Original Article

Tcf7l1 promotes transcription of Kruppel-like factor 4 during *Xenopus* embryogenesis

Qing Cao[1,✉], Yan Shen[1], Wei Zheng[1], Hao Liu[1], Chen Liu[2,✉]

[1] *College of Medicine, Henan University of Science and Technology, Luoyang, Henan 471023, China;*
[2] *Department of Developmental Genetics, Nanjing Medical University, Nanjing, Jiangsu 211166, China.*

Abstract

Kruppel-like factor 4 (Klf4) is a zinc finger transcription factor and plays crucial roles in *Xenopus* embryogenesis. However, its regulation during embryogenesis is still unclear. Here, we report that Tcf7l1, a key downstream transducer of the Wnt signaling pathway, could promote *Klf4* transcription and stimulate *Klf4* promoter activity in early *Xenopus* embryos. Furthermore, cycloheximide treatment showed a direct effect on *Klf4* transcription facilitated by Tcf7l1. Moreover, the dominant negative form of Tcf7l1 (dnTcf7l1), which lacks *N*-terminus of the β-catenin binding motif, could still activate *Klf4* transcription, suggesting that this regulation is Wnt/β-catenin independent. Taken together, our results demonstrate that Tcf7l1 lies upstream of Klf4 to maintain its expression level during *Xenopus* embryogenesis.

Keywords: Kruppel-like factor 4 (Klf4), Tcf7l1, transcription regulation, *Xenopus* laevis

Introduction

Klf4, a zinc finger containing transcription factor of the Kruppel-like factor family, plays critical roles in stem cell biology and early embryogenesis. As a pluripotency factor, Klf4 is required for maintaining pluripotency, self-renewal of embryonic stem (ES) cells and reprogramming of differentiated cells[1–2]. In fact, knockdown of *Klf4* results in the differentiation of ES cells[3–4]. During *Xenopus* embryogenesis, *Klf4* is expressed maternally and zygotically[5], which is vital for germ layer formation and body axis patterning. Overexpression of *Klf4* promotes neural precursor and endoderm formation, whereas knockdown of *Klf4* leads to the failure of three germ layer differentiation[5–6]. Due to its important functions in cell fate determination in both ES cells and early embryos, Klf4 should be maintained at a correct level so that cell differentiation could continue properly. A few attempts so far have been done on the transcription regulation mechanism of Klf4 in ES cells, some cancer cells and adult cells[7–9]. It has been reported that P53[10], CDX2[11], histone modification[12], microRNA[13–15], BMPs and TGFβs[16–17] could regulate *Klf4* expression. Besides the mechanism described above, Klf4 could upregulate its own transcription by binding to its promoter[1,18]. Although the previous studies have provided some clues of the regulation mechanism of *Klf4*, there is still

✉Corresponding author: Dr. Qing Cao, College of Medicine, Henan University of Science and Technology, Luoyang, Henan 471023, China, Tel: 0379-64830345, E-mail:caoqing@haust.edu.cn; Dr. Chen Liu, Department of Developmental Genetics, Nanjing Medical University, Nanjing, Jiangsu 211166, China, Tel/Fax: 025-86869463/ 025-86869466, E-mail: liuchen@njmu.edu.cn.

little information available in literature about the regulation of *Klf4* transcription during early embryogenesis.

Tcf7l1 (also known as Tcf3) is a key downstream transducer of the Wnt signaling pathway, which is required for early body axis specification and mesoderm induction[19–23]. It is maternally expressed in *Xenopus* embryos and plays dual functions (repressor or activator) in regulating gene expression[24]. Depletion of Tcf7l1 showed headless phenotype in both zebrafish and *Xenopus* embryos[25–26]. Furthermore, Tcf7l1 is reported as an integral component of the regulatory circuitry of ES cells[27]. In ES cells, Klf4 and a few other transcription factors, e.g. Oct4, Sox2, Myc and Nanog, etc., comprise a core genetic circuitry to maintain pluripotency and self-renewal of ES cells[1]. Recently, it has been demonstrated that Tcf7l1 is involved in regulation of *Oct4*, *Sox2*, *Nanog* and *Klf4* transcription in ES cells[27–28]. Embryogenesis is a process of cell differentiation by loss of pluripotency and the pluripotency factors play important roles in this process[6]. Based on the research in ES cells described above, we aim to know whether Tcf7l1 likewise regulates *Klf4* transcription *in vivo*.

