Latent TGF-beta binding protein-1 plays an important role in craniofacial development

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Abstract

Objective: This study aims to replicate the phenotype of *Ltbp*1 knockout mice in zebrafish, and to address the function of *LTBP1* in craniofacial development. Methods: Whole mount in situ hybridization (WISH) of *ltbp1* was performed at critical periods of zebrafish craniofacial development to explore the spatial-temporal expression pattern. Furthermore, we generated morpholino based knockdown model of *ltbp1* to study the craniofacial phenotype. Results: WISH of *ltbp1* was mainly detected in the mandibular jaw region, brain trunk, and internal organs such as pancreas and gallbladder. And *ltbp1* colocalized with both *sox9a* and *ckma* in mandibular region. Morpholino based knockdown of *ltbp1* results in severe jaw malformation. Alcian blue staining revealed severe deformity of Meckel's cartilage along with the absence of ceratobranchial. Three-dimension measurements of *ltbp1* morphants jaws showed decrease in both mandible length and width and increase in open mouth distance. Expression of cartilage marker *sox9a* and muscle marker ckma was decreased in *ltbp1* morphants. Conclusions: Our experiments found that *ltbp1* was expressed in zebrafish mandibular jaw cartilages and the surrounding muscles. The *ltbp1* knockdown zebrafish exhibited phenotypes consistent with *Ltbp1* knockout mice. And loss of *ltbp1* function lead to significant mandibular jaw defects and affect both jaw cartilages and surrounding muscles.

Keywords: *LTBP1*. Craniofacial anomalies. Developmental biology. Zebrafish.

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Introduction

The latent transforming growth factor-β binding protein-1 (*LTBP-1*) is an extracellular matrix protein that is structurally similar to the fibrillin family. There are several human and mouse *Ltbp1* splice variants including a short (*Ltbp-1S*) and long (*Ltbp-1L*) form, which may arise from the two separated promoters, alternative RNA splicing, and differential proteolytic processing.[1] *LTBP1* is widely expressed among species in variable amounts in different tissues, including heart, lung, liver, placenta, skeletal muscle, and bone.[2,3] Current researches suggest that *LTBP1* is a multifunctional protein that bind latent transforming growth factor-β (TGF-β) and regulate its function in bone and other connective tissues.[4-10] *LTBP1* is reported to ease the secretion of latent TGF-β and modulate the activation of latent TGF-β.[11-16] It is considered as a stable component of extracellular fibrillar structure that play a critical role in the storage and release of TGF-β as a large latent TGF-β complex.[17-19] In addition to regulating the function of TGF-β, *LTBP1* function as a structural component of connective tissue microfibrils.[20] Studies focusing on its function as matrix proteins find that *LTBP1* colocalize with fibrillin 1 in microfibrillar structures not only in the extracellular matrix of primary osteoblasts but also at the surface of newly forming osteoid and bone, suggesting that *LTBP1* play an important role in the formation of bone and connective tissue.[8]

The important function of *LTBP1* has been investigated by a series of studies. Targeted deletion of exon 1 or 2 led to *Ltbp-1L*-null mice dying shortly after birth from defects in heart organogenesis.[21] These severe cardiac deformities include persistent truncus arteriosus and interrupted aortic arch, which are associated with imperfect function of cardiac neural crest cells. Similarly, targeted deletion of exon 8 to generate *Ltbp1L* knockout mice died perinatally, these mice also present heart defects including persistent truncus arteriosus and interrupted aortic arch which are similar to *Ltbp1L*-null mice.[22] These studies indicate the crucial role of *LTBP1* in the development of the cardiovascular system. Another *Ltbp1* knockout mice is manipulated by targeted deletion of exon 5, which is the first exon shared by the *Ltbp1L* and *Ltbp1S*.[23] These total knockout mice are viable and fertile, they exhibit craniofacial abnormalities consisting of more compact head structure with shorten maxilla and mandible. Furthermore, the total *Ltbp1* knockout mice have shortened long bones. Moreover, *LTBP1* knockout mice are less prone to hepatic fibrogenesis.[23] These phenomena suggest that loss of *LTBP1* function lead to potential changes of the biological activity of TGF-β in fibrogenesis action. Researchers also used the deletion of *Ltbp1L* exon 5 knockout model to address the function of Ltbp1 in female fertility.[24] Since *Ltbp1* knockout female mice showed impaired reproduction with subfertility and ovarian cyst formation, the interruption of TGF-β function which would lead to defective follicular wound healing was suggested as the potential cause.

It is known that *LTBP1* is critically required for the cardiovascular system organogenesis, bone and connective tissue formation, female reproduction, and craniofacial development.[21-24] However, the discrepancy of phenotypes in *Ltbp1* gene-manipulated mice is difficult to explain. Moreover, the function of *LTBP1* in craniofacial development remains unclear. Since the gene function, fundamental signaling pathways and cellular events in craniofacial morphogenesis have proven to be highly conserved among vertebrates from zebrafish and mouse to man,[25,26] we chose zebrafish to observe the phenotype in another model and to address the function of *LTBP1* in craniofacial aspect. We performed whole mount in situ hybridization (WISH) of *ltbp1* in critical periods of zebrafish craniofacial development to explore the spatial-temporal expression pattern. Furthermore, we generated morpholino based knockdown model of *ltbp1* to study the craniofacial phenotype.

