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Molecular characterization of sdf1 and cxcr4 in the Mozambique tilapia, *Oreochromis mossambicus*

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ABSTRACT

Animal sexual reproduction relies on primordial germ cells (PGCs), the predecessors of the germ cell lineage, giving rise to either spermatogonia or oogonia after the completion of gonadal differentiation. There is limited information on the mechanism of PGC migration leading to the formation of the primordial gonad in Perciform fish. *Oreochromis mossambicus*, a tilapiine species, was investigated that is a commercially important aquaculture species in many parts of the world while in other areas it has become an invasive pest. Key components involved in PGC migration were identified, including the stromal-cell derived factor 1 (*Om-sdf*1a, *Om-sdf*1b) and the CXC receptor 4 (*Om-cxcr4*): both share conservation with existing model species. The spatial gene expression profiles were determined through transcript and protein analysis and displayed distinct localisation within the region of the developing gonad in larvae and within the adult gonads of certain cell populations. A recombinant Om-sdf1a was produced in *Escherichia coli* that activates Om-cxcr4 using a BRET-based yeast *in vitro* assay system, suggesting that it is structurally similar to the native Om-sdf1a and is appropriate for further structural studies. This study has improved understanding of the molecular basis of tilapia reproduction through investigation of gonad development, which may be important in the progression towards reproductive suppression methods to control tilapia populations in the wild. In addition, this research will facilitate developments in germ cell transplantation, an innovative technique that harnesses germ cell migration and allows the uptake of foreign germ cells, which differentiate to produce sperm or ova.

1. Introduction

Cell migration is a process fundamental for embryonic development, organ development, homeostasis and immune response (Tsujioka, 2011). Accordingly, improper migration is associated with organ failure, cancer metastasis, immune deficiency and reproductive dysfunction (Ulrich and Heisenberg, 2009). Migratory cells have been shown to respond to external signalling molecules, such as chemokines which guide them to their destination (Ulrich and Heisenberg, 2009).

Chemokines are a group of cytokine proteins found in vertebrates, named for their ability to induce chemotaxis (cell migration) (Alejo and Tafalla, 2011). Stromal-cell derived factor 1 (sdf1, also known as CXCL12) is a chemokine that has been implicated in a wide variety of body functions and disorders, including primordial germ cell (PGC) migration (Doitsidou et al., 2002; Raz, 2002; Roland et al., 2003). PGCs are the precursors to sperm and

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eggs, and develop at distal regions of the embryo, which requires migration, with the assistance of sdf1, to the developing gonads (Cinalli et al., 2008). The PGC migration has provided an excellent system to analyse the principles of chemotaxis; sdf1 appears to be secreted by somatic cells at the gonad layer. The sdf1 then binds to CXC receptor 4 (cxcr4), a G protein-coupled receptor (GPCR) which is located on the surface membranes of PGCs, facilitating PGC movement in the direction of sdf1 binding (Roland et al., 2003). Additionally, sdf1 can be sequestered and degraded by somatic cells positioned en route to the gonad layer (Richardson and Lehmann, 2010). These somatic cells express CXC receptor 7 (cxcr7) gene, which serves to create a concentration gradient for PGC's to follow (Richardson and Lehmann, 2010).

Knowledge of the PGC migration in fish is derived primarily from studies in zebrafish (*Danio rerio*), where sdf1 and cxcr4 have key roles in PGC migration and gonad development (Doitsidou et al., 2002; Raz, 2002; Boldajipour et al., 2008). In the Japanese rice fish (medaka, *Oryzias latipes*) two isoforms of sdf1 (sdf1a and sdf1b) guide the direction of PGC movement (Herpin et al., 2008). Both genes are expressed in the lateral plate mesoderm yet during late embryonic development cell-specific gene expression suggests sub-functionalization of sdf1a and sdf1b (Kluver, 2007). Recently, an sdf1 gene (*Slal-sdf1*) was described within the Yellowtail kingfish (*Serolii lalandi*), which is expressed throughout larval development, during PGC migration (Fernandez et al., 2015). As a phylogenetically distinct perciform, the Yellowtail kingfish sdf1 has provided a clearer understanding into the broad regulatory role for this migration component.