In this paper, we aimed to investigate the effect of Tcf7l1 on *Klf4* transcription during *Xenopus* early embryogenesis through gain of function and loss of function analyses. First, we found that *Klf4* expression is strongly upregulated in response to overexpression of *Tcf7l1* both in whole embryos and animal caps. Secondly, knockdown of Tcf7l1 leads to reduction of *Klf4* expression. Thirdly, dual-luciferase reporter assay showed that *Klf4* promoter activity is dramatically stimulated by Tcf7l1. Finally, we demonstrated that the transcription of *Klf4* promoted by Tcf7l1 is a direct effect and seems to be Wnt/β-catenin independent. In summary, our results suggest that Tcf7l1 lies upstream of Klf4 and is required for *Klf4* transcription during *Xenopus* embryogenesis.

Material and methods

Embryos and explants

Xenopus laevis embryos were obtained, cultured and staged as previously described[5]. Animal caps were excised from stage 8 embryos and collected at stage 10.5. To block protein translation, embryos were incubated in 0.1 × MBSH (1×MBSH: 88 mmol/L NaCl, 2.4 mmol/L NaHCO$_3$, 1 mmol/L KCl, 0.82 mmol/L MgSO$_4$, 0.41 mmol/L CaCl$_2$, 0.33 mmol/L Ca(NO$_3$)$_2$, 10 mmol/L HEPES, pH-7.4) containing cycloheximide (CHX) at 25 μg/mL from stage 7 to stage 10.5 (gastrulation).

In vitro transcription, morpholino oligonucleotides and microinjection

To prepare mRNAs for microinjection, plasmids pCS2 + Tcf7l1 and pCS2 + dnTcf7l1 were linearized by *Not*I, transcribed with Sp6 mMessage mMachine kits (Ambion) and cleaned up with RNeasy Kit (Qiagen). An antisense morpholino oligonucleotide (MO), Tcf7l1 MO: 5′-CGCCGCTGTTTAGTTGAGGCATGA-3′, was used to knock down *Xenopus laevis* Tcf7l1 as previously reported[19]. The standard control MO (ctrlMO) 5′-CCTCTTACCTCAGTTACAATTTATA-3′ against the human *β-globin* gene was used as a control. All MOs were purchased from GeneTools. Injected doses of mRNA or MOs are described in the text.

Whole-mount *in situ* hybridization

A digoxigenin-labeled probe of Klf4 was prepared by digesting plasmids pCS2 + Klf4 with *Cla*I, transcribing with T7 RNA polymerase (Fermentas) and cleaned up with RNeasy Kit (Qiagen). *Xenopus laevis* embryos were fixed in 1×HEMFA (0.1 mmol/L HEPES, 2 mmol/L EGTA, 1 mmol/L MgSO$_4$, 4% formaldehyde, pH7.4) for 2 hours and dehydrated twice in absolute ethanol for 10 minutes. Whole-mount *in situ* hybridization was carried out according to standard protocols of Harland (1991)[29] except for using BM purple (Roche) instead of NBT/BCIP for chromogenic reaction.

cDNA synthesis and quantitative RT-PCR

Total RNA was extracted from embryos or animal caps with Trizol (Qiagen). First strand cDNA was synthesized from 2 μg total RNA with RevertAid™ First Strand cDNA Synthesis kit (Fermentas). cDNA was used at a dilution of 1:40 for Quantitative RT-PCR (qPCR). Amplification parameters were as follows: one cycle of predenaturation at 95°C for 10 senconds, followed by 40 cycles of denaturation at 95°C for 5 seconds, annealing and extension at 60°C for 31 seconds and an additional cycle for the melting curve. Crosspoints were calculated using ABI 7300 system SDS software and normalized according to the expression level of ODC (ornithine decarboxylase) included in each run. Final results were presented as histograms with relative units. qPCR primers are listed below: *Klf4* (F): 5′-GAGAGGTGGAGGGAAGATCCA-3′; *Klf4* (R): 5′-CCAAACCATCATAAGCACGAGAC-3′; *ODC* (F): 5′-CAAAGCTTGTTCTACGCATAGCA-3′; *ODC*(R): 5′- GGTGGCACCAAATTTCACACT-3′.