This study aims to replicate the phenotype of *Ltbp1* knockout mice in zebrafish, and to address the function of *LTBP1* in craniofacial development.

Methodology

Zebrafish husbandry

Wild-type zebrafish (Tuebingen) were maintained with standard techniques.[27] All experiments were performed in compliance with the Animal Ethics Committee.

Zebrafish embryos were collected from natural mating and kept at 28.5°C. About 300 male/female zebrafish embryos were used for detection of *ltbp1* expression pattern at five experimental periods: 1 cell, sphere, shield, 1 day postfertilization (DPF), and 3 DPF

stages. About 1800 male/female zebrafish embryos were used for *ltbp1* knock-down phenotype study. The animals were divided into three experimental groups: 1. control group (wild-type zebrafish injected with control morpholino); 2. *ltbp1* knock-down group (wild-type zebrafish injected with *ltbp1* morpholino); 3. Rescue group (wild-type zebrafish co-injected with *ltbp1* morpholino and synthesized *ltbp1* mRNA). Each group was evaluated at two experimental periods: 3 DPF and 5 DPF stages.

Microinjection of morpholino antisense oligo and mRNA

Morpholino antisense oligos (MO) were designed to block translation and synthesis by Gene Tools, Inc. The *ltbp1*-MO was targeted to the 5′UTR of the *ltbp1* mRNA, and the *ltbp1*-MO was bound to the 5′UTR (including ATG) of the *ltbp1* mRNA. A standard control-MO was used as negative control (Table 1).[28] Full-length cDNA was used and amplified to test knockdown specificity of the MO.[26,28] Mutations in *ltbp1*-mRNA were introduced within the first 12 bases of open reading frame (ORF) (ATGCTgGTcTGt) without changing the sequences of amino acid so that mutated *ltbp1* mRNA could mimic *ltbp1* mRNA and it would not anneal to *ltbp1*-MO. The MOs and synthesized mRNA were injected into the yolk of embryos at the 1-cell stage. Primers used are described in Table 2. Zebrafish embryos at the 1-cell stage were injected with 1 nL of *ltbp1*-MO at a concentration ranging from 0.1 to 1 mM and a control-MO was used as a negative control. The final dosages of *ltbp1*-MO are 0.25 pmol, 0.5 pmol, and 0.75 pmol. Zebrafish embryos at the 1-cell stage were co-injected with *ltbp1*-mRNA(100 pg/embryo) and *ltbp1*-MO(0.75 pmol/embryo) for the rescue experiments.

Whole mount *in situ* hybridization

Probes for *ltbp1*, *sox9a*, and *ckma* were cloned by polymerase chain reaction with primers presented in Table 3, whole mount in situ hybridization (WISH) was performed as described.[29] The stained embryos were photographed with a Nikon SMZ1500 stereomicroscope.

Alcian blue staining

Embryos were fixed at 5 DPF, and cartilages were visualized by staining with alcian blue (SigmaAldrich; as described by Kimmel, et al.[30] (1998) without using the 1.67% trypsin to digest the tissue.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). At 5 DPF, embryos were categorized according to zebrafish jaw malformation: normal, embryos with normal cartilages compared to uninjected embryos; mild, all cartilages are generally smaller and Meckel's cartilage is mildly malformed as well as positioned ventrally, leading to mandibular jaws opened along with the defects of ceratobranchial; moderate, deformities of Meckel's cartilage are severe, jaws are widely opened and the ceratobranchial is almost completely absent; severe, increased severity of the Meckel's cartilage distortion and the ceratobranchial is completely absent; malformed, embryos with malformed general

Table 1- Morpholino antisense oligos

Morpholino	Sequence
ltbp1-MO	5'-AAACAATGATGTCCCACACGAGCAT-3
control-MO	5'-CCTCTTACCTCAGTTACAATTTATA- 3

Table 2- Primers for the plasmid clone

Construct	Primer	Sequence 5'-3'
ltbp1-mRNA	*ltbp1*-mm-ATG-EcoRI	GGAATTCcaccATGCTGGTCTGTGACATCATTGTT
	ltbp1-R-XhoI	CCGCTCGAGTCACTCTGTGCCCGTACTGGT

Table 3- Primers for the probes

probe	Primer	Sequence 5'-3'
ltbp1	PF-*ltbp1*-11	ATGCCAGCTCTTTGGAAGTGA
	PR-*ltbp1*-743-T7	taatacgactcactatagggGTTGATATCCACGCAGGCCA
sox9a	PF-*sox9a*-167	CTCCTCGACCCCTACCTGAA
	PR-*sox9a*-761-T7	taatacgactcactatagggAGATGTGGGTCTGTTCGCTG
ckma	PF-*ckma*-404	GTCACGGTGGATACAAGGCA
	PR-*ckma*-1246-T7	taatacgactcactatagggTCATGCTGTCGATGGACTCG

morphology. The malformed category was excluded when the percentage of cartilage was estimated. The percentage of cartilage malformation was estimated by considering the total number of mild, moderate, and severe category, and then dividing it by the total number of normal, mild, moderate, and severe category. The mean grey value of WISH staining on the mandibular cartilage (outlined by red dot line) and mandibular surrounding muscle (outlined by red dot line) was measured via ImageJ software. Measurements of lower jaw regions were conducted, including open mouth distance (Figure 3B), mandible length (Figure 3D), and mandible width (Figure 3F), the red double arrow represents the corresponding length. Each specific length was obtained by comparing the length of red double arrow with a scale bar that corresponds with the magnification of the alcian blue images.

