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Original research

Essential roles of *stat5.1*/*stat5b* in controlling fish somatic growth

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ABSTRACT

Signal transducer and activator of transcription 5b (STAT5b) has been identified as a key downstream mediator of growth hormone (GH) signaling in somatic growth of mammalian. However, the corresponding homologue gene of *Stat5b* is unknown in fish species. In this study, we generated loss-of-function mutants in *stat5.1* and *stat5.2*, two *stat5* homologues existing in zebrafish. In *stat5.1*-deficient zebrafish, a significant reduction of body length and body weight was detected in the embryos/larvae and adults compared with the wild-type control fish, and sexual size dimorphism in adult zebrafish was also eliminated. However, the *stat5.2*-deficient zebrafish displayed a normal developmental phenotype during all lifespan. Chromatin immunoprecipitation combined with deep sequencing (ChIP-seq) method was adopted to further investigate the potential transcriptional targets of Stat5 protein and cast much light upon the biological function of Stat5. We identified more than 800 genes as transcriptional targets of Stat5 during zebrafish embryogenesis. KEGG analysis indicated that the Stat5 target gene network is predominantly linked to the metabolic pathways, neuroactive ligand-receptor interaction and JAK-STAT signaling pathways. Further validation studies suggested that Stat5.1 protein could directly regulate the expression of *gh1*, and *stat5.1*-mutated zebrafish showed a reduction of *gh1* mRNA level. In the present study, *stat5.1* was revealed as the corresponding homologue gene of *Stat5b* in fish species. Additionally, we found a novel molecular interaction between Stat5.1/Stat5b and GH, and unraveled a positive feedback loop Stat5.1-GH-Stat5.1 which is necessary for somatic growth and body development in zebrafish.

1. Introduction

Growth hormone (GH) is a key hormone that regulates somatic growth and metabolism by inducing the expression of multiple signaling pathways including insulin-like growth factor (IGF) and Jak2-STAT5 signaling pathways (Ahmed and Farquharson, 2010). In mammals, there are two *Stat5* homologues, *Stat5a* and *Stat5b*, which are clustered on the same chromosome and have more than 90% similarity in amino acid sequences in a species (Siveen et al., 2014). *Stat5a*-deficient mice developed normally except that the pregnant females failed to lactate after parturition that could not be compensated by Stat5b activation (Liu et al., 1997). In human patient, homozygous mutation in the *STAT5b* gene resulted in IGF-1 deficiency and impairment of body growth (Kofoed et al., 2003). Sexual size dimorphism, the significant growth difference between female and male, has been observed in mouse since males grow much faster than females. Loss of Stat5b function in mouse caused reduced sexual size dimorphism and altered expression levels of sex-biased genes (Udy et al., 1997; Zhang et al., 2012).

Somatic growth is a complex trait in teleosts that is normally regulated by the GH/IGF axis genes expressed in the hypothalamus-pituitary-gonad (HPG) axis (Ma et al., 2016). The higher expression of GH/IGF axis genes correlates with the faster growth rate in multiple farmed fish species (Cruz et al., 2006; Zhang et al., 2006; Kaneko et al., 2011; Zhong et al., 2012). For example, *gh*-transgenic coho salmon (*Oncorhynchus kisutch*) and common carp (*Cyprinus carpio*) showed a substantially faster growth rate than control fish (Zhu et al., 2013; Johnston et al., 2014). However, studies regarding the association of Jak2-STAT5 signaling pathway with somatic growth of fish are still lacking. Moreover, sexual size

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dimorphism has been observed in many fish species and has potential applications in farmed fish, such as tilapia (*Oreochromis niloticus*) and yellow catfish (*Pelteobagrus fulvidraco*) (Dan et al., 2013; Mei and Gui, 2015). To date, the molecular mechanism underlying sexual size dimorphism remains unknown in fish species.

As a perfect model animal, zebrafish has been chosen to study the function of Stat5 (Chen et al., 2014). In zebrafish, there are two *stat5* homologues, named *stat5.1* and *stat5.2*, which are located on different chromosomes (Lewis and Ward, 2004; Xiong et al., 2017). Sexual size dimorphism has been observed in adult zebrafish since females have larger body size and heavier weight than males. However, the corresponding functions of *stat5.1* and *stat5.2* in somatic growth in fish species are not clear. Here, we investigated the role of Stat5 in fish species by creating *stat5.1* and *stat5.2* mutant zebrafish lines using CRISPR/Cas9 and TALEN technologies, respectively. We found that *stat5.1* in teleosts has a similar function to that of *Stat5b* in mammals. Chromatin immunoprecipitation combined with deep sequencing (ChIP-seq) analysis was further conducted to identify the Stat5.1/Stat5b target genes during embryonic development of zebrafish.

2. Results

2.1. Establishment of stat5.1-mutated zebrafish line

To study the function of *stat5.1* during zebrafish development, CRISPR/Cas9 system was used to generate *stat5.1*-mutated zebrafish. The sgRNA targeting site was chosen in the seventh exon. The sequencing results showed that several types of mutations were passed through the germline (data not shown). Finally, two *stat5.1* mutant lines were established, which contained a 13-bp and 16-bp deletion, respectively, named *stat5.1*Δ13 and *stat5.1*Δ16. (Fig. 1A). Zebrafish Stat5.1 protein consists of four functional domains including STAT_int, STAT_alpha, STAT_bind and SH2. The deletions in *stat5.1*Δ13 and *stat5.1*Δ16 resulted in frame shift which caused premature stop codons (Fig. 1B). To verify the loss of *stat5.1* function in mutant zebrafish, qRT-PCR was conducted to examine the expression of *stat5.1* mRNA in *stat5.1*Δ13 mutants. As shown in Fig. 1C, the expression of *stat5.1* mRNA in *stat5.1*Δ13 mutants decreased by 63.2% and 74.2% compared with that in wild-type zebrafish at 48 and 72 hours post fertilization (hpf), respectively.