Construction of *Xenopus Klf4* promoter reporter plasmid

To further study the transcription mechanism, we

need to isolate the promoter sequence of *Klf4*. Because the genomic data for *Xenopus laevis Klf4* gene is not available, we chose *Xenopus tropicalis* to do such an experiment, which is a close relative of *Xenopus laevis* and whose genomic DNA is completely sequenced. It has been reported that genes and regulatory mechanism are conserved between *Xenopus laevis* and *Xenopus tropicalis*. Since *Xenopus laevis* Klf4 and *Xenopus tropicalis* Klf4 have 94% similarities[5], we used *Xenopus tropicalis Klf4* promoter sequence to carry out luciferase assay. The DNA of Klf4 promoter region $-2144/+70$ (transcription start as $+1$) was amplified from a library of *Xenopus tropicalis* genomic DNA and was subcloned to pGL3-basic to generate Klf4Luc $(-2144/+70)$.

Luciferase assay

To perform luciferase assays on *Xenopus* embryos, promoter reporter plasmid DNAs and mRNAs or MOs were co-injected into equatorial region of all blastomeres at 2 or 4-cell stage. Embryos were collected at the gastrula stage, and homogenized in $1\times$passive lysis buffer (Promega) by using tips. Homogenates were incubated at room temperature for 10 minutes and centrifuged at 12,000 r/min for 10 minutes at 4°C. Then, the supernatants were transferred to fresh tubes and used for luciferase assay. Luciferase activity was measured with 20 μL lysate of each sample and 100 μL of luciferase substrate (Promega) using a Lumat LB 9507 luminometer (Berthold). Luciferase activities are presented as diagrams with relative units compared to the value of plasmid DNA injection alone. Each reporter assay was carried out at least 4 times independently.

Statistical analysis

Statistical analysis was calculated using Graphpad Prism5 software. Data were presented as mean±SD. Differences between groups were determined by Student's *t*-test with significance at $P\leq0.05$ (* indicate $P<0.05$ and ** indicate $P<0.01$).

Results

Tcf7l1 promotes *Klf4* transcription in early *Xenopus* embryos

First, the effect of Tcf7l1 on *Klf4* transcription was examined through whole-mount *in situ* hybridization in early *Xenopus* embryos by loss of function and gain of function. During gastrulation, the expression level of Klf4 was very low in uninjected control embryos, while *Klf4* transcription level was dramatically upregulated in the animal pole and the lateral region in embryos injected with *Tcf7l1* mRNA (**Fig. 1A**, **C**). Conversely, when endogenous Tcf7l1 was knocked down by injecting Tcf7l1 MO, which was described previously[19], *Klf4* transcription was eventually diminished. At the tailbud stage, *Klf4* was specifically expressed in the cement gland. Embryos injected with Tcf7l1 MO showed a headless phenotype with no cement gland formation, and *Klf4* expression was severely downregulated (**Fig. 1B**, **C**). Our results showed that Tcf7l1 dramatically activated *Klf4* transcription. Loss of Tcf7l1 function leads to downregulation of *Klf4* in *Xenopus* embryos.

Second, in order to confirm the phenotype, *Klf4* expression in the whole embryos and animal caps was
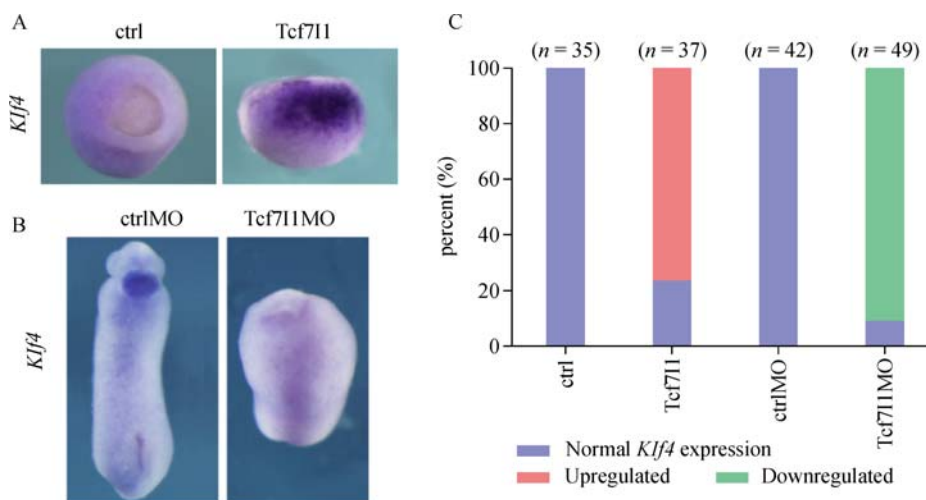