Data are expressed as mean ± standard deviation (SD). Comparison of different groups was performed by unpaired t-test to estimate data measurements. P-value less than 0.05 (P<0.05) was considered statistically significant.

Results

ltbp1 expresses at mandibular arch skeleton

Since previous studies indicated that *LTBP1* is a crucial gene in the early embryo development and it is widely expressed in several systems, we suspected that *LTBP1* was expressed in craniofacial tissues. However, the expression pattern of *LTBP1* in craniofacial tissues remains unclear. Therefore, we decided to explore the expression pattern of *LTBP1* zebrafish ortholog in craniofacial tissues.

According to WISH of *ltbp1* on zebrafish embryos, maternal transcripts were detected during the early developmental stage from 1 cell (Figure 1A) to shield (Figure 1C). At pharyngula period (1 DPF), *ltbp1* expressed at pharyngeal arches, brain, the most part of trunk, and the origin of internal organs (Figure 1D). In the developmental stage of protruding mouth (3 DPF), *ltbp1* was mainly detected in the mandibular jaw region and brain, but reduced expression also existed in the trunk and internal organs such as pancreas and gallbladder (Figure 1E, F). We performed 2-color WISH of *ltbp1* with cartilage marker *sox9a* and muscle marker *ckma,* respectively, to further explore

the expression pattern of *ltbp1* in the mandibular jaw region. *sox9a* is a marker of cranial neural crest that differs from cartilage, and *sox9a* participates in both determination of crest-derived chondrogenic lineages and morphogenesis of cartilage (2-3 DPF)[31]. *ckma* is a terminal differentiation marker for skeletal muscle,[32] and *ckma* was expressed in muscles of the trunk, pectoral fin, head, and heart.[33] According to the observation of the tissue section of zebrafish jaw, *ltbp1* colocalized with both *sox9a* (Figure 1G-N) and *ckma* (Figure 1J-L). The results indicated that *ltbp1* was expressed in craniofacial tissues, and it was mainly concentrated in the zebrafish jaw cartilages and its surrounding muscles.

Loss of *ltbp1* function Results in Severe Jaw Malformation

Based on the fact that *ltbp1* is expressed in the zebrafish mandibular jaw region, we speculated that *ltbp1* play a role in the formation of mandibular jaw. The loss of *ltbp1* function was mediated by morpholino knockdown to assess the function of *ltbp1* during craniofacial development and mandibular jaw formation. We designed translation-blocking morpholino antisense oligonucleotides (MO) for *ltbp1* targeting regions in the 5'-UTR of the mRNA. MO with concentration ranging between 0.1 and 1 mM was injected 1 nL into zebrafish embryos in 1-cell stage, and a control-MO was used as a negative control. Embryos injected with control-MO were indistinguishable from uninjected embryos. Embryos injected with *ltbp1*-MO exhibited several defects, including severe jaw malformation, pericardial edema, generally shortened body length and microphthalmia. At 5 DPF, *ltbp1* morphants presented significantly underdeveloped jaw regions and the mandibular jaws were widely opened (Figure 2E-F, I-J and M-N). We performed alcian blue staining to visualize the jaw cartilage skeleton. The staining revealed severe deformity of Meckel's cartilage along with the absence of ceratobranchial (Figure 2C-D, G-H, K-L and O-P). Further measurements of lower jaw regions were conducted, including open mouth distance (Figure 3B), mandible length (Figure 3D) and mandible width (Figure 3F), in order to assess the jaw defects. The measurement of this three dimensions showed that *ltbp1* morphants decreased in both mandible length and width and it increased in open mouth distance compared to the control-MO group (Figure 3A,C,E).

Interestingly, as *ltbp1* MO dosages increase, both penetrance and severity of the jaw defects increases (Figure 2Q).

Rescue experiments with full-length *ltbp1* mRNA were carried out to confirm the specificity of *ltbp1* knockdown phenotype. Co-injection of nonhomologous *ltbp1* mRNA with *ltbp1* MO significantly rescued the jaw defects (Figure 4A-D). The rescued *ltbp1*-morphants presented increased jaw length and width, whereas their open mouth distance (Figure 4E-H) decreased, and also decreased the rescue of ceratobranchial

(Figure 4C). As most phenotypes were rescued when the mRNA encoding for *ltbp1* were co-injected in the *ltbp1* MO, the off-targeting effect of *ltbp1* MO was ruled out, indicating the specificity of *ltbp1* knockdown phenotype.