A greater understanding of the molecular components of PGC migration processes can provide a way with which we may manipulate reproduction in cultured species populations. For example, this knowledge has implications in the innovative application of germ cell transplantation. Germ cell transplantation refers to the method of transplanting early stage germ cells, such as PGCs, from one organism into another (Majhi et al., 2009). If the recipient organism is at an embryonic state, and undergoing PGC migration, then donated germ cells may also utilise the recipient's migration cues and colonise its gonads, differentiating into either sperm or eggs (Majhi et al., 2009). This can result in the remarkable occurrence of one species producing the gametes of another; the mating of two such organisms can result in the recipient species spawning offspring of the donor species (Yoshizaki et al., 2011). Molecular studies involving gonad development also provide knowledge that may be used to inhibit development for the purpose of pest control (Smith and Walker, 2004).

Oreochromis species (common name tilapia) represent an excellent experimental model to undertake germ cell migration studies in perciforms. Tilapia are relatively fast growing, compared to many perciforms, are small in size at sexual maturity, easy to culture and of economic importance (Lacerda et al., 2008). There has also been an increase in the availability of genomic and transcriptomic resources for *O. niloticus*. In this study, *sdf1* (Om-*sdf1*) and *cxcr4* (Om-*cxcr4*) gene sequences were identified in *O. mossambicus* and the spatial distribution was visualised using *in situ* hybridisation (ISH) and immunolocalisation in embryos and adult gonads. A recombinant Om-sdf1a has been generated in a bacterial expression system and tested for receptor binding in a yeast system, as a prelude to a future *in vitro* chemotaxis assay, as well as structure determination.

2. Materials and methods

2.1. Gene identification and analysis

The *Om-sdf1* and *Om-cxcr4* sequences were obtained from transcriptome resources provided in GenBank SRA file SRP057722. *Om-sdf1* and *Om-cxcr4* sequences were confirmed and homologous genes were identified using the NCBI basic local alignment search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Open reading frames and conceptual amino acid sequences were found using the NCBI ORF finder (http://sdf.ncbi.nlm.nih.gov/projects/gorf/). Signal peptides were predicted using the Signal P server (http://sdf.cbs.dtu.dk/services/SignalP/). The mature protein regions were aligned with the complementary region of other teleost fish using GeneDoc software (version 2.7.0). A neighbour-joining tree was constructed *via* MEGA using the number of distances method with pairwise deletion (Saitou and Nei, 1987; Tamura et al., 2011). Protein secondary structure predictions were made using the Assisted Model Building with Energy Refinement (AMBER) 11 (Case et al., 2014) with force field parameters ff13. Each structure was heated to 315 K over 50 ps to avoid being kinetically trapped in local minima, and then subjected to unrestrained MD simulations at 315 K for the purpose of complex equilibration, in which the structure was sampled every picosecond (Wang et al., 2011). The simulation was continued until the backbone root mean square deviation (RMSD) of structures within a reasonable time range was $\leq$2–3 Å

2.2. Animal maintenance and tissue collection

The *O. mossambicus* specimens were obtained from the Department of Agriculture and Fisheries, Bribie Island, and Sommerset Dam (both Queensland, Australia), under permit number 162882. Adults were kept in 401 broodstock holding systems and induced to spawn in separate aquariums by increasing water temperature to 28 °C. After spawning and fertilisation, embryos were collected at 4 days post hatch (DPH). Embryos were euthanized with an overdose (>100 mg/L) of tricaine methanesulfynate MS-222 and placed into RNAlater (Qiagen, Chadstone, Vic) or fixative (4% paraformaldehyde in phosphate buffered saline; PBS). For collection of adult gonad tissue, animals were sacrificed using tricaine methanesulfynate MS-222, the gonad removed and placed into RNAlater (Qiagen) or 4% paraformaldehyde in PBS.

2.3. Histology

Histology was performed to identify cell and tissue morphology within *O. mossambicus* larvae (10 DPH) and adult