Biochemical and Biophysical Research Communications 430 (2013) 1175–1181

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

The transition of mouse pluripotent stem cells from the naïve to the primed state requires Fas signaling through 3-*O* sulfated heparan sulfate structures recognized by the HS4C3 antibody

Kazumi Hirano [a], Toin H. Van Kuppevelt [b], Shoko Nishihara [a,*]

[a] Laboratory of Cell Biology, Department of Bioinformatics, Faculty of Engineering, Soka University, 1-236 Tangi-cho, Hachioji, Tokyo 192-8577, Japan
[b] Department of Biochemistry, Nijmegen Center for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, 280 P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 27 November 2012
Available online 8 December 2012

Keywords:
Mouse embryonic stem cells
Mouse epiblast stem cells
Naïve state
Primed state
Fas signaling
Heparan sulfate

ABSTRACT

The characteristics of pluripotent embryonic stem cells of human and mouse are different. The properties of human embryonic stem cells (hESCs) are similar to those of mouse epiblast stem cells (mEpiSCs), which are in a later developmental pluripotency state, the so-called "primed state" compared to mouse embryonic stem cells (mESCs) which are in a naïve state. As a result of the properties of the primed state, hESCs proliferate slowly, cannot survive as single cells, and can only be transfected with genes at low efficiency. Generating hESCs in the naïve state is necessary to overcome these problems and allow their application in regenerative medicine. Therefore, clarifying the mechanism of the transition between the naïve and primed states in pluripotent stem cells is important for the establishment of stable methods of generating naïve state hESCs. However, the signaling pathways which contribute to the transition between the naïve and primed states are still unclear. In this study, we carried out induction from mESCs to mEpiSC-like cells (mEpiSCLCs), and observed an increase in the activation of Fas signaling during the induction. The expression of *Fgf5*, an epiblast marker, was diminished by inhibition of Fas signaling using the caspase-8 and -3 blocking peptides, IETD and DEVD, respectively. Furthermore, during the induction, we observed increased expression of 3-*O* sulfated heparan sulfate (HS) structures synthesized by HS 3-*O*-sulfotransferase (3OST), which are recognized by the HS4C3 antibody (HS4C3-binding epitope). Knockdown of *3OST-5* reduced Fas signaling and the potential for the transition to mEpiSCLCs. This indicates that the HS4C3-binding epitope is necessary for the transition to the primed state. We propose that Fas signaling through the HS4C3-binding epitope contributes to the transition from the naïve state to the primed state.

1. Introduction

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of pre-implantation blastocysts [1,2], have the characteristic features of self-renewal and pluripotency and are able to differentiate into all cell types deriving from the different germ layers [3–5].

Mouse ESCs (mESCs) are defined as "naïve" pluripotent stem cells because of their functional similarity to the pre-implantation epiblast [6]. Their self-renewal is maintained by leukemia inhibitory factor (LIF)/signal transducer and activator of transcription 3 (STAT3) signaling as well as MAPK/ERK kinase (MEK)/glycogen synthase kinase 3 (GSK3) inhibition, conditions known as LIF/2i [7]. Mouse epiblast stem cells (mEpiSCs) are distinguished from mESCs by the difference in the developmental stage, namely that they are derived from post-implantation blastocysts. The pluripotent state of mEpiSCs has been described as "primed" based on their post-implantation epiblast-like transcriptional and epigenetic properties [6,8,9]. mEpiSCs are maintained by Nodal/Activin/Smad2 and fibroblast growth factor 2 (FGF2)/extracellular signal-regulated kinase (ERK) signaling [8,9].

Human pluripotent stem cells, human ESCs (hESCs) and human induced pluripotent stem cells (hiPSCs), share defining features with mEpiSCs being in the primed state rather than the mESCs like naïve state. Therefore, hESCs and hiPSCs are believed to be primed pluripotent stem cells [8,9]. Unlike mESCs, hESCs proliferate slowly and have vulnerability to single-cell dissociation [10,11]. Therefore, hESCs and hiPSCs are difficult to rapidly expand and genetically manipulate. To overcome these critical problems and allow their application in regenerative medicine, it is necessary to elucidate the molecular mechanisms of the transition between the naïve and primed states. However, to date the signaling pathways contributing to the transition remain unclear.

* Corresponding author. Fax: +81 42 691 8140.
E-mail address: shoko@soka.ac.jp (S. Nishihara).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.005

Heparan sulfate (HS) is a sulfated glycan, abundantly expressed and having functions in mESCs [12–18]. The sulfation pattern of HS is dynamically changed by alteration of the expression of elongation enzymes and various sulfotransferases in accordance with differentiation of mESCs [17–20]. We have reported that HS and sulfation thereof contribute to self-renewal and differentiation of mESCs by regulating BMP, Wnt, and FGF signaling [12,17]. Furthermore, we found that 3-*O* sulfated HS structures synthesized by HS 3-*O*-sulfotransferase-5 (3OST-5), named the "HS4C3-binding epitope", were increased, and that these structures regulated Fas signaling in differentiated mESCs in the absence of LIF [20].

Here, we carried out induction from mESCs in the naïve state to mEpiSC-like cells (mEpiSCLCs) in the primed state to analyze the transition from the naïve to the primed state. The expression of the HS4C3-binding epitope increased and regulated Fas signaling during the transition. We propose that Fas signaling through the HS4C3-binding epitope contributes to the transition from the naïve to the primed state.