Loss of *ltbp1* function Lead to Abnormal Cartilage and Muscle during Jaw Development

Since *ltbp1* is expressed in both jaw cartilages and jaw surrounding muscles, and the loss of *ltbp1* function affected jaw development, we examined the two tissue
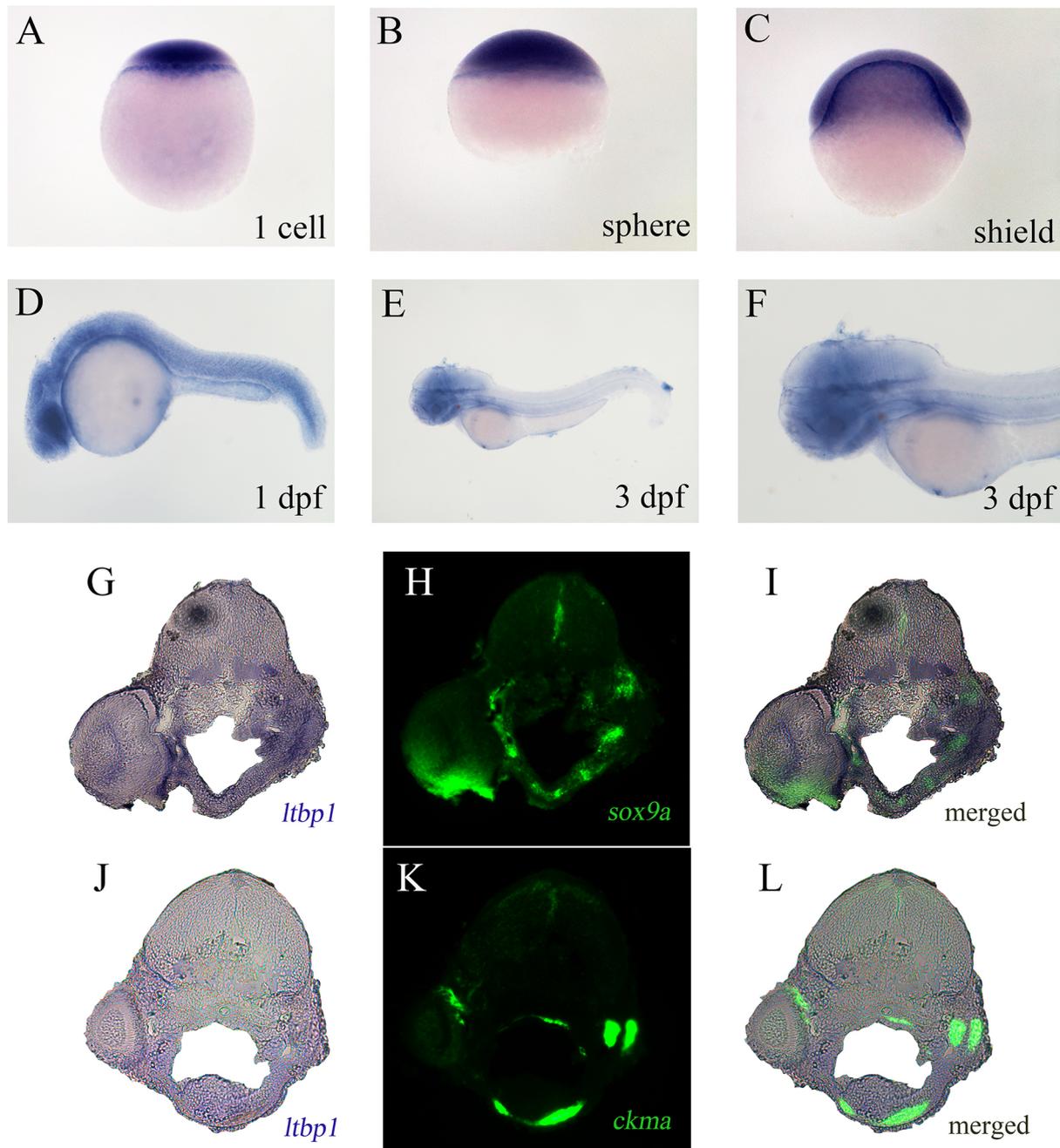
(A–F) Expression patterns analyzed by WISH with *ltbp1* probes in the stages indicated (developmental stage: 1 cell , sphere, shield, 1 DPF and 3 DPF). Views are lateral. (G–L) Double-dye WISH of *ltbp1* with either *sox9a* or ckma fluorescence-probe. *ltbp1* colocalized with both *sox9a* (G–I) and *ckma* (J–L) in the 3-DPF embryo transections.

