Developmental Biology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

From the Society for Developmental Biology

Cdx2 represses Oct4 function via inducing its proteasome-dependent degradation in early porcine embryos

Gerelchimeg Bou [1], Shichao Liu [1], Jia Guo, Yueming Zhao, Mingju Sun, Binghua Xue, Jiaqiang Wang, Yanchang Wei, Qingran Kong, Zhonghua Liu *

College of Life Science, Northeast Agricultural University, Harbin 150030, China

ARTICLE INFO

Article history:
Received 25 November 2015
Received in revised form
17 December 2015
Accepted 18 December 2015

Keywords:
Pig
Early embryo
Cdx2
Oct4
Molecular interaction
Proteasomal degradation

ABSTRACT

Reciprocal repression of inner cell mass specific factor OCT4 and trophectoderm specific factor CDX2 promotes mouse first lineage segregation. Studies in mouse embryonic stem (ES) cells revealed that they bind to each other's regulatory regions to reciprocally suppress transcription, additionally they form protein complex for mutual antagonism. However, so far the molecular interaction of Oct4 and Cdx2 in other mammal's early embryo is not yet investigated. Here, over-expression of Cdx2 in early porcine embryo showed CDX2 represses Oct4 through neither the transcriptional repression nor forming repressive complex, but promoting OCT4 nuclear export and proteasomal degradation. The results showed novel molecular regulation of CDX2 on Oct4, and provided important clues for clarifying the mechanism of interaction between CDX2 and Oct4 in embryo of mammals other than mouse.

1. Introduction

POU family transcription factor OCT4 (encoded by Pou5f1) and the homeodomain transcription factor CDX2 separately play core roles in regulating inner cell mass (ICM) and trophectoderm (TE) commitment. In blastocysts of mammals including mouse, human, cattle and pig, Cdx2 is specifically expressed in the TE, while Oct4 is expressed in both ICM and TE for a short or long, species-specific period and finally restricted to the ICM (Hall et al., 2009; Kirchhof et al., 2000; Kuijk et al., 2008; Szczepanska et al., 2011; Yeom et al., 1996). Oct4 or Cdx2 null mouse embryos die around implantation, respectively due to dysfunctional ICM and TE (Blij et al., 2012; Le Bin et al., 2014; Nichols et al., 1998; Strumpf et al., 2005; Wu et al., 2013). *In vitro*, Oct4 over-expression represses Cdx2 transcription in trophoblast stem (TS) cells and convert TS into embryonic stem (ES) cells (Wu et al., 2011), while Cdx2 over-expression (Niwa et al., 2005; Tolkunova et al., 2006), like Oct4 knockdown (Niwa et al., 2000; Velkey and O'Shea, 2003), represses Oct4 transcription in ES cells and induces its differentiation into TS cells.

Mutual antagonism between these two factors has been investigated in mouse ES cells ectopically expressing Cdx2 (Niwa et al., 2005). In this study, the authors drew three main conclusions: first, Oct4 and Cdx2 play decisive roles in the cell fate conversion between ES and TS/TE cells; second, Cdx2 and Oct4 reciprocally represses each other's gene transcription; third, CDX2 and OCT4 form a repressor complex that reciprocally represses their target genes in ES cells. Thus, they proposed that reciprocal inhibition between Cdx2 and Oct4 should contribute to the first differentiation event of mammalian embryonic development, due to the co-expression of Cdx2 and Oct4 prior to blastocyst formation (Dietrich and Hiiragi, 2007), and their eventual specification in different domains, with CDX2 in the outer side cells – TE and OCT4 in the inner side cells – ICM. Together with dysfunctional TE and increased Oct4 expression in TE cells of Cdx2 deficient mouse blastocysts (Jedrusik et al., 2010; Strumpf et al., 2005; Wu et al., 2010), the increased Cdx2 expression in Oct4 deficient mouse embryos (Wu et al., 2013) further confirmed that molecular interaction between Cdx2 and Oct4 in early embryos is critical to ICM and TE segregation. In contrast to mouse, very few studies were carried out in other mammals to explore the molecular mechanisms underlying first lineage segregation. It is clear now that Cdx2 possesses restricted expression in TE cell of almost all the tested mammals, and Oct4 strongly expresses in ICM. However, existing Cdx2 knockdown studies, one in monkey and one is cattle warned us that the molecular interaction between Cdx2 and Oct4 in these species' early embryos might be different from mouse, because Cdx2 knockdown has failed to elevate Oct4 transcription in blastocysts (Berg et al., 2011; Jedrusik et al., 2010; Sritanaudomchai et al., 2009). Consistently, we also found that

