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Oct4 was a novel target of Wnt signaling pathway

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Abstract The specific expression of Oct4 during early mouse development is required for the correct maintenance of pluripotent cells, and the regulatory control of the Oct4 expression is important. Wnt signaling could have multiple and/or complex effects on embryonic stem (ES) cells characteristics. Elucidation of the molecular mechanisms affecting Wnt signaling in ES cells could provide a better understanding of how these effects occur. The purpose of this study was to determine whether Oct4 was regulated by Wnt signaling in undifferentiated ES cells. Here, we report Oct4 as a novel target of β-catenin-mediated transcription. First, we observe that Wnt signaling pathway is activated in undifferentiated mouse ES cells. In 239T cells, Oct4 promoter was regulated by β-catenin. Through promoter mapping and chromatin immuno-precipitation assays, we found that Oct4 is a direct target of β-catenin/TCF-mediated transcription and the binding site at $-875/-881$ of Oct4 promoter is critical for b-catenin/TCF-dependent expression regulation. We further detect the expression of Oct4 in treatment with glycogen syntheses kinase (GSK)-3-specific inhibitor in mouse ES cells and HepG2 cells. We found that GSK-3-specific inhibitor can maintain the expression of Oct4 in ES cells and can enhance the expression of Oct4 in HepG2 cells. Our results suggest that Oct4 might be a novel target of β-catenin/TCF-mediated downstream gene in Wnt-activated cells.

Keywords Oct4 · β-Catenin · Stem cell

Introduction

The canonical Wnt/β-catenin signaling pathway is involved in tumor genesis in various tissues such as colon and liver as well as in embryogenesis [1]. In the absence of Wnt signaling, a multi-protein complex composed of glycogen syntheses kinase-3β (GSK-3β), Axin, and the tumor suppressor adenomatous polyposis coli (APC) phosphorylates β-catenin, leading to its ubiquitin-dependent degradation [2]. The Wnt signaling is activated by the binding of secreted Wnt ligands to the trans-membrane receptor frizzled (Fzd), which then blocks the function of this multi-protein complex and stabilizes β-catenin. The stabilized β-catenin is then able to translocate to the nucleus, form a complex with TCF/LEF, and induce expression of a variety of TCF/LEF target genes [3].

The activity of β-catenin/TCF is frequently deregulated, resulting in the suppression of genes whose expression has significant consequences on embryogenesis [4]. Therefore, identifying the target genes of Wnt signaling is important for understanding the β-catenin-mediated embryogenesis. It has been reported that activation of Wnt signaling by 6-bromoindirubin-3′-oxime (BIO), a pharmacological GSK-3-specific inhibitor can maintain the pluripotency in human and mouse embryonic stem (ES) cells, but little of

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the maintenance mechanism has been revealed [5]. Similarly, activation of the Wnt pathway by mutations affecting the APC protein inhibits in vitro mESC differentiation and neural differentiation in teratoma assays [6]. In addition, treatment with Wnts provided a transient stimulation of human ESC proliferation [7]. These observations has suggested that Wnt signaling could have multiple and/or complex effects on ES cells characteristics and that elucidation of the molecular mechanisms affecting Wnt signaling in ES cells could provide a better understanding of how these effects occur. It remains unknown whether the Wnt signaling pathway functionally affects the important transcription factors such as Nanog, Oct4, and Sox2 in regulating stem cell self-renewal.

Oct4 is a member of the Pit, Oct, and Unc family of transcription factors. When the embryos develop into the blastocyst, the trophectoderm is formed in the outer layer of the embryos as the first cell lineage specification [8]. In vitro, Oct4 is expressed in undifferentiated ES and embryonal carcinoma cells and is down-regulated when these cells are induced to differentiate by retinoic acid (RA) treatment or the removal of leukemia inhibitory factor [9, 10]. These remarkable expression patterns of Oct4 during early mouse development and the undifferentiated cell lines suggest that Oct4 plays an important role in maintaining pluripotency of cells.

Because the specific expression of Oct4 during early mouse development is required for the correct maintenance of pluripotent cells, the regulatory control of the Oct4 expression has been studied extensively. The purpose of this study was to determine whether Oct4 was regulated by Wnt signaling in undifferentiated ES cells. A number of β-catenin/TCF target genes have been reported, which play critical roles in carcinogenesis by affecting cell growth and cell cycling (c-MYC, CCND1, c-Jun, Fra-1, Gastrin, WISP-1, and ITF-2), cell survival (Id2, MDR1, and COX2), and invasion and tumor dissemination (MMP7, LAMC2, and VEGF). Most of these genes have one or more TCF/LEF-binding elements in their promoter which are composed of a highly conserved consensus sequence 5′-(A/T)(A/T)CAA(A/T)G-3′ [11]. In this study, we constructed 2.2 kbp of the mOct4 5′ upstream region and identified a *cis*-regulatory element that may be an important determinant for the transcriptional activity of the mOct4 promoter. The consensus sequence 5′-CTTTGAA-3′ is important to the activation of mOct4 by stable forms of β-catenin (β-catenin S37/A and ΔN-cat). Our studies also showed that LEF1 may be a co-activator of β-catenin in regulating the expression of Oct4. These observations suggested that Wnt signaling could maintain the expression of Oct4 in undifferentiated mouse ES cells. And it also provided a better understanding of the molecular mechanisms affecting Wnt signaling in ES cells.