Figure 1- Expression patterns of *ltbp1*

in *ltbp1*-morphants during jaw formation. Because 3 DPF is the stage of protruding-mouth stage, when the developing cartilages of the pharyngeal skeleton such as Meckel's cartilage, has grown dramatically, we chose 3 DPF as developmental stage to examine the effects of *ltbp1* knockdown. We conducted WISH of *sox9a* and *ckma* respectively on control-MO embryos, *ltbp1*-morphants, as well as *ltbp1* rescued morphants at 3 DPF. The expression of *sox9a* was detected in mesenchymal condensation of pharyngeal arches
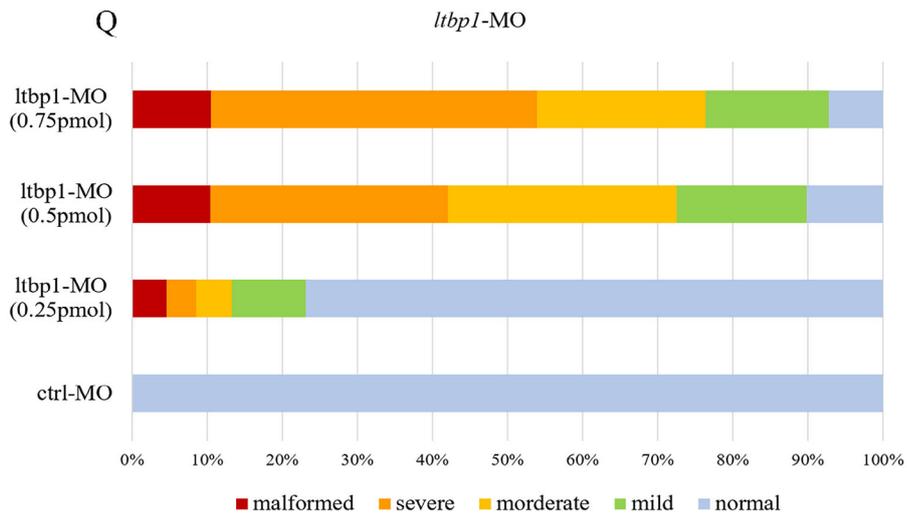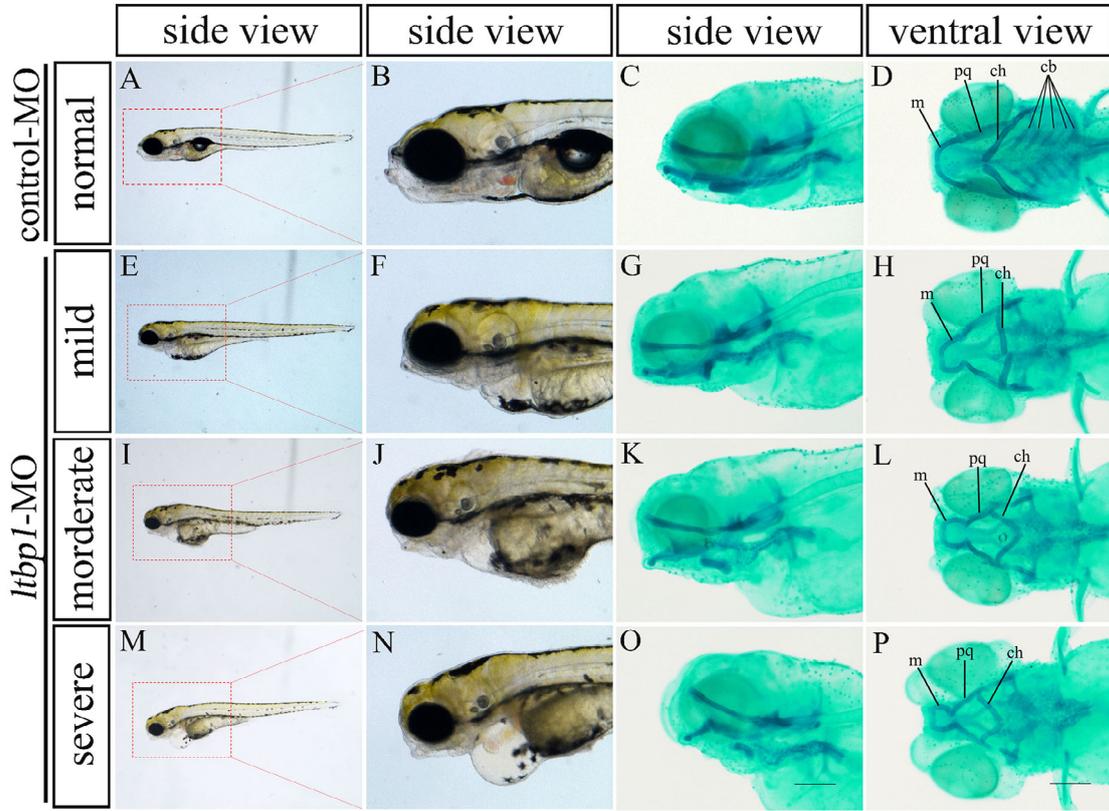
Injection of *ltbp1*-MO caused marked jaw defects compared to standard control embryos at 5 DPF (A,E,I ,M and B,F,J,N). Larvae at 5 DPF were stained with alcian blue to observe craniofacial cartilage structures (C,G,K,O and D,H,L,P). Views are lateral and ventral. (Q) Quantification of craniofacial phenotypes of *ltbp1* morphant. We defined five categories regarding jaw malformation of zebrafish: (A-D) normal, embryos with normal cartilages compared to uninjected embryos; (E-H) mild, all cartilages are generally smaller, and Meckel's cartilage is mildly malformed as well as positioned ventrally, leading to opened mandibular jaws as well as defects of ceratobranchial; (I-L) moderate, deformities of Meckel's cartilage are more severe and jaws are widely opened, and the ceratobranchial are almost completely absent; (M-P) severe, severe distortion of Meckel's cartilage and the ceratobranchial are completely absent; malformed, embryos with malformed general morphology. ch, ceratohyal; m, Meckel's cartilage; pq, palatoquadrate; cb, ceratobranchial. Scale bars: 200 µm (C-D, G-H, K-L and O-P).