* Corresponding author.
E-mail address: liu086@yahoo.com (Z. Liu).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.ydbio.2015.12.014
0012-1606/

Cdx2 knockdown in early porcine embryos does not change Oct4 mRNA level at blastocyst stage. Moreover, Oct4 has longer period expression in TE cells of some species like cattle, pig, even human (Cauffman et al., 2005; Chen et al., 2009; Hall et al., 2009; Kirchhof et al., 2000; van Eijk et al., 1999). Berg et al. (2011) found that the loss of Tcfap2 binding site in upstream regulatory sequences of cattle Oct4 gene accounts for its prolonged expression TE cells, while in mouse, transcription factor Tcfap2 can mediate a Cdx2-independent repression of Oct4 in TE cells. All these collectively implied that in early development of different mammalian species, there is diversity in molecular regulation of core genes like Cdx2 and Oct4. It is believed that more understanding on the regulation and interaction of Cdx2 and Oct4 in early development of mammals other than mouse will enrich our knowledge about mammalian embryonic development (Rossant, 2011). Therefore, to explore the regulatory interaction between Cdx2 and Oct4 in early porcine embryos, we studied the regulatory effects of Cdx2 overexpression on Oct4 expression and function during early development. Previous study in mice over-expressing a Cdx2 transgene under the control of its own promoter did not find any remarkable changes in early embryonic development, but rather a shift in axial patterning during later stages (Gaunt et al., 2008). Additionally, they did not investigate the effect of Cdx2 on Oct4 expression in early embryos. It is possible that ectopic Cdx2 driven from its own promoter is not expressed robustly in early embryos and does not yield any effects until the 16-cell stage, when its natural expression is elevated (Jedrusik et al., 2010; Wu et al., 2010). Thus, in the present study, we used the method of quantitative injection of *Cdx2* mRNA into porcine early embryos to facilitate investigation of the regulatory effects of Cdx2 on Oct4. To our knowledge it is the first time that the molecular interaction of Cdx2 and Oct4 has been investigated in early mammalian embryos, and our results shed light on a new model involving Oct4 and Cdx2 interaction in porcine embryo just before ICM and TE lineage segregation.

2. Material and methods

2.1. Recovery and culture of porcine oocytes, in vitro fertilization (IVF) procedures, and embryonic culture

Ovaries were collected from a slaughterhouse and transported to lab in 0.9% saline at 37 °C. Follicles 3–5 mm in diameter was aspirated to obtain cumulus–oocyte complexes. Oocytes were matured as previously described (Liu et al., 2008). Animal work followed the guidelines of Animal Care and Use Committee of the Northeast Agricultural University. *IVF* and embryonic culture were same as previously described (Bou et al., 2014).

2.2. siRNA and mRNA production and injection into porcine zygotes

For overexpression via mRNA injection, C*dx2* (936 bp, with primers: F:ATGTACGTGAGCTACCTCCTGGACAAGGAC; R:CTGGGTGACGGTGGGGTTTAACACGC) and *Oct4* (1083 bp, with primers: F:ATGGCGGGACACCTGGCTTCCG; R:TCAGTTTGAATGCATGGGGGAGC), their full-length ORF of DNA construct were cloned from cDNA of porcine blastocyst with PCR method and inserted into MCS of pMACS Kk.HA. (N) plasmid backbone (Miltenyi Biotec). To obtain the dominant negative mutated Oct4 mRNA, after adding a heterogeneous nuclear localization signal (NLS) in MCS behind C-terminal of Oct4 in pMACS Kk.HA. (N)-Oct4 plasmid, the targeted mutation in original NLS was generated by PCR-based site-directed mutagenesis through amplifying the pMACS Kk.HA. (N)-Oct4-NLS plasmid with primers bearing mutations (F:CCAGAGTCGACACAAGTATCGAGAAC; R:AAAGTCGACTCTGGATCCGGCCTGCACGAGGGT). As a control, EGFP (702 bp, F:ATGGTGAGCAAGGGCGAGGAGCT; R:TCACTTGTACAGCTCGTCCATGCCGAG) was cloned from pEGFP-C1 plasmid by PCR method and constructed into pMACS Kk.HA. (N) plasmid. The full-length *Cdx2*, *Oct4*, *dnOct4* and *EGFP* mRNA were synthesized in vitro using RIBOMAX Large Scale RNA Production System T7 kit (Promega) and according to the manufacture's instruction followed by the quality assessment via gel analysis.