Fig. 1 Klf4 expression is upregulated by Tcf7l1. A: 300 pg of *Tcf7l1* mRNA was injected into 2 or 4-cell stage embryos. Then, the embryos were collected at stage 10.5 for whole-mount *in situ* hybridization by using *Klf4* probe. B: 2 or 4-cell stage embryos were injected with 40 ng ctrlMO or Tcf7l1MO and were collected at stage 28 to test *Klf4* expression. C: Quantification of embryos with normal or altered gene expression observed in A and B in two experiments.

tested by using qPCR. Overexpression of *Tcf7l1* led to significant upregulation of *Klf4* in both whole embryos and animal caps (**Fig. 2A**, **B**). In contrast, *Klf4* expression was dramatically reduced in embryos with knockdown of Tcf7l1 (**Fig. 2C**). The qPCR results confirmed that *Klf4* expression was indeed regulated by Tcf7l1.

Tcf7l1 stimulates *Klf4* promoter activity

To further study the regulatory mechanism of Tcf7l1 on *Klf4* transcription, we performed luciferase reporter assay. *Xenopus tropicalis Klf4* promoter sequence −2144/+70 was subcloned into PGL3-basic vector and the luciferase reporter, Klf4Luc (−2144/+70), was constructed. In embryos, Klf4Luc (−2144/+70) was significantly stimulated by overexpression of *Tcf7l1* because the luciferase activity was much higher in embryos injected with both the reporter plasmid and *Tcf7l1* mRNA than in embryos injected with the reporter alone (**Fig. 3A**). The stimulation was specific since the vector pGL3-basic, which was used for making the reporter construct, was not stimulated by overexpression of *Tcf7l1* (**Fig. 3B**). On the contrary, when Tcf7l1 MO was injected into the embryos, luciferase activity

was decreased appreciably (**Fig. 3C**). These series of experiments clearly demonstrated that Tcf7l1 stimulates *Klf4* promoter activity.

Tcf7l1 promotes *Klf4* transcription in the absence of protein translation

To further examine whether Tcf7l1 regulated *Klf4* transcription directly or not, we performed CHX treatment to block protein translation. Overexpression of *Tcf7l1* with or without CHX both showed dramatic upregulation of *Klf4* expression (**Fig. 4A**, **B**). The results suggested that even in the absence of protein translation, Tcf7l1 was still able to promote *Klf4* transcription. Furthermore, overexpression of a dominant negative form of Tcf7l1(dnTcf7l1), which does not contain the β-catenin binding motif, showed a similar effect on *Klf4* transcription suggesting that without β-catenin, Tcf7l1 could still activate *Klf4* expression.

Discussion

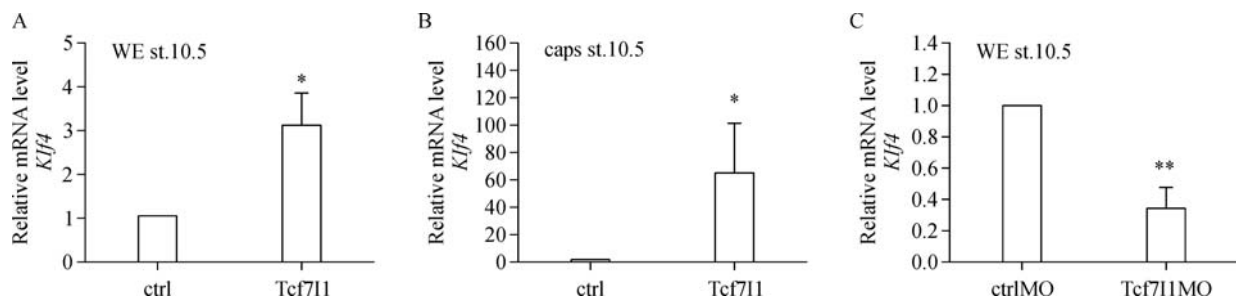Klf4 plays important roles not only in maintaining pluripotency of embryonic stem cell, but also in early

