the cDNA with the use of TdT and dCTP (Invitrogen). The 5′-end of *gcSqt* was obtained by two rounds of nested PCR with the use of the primer sqt5N1 and Abridged Anchor Primer (Invitrogen), and sqt5N2 and Abridged Universal Amplification Primer (AUAP; Invitrogen). For 3′-RACE, first strand cDNA was synthesized with the adapter primer (AP, Invitrogen). The 3′-end of *gcSqt* was also amplified by two rounds of nested PCR with the use of the primers of sqt3N1 and AUAP, followed by sqt3N2 and AUAP. The PCR fragments were sequenced and the full-length cDNA sequence was assembled. Finally, we amplified the full-length coding sequence of gcSqt with Phusion High-Fidelity DNA Polymerase (Finnzymes, Espoo, Finland) with the use of the primers squintF and squintR (Supplementary Table 1) to confirm the sequence.