To deplete porcine *Oct4*, the online tool BLOCK-iT RNAi Designer was used to design Stealth siRNA. Two Oct4 specific Stealth RNAi oligonucleotides and one scrambled siRNA duplex as control were used in this study (Osi1: F:UCGAACAAUUUGCCAAGCUCCUAAA; R:UUUAGGAGCUUGGCAAAUUGUUCGA; Osi2: F:GGCAAACGAUCAAGCAGUGACUA

UU; R:AAUAGUCACUGCUUGAUCGUUUGCC; Scrambled siRNA: F:GGCCAAGCUAACGGAGUCAUAAAUU; R:AAUUUAUGACUCCGUUAGCUUGGCC). Then siRNA and mRNA microinjections were carried out with an Eppendorf FemtoJet microinjector and Narishige NT-88NE micromanipulators. For injection, glass capillary femtotip II (Eppendorf) was loaded with 5 μl of RNA (siRNA: 20 nM) by microloader (Eppendorf), and about 30 pl of solution was injected into the cytoplasm of porcine zygotes.

2.3. RNA preparation and quantitative PCR (qPCR)

RNA was extracted with PureLink Micro-to-Midi total RNA purification kit (Invitrogen) and reverse transcribed with the High capacity cDNA reverse transcription kits (Applied BioSystems) using random primers or the First strand cDNA kit (Fermentas) for gene specific primers (Cdx2 specific RT primer: R:ATCTCCTTACTGGCAGAAGGTCAG; Oct4 specific RT primer: R:TCAAGAGCATCATTGAACTTCACC). SYBR Premix Ex Taq for Perfect Real Time (Takara) was used for qPCR with oligonucleotide primers (Oct4 qPCR: 163 bp, F:GAAGCTGGACAAGGAGAAGCTGGAG; R:ATGGTCGTTTGGCTGAACACCTTC; Cdx2 qPCR: 117 bp, F:AGTCGCTACATCACCATTCGGAG; R:GCTGCTGTTGCTGCAACTTCTTC; 18 S rRNA qPCR: 149 bp, F:TCCAATGGATCCTCGCGGAA; R:GGCTACCACATCCAAGGAAG). Data from three replicates were analyzed by $^{\Delta\Delta}$Ct method after normalization with 18 s rRNA level (Kuijk et al., 2007).

2.4. MG132 treatment of porcine embryos

0.5 μl of 5 mM MG132 (carbobenzoxyl leucinyl leucinyl leucinal-Hl, at a final concentration of 5 μM) was added into 500 μl culture medium of one day post fertilization embryos. Then after 24 or 48 h, the embryos were collected for further WB or IF analysis. The MG132 was dissolved in dimethyl sulfoxide (DMSO), thus the control embryos were supplemented with the same amount of DMSO.

2.5. Immunofluorescence assays (IF) and Western blot (WB) assay

IF and WB was conducted in conventional procedures (Xu et al., 2009a). Quantity One software (BioRad) was used to analyze the results. Primary antibodies include mouse monoclonal to Cdx2 (MU392A-UC, Biogenex, IF: 1:50, WB: 1:200); goat polyclonal to Oct4 (N19) (sc-8628, Santa Cruz, IF: 1:50, WB: 1:200); mouse monoclonal to Beta-Actin (A1978, Sigma, WB: 1:500).

2.6. Co-immunoprecipitation (Co-IP)

Co-IP experiments were performed using the Pierce Co-Immunoprecipitation Kit (26149, Pierce) as per the manufacturer's instructions. Briefly, embryo lysates were then applied to columns containing 1 μg immobilized antibodies (Rabbit polyclonal to HA tag: ab9110, Abcam, IF: 1:1000, Co-IP: 1 μg per column or rabbit IgG: A7016, Beyotime, Co-IP: 1 μg per column) covalently linked to

Please cite this article as: Bou, G., et al., Cdx2 represses Oct4 function via inducing its proteasome-dependent degradation in early porcine embryos. Dev. Biol. (2015), http://dx.doi.org/10.1016/j.ydbio.2015.12.014