Fig. 2 **Tcf7l1 promotes *Klf4* transcription in both whole embryos and animal caps.** A: 300 pg of *Tcf7l1* mRNA was injected into 4-cell stage embryos, control and injected embryos were collected at stage 10.5. B: For animal cap assay, animal caps were cut at stage 8 and were collected until control embryos reached stage 10.5. C: ctrlMO and Tcf7l1 MO were injected at 40 ng, respectively, and embryos were collected at stage 10.5 for qPCR. Data were presented as mean±SD. Differences between groups were determined by Student's *t*-test. * indicates *P* < 0.05. ** indicates *P* < 0.01. qPCR was carried out in three experiments.
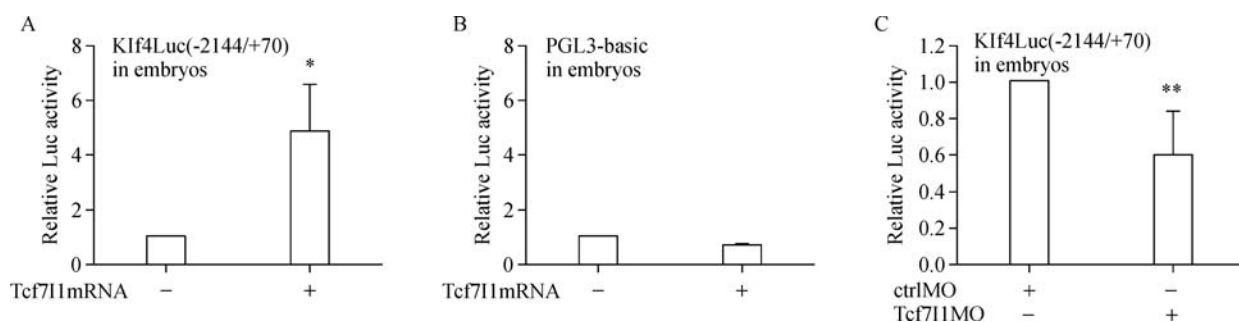