Experimental procedures

Cell culture and transfection

NIH3T3 and 293T cells were cultured in Dulbecco's modified Eagles medium (Invitrogen, CA) supplemented with 10% fetal bovine serum (Hyclone, UT) and 100 μg/ml antibiotics (penicillin and streptomycin, Invitrogen, CA). CGR8 ES cells were cultured on 0.1% gelatin-coated substrates in ES medium consisting of GMEM (Sigma, MO) supplemented with 20% ES-qualified fetal bovine serum (Hyclone, UT), 100 mM non-essential aminoacids (Invitrogen, CA), 10^{-4} β-mercaptoethanol (Sigma, St. Louis, MO), 0.224 μg/ml L-glutamine (Invitrogen, CA), and 1,000 units ml^{-1} human recombinant LIF (CHEMICON, CA). 293T cells were transfected by calcium phosphate co-precipitation methods. NIH3T3 and CGR8 ES cells were transfected by lipofectamine 2000 (Invitrogen, CA).

Plasmid construction and reporter assays

Mouse Oct4 promoter fragment was amplified by PCR from the mouse liver genome DNA and inserted to the *Sma*I site of a promoter less luciferase reporter vector pGL3-Basic (Promega, Madison, WI). The primers for Oct4p were: forward: 5′-acaggactgctgggctgcag-3′; reverse: 5′-gtggaaagacggctcacct-3′. The primer for deletion was 5′-ggc actgttccacaatgaatg-3′. The LEF reporter, β-catenin S37A, ΔN-catenin, TCF3, and TCF4 were kindly provided by Dr. Yeguang Chen. LEF1 and CatC-LEF1 were provided by Dr. Rudolf Grosschedl. For reporter assays, transfection efficiencies were normalized with *Renilla* plasmid as internal references, and DNA concentrations were kept constant with empty expression vector. Cells were harvested 48 h after transfection, and luciferase activity was measured by using Dual-luciferase (Promega, WI).

Cell staining

For cell immunostaining, cells grown on coverslips were fixed with 2% paraformaldehyde in PBS, washed, blocked in 10% normal goat serum, and then stained with first antibodies including: mouse β-catenin antibody (Santa Cruz, SC-59737, CA), mouse Oct4 antibody (Santa Cruz, SC-5279, CA), Secondary antibody is goat anti-mouse TRITC (Southern Biotech). The images were captured using the Olympus FV500 system.

Chromatin immunoprecipitation assay

For Chip assays, CGR8 cells were cultured in 10 cm dish. The cells were washed by PBS and then incubated in 1% formaldehyde of PBS for 10. Then 0.125 M glycine was

added to stop the reaction. The cells were then washed twice with PBS and collected in 0.5 ml PBS and 1× protease inhibitor. After the centrifugation (2,000 rpm, 2 min, 4°C), the cells were lysed in 0.6-ml Chip sonication buffer (1% Triton X-100, 0.1% Deoxycholate, 50 mM Tris 8.1, 150 mM NaCl, 5 mM EDTA, and 1× protease inhibitor) and sonicated to an average fragment length of 600 bp. The complexes were absorbed in 40 µl protein A/G Plus-Agarose (Santa Cruz, CA) and 20 µg Herring Sperm DNA (Promega) per sample and incubation for 1–2 h at 4°C. The complexes were eluted by 500 µl elution buffer. Then the cross-links were reversed by adding 20 µl NaCl per. After digestion with proteinase K for 1 h at 55°C, DNAs were extracted and precipitated before being analyzed by PCR using the primers: Oct-4 chip F1: 5′-gtggggaggttgtagcccgaccctgc-3′; Oct-4 chip R1: 5′-tggaagacctaaataaaggcagcgac-3′; Oct-4 chip F2: 5′-cacatgtgctatgtgtagctgtgtgt-3′; Oct-4 chip R2: 5′-cagtggctctaggtgccctgggggc-3′; Oct-4 chip F3: 5′-ctctggggaccaggattgtccagcca-3′; Oct-4 chip R3: 5′-aggccggggcctctttcctcaagatt-3′.

Reverse-transcriptase (RT)-PCR

Cytoplasmic RNAs were extracted from lysates of cells treated with TRIzol reagent (Invitrogen, CA) and quantified by spectrophotometry. First strand cDNA was synthesized using reverse transcriptionase from Invitrogen (CA). PCR reactions were then performed to amplify the intended genes using conditions such as 95°C/3 min, 94°C/30 s, 58°C/40 s, 72°C/45 s for 25–32 cycles.

