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The expression of natriuretic peptide receptors in developing zebrafish embryos

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ABSTRACT

There are three isoforms of natriuretic peptide (NP) specific cell surface receptor: NP receptor-A (NPRA), receptor-B (NPRB), and receptor-C (NPRC). They are also known as NPR1, NPR2 and NPR3, respectively. NPs and their receptors were revealed to involve in diverse cellular and physiological processes including renal, cardiovascular, neuronal, and immunological aspects. However, the systematic analysis of the expression of these receptors in non-mammalian vertebrates is thus far lacking. In this study, two versions of the *npr1* gene (*npr1a* and *npr1b*) in zebrafish was identified. Multiple sequences alignment analysis showed that zebrafish NPRs shared high homologies with NPRs of other species and possessed a typical signature domain of NPRs. The results of whole mount *in situ* hybridization and reverse transcription polymerase chain reaction analysis revealed that at embryonic stages, *npr1a* was mainly expressed in tectal ventricle, brian, heart and retina, whereas *npr1b* was broadly present in anterior pronephric duct. Unlike *npr1*, *npr2* mainly expressed in branchial arches and neural tube during embryonic development. However, *npr3* was expressed in pronephric ducts and corpuscle of stannius in zebrafish embryos at 72 hpf. In adults, we demonstrated that all the three NP receptors were highly existed in brain and kidney. Overall, these findings will provide an important basis for the functional analysis of NPs and its receptor during embryonic development.

1. Introduction

The natriuretic peptides (NPs) family includes three well-characterized peptides: atrial (A-type) natriuretic peptide (ANP) (de Bold et al., 1981), brain (B-type) natriuretic peptide (BNP) (Sudoh et al., 1988), and C-type natriuretic peptide (CNP) (Heublein et al., 1992). ANP and BNP are synthetized mainly in the heart and to a lesser extent in other organs, and then act on the cardiomyocytes and other cell types by paracrine effects or the remote organs such as the kidneys, adrenal glands and vasculature by endocrine effects, whereas CNP is mostly produced by endothelial and renal cell (Levin et al., 1998; Cataliotti et al., 2002; Chen et al., 2005; Becker et al., 2014). The three NPs mediate natriuretic, diuretic and vasorelaxant largely directed to reduce blood pressure and maintain fluid volume homeostasis (de Bold et al., 1981; de Bold, 1985; Brenner et al., 1990).

The functions of NPs are mediated by their receptors (natriuretic peptide receptors, NPRs), one kind of transmembrane proteins, which are vital for the signal delivery after extracellular binding of a NPs (Nankervis et al., 2007). It is well known that interaction of NPs with their receptors plays a central role in physiology and pathophysiology of hypertension and cardiovascular disorders (Pandey, 2005; Madiraju et al., 2018). There are three isoforms of NPRs: NPR-A, NPRB, and NPRC, which are also known as guanylyl cyclase-A (GC-A), guanylyl cyclase-B (GC-B), and the clearance receptor, or as NPR1, NPR2, and NPR3, respectively (Hagiwara et al., 1995; Pandey, 2005; Potter et al., 2006; Becker et al., 2014). Generally, NPRA responds specifically to ANP and BNP, whereas CNP is the preferential ligand for NPRB (Nankervis et al., 2007). The three receptors have conserved catalytic and regulatory domains and divergent ligand binding domains, and were functionally divided into two major types (Hagiwara et al., 1995; Pandey, 2005; Madiraju et al., 2018). One type of receptors consists of NPRA and NPRB, which are members of membrane guanylate cyclase (GCase) receptor family containing GC domains in their cytoplasmic C-terminal regions and synthesize cGMP in response to hormone binding. Each of the receptor seems to be present as a tetramer, formed via disulfide bonds, on the plasma membrane and have a relative molecular weight mass of 130–180 kDa (Anand-Srivastava, 2005). The other type of receptor contains NPRC which can binds all NPs and exists as a