Figure 2- Depletion of ltbp1 causes severe jaw malformation in zebrafish

and jaw cartilages in control and rescued morphants, while significantly decreased in *ltbp1*-morphants (Figure 5A-C and G). Similarly, expression of *ckma* was shown in mandibular region of craniofacial muscle in control and rescued morphants, but severely reduced in *ltbp1*-morphants (Figure 5D-F and H). The WISH of *sox9a* and *ckma* indicated that *ltbp1* function loss affected jaw development in both jaw cartilage and
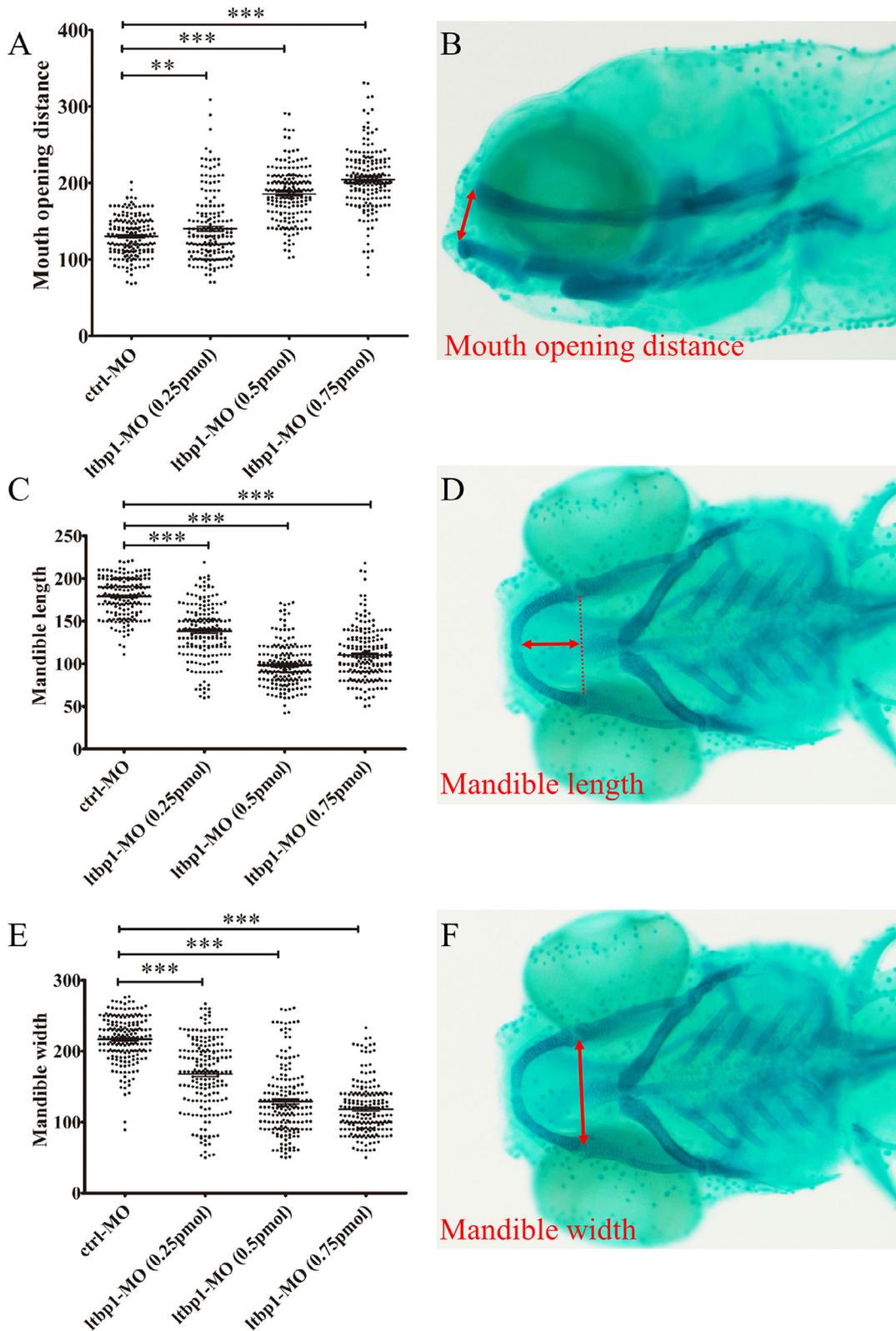
Figure 3- Three dimensions (3D) measurements of mandibular jaw malformation

