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SciVerse ScienceDirect
Journal of Genetics and Genomics 39 (2012) 463−471
JGG

ORIGINAL RESEARCH

Kzp Regulates the Transcription of *gata2* and *pu.1* during Primitive Hematopoiesis in Zebrafish Embryos

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Received 15 May 2012; revised 24 June 2012; accepted 28 June 2012
Available online 5 September 2012

ABSTRACT

Kaiso zinc finger-containing protein (Kzp), a maternally-derived transcription factor, controls dorsoventral patterning during zebrafish gastrulation. Here, we uncovered a new function for Kzp in zebrafish embryonic primitive hematopoiesis. The depletion of *kzp* led to defects in primitive hematopoiesis including the development of the erythroid and myeloid lineages. On the other hand, overexpression of *kzp* caused the ectopic expression of *gata1, gata2*, and *pu.1*. Chromosome immunoprecipitation assays revealed that Kzp protein directly binds to *gata1*, *gata2*, and *pu.1* promoters. Interestingly, the ectopic expression of *gata2* was able to rescue the erythroid, but not the myeloid lineage in *kzp*-depleted zebrafish embryos. *gata1* expression controlled by Kzp was dependent on *gata2* during primitive erythropoiesis. Our results indicate that Kzp is a critical transcriptional factor for the expression of *gata2* and *pu.1* to modulate primitive hematopoiesis.

KEYWORDS: *kzp*; Hematopoiesis; Erythropoiesis; Myelopoiesis; Zebrafish

1. INTRODUCTION

Over the years, zebrafish has proven to be an excellent vertebrate model system for studying the precise molecular mechanisms of hematopoiesis (Berman et al., 2005; Juarez et al., 2005). As in mammals, primitive and definitive hematopoiesis of the zebrafish embryo occurs in anatomically different regions and at different developmental stages, making them easily distinguishable due to lineages they give rise to in different locations (Orkin and Zon, 2008). Embryonic primitive hematopoiesis gives rise to primitive erythrocytes, which arise from the intermediate cell mass, and to endothelial cells and macrophages derived from cephalic mesoderm. On the other hand, definitive hematopoiesis produces erythromyeloid progenitors in the posterior blood island at first, and later, hematopoietic stem cells (HSCs) in the aorta-gonad-mesonephros region (Berman et al., 2005). In zebrafish, embryonic primitive hematopoiesis begins at the 3- to 5-somite stage before blood circulation starts at about 24 h post-fertilization (hpf). Hematopoietic cells are derived first from the hemangioblast and then from HSCs.

Several genes are critical for the expansion, commitment, and differentiation of the hemangioblast, HSCs, and progenitors (*scl*, *lmo2*, *flk1*, *gata2*, *c-myb, and runx1*), the erythroid lineage (*gata1* and *β-globin*), and the myeloid lineage (*pu.1*, *lcp1*, *and mpo*) (El Omari et al., 2011; Tijssen et al., 2011). *scl* and *gata2* are critical for the specification of primitive progenitors, which produce erythroid and myelopoiesis lineages in the early stage (Juarez et al., 2005; De Pooter et al., 2006). *gata1* plays a key role in erythroid differentiation (Rodriguez et al., 2005). The transcription factor *pu.1* is involved in myelopoiesis/granulopoiesis and negatively regulates the expression of erythroid genes (Mak et al., 2011). While *β-globin* is specifically expressed in erythrocytes, *lcp1* and *mpo* genes are markers of macrophage and granulocytes, respectively (Meijer et al., 2008).

When Kzp (Kaiso zinc finger-containing protein), a DNA-binding zinc finger homeodomain protein, was first cloned

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http://dx.doi.org/10.1016/j.jgg.2012.08.003

from a zebrafish expression library, it was not known whether it is involved in hematopoiesis. It was identified as a crucial transcription factor controlling dorsoventral patterning during zebrafish gastrulation (Yao et al., 2010). Previous results provided the first known function for Kzp as the promoter of ventroposterior patterning at the early stage, a function achieved by direct binding of Kzp to the *Wnt8* promoter to regulate its expression. Interestingly, we also noticed that *gata2* was dramatically downregulated in *kzp*-depleted zebrafish embryos in the microarray experiment (data not shown). The transcription factor *gata2* plays a key role in hematopoiesis and gene regulation during erythropoiesis in zebrafish embryos (Kitajima et al., 2002; Ohneda et al., 2002). Therefore, we hypothesized that Kzp might be involved in zebrafish hematopoiesis, possibly *via* the modulation of *gata2*. Our results in this study indicate that the depletion and overexpression of *kzp* affect the development of progenitors and the expression of lineage-specific hematopoietic markers during primitive hematopoiesis. We demonstrated that Kzp contributes to primitive hematopoiesis by controlling the expression of *gata2* and *pu.1* genes directly and is not required for early angiogenesis in zebrafish embryos.

2. MATERIALS AND METHODS

2.1. Zebrafish embryos

Embryos from wild-type zebrafish (*Danio rerio*) and Tg(*flk1*:GFP) transgenic strain were used. Zebrafish were raised at 28.5°C and embryos were collected and staged as described (Kimmel et al., 1995).