Real-time RT-PCR analysis

Two microgram of total RNA was reverse transcribed in a final volume of 20 µl as previously described. PCR reactions were undertaken using the Realtime PCR Master Mix (SYBR GREEN) Reagent Kit (TOYOBO), according to the manufacturer's protocol. PCR was running in 15-µl total volume for 45 cycles. Primer sequences were:

Actin F: 5′-AGTGTGACGTTGACATCCGT-3′;

Actin R: 5′-TGCTAGGAGCCAGAGCAGTA-3′;

Mus Frizzled 1 F: 5′-GCGCCTCTCTTCGTTTATCTGT-3′;

Mus Frizzled 1 R: 5′-TCTCTGTCTTGGTGCCGTCAT-3′;

Mus Frizzled 4 F: 5′-CAGAACGACCACAACCACATG-3′;

Mus Frizzled 4 R: 5′-CCCACGGAGTGGCACTCTT-3′;

Mus Frizzled 5 F: 5′-TGGATTGGCCTGTGGTCTGT-3′;

Mus Frizzled 5 R: 5′-GCACCAAGAATCCCAGTGACA-3′;

Mus Frizzled 6 F: 5′-CCTCTTAGCCGGCATCATCTC-3′;

Mus Frizzled 6 R: 5′-TGGTTCCGGCCATCATG-3′;

Mus Frizzled 7 F: 5′-CCAGGTGGATGGTGACCTAC-3′;

Mus Frizzled 7 R: 5′-GACGTCCCGATGAAGAGGTA-3′.

Results

Wnt signaling pathway is activated in undifferentiated mouse ES cells

The reception and transduction of Wnt signal involves two families of cell surface receptors. One is Fzd receptor gene family; the other is LRP receptor gene family. The expression of Fzd receptors is tissue-specific [12]. The expression level of Fzd receptors is a marker of the response ability of a cell line to Wnt signal. By RT-PCR, we compared the expression of nine members of the Fzd family (Fzd 1–9) in ES and in mouse fibroblast (NIH3T3) cells. We detected the expression of Fzd 1, 4, 5, 6, and 7 in mouse ES cells, but could not detect any of them in NIH3T3. We further use real-time PCR to prove that the expression of these genes in ES cells is much higher than in NIH3T3 cells, especially the expression of Fzd 1 whose expression in ES cells is 300-folds higher than NIH3T3 cells (Fig. 1B). As reported before, Fzd 1 is a main receptor whose role in Wnt signaling pathway was clearly elucidated. The tissue-specific expression of Wnt receptors implied that Wnt signaling pathway might be functional in ES cells.

β-Catenin was reported as a member of adhesion junction. It was later discovered as a key element which has regulative activity in Wnt signaling pathway. β-Catenin can activate the transcription of target genes by binding another component in Wnt pathway—TCF/LEF1 [13]. The amount and localization of β-catenin can reflect the activation status of classic Wnt pathway. The immunostaining of β-catenin showed that β-catenin accumulated in both nuclear and cytoplasmic regions in undifferentiated ES cells. However, in differentiated ES cells, the immunostaining of β-catenin spread beyond the nuclear and cytoplasmic regions and into the membrane region (Fig. 1C). The activation level of Wnt reporter gene can also reflect the activation status of this signal pathway. Figure 1D showed that LEF1 reporter gene had different activity levels in ES and NIH3T3 cells, and its activity in ES cells was obviously decreased after differentiation.

Regulation of Oct4 promoter by β-catenin

As is shown in Fig. 3a, we found three potential TCF/LEF-binding sites in the 2.2 kb promoter upstream of Oct4 gene.