Red double arrow represents the (B) mouth-opening distance, (D) mandible length, and (F) mandible width. Scatter plots showed an increase of mouth-opening distance and a decrease of mandible length and width in (A,C,E) *ltbp1*-morphant. N=3, n=60 per group, **P<0.01, ***P<0.001.
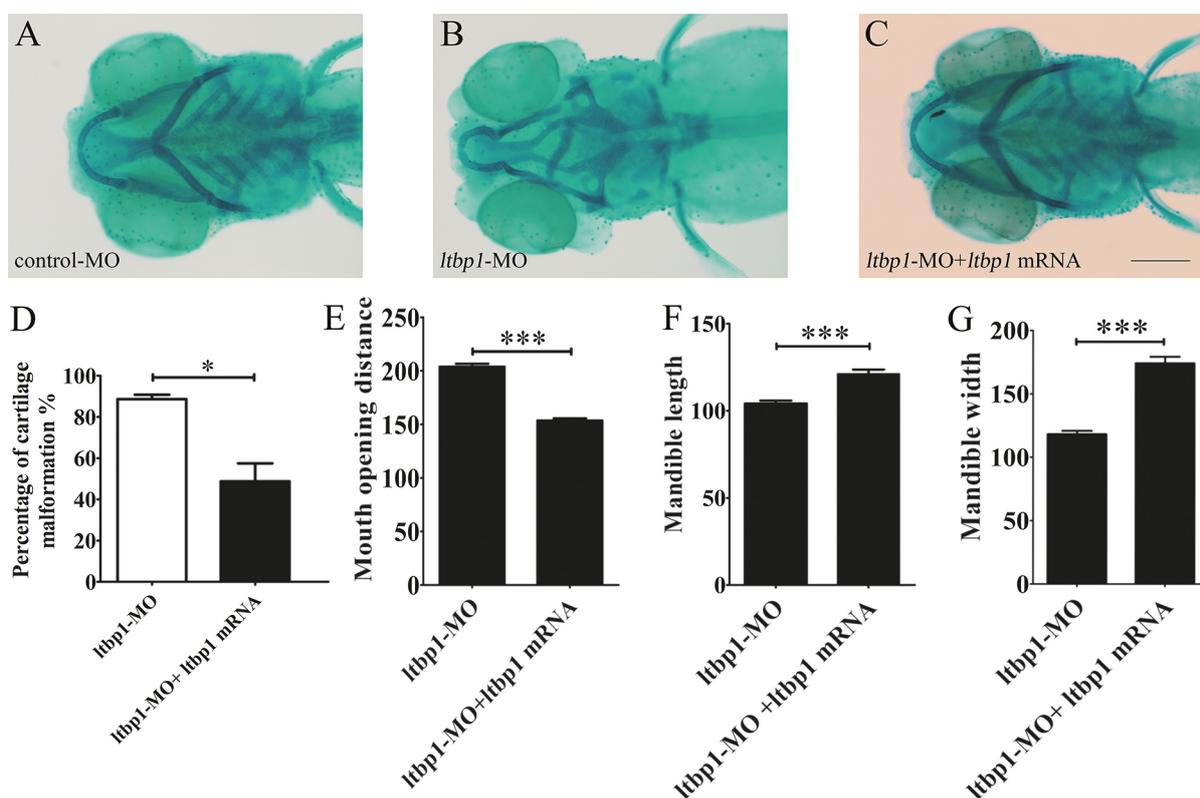
RNA rescue experiments for *ltbp1* knockdown in zebrafish using *ltbp1* mRNA. (A-C) Embryos were stained with alcian blue at 5 DPF to visualize craniofacial cartilage structures. All views are ventral. Jaw malformation induced by *ltbp1* knockdown was significantly rescued by the co-injection of *ltbp1* mRNA (D), including reduction in mouth opening distance (F), recover in mandible length (G) and width (H). (E-G) Data represent the mean ± SD, N=3, n=60 per group, (*P<0.05, ***P<0.001). Error bars indicate standard error. Scale bars: 200 µm (A–C). MO, morpholino antisense oligo.

Figure 4- Specificity of ltbp1 knockdown phenotype

muscle aspects.

Discussion

LTBP1 is an important extracellular matrix protein that regulates the bioactivity of TGF-β and also exists as microfibrillar structure of bone and connective tissue. According to recent researches, the expression of *ltbp1* is widely distributed in different tissues.[2,3] Gene disruption experiments have shown that *LTBP1* play roles in several fields, including the development and formation of cardiovascular system, craniofacial system, reproduction system as well as functions in both bone and connective tissue.[21-24] In this study, we explored the expression pattern of *ltbp1* using zebrafish. The results of *ltbp1* WISH showed detectable maternal transcripts from 1 cell to shield stage, which indicated that *ltbp1* is necessary during the early embryo development. In the pharyngula period (1 DPF), *ltbp1* was extensively expressed in the pharyngeal arches, brain, most part of trunk and the origin of some internal organs. In the developmental stage of protruding mouth (3 DPF), *ltbp1* expression was mainly in the mandibular jaw region and brain, while reduced expression was also present in the trunk and internal organs such as pancreas and gallbladder. The *ltbp*1 WISH results are according with previously reported widespread tissue expression. Since *ltbp1* is strongly expressed in the mandibular jaw region at 3 DPF, and the zebrafish jaw region mainly consists of cartilages and muscles. We carried out 2-colour WISH of *ltbp1* with cartilage marker *sox9a* and muscle marker *ckma* in order to specify the target tissue of *ltbp1* expression. The results showed that *ltbp1* colocalized with both *sox9a* and *ckma,* indicating that *ltbp1* concentrates in the zebrafish jaw cartilages and its surrounding muscles.