Fig. 3 **Tcf7l1 stimulates Klf4Luc(−2144/+70) activity.** A and B: 40 pg of Klf4Luc (−2144/+70) or PGL3-basic plasmid was injected alone or together with 300 pg *Tcf7l1* mRNA, embryos were collected at stage 10.5 for luciferase assay. C: 40 pg Klf4Luc (−2144/+70) was co-injected with 20 ng of ctrlMO or Tcf7l1MO, respectively, embryos were collected at stage 10.5 for luciferase assay. Luciferase reporter assay was carried out at 5 (A, B) or 4 (C) independent times. Data were presented as mean±SD. Differences between groups were determined by Student's *t*-test. * indicates *P* < 0.05. ** indicates *P* < 0.01.
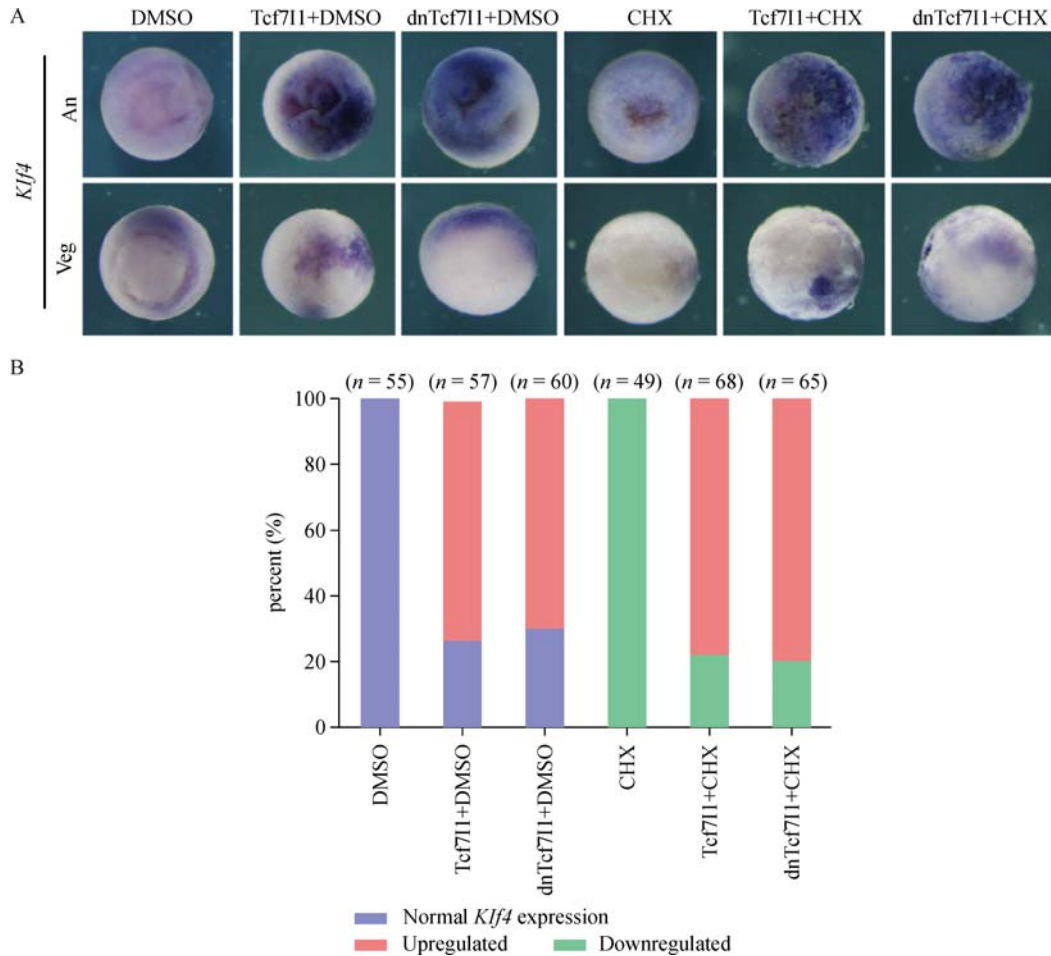
Fig. 4 Cycloheximide (CHX) treatment shows that *Klf4* expression is directly upregulated by Tcf7l1. A: *Klf4* expression is shown in response to injection of *Tcf7l1* mRNA or *dnTcf7l1* mRNA, to treatment with CHX, to injection of *Tcf7l1* mRNA or *dnTcf7l1* mRNA together with CHX treatment. Embryos were orientated to animal view (An) and vegetal view (Veg). *Tcf7l1* mRNA or *dnTcf7l1* mRNA was injected at 300 pg of each embryo. B: Quantification of embryos with normal or altered gene expression observed in three experiments.

embryogenesis. In our previous work, Klf4 could induce endoderm differentiation through regulating Nodal/activin signaling pathway[5]. Zhang *et al*. and Evans *et al*. have reported that Klf4 could interact with β-catenin and Tcf7l2 (formerly named TCF4) in color-ectal cancer cells and inhibit body axis formation in *Xenopus* embryos[30–31]. Feedback loops among differ-ent signals are frequently present during embryogenesis and are essential for maintaining the homeostasis of signals, so that they can function in the correct time, space and at correct levels. Since Klf4 has the ability to regulate Wnt and Nodal/activin signals[5,30–31], which play key roles in germ layer differentiation and body axis formation, we aim to know whether Nodal/activin or Wnt pathways could regulate *Klf4* transcription. So we examined the regulatory effect of the key nuclear signal transducers in Nodal/activin or Wnt signaling, especially FAST1 and TCF/LEF. Our results showed that overexpression of *FAST1* in embryos did not generate significant effect on *Klf4* transcription, as

revealed by whole-mount *in situ* hybridization (**Supple-mentary Fig. 1**, available online). Nevertheless, *Klf4* transcription level was augmented strongly in embryos injected with *Tcf7l1* mRNA.