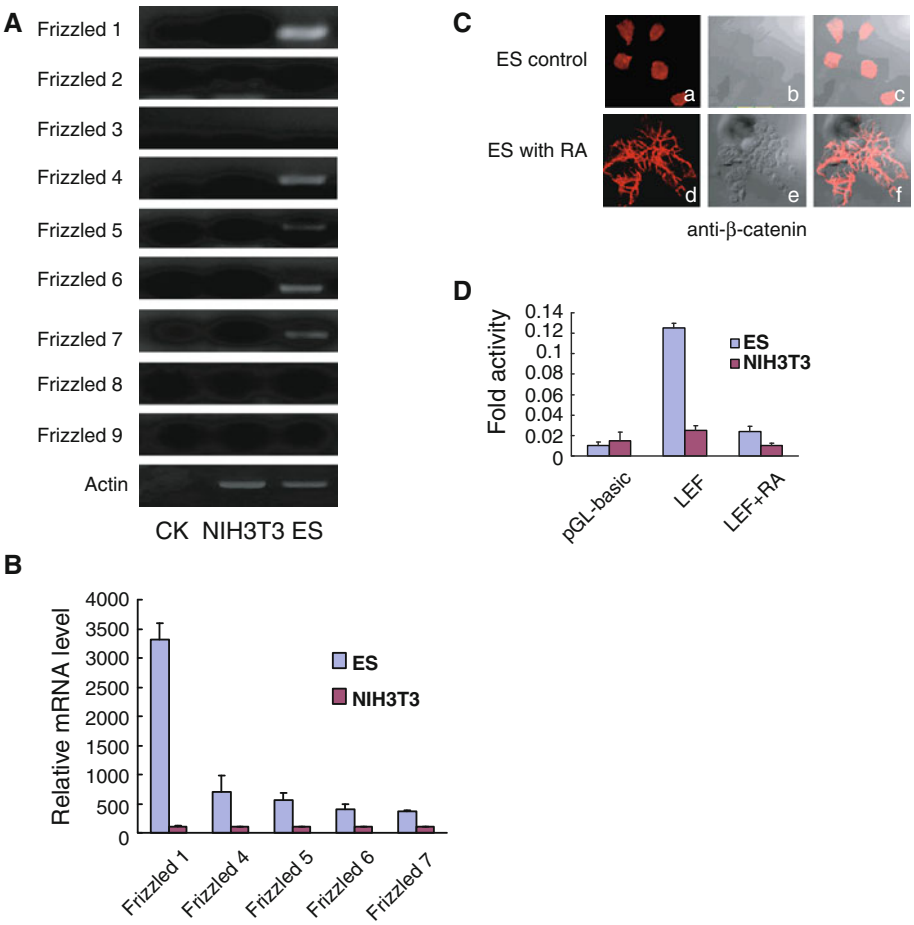

Fig. 1 Wnt signaling pathway is activated in undifferentiated mouse ES cells. **A** RT-PCR analysis of Fzd receptors in NIH3T3 and mouse ES cells. **B** Real-time PCR analysis of Fzd 1, 4, 5, 6, 7 in NIH3T3 and mouse ES cells. All samples are normalized to β-actin. Data are the average of three assays, *bars* represent mean ± sem. **C** Confocal images of mouse ES cells grown under the indicated conditions and incubated with β-catenin-specific antibody. *RA* retinoic acid. **D** Luciferase reporter assay in NIH3T3 and mouse ES cells. Cells were transfected with Wnt reporter LEF plasmid constructs and treated with RA. pGL-basic was used as control. Data are the average of three assays, *bars* represent mean ± sem

We used PCR technique to amplify this fragment from mouse genome DNA and cloned it into pGL-basic vector containing a luciferase gene (Fig. 2a). Figure 2b showed that the activity of this promoter could reflect the expression pattern of endogenous Oct4, its activity was high in ES and F9 cells, while low in NIH3T3 cells.

β-Catenin has 130 amino acids in its N-terminus, which is rich in Ser/Thr residues and controls its stability. Its C-terminus is composed of 100 amino acids, responsible for the activation of target genes. The most important structure of β-catenin is its 12 Arm repeats, which form a bar-shape supercoiled structure to prevent the protein from degradation, playing an important role in its binding with other proteins like cadherin, APC, and TCF/LEF1 [14]. Wild-type β-catenin is inappropriate for transfection because of its easy degradation. We used a β-catenin mutant, S37/A, which can stably exist in cells. Figure 2c showed that with the increase of β-catenin S37/A in 293T cells, the activity of Oct4 promoter increased.

We further searched possible co-factors of β-catenin in the regulation of Oct4 promoter. Co-transfection experiments showed that LEF1 had a synergistic effect with β-catenin in the activation of Oct4 promoter (Fig. 2d). When transfected alone, β-catenin activated Oct4 promoter by five-fold while LEF1 activated the same by eight-fold. When the two were co-transfected, the activity of Oct4 promoter could be activated 43-fold. Compared to LEF1, neither TCF3 nor TCF4 had obvious effects on Oct4 promoter. Therefore, we propose that the transcriptional factor which plays a role in Oct4 regulation by Wnt is LEF1 rather than TCF3 or TCF4.

We further did activation experiments of Oct4 promoter by other forms of β-catenin and LEF1. ΔN-cat is the N-terminals deletion mutant of wild-type β-catenin, while catC-LEF1 is the fusion protein of the C-terminus of wild-type β-catenin and full-length LEF1. Figure 2f showed that either ΔN-cat alone or its combination with LEF1 could activate Oct4 promoter to a high level. The fusion protein catC-LEF1 can enhance the activity to 50-folds. Those results proved our hypothesis that β-catenin and LEF1 co-regulate Oct4 promoter.