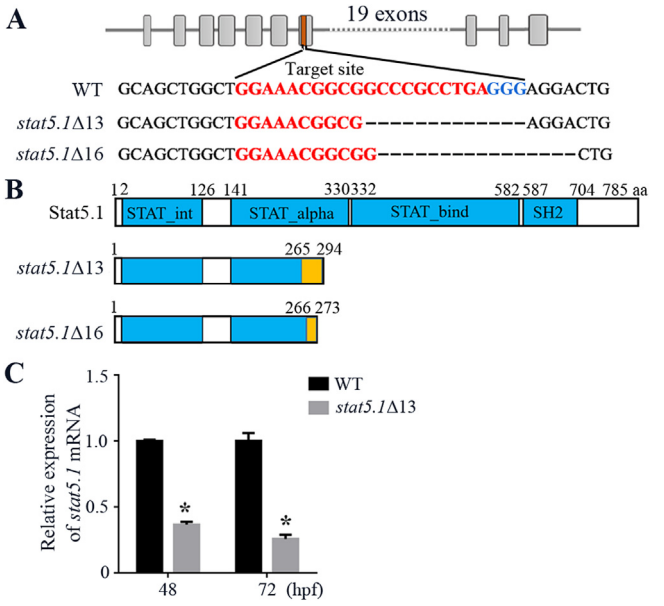

Fig. 1. Generation of *stat5.1*-mutated zebrafish. **A**: Schematic representation of zebrafish *stat5.1* (top) and its two mutated types (middle and bottom). The thin lines and grey boxes represent the introns and exons, respectively. The sgRNA target sequence is shown in red, followed by a PAM sequence "GGG" shown in blue. **B**: Illustration of deduced protein structures of wild-type *stat5.1* (top) and two mutated *stat5.1* (middle and bottom). **C**: Comparison of *stat5.1* mRNA levels between wild-type and *stat5.1*Δ13 zebrafish at 48 and 72 hpf. Significant difference was determined by two-tailed *t*-test ($^*P < 0.05$).

2.2. Stat5.1 is required for normal somatic growth in zebrafish embryos/larvae

Although homozygous *stat5.1*Δ13 mutant larvae develop normally, their body size was smaller than that of wild-type larvae (Fig. 2A), and a significant reduction of body length was also detected in *stat5.1*Δ13 mutant larvae compared with wild-type larvae at 96 hpf (Fig. 2B), suggesting that *stat5.1* is required for normal somatic growth of zebrafish embryos/larvae. Moreover, qRT-PCR analysis showed that *gh1* mRNA levels in *stat5.1*Δ13 mutants were significantly reduced to 38.1%, 42.2%, 19.3% and 63% of wild-type zebrafish at 48, 72, 96 and 120 hpf, respectively (Fig. 2C), and whole mount *in situ* hybridization on embryos at 48 and 72 hpf further demonstrated a dramatic reduction of *gh1* mRNA in pituitary in *stat5.1*Δ13 mutants (Fig. 2D), suggesting that loss of *stat5.1* function leads to a decrease in *gh1* mRNA expression.

2.3. Stat5.1 regulates somatic growth and sexual size dimorphism in adult zebrafish

At 3 months post fertilization (mpf), both *stat5.1*Δ13 and *stat5.1*Δ16 homozygous male and female mutant zebrafish at F_2 generation displayed smaller body size compared with wild-type zebrafish (Fig. 3A and B), whereas there was no obvious difference in body size between the *stat5.1* heterozygous and wild-type zebrafish (data not shown). *Stat5.1*Δ13 was then selected to conduct the following experiments. The somatic growth of F_3 generation of *stat5.1*Δ13 mutant and wild-type zebrafish were evaluated at 1, 2 and 3 mpf, respectively (Fig. 3C and D). At 1 mpf, it was hard to discriminate males and females by morphological features, and no obvious difference in body size was observed between different individuals. For wild-type zebrafish, although there was no difference in body weight between males and females at 1 and 2 mpf, the body weight of females was 9.9% heavier than that of males at 3 mpf ($^*P < 0.05$). The growth deficiency of *stat5.1*Δ13 mutant zebrafish was detected as early as at larval stage. Regardless of the sex, the average weight of *stat5.1*Δ13 mutant zebrafish was about 61.0%, 41.90% and 23.8% lower than that of wild-type zebrafish at 1, 2 and 3 mpf, respectively. Strikingly, no significant difference of body weight was observed between females and males at all three ages when *stat5.1* was mutated. Our data indicate that *stat5.1* is not only required for normal somatic growth, but also essential for sexual size dimorphism in adult zebrafish.

2.4. Stat5.2-mutated zebrafish display a normal developmental phenotype

stat5.2 mutant zebrafish was generated by TALEN biotechnology. The targeting sites were chosen in the second exon. Finally, two *stat5.2* mutant lines with 5-bp deletion (named *stat5.2*Δ5) and 13-bp deletion (named *stat5.2*Δ13), respectively, were established (Fig. 4A). The deletions in *stat5.2*Δ5 and *stat5.2*Δ13 zebrafish led to premature stop codons in the STAT_int domain (Fig. 4B). Results of qRT-PCR indicated that the expression of *stat5.2* mRNA in *stat5.2*Δ5 was reduced to 20.5% and 10.5% of wild-type zebrafish at 48 and 72 hpf, respectively (Fig. 4C; $^*P < 0.05$). All of the F_2 generation