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Identification and characterization of a motilin-like peptide and its receptor in teleost

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ABSTRACT

Although putative *motilin* receptor sequences have been reported in teleost, there is no proof for the existence of the *motilin* gene in teleost. In this study, we have identified a *motilin-like* gene in the genome of several fish species and cloned its cDNA sequence from zebrafish. The zebrafish motilin-like precursor shares very low amino acid (aa) identities with the previously reported motilin precursors. Processing of the zebrafish motilin-like precursor may generate a 17-aa C-terminal amidated mature peptide, the motilin-like peptide (motilin-LP). A putative zebrafish motilin receptor (MLNR) was also identified in zebrafish. In cultured eukaryotic cells transfected with the zebrafish MLNR, zebrafish motilin-LP could enhance both CRE-driven and SRE-driven promoter activities. Tissue distribution studies indicated that the zebrafish *motilin-like* gene is mainly expressed in the intestine and liver while the zebrafish *MLNR* gene is highly expressed in brain regions, suggesting that motilin-LP behaves like other gut hormones to regulate brain functions. These data suggest that the presence of a unique motilin/MNLR system in teleost.

1. Introduction

Motilin, a 22-aa peptide, was originally isolated from the porcine intestine in 1972 (Brown et al., 1972). In humans, this peptide is mainly produced in the endocrine cells of the gastrointestinal mucosa to induce gastric contractile activity (Husebye, 1999; Poitras and Peeters, 2008). Its physiological effects are mediated by its cognate receptor GPR38 which was deorphanized in 1999 as the motilin receptor (MLNR) Feighner et al., 1999. Ghrelin, another gastric peptide related to motilin, was isolated from the rat stomach (Kojima et al., 1999). Ghrelin is the endogenous ligand for growth hormone secretagogue receptor (GHSR) (Kojima et al., 1999) which shares 53% aa identity with MLNR in humans (McKee et al., 1997; Smith et al., 2001). MLNR, GHSR and other three related receptors, including neurotensin receptor (NTSR), neuromedin U receptor (NMUR) and GPR39 are members of the GHSR receptor gene family. Similar to their receptors, motilin and ghrelin also share identifiable sequence similarities (Asakawa et al., 2001; Tomasetto et al., 2000) and are classified into the ghrelin gene family (Smith et al., 2001). Both motilin and ghrelin play important roles in regulating gastrointestinal (GI) functions (Sanger, 2008) and their receptors are important drug targets for GI diseases (Poitras and Peeters, 2008; Sanger, 2008).

The motilin and MLNR cDNA sequences have been cloned from several mammalian species (Feighner et al., 1999; Huang et al., 1999; Ohshiro et al., 2008) and chicken (Huang et al., 1999). In rodents, both motilin and its receptor are pseudogenes (He et al., 2009; Huang et al., 1999). The existence of motin/MLNR system in teleost is controversial because neither the *motilin* gene has been identified in teleost (Ohshiro et al., 2008) nor mature motilin peptide could be detected by immunohistochemistry (De Girolamo et al., 1999; Olsson et al., 2008; Pan and Fang, 1993). However, a putative MLNR was reported in pufferfish (Olsson et al., 2008; Palyha et al., 2000).

Our previous studies indicated the existence of a functional ghrelin/GHSR system in teleost (Chan and Cheng, 2004; Yeung et al., 2006; Zhang et al., 2008). In this study, we have investigated the presence of a related motilin/MLNR system in teleost through bioinformatics, molecular cloning and functional studies.

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Table 1
Primers used in this study.

Primers	Sequence (5′–3′)
Motilin-like-F	CTCAACATCTCACAGCACTG
Motilin-like-R	CTCCATCCTCTTCTGGAGAC
MNLR-F	GCCGAAAGTTGTGGAAGAGT
MNLR-R	CAGGTAGAAGAGCACCATCGAG
18S-F	CAAACAAGGCAAACACAGAC
18S-R	CATAGGACCAGTGCTGGGAC

2. Materials and methods

2.1. Animals and chemicals

Zebrafish were obtained from a local fish farm in Guangzhou, China. All animal experiments were conducted in accordance with the guidelines and approval of the respective Animal Research and Ethics Committees of the Sun Yat-Sen University and the Chinese University of Hong Kong.