Localization of Wnt responsible element between −881/−875 in Oct4 promoter

Oct4 promoter has three TCF/LEF-binding elements which are composed of a highly conserved consensus sequence 5′-(A/T)(A/T)CAA(A/T)G-3′. To localize the DNA sequence

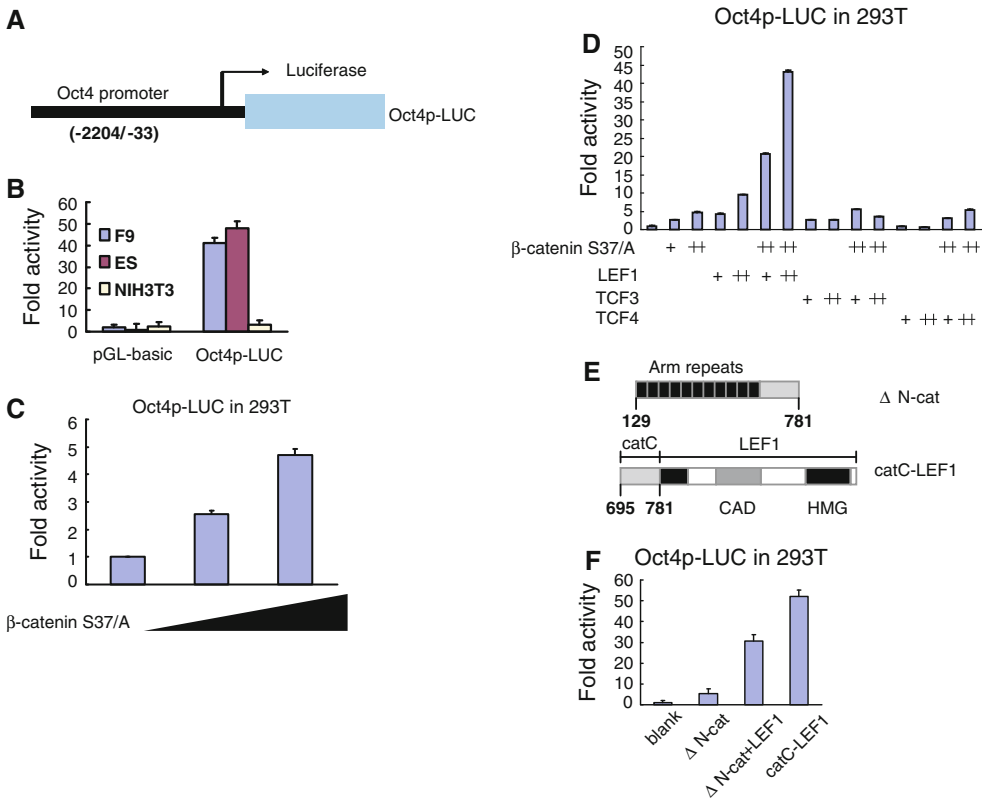

Fig. 2 Regulation of Oct4 promoter by β-catenin. **a** 5′-Flanking sequence from −2,204/−33 of mouse Oct4 gene was constructed to drive luciferase gene expression. **b** Activity of Oct4 promoter in NIH3T3, F9 and mouse ES cells. pGL-basic was used as control. **c** Dose-dependent response of Oct4 promoter to activated β-catenin. Oct4 promoter was co-transfected with increasing amount of β-catenin to 293T cells, and the luciferase activity was measured from cell lysate. **d** Oct4 promoter and β-catenin were Co-transfected with LEF1, TCF3 and TCF4 to 293T cells. The luciferase activity was measured from cell lysate. **e** Schematic presentation of ΔN-cat protein and catC-LEF1. **f** Oct4 promoter was co-transfected with ΔN-cat, ΔN-cat and LEF1, catC-LEF1 to 293T cells. All data from Fig. 2b–f are the average of three assays, *bars* represent mean ± sem

that is responsive to Wnt mediated activation, we generated a promoter deletion construct as shown in Fig. 3a. To further identify the position of the response element, we did a deletion which only kept the binding site between −875 and −881. The results of transient transfections are shown in Fig. 3b, in which β-catenin still activate the deletion of Oct4 promoter in a similar manner as the wild-type promoter.

Comparative sequence analysis showed that this potential TCF-LEF site between −875 and −881 of mouse Oct4 promoter was conservative among human, rat, bovine, and mouse (Fig. 3c). Our results showed that β-catenin may bind to the Oct4 promoter at the −875/−881 position.

To confirm the binding of Oct4 promoter by β-catenin in ES cells, we performed a CHIP assay in ES cells. We designed three pairs of PCR primers separately at the three potential sites (Fig. 3d). The result was shown in Fig. 3e. The three fragments could be detected in Input, verifying the quality of samples before immuno-precipitation. Using PCR to test samples after immuno-precipitation, we found that fragments Oct4 promoter could only be amplified from DNA added β-catenin antibodies, not IgG antibodies. Only the third pair of primers could amplify the fragment, showing that β-catenin could bind specifically and directly to the −881/875 position of mouse Oct4 promoter.

The expression of Oct4 was maintained by a GSK-3-specific inhibitor in mouse ES cells

We have previously showed that the Oct4 promoter was regulated by β-catenin in 293T cells, but we do not know if endogenous Oct4 can be sustained by Wnt signaling pathway. BIO, initially derived from Tyrian purple are selective and potent inhibitors of GSK-3 which can function well in ES cells [5]. First, we monitored the activity of Oct4 promoter in mouse ES cells. Compared with LIF-treated cell, the activity of Oct4 promoter was slightly upregulated in BIO-treated cells. Transient transfection with β-catenin, the activity of Oct4 promoter was also upregulated compared with none LIF-treated cells (Fig. 4A).