In the present study, we focus on the regulatory effect of Tcf7l1 on *Klf4* expression. We found that *Klf4* expression is dramatically decreased in embryos with Tcf7l1 knockdown. On the contrary, overexpression of *Tcf7l1* in whole embryos and animal caps increased *Klf4* expression significantly by both qPCR and whole-mount *in situ* hybridization. These results suggest that Tcf7l1 could promote *Klf4* transcription during *Xenopus* embryogenesis. *Xenopus* germ layer formation is a zygotic event and does not begin until mid-blastula (stage 8.5), so zygotic gene transcription has not started at stage 7. To further study the effect of Tcf7l1 on *Klf4* is direct or not, we performed CHX treatment. Activation of *Klf4* was still observed when *Tcf7l1*-injected embryos were treated with CHX form stage 7 to stage 10.5. It is suggested that activation of *Klf4* by Tcf7l1 was a direct

effect and not through activation of other zygotic genes to regulate *Klf4* transcription. In addition, luciferase reporter assays showed that overexpression of *Tcf7l1* stimulates *Klf4* transcription. Based on our data, we have demonstrated that Wnt downstream transducer Tcf7l1 lies upstream of Klf4 and promotes *Klf4* transcription.

Tcf7l1 transduces Wnt signaling *via* interaction with β-catenin, but the regulatory effect seemed to be Wnt/β-catenin independent, because overexpression of β-catenin in embryos didn't change *Klf4* expression significantly (***Supplementary Fig. 2***, available online). Moreover, a dominant-negative form of Tcf7l1 (dnTcf7l1) that does not bind β-catenin showed the similar effect on *Klf4* transcription. This suggested that Tcf7l1 could activate *Klf4* transcription and the effect seems to be Wnt/β-catenin independent. However, the details of how Klf4 is regulated by Tcf7l1 are still unclear and need to be further explored. Embryogenesis is a process regulated *via* differentiation signals and the pluripotency factors, so our studies have provided a deeper understanding of the regulation of Klf4 *via* differentiation signals during early embryogenesis.

References

[1] Kim J, Chu J, Shen X, et al. An extended transcriptional network for pluripotency of embryonic stem cells[J]. *Cell*, 2008, 132(6): 1049–1061.

[2] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors[J]. *Cell*, 2006, 126(4): 663–676.

[3] Aksoy I, Giudice V, Delahaye E, et al. Klf4 and Klf5 differentially inhibit mesoderm and endoderm differentiation in embryonic stem cells[J]. *Nat Commun*, 2014, 5: 3719.

[4] Jiang J, Chan YS, Loh YH, et al. A core Klf circuitry regulates self-renewal of embryonic stem cells[J]. *Nat Cell Biol*, 2008, 10 (3): 353–360.

[5] Cao Q, Zhang X, Lu L, et al. Klf4 is required for germ-layer differentiation and body axis patterning during Xenopus embryogenesis[J]. *Development*, 2012, 139(21): 3950–3961.

[6] Cao Y. Regulation of germ layer formation by pluripotency factors during embryogenesis[J]. *Cell Biosci*, 2013, 3(1): 15.

[7] Tian C, Yao S, Liu L, et al. Klf4 inhibits tumor growth and metastasis by targeting microRNA-31 in human hepatocellular carcinoma[J]. *Int J Mol Med*, 2017, 39(1): 47–56.

[8] Fadous-Khalifé MC, Aloulou N, Jalbout M, et al. Krüppel-like factor 4: A new potential biomarker of lung cancer[J]. *Mol Clin Oncol*, 2016, 5(1): 35–40.

[9] Zhao Q, Cai W, Zhang X, et al. RYBP Expression is regulated by KLF4 and Sp1 and is related to hepatocellular carcinoma prognosis[J]. *J Biol Chem*, 2017, 292(6): 2143–2158.

[10] Yoon HS, Chen X, Yang VW. Kruppel-like factor 4 mediates p53-dependent G1/S cell cycle arrest in response to DNA damage[J]. *J Biol Chem*, 2003, 278(4): 2101–2105.

[11] Dang DT, Mahatan CS, Dang LH, et al. Expression of the gut-enriched Krüppel-like factor (Krüppel-like factor 4) gene in the human colon cancer cell line RKO is dependent on CDX2[J]. *Oncogene*, 2001, 20(35): 4884–4890.

[12] Chen ZY, Rex S, Tseng CC. Krüppel-like factor 4 is transactivated by butyrate in colon cancer cells[J]. *J Nutr*, 2004, 134(4): 792–798.