Considering that the function of a gene is usually associated with its expression pattern, we hypothesize *ltbp1* may have its function in the craniofacial development of zebrafish. According to previous studies, different strategies of *Ltbp1* knockout mice presented several phenotypes, including heart defects, craniofacial deformities, and impaired reproduction.[21-24] However, the discrepancy of the variable phenotypes

Bright-field views of in situ hybridization of cartilage marker *sox9a* and muscle marker *ckma* expression in zebrafish larvae at 72 hour postfertilization (hpf). *sox9a* expression in (A) control-MO, (B) *ltbp1*-MO, (C) *ltbp1*-MO with *ltbp1* mRNA from ventral view. *sox9a* expression was reduced in mandibular cartilage (outlined by red dot line) of 72-hpf (B) *ltbp1*-morphant. The reduction of *sox9a* expression in morphants was rescued by co-injection of (C) *ltbp1* RNA. (G) *sox9a* expression showed significant differences among morphants and control embryos. *ckma* expression in (D) control-MO, (E) *ltbp1*-MO, (F) ltbp1-MO with *ltbp1* mRNA from ventral view. *ckma* expression was reduced in mandibular surrounding muscle (outlined by red dot line) of 72-hpf (E) *ltbp1*-morphant. The reduction of *ckma* expression in morphants was rescued by co-injection of (C) *ltbp1* RNA. (H)*ckma* expression showed significant differences among morphants and control embryos. (G,H)N=3,n=15 per group, (**P<0.01***P<0.001). Scale bar: 200 μm (A–F). MO, morpholino antisense oligo.

Figure 5- Loss of ltbp1 function affects formation of both craniofacial cartilage and muscle

in *Ltbp1* gene-manipulated mice is complicated and ambiguous. To repeat the phenotype in another model, we performed morpholino based knockdown of *ltbp1* using zebrafish.

Generally, *ltbp1* morphants presented shortened body length, heart edema, and severe jaw malformation. At 5 DPF, *ltbp1* morphants manifested significantly underdeveloped jaw regions and the mandibular jaw was widely opened. These phenotypes are consistent with the observation of *Ltbp1* knockout mice which were built by different strategies.

In this study, we focused on jaw defects of *ltbp1* morphants, since this craniofacial aspect is the most significant observed in this phenotype, remaining unclear. Alcian blue staining of 5 DPF *ltbp1* morphants revealed severe deformity of Meckel's cartilage as well as the absence of ceratobranchial. We conducted three dimensional measurements of lower jaw regions to assess the jaw defects, and the *ltbp1* morphants decreased both mandible length and width and their open mouth distance increased. Similar phenotype was previously observed in the *Ltbp1*-null mice reported by Drews, et al.[23] (2008), which showed modified facial profile with shortened snouts and

shortened maxilla and mandible. We also found that both penetrance and severity of *ltbp1*-morphants defects of jaw increases as the dosages of *ltbp1* MO increases. Meanwhile, most phenotypes were rescued by co-injection of nonhomologous *ltbp1* mRNA with *ltbp1* MO, therefore the off-targeting effect of *ltbp1* MO was ruled out, indicating the specificity of *ltbp1* morphant phenotype. Since *ltbp1* is expressed in both jaw cartilages and surrounding muscles, we speculated that the jaw deformities could derive from the defects of either or both the two tissues. In order to address possible major aspect, we checked both cartilage and muscle markers in *ltbp1*-morphants. Both expression of *sox9a* and *ckma* was significantly decreased in *ltbp1*-morphants compared to control-MO and *ltbp1* rescued morphants, indicating that knockdown of *ltbp1* affected jaw development in both jaw cartilage and muscle aspects.

Considering that some previous studies have found that latent TGF-β is present in the matrix of chondrocytes and that *LTBP1* is responsible for storing TGF-β complex in the matrix.[6,7] TGF-β1 and their receptors have been found to be related to zebrafish craniofacial bone and cartilage development.[34] The cartilage development may be affected by indirect regulating function of *LTBP1* and TGF-β1. Besides, *LTBP1* may directly affect chondrocytes as an important extracellular matrix protein. Furthermore, *LTBP1* may participate in the muscle formation as microfibrillar structures of connective tissue[8]. Future studies will investigate the biological activity of TGF-β and extracellular fibrillar structure whether they are compromised or not. Although the specific mechanisms require further research, our results have extended the understanding of the important role of *LTBP1* especially in craniofacial development, easing future investigations on the mechanisms of *LTBP1* gene function.

Conclusion

Our experiments found that *ltbp1* is expressed in zebrafish mandibular jaw cartilages and the surrounding muscles in addition to the previously reported tissues. Also, the *ltbp1* knockdown zebrafish presented phenotypes consistent with *Ltbp1* knockout mice. Lastly, loss of *ltbp1* function lead to significant mandibular jaw defects and affect both jaw cartilages

and surrounding muscles.

Authors' contributions

Xiong, Yiting: Conceptualization (Equal); Data curation (Equal); Formal analysis (Equal); Funding acquisition (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Resources (Equal); Software (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing-original draft (Equal); Writing-review & editing (Equal). **Sun, Rongrong**: Conceptualization (Equal); Methodology (Equal); Project administration (Equal); Supervision (Equal); Writing-original draft (Equal); Writing-review & editing (Equal). **LI, Jingyu**: Data curation; (Equal); Formal analysis (Equal). **Wu, Yue:** Data curation (Equal); Formal analysis (Equal). **Zhang, Jingju**: Conceptualization (Equal); Methodology (Equal); Project administration (Equal); Supervision (Equal); Writing-original draft (Equal); Writing-review & editing (Equal)

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