Subsequently, we compared the expression of Oct4 in mouse ES cells between none LIF-treated cell and BIO-treated cell. As shown in Fig. 4B, the expression of

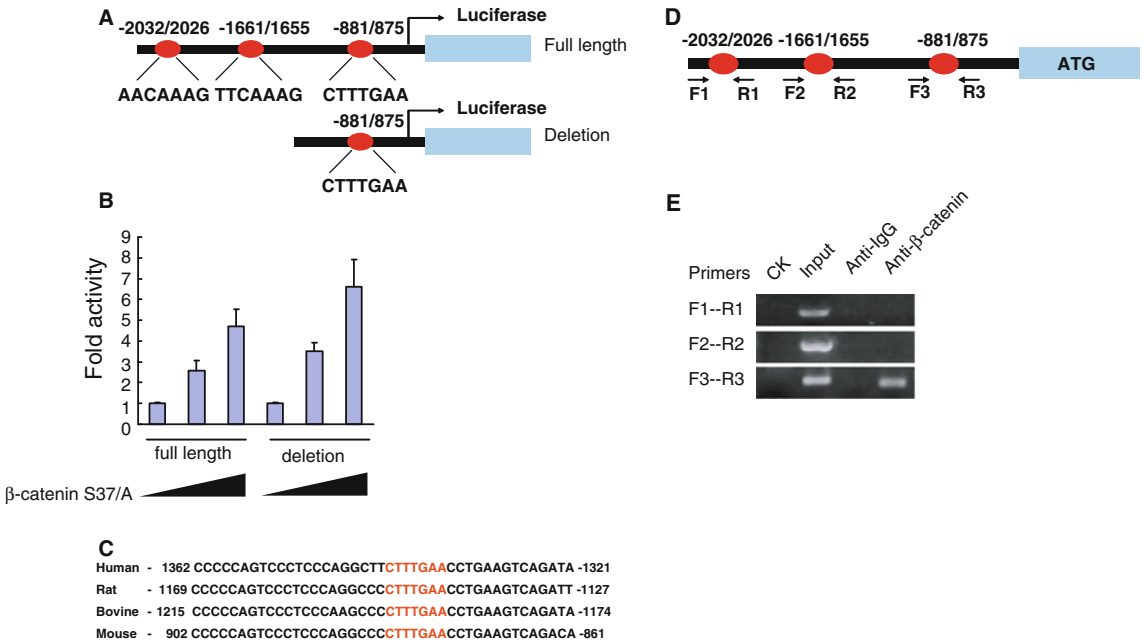

Fig. 3 Localization of Wnt responsible element between −875 and −881 in Oct4 promoter. **a** Schematic presentation of full-length Oct4 promoter which contains three potential binding site and deletion Oct4 promoter which contains one potential binding site. **b** Dose-dependent response of full length and deletion Oct4 promoter to activated *β*-catenin. Full length and deletion Oct4 promoter were co-transfected with increasing amount of *β*-catenin to 293T cells. Data are the average of three assays, *bars* represent mean ± sem. **c** Sequence comparison of Oct4 promoter sequence in four species. Location in mouse Oct4 promoter −881/−875 was highlighted. **d** Schematic presentation of three potential binding sites in Oct4 promoter. *Arrows* indicate the positions of primers used for chromatin immunoprecipitation assay. **e** Chromatin immunoprecipitation (ChIP) assay for the binding of *β*-catenin to Oct4 promoter. Anti-IgG was used as negative control

Oct4 decreased gradually after withdrawing of LIF in cell culture, but in BIO-treated cell, the expression of Oct4 was still at the same level compared with the day 0 cells. We also observed the expression of Oct4 in Wnt signal activated HepG2 cells. With the treatment of BIO, the expression of Oct4 was increased.

At last, LIF-treated cell, none LIF-treated cell and BIO-treated cell were immuno-stained with Oct4 antibody. Compared with LIF-treated cell, BIO-treated cell maintained normal Oct4 expression and normal ES morphology. On the contrary, none LIF-treated cell had very weak Oct4 signal and with a significant differentiated morphology (Fig. 4C).

Discussion

Stem cells have great application potential in the future of medicine based on their abilities to regenerate virtually all organs and tissues. However, the cellular and molecular mechanism controlling stem cell pluripotency remains largely unknown. Oct4 protein has attracted considerable attention as a key regulator of stem cell pluripotency and cell differentiation [15]. Oct4 functions to maintain the inner cell mass/epiblast lineage and establish the extraembryonic ectoderm through fgf4 expression [16, 17]. As a transcription factor, Oct4 has been shown to activate the expression of several downstream genes involved in cell proliferation and differentiation [18, 19]. Despite the fact that a regulative network is discovered among transcriptional factors in ES cells, Oct4, Sox2, and Nanog, the manner in which Oct4 is regulated by other signaling pathways remains unclear [20]. Therefore, understanding the regulative mechanisms of Oct4 by other upstream elements can contribute a lot to the study of pluripotency of ES cells.