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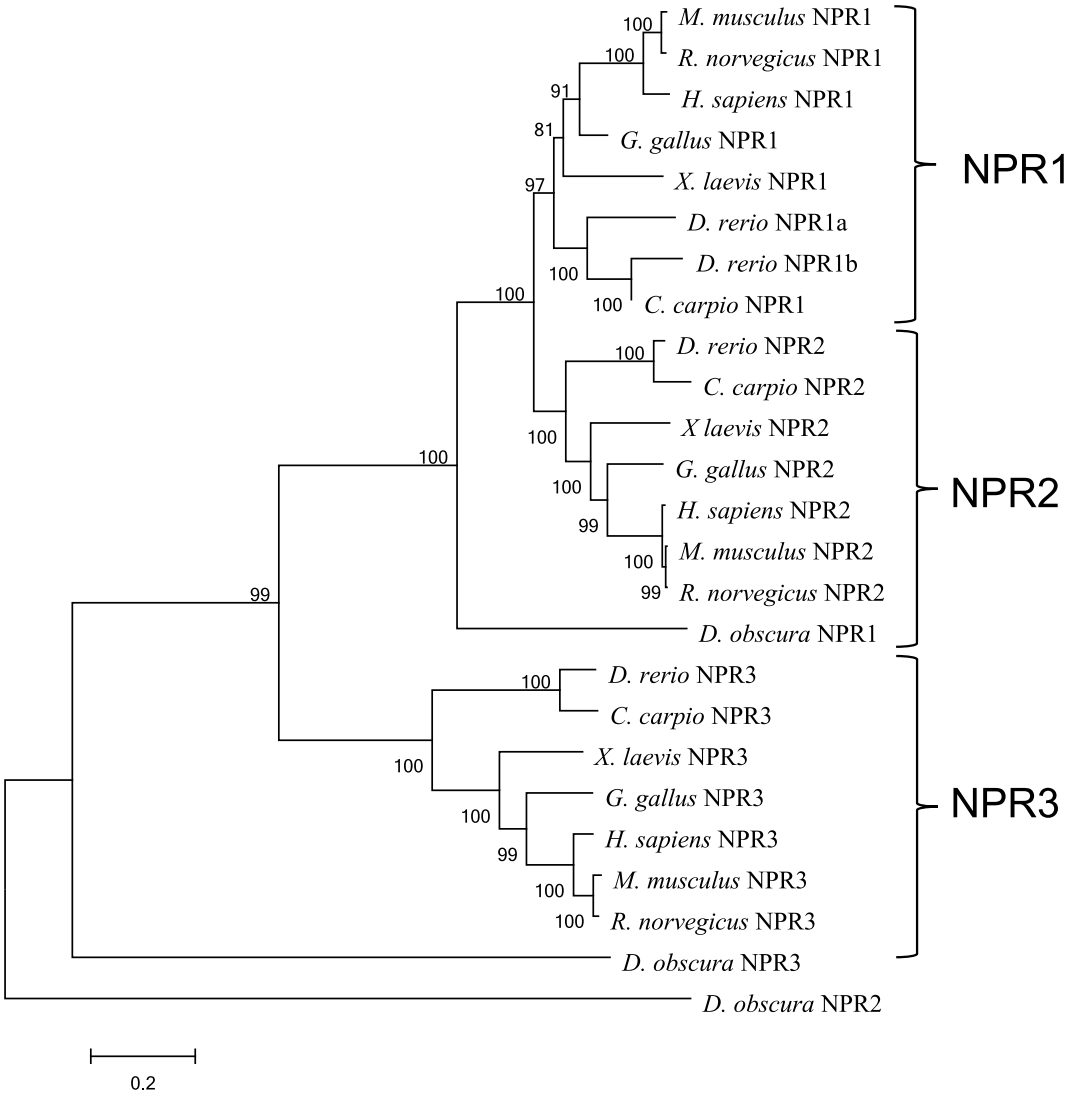

Fig. 1. Phylogeny of NPRs family members. A neighbor-joining tree was produced with MEAG5.0 software displaying the relationships of NPRs family member full-length amino acid sequences. One thousand bootstraps were carried out to check the repeatability of the result. GenBank accession numbers: *Danio rerio* (*D. rerio npr1a*) (CAK10930.1); *Cyprinus carpio* (*C. carpio npr1a*) (XP_018932300.1); *Xenopus laevis* (*X. laevis npr1a*) (NP_001083703.1); *Mus musculus* (*M. musculus npr1a*) (NP_032753.5); *Rattus norvegicus* (*R. norvegicus npr1a*) (NP_036745.1); *Homo sapiens* (*H. sapiens npr1a*) (NP_000897.3); *Gallus gallus* (*G. gallus npr1a*) (XP_015153964.1); *Drosophila obscura* (*D. obscura npr1a*) (XP_022218804.1); *D. rerio npr2* (XP_009300100.1); *C. carpio npr2* (XP_018958163.1); *X. laevis npr2* (NP_001084176.1); *M. musculus npr2* (NP_776149.1); *R. norvegicus npr2* (NP_446290.1); *H. sapiens npr 2* (NP_003986.2); *G. gallus npr2* (XP_003642967.1); *D. obscura npr2* (XP_022222015.1); *D. rerio npr3* (XP_005165413.1); *C. carpio npr3* (XP_018933403.1); *X. laevis npr3* (AAI70137.1); *M. musculus npr3* (NP_001034270.1); *R. norvegicus3* (NP_037000.1); *H. sapiens npr3* (NP_001191304.1); *G. gallus npr3* (XP_003643022.1); *D. obscura npr3* (XP_022218817.1).

monomers or homodimer, and has a single membrane-spanning domain with a very short intracellular tail (Anand-Srivastava, 2005).

In last three decades, natriuretic peptides and their receptors were revealed to involve in diverse cellular and physiological processes including renal, cardiovascular, neuronal, and immunological aspects in health and disease (Toop and Donald, 2004). Much is known about the biochemistry and physiology of these NPRs in mammalian species. However, the systematic analysis of the expression of these receptors in non-mammalian vertebrates is thus far lacking. Currently, we analyzed the expression patterns of NPRs in zebrafish at embryonic and adult stage using whole mount *in situ* hybridization (WISH) and reverse transcription polymerase chain reaction (RT-PCR).

2. Results

2.1. Characterization of the zebrafish npr genes and phylogenetic analysis

There are 3 *NPR* genes, *NPR1*, *NPR2* and *NPR3* in mammals, whereas there are four *npr* genes in zebrafish, including 2 versions of *npr1s*: *npr1a* (localized in Chromosome 19) and *npr1b* (localized in Chromosome 16), *npr2* (mapped to chromosomes 5) and *npr3* (mapped to chromosomes 5). Multiple alignments of the NPR protein sequences showed that all the four kinds NPR sequences shared high homologies with NPRs of other species and possessed the typical signature domain of NPRs (Lelièvre et al., 2006). Moreover, a transmembrane spanning domain was found in the NPRs of zebrafish, compatible with the presence of the region of hydrophobic residues (Supplemental Fig. 1-3). Interestingly, the NPR3 sequence also showed a putative intracellular Gi coupling domain in C-terminus end, which was highly homologous with analogous sequences in other species (Supplemental Fig. 3).