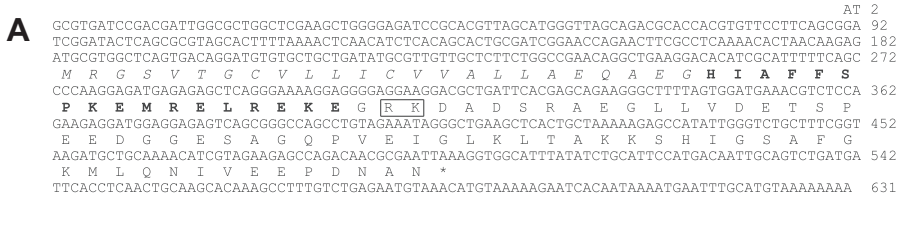
Zebrafish motilin-LP was synthesized by GL Biochem (Shanghai, China). The purity of the synthesized peptide was >95% as determined by analytical HPLC.

2.2. Data mining and sequence analysis

An EST candidate encoding for the zebrafish motilin-like precursor was identified by searching the non-human, non-mouse ESTs databases using tblastn tool (http://www.ncbi.nlm.nih.gov). We further searched for such *motilin-like* gene candidates from other genome-sequenced teleostean species on the Ensembl genome database (http://www.ensembl.org) and UCSC genome browser (http://genome.ucsc.edu) using the zebrafish motilin-like sequence. Genomic regions covering putative *motlin-like* gene were subjected to open reading frame (ORF) prediction using Genscan (http://genes.mit.edu/GENSCAN.html). Synteny analysis was performed on the release 56 version of Ensembl database (http://www.ensembl.org). The signal peptide and the neuropeptide prohormone cleavage sites were predicted using SignalP 3.0 (Bendtsen et al., 2004) and Neuropred software (Southey et al., 2006), respectively. Multiple sequence alignments were performed using ClustalW (Thompson et al., 1994) and phylogenetic trees were constructed by MEGA3.1 using the neighbor-joining method (Kumar et al., 2004).

2.3. Cell culture, transfection and functional assays

The ORF of the putative zebrafish MNLR was subcloned into the pcDNA3.1 expression vector (Invitrogen). COS-7 Cells were maintained at 37 °C in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). All media were supplemented with antibiotics (10 U/ml penicillin and 100 μg/ml streptomycin). Twenty hours before transfection, 1.5×10^5 cells/well were seeded into 24-well tissue-culture plates. Five hundred ng of pSRE-Luc, pCRE-luc or pc-fos-luc reporter plasmid (Stratagene, La Jolla, CA), 50 ng of pcDNA-zebrafish-MNLR and 50 ng of pRL-CMV (for normalization of transfection efficiency) containing the *Renilla* luciferase reporter gene were co-transfected into the cells in 250 μl serum-free medium using Lipofectamine reagent (Invitrogen). Six hours after transfection, cells were incubated with



C

Chicken	Dog	Human	Medaka	Stickleback	*Takifugu*	*Tetraodon*	Zebrafish	
	32.2	31.3	12.8	15.7	18.3	16.2	9.6	**Chicken**
		73.0	9.2	11.1	12.5	11.4	9.6	**Dog**
			7.3	10.4	11.5	12.4	8.7	**Human**
				29.4	31.7	28.6	6.7	**Medaka**
					51.0	47.6	4.8	**Stickleback**
						80.8	9.5	***Takifugu***
							7.7	***Tetraodon***
								Zebrafish

Fig. 1. Sequence analysis of motilin-like precursors. (A) The nucleotide sequences and the deduced amino acid sequences of the zebrafish motilin-like precursor. (B) Comparison of aa sequences of teleostean motilin-LP precursors with tetrapodal motilin precursors. Sequences were aligned by the ClustalW program. Identical sequences are indicated by asterisks. Gaps (indicated by hyphens) are introduced in some sequences to maximize alignment. The predicted signal peptides are in italics. The mature peptide (motilin-LP) is in bold and the putative cleavage sites are boxed. (C) The aa percentage identities of teleostean motilin-LP precursors and motilin precursors of other species.