Wnt signaling pathway plays an important role in embryogenesis, cell differentiation, and morphogenesis of tissues and cells [21]. It is reported that a specific inhibitor of GSK-3 can maintain the pluripotency in mouse and human ES cells, but the underlying mechanism needs further studies [5]. Similarly, another report shows that the mutation of APC can inhibit the differentiation of mouse ES cells by activating Wnt pathway [6]. Those studies imply the multi-effect of Wnt pathway in the maintenance of ES cells properties. However, we are still unclear about whether Wnt pathway can directly affect transcription factors specifically highly expressed in ES cells, such as Nanog, Oct4 and Sox2.

Both Oct4 and Wnt pathway play important roles in maintaining the pluripotency of ES cells, but their

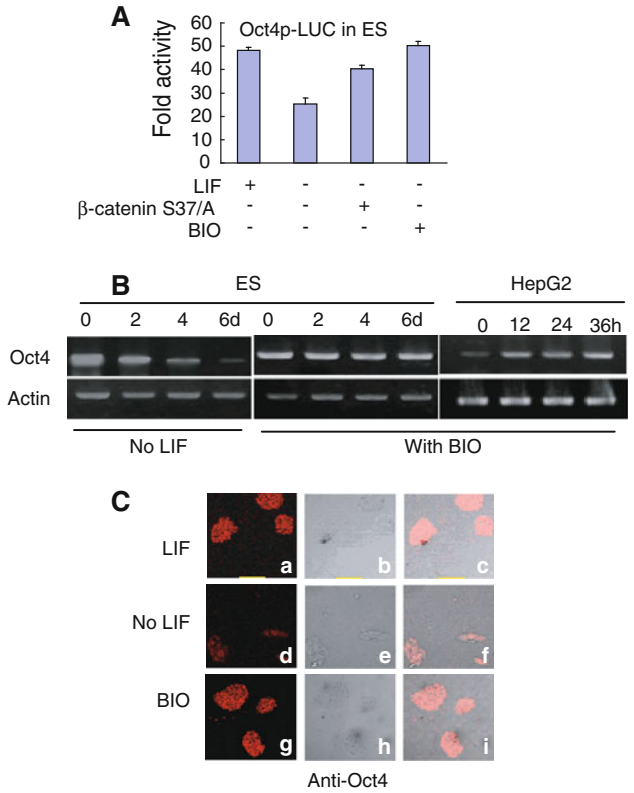

Fig. 4 The expression of Oct4 was maintained by a GSK-3-specific inhibitor. **A** The activity of Oct4 promoter in ES cells treated with BIO and co-transfected with β-catenin. Data are the average of three assays, *bars* represent mean ± sem. **B** Effect of BIO on the expression of Oct4. BIO-treated ES cells and HepG2 cells were harvested at indicated time points and analyzed by RT-PCR. Actin was used as control. **C** Immuno-staining of Oct4 in ES cells. LIF-treated cell, none LIF-treated cell, and BIO-treated cell were incubated with Oct4 antibody. Compared with LIF-treated cell, BIO-treated cell maintained normal Oct4 expression and normal ES morphology. None LIF-treated cell had very weak Oct4 signal and with a significant differentiated morphology

mechanisms need further study. However, Wnt pathway gives us some hints on the role played by Oct4. APC, a part of an anti-oncogene, affects the relationship between Wnt receptors and β-catenin in the nucleus. By measuring changes of β-catenin activity, we defined APC mutations of different levels and found that they have different effects on cell differentiation [6]. In a similar way, different expression levels of Oct4 have different effects on ES cell differentiation [22]. Therefore, Wnt pathway and Oct4 are possibly connected in determining the cell fate in the differentiation and developmental processes.

Our research results suggest that 2.2 kb of Oct4 promoter can activate the specific expression of Oct4. The activity of Oct4 is regulated by Wnt pathway. We identified a conserved TCF/LEF element at the position of −875/−881 upstream. Our research proves that the coactivator of β-catenin on Oct4 promoter is LEF1, which is different from the coactivator of β-catenin in regulating Nanog, TCF3. This shows the diversity and complexity of downstream genes in Wnt pathway. On a cellular level, we further proved that GSK-3 inhibitor can sustain the activity of Oct4 promoter, providing us with more information in understanding how Wnt pathway maintains the pluripotency of ES cells.