[13] Li J, Dong J, Zhang ZH, et al. miR-10a restores human mesenchymal stem cell differentiation by repressing KLF4[J]. *J Cell Physiol*, 2013, 228(12): 2324–2336.

[14] Lin CC, Liu LZ, Addison JB, et al. A KLF4-miRNA-206 autoregulatory feedback loop can promote or inhibit protein translation depending upon cell context[J]. *Mol Cell Biol*, 2011, 31(12): 2513–2527.

[15] Xu N, Papagiannakopoulos T, Pan G, et al. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells[J]. *Cell*, 2009, 137(4): 647–658.

[16] King KE, Iyemere VP, Weissberg PL, et al. Krüppel-like factor 4 (KLF4/GKLF) is a target of bone morphogenetic proteins and transforming growth factor beta 1 in the regulation of vascular smooth muscle cell phenotype[J]. *J Biol Chem*, 2003, 278(13): 11661–11669.

[17] Davis-Dusenbery BN, Chan MC, Reno KE, et al. down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-β and bone morpho-genetic protein 4[J]. *J Biol Chem*, 2011, 286(32): 28097–28110.

[18] Dang DT, Zhao W, Mahatan CS, et al. Opposing effects of Krüppel-like factor 4 (gut-enriched Krüppel-like factor) and Krüppel-like factor 5 (intestinal-enriched Krüppel-like factor) on the promoter of the Krüppel-like factor 4 gene[J]. *Nucleic Acids Res*, 2002, 30(13): 2736–2741.

[19] Liu F, van den Broek O, Destrée O, et al. Distinct roles for Xenopus Tcf/Lef genes in mediating specific responses to Wnt/β-catenin signalling in mesoderm development[J]. *Development*, 2005, 132(24): 5375–5385.

[20] Merrill BJ, Pasolli HA, Polak L, et al. Tcf3: a transcriptional

regulator of axis induction in the early embryo[J]. *Development*, 2004, 131(2): 263–274.

[21] Hoffman JA, Wu CI, Merrill BJ. Tcf7l1 prepares epiblast cells in the gastrulating mouse embryo for lineage specification[J]. *Development*, 2013, 140(8): 1665–1675.

[22] Sorrell MR, Dohn TE, D'Aniello E, et al. Tcf7l1 proteins cell autonomously restrict cardiomyocyte and promote endothelial specification in zebrafish[J]. *Dev Biol*, 2013, 380(2): 199–210.

[23] Shy BR, Wu CI, Khramtsova GF, et al. Regulation of Tcf7l1 DNA binding and protein stability as principal mechanisms of Wnt/β-catenin signaling[J]. *Cell Rep*, 2013, 4(1): 1–9.

[24] Molenaar M, van de Wetering M, Oosterwegel M, et al. XTcf-3 transcription factor mediates β-catenin-induced axis formation in Xenopus embryos[J]. *Cell*, 1996, 86(3): 391–399.

[25] Kim CH, Oda T, Itoh M, et al. Repressor activity of Headless/Tcf3 is essential for vertebrate head formation[J]. *Nature*, 2000, 407(6806): 913–916.

[26] Hikasa H, Ezan J, Itoh K, et al. Regulation of TCF3 by Wnt-dependent phosphorylation during vertebrate axis specification [J]. *Dev Cell*, 2010, 19(4): 521–532.

[27] Cole MF, Johnstone SE, Newman JJ, et al. Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells[J]. *Genes Dev*, 2008, 22(6): 746–755.

[28] Park MS, Kausar R, Kim MW, et al. Tcf7l1-mediated transcriptional regulation of Krüppel-like factor 4 gene[J]. *Anim Cells Syst*, 2015, 19: 16–29.

[29] Harland RM. Appendix G: In situ hybridization: an improved whole-mount method for Xenopus embryos[J]. *Methods Cell Biol*, 1991, 36: 685–695.

[30] Evans PM, Chen X, Zhang W, et al. KLF4 interacts with β-catenin/TCF4 and blocks p300/CBP recruitment by β-catenin [J]. *Mol Cell Biol*, 2010, 30(2): 372–381.

[31] Zhang W, Chen X, Kato Y, et al. Novel cross talk of Kruppel-like factor 4 and β-catenin regulates normal intestinal home-ostasis and tumor repression[J]. *Mol Cell Biol*, 2006, 26(6): 2055–2064.