2.2. Morpholino and plasmid construction

kzp morpholino (MO) used was 5′-TGCCTCCTCTGTGCCC TCTCCCATC-3′ as described previously (Yao et al., 2010). The coding region of *gata2* was amplified with the primer pair 5′-CTCGAGCATTTTCTAAGCGCGGACAC-3′ and 5′-CTCG AGGTCCAAACCCGACGTGAACC-3′. The PCR fragment was inserted into pcDNA3.1 digested with *Xho* I. pcDNA3.1-*gata2* plasmid linearized at a 3′ *Xba* I site was used for mRNA synthesis. The PCR fragment corresponding to *kzp* coding region was generated and cloned into pcDNA-FLAG for zebrafish embryo overexpression as described previously (Yao et al., 2010).

2.3. mRNA synthesis

Capped mRNAs and probes were synthesized *in vitro* using the mMESSAGE mMACHINE kit (Ambion, AM1344, Life Technologies, USA) according to the manufacturer's protocol. Antisense RNA probe for *in situ* hybridization was synthesized in the presence of digoxigenin-UTP.

2.4. Microinjection

Microinjection was carried out as described previously (Nasevicius and Ekker, 2000). Embryos were injected with 1 ng *kzp* MO, 100 pg *kzp* DNA, and 15 pg *gata2* mRNA, respectively.

2.5. Whole-mount *in situ* hybridization (WISH)

WISH was performed as described previously (Cvejic et al., 2011). *gata2*, *lmo2*, and *c-myb* cDNAs were synthesized using the following primer pairs: *gata2*, 5′-GCCTGTACCAC AAGATGAACG-3′ and 5′-GTACAGCTCAGCAACATCGC-3′; *lmo2*, 5′-CAAATGCGCTGAATTATACCTG-3′ and 5′-TCT TGAGCTCCAATTAAAGCTA-3′; *c-myb*, 5′-AAATATTCAT CCGTCAGACACC-3′ and 5′-AATGGCAACACTACTAAAC TGG-3′. *gata1*, *scl*, *pu.1*, *β-globin*, *lcp1*, and *mpo* cDNAs were generous gifts from Dr. Zhong Hanbing (Peking University, China).

2.6. RT-PCR analysis

RNA was extracted from embryos at the 10-somite stage using TRIzol (Invitrogen, USA). Total RNA was reverse transcribed into cDNA using PrimeScript RT Reagent Kit (TaKaRa, Japan). The following primer pairs were used for RT-PCR analysis: *scl*, 5′-CAGCCATAAGGTGCAGACCA-3′ and 5′-CCAGACGCAGGATCTCGTTC-3′; *gata2*, 5′-TACA CAGTCCCGGTATTCCC-3′ and 5′-CCCGAAGAGGACTA CATCCC-3′; *gata1*, 5′-GCCACTACCTCTGTAATGC-3′ and 5′-GAATACGCTCCTACATGCTC-3′; *β-globin*, 5′-TGTC TTATCGTGTACCCCTG-3′ and 5′-CATCTGAGCAGCAA CAAC-3′; *c-myb*, 5′-AAGTCAGCCCAACTCCGCTA-3′ and 5′-ACAATGCCAACCGAACTGTCC-3′; *pu.1*, 5′-GCACAAA GAGGTTTTAGCCAA-3′ and 5′-ACTTCATTAATGCGAA GGTGT-3′; *lmo2*, 5′-TCTTTCTGAAGGCCATCGAG-3′ and 5′-ATGAGCAGGTAACGATCTCCA-3′.

2.7. Chromosome immunoprecipitation (ChIP) assay

ChIP was performed essentially as described before (Boyd and Farnham, 1999; Yao et al., 2010). Briefly, about 200 embryos were injected with Kzp-FLAG plasmid at the one-cell stage and harvested at the 10-somite stage. The harvested embryos were adjusted to 1% formaldehyde, homogenized, and then, sonicated to generate 300−500 bp chromatin fragments. Fragmented chromatin was immunoprecipitated with FLAG antibody or IgG antibody. Nucleic acids were precipitated with ethanol and used for PCR. The following PCR primers were used: for the *gata1* promoter, 5′-ATAATATTCAAAATGGAAAGCG-3′ and 5′-ACCGTATTTATGAACTTACCTG-3′; for the *gata2* promoter, 5′-CGCTGTCATCACCCATCAGA-3′ and 5′-CCCGCTTTTC ATTTCAACCAAC-3′; for the *pu.1* promoter, 5′-AATCAA CAATTTGCCCACA-3′ and 5′-CTGCGTAAAACATATGCT GA-3′; for the *pu.1* intron 1, 5′-AAGCCCTTGAGAAAGCA TC-3′ and 5′-CATAAGGCACATTAGGCAAC-3′.

3. RESULTS

3.1. Kzp modulates primitive hematopoiesis in zebrafish embryos

To investigate the role of Kzp in the early stage of primitive hematopoiesis, *kzp* MO or *kzp* DNA was injected into zebrafish