Oct4 is known to interact with other transcription factors to activate and repress gene expression in mouse ES [23]. For example, it can heterodimerize with the HMG-box transcription factor, Sox2, to affect the expression of several genes in mouse ES cells [24, 25]. Oct4, Sox2, and Nanog are thought to be central to the transcriptional regulatory hierarchy specifying ES cell identity because of their unique expression patterns and their essential roles during early development [8, 26, 27]. By showing the activation of Oct4 by Wnt pathway in our article, we provide new insight into the underlying mechanism in which Wnt pathway maintains the pluripotency of mouse ES cells. Further studies are still required to illustrate the network of mechanisms Wnt pathway uses to maintain the pluripotent state of mouse ES cells.

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References

1. Cadigan KM, Nusse R (1997) Wnt signaling: a common theme in animal development. Genes Dev 11:3286–3305
2. Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA (2001) Novel mechanism of Wnt signaling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol 3:683–686
3. Aulehla A, Wehrle C, Brand-Saberi B, Kemler R, Gossler A, Kanzler B, Herrmann BG (2003) Wnt3a plays a major role in the segmentation clock controlling somatogenesis. Dev Cell 4:395–406
4. Aubert J, Dunstan H, Chambers I, Smith A (2002) Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. Nat Biotechnol 20:1240–1245
5. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH (2004) Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med 10:55–63
6. Kielman MF, Rindapaa M, Gaspar C, van Poppel N, Breukel C, van Leeuwen S, Taketo MM, Roberts S, Smits R, Fodde R (2002) Apc modulates embryonic stem-cell differentiation by controlling the dosage of beta-catenin signaling. Nat Genet 32:594–605
7. Dravid G, Ye Z, Hammond H, Chen G, Pyle A, Donovan P, Yu X, Cheng L (2005) Defining the role of Wnt/beta-catenin signaling in the survival, proliferation, and self-renewal of human embryonic stem cells. Stem Cells 23:1489–1501
8. Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P (1990) New type of POU domain in germ line-specific protein Oct-4. Nature 344:435–439
9. Brehm A, Ohbo K, Scholer HR (1997) The carboxy-terminal transactivation domain of Oct-4 acquires cell specificity through the POU domain. Mol Cell Biol 17:154–162

10. Scholer HR, Dressler GR, Balling R, Rohdewohld H, Gruss P (1990) Oct-4: a germline-specific transcription factor mapping to the mouse t-complex. EMBO J 9:2185–2195
11. Roose J, Clevers H (1999) TCF transcription factors: molecular switches in carcinogenesis. Biochim Biophys Acta 1424:M23–M37
12. Huang HC, Klein PS (2004) The Frizzled family: receptors for multiple signal transduction pathways. Genome Biol 5:234
13. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R (1997) Beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J 16:3797–3804
14. Kang DE, Soriano S, Xia X, Eberhart CG, De Strooper B, Zheng H, Koo EH (2002) Presenilin couples the paired phosphorylation of beta-catenin independent of axin: implications for beta-catenin activation in tumorigenesis. Cell 110:751–762
15. Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Scholer HR, Smith A (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 95:379–391
16. Ambrosetti DC, Scholer HR, Dailey L, Basilico C (2000) Modulation of the activity of multiple transcriptional activation domains by the DNA binding domains mediates the synergistic action of Sox2 and Oct-3 on the fibroblast growth factor-4 enhancer. J Biol Chem 275:23387–23397
17. Yuan H, Corbi N, Basilico C, Dailey L (1995) Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. Genes Dev 9:2635–2645
18. Gu P, Goodwin B, Chung AC, Xu X, Wheeler DA, Price RR, Galardi C, Peng L, Latour AM, Koller BH, Gossen J, Kliewer SA, Cooney AJ (2005) Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development. Mol Cell Biol 25:3492–3505
19. Chew JL, Loh YH, Zhang W, Chen X, Tam WL, Yeap LS, Li P, Ang YS, Lim B, Robson P, Ng HH (2005) Reciprocal transcriptional regulation of Pou5f1 and Sox2 via the Oct4/Sox2 complex in embryonic stem cells. Mol Cell Biol 25:6031–6046
20. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122:947–956
21. Garcia-Castro MI, Marcelle C, Bronner-Fraser M (2002) Ectodermal Wnt function as a neural crest inducer. Science 297:848–851
22. Niwa H, Miyazaki J, Smith AG (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 24:372–376
23. Pesce M, Scholer HR (2001) Oct-4: gatekeeper in the beginnings of mammalian development. Stem Cells 19:271–278
24. Botquin V, Hess H, Fuhrmann G, Anastassiadis C, Gross MK, Vriend G, Scholer HR (1998) New POU dimer configuration mediates antagonistic control of an osteopontin preimplantation enhancer by Oct-4 and Sox-2. Genes Dev 12:2073–2090
25. Nishimoto M, Fukushima A, Okuda A, Muramatsu M (1999) The gene for the embryonic stem cell coactivator UTF1 carries a regulatory element which selectively interacts with a complex composed of Oct-3/4 and Sox-2. Mol Cell Biol 19:5453–5465
26. Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R (2003) Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev 17:126–140
27. Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, Smith A (2003) Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell 113:643–655