2. Materials and methods

2.1. Construction of expression vectors

We generated siRNA expression plasmids that targeted *3OST-5* or *EGFP* as a negative control by inserting the appropriate dsDNAs between the *Bam*HI and *Hin*dIII sites of pSUPER.retro.puro (OligoEngine). The siRNA sequences used for RNA interference (RNAi) were designed as described previously [20] using "siDirect": EGFP, 5′-GATCCCGCCACAACGTCTATATCATGGGGAAAATCCATGATATAGA CGTTGTGGCTTTTTTGGAAA-3′; *3OST-5–1*, 5′-GATCCCGTAGACCCCT CCGTCATTACCGCTTCCTGTCACGGTAATGACGGAGGGGTCTACTTTTT TA-3′; *3OST-5–2*, 5′-GATCCCCGGTTAGGACCAGCATATACAGCTTCC TGTCACTGTATATGCTGGTCCTAACCGTTTTTTA-3′. We performed experiments using these two constructs that target *3OST-5* (*3OST-5–1* and *3OST-5–2* expressed different siRNAs targeting *3OST-5*), and obtained consistent results with these two constructs. We show the results using the construct including the *3OST-5–1* sequence in this manuscript.

2.2. Cell culture

R1 [21] mESC line was maintained on mouse embryonic fibroblasts (MEFs) inactivated with 10 μg/ml mitomycin C (Sigma) in ESC medium (DMEM supplemented with 15% FBS {Hyclone}, 1% penicillin/streptomycin {Gibco}, 0.1 mM 2-mercaptoethanol {Gibco}, and 0.1 mM non-essential amino acids {Gibco}) with 1000 U/ml LIF (Chemicon). The R1 line was a gift from Dr. Seiji Hitoshi (Shiga Univ. Medical Science, Japan). MEFs were prepared from embryos at embryonic day 14.5 (E14.5; ICR).

Stable knockdown of *3OST-5* mRNA was performed as follows. To produce retrovirus, the pSUPER.retro.puro constructs were transfected into ecotropic virus-packaging (PLAT-E) cells. Supernatants, that contained virus and were derived from these PLAT-E cultures, were mixed with 8 μg/ml polybrene (Sigma) and the virus/polybrene mixtures were incubated with mESCs for 24 h. After infection, the cells were replated with ESC medium containing LIF and 2 μg/ml puromycin and cultured for 5–7 days.

2.3. Induction of mouse epiblast stem cell-like cells (mEpiSCLCs)

We carried out induction from mESCs to mEpiSCLCs as described previously [22]. In brief, mESCs were harvested and 2×10^5 cells per dish were replated on 35 mm tissue culture dishes precoated with 15 μg/ml fibronectin (Sigma) in ESC medium containing LIF. After 24 h of culture, the medium was replaced with serum-free medium consisting of DMEM/F12 supplemented with 48% Neurobasal (Invitrogen), 1% N2 supplement (Invitrogen), 2% B27 supplement (Invitrogen), 1 mM L-glutamine (Gibco), 1% penicillin/streptomycin, 0.1 mM 2-mercaptoethanol, 0.1 mM nonessential amino acids, and 5 mg/ml bovine serum albumin (BSA). Then 20 ng/ml activin A (Wako) and 12 ng/ml FGF2 (Wako) were added to the serum-free medium. Thereafter, cells were passaged every 2–3 days using collagenase IV (Invitrogen). To analyze the inhibition of Fas signaling, the cells were cultured in medium containing 20 μM Ac-IETD-CHO or 20 μM Ac-DEVD-CHO (Peptide Institute Inc). Ac-IETD-CHO and Ac-DEVD-CHO are inhibitors of caspase-8 and caspase-3, respectively. Alkaline phosphatase was detected following the manufacture's instructions (Nacalai Tesque).

2.4. Immunostaining

Cells were fixed with 4% paraformaldehyde, washed, and subsequently blocked with PBS containing 5% BSA, 0.05% Triton X-100, and 1% normal goat serum. For immunostaining against HS4C3-binding epitope, after washing, the cells were stained with a vesicular stomatitis virus (VSV)-tagged phage-display anti-HS antibody HS4C3 [20,23]. Then, the cells were washed again and stained with mouse anti-VSV glycoprotein antibody (Sigma). Finally, the cells were washed and stained with the antibody goat alexa fluor 488-conjugated anti-mouse IgG (Invitrogen). For immunostaining against Fas, anti-Fas antibody M-20 (Santa Cruz) was used as primary antibody, and detected by secondary antibody goat alexa fluor 488-conjugated anti-rabbit IgG (Molecular Probes). Immunofluorescence images were taken using an LSM5Pascal confocal laser scanning microscope (Carl Zeiss).

2.5. Real-time PCR

Total RNA was isolated from cells using TRIzol® Reagent (Invitrogen) and reverse transcribed using an oligo-dT primer (Invitrogen) and a Superscript II First Strand Synthesis Kit (Invitrogen). For real-time PCR reactions, FastStart Universal SYBR Green Master (Roche) was used. Real-time PCR was performed using an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems). The relative amounts of each mRNA were normalized against the amount of *β-actin* mRNA in the same sample. Primer sets for real-time PCR are listed in Supplementary Table 1.

2.6. Immunoblotting

Cells were lysed with lysis buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM Na_3VO_4, 10 mM NaF, and protease inhibitors). Samples containing 5 or 10 μg of cell lysate were separated by 10% or 15% SDS–PAGE and transferred onto PVDF membranes (Millipore). After blocking, the membranes were incubated with antibodies against cleaved (activated) caspase-3, cleaved (activated) caspase-8, cleaved PARP (Cell Signaling Technology), and β-actin (Sigma). The membranes were then incubated with the appropriate peroxidase-conjugated secondary antibodies (Cell Signaling Technology), washed and developed with ECL Plus reagents (GE Healthcare).

3. Results

3.1. Fas signaling is activated during induction to mouse epiblast stem cell-like cells (mEpiSCLCs)

We carried out induction from mESCs to mEpiSCLCs according to the protocol established by Guo et al. [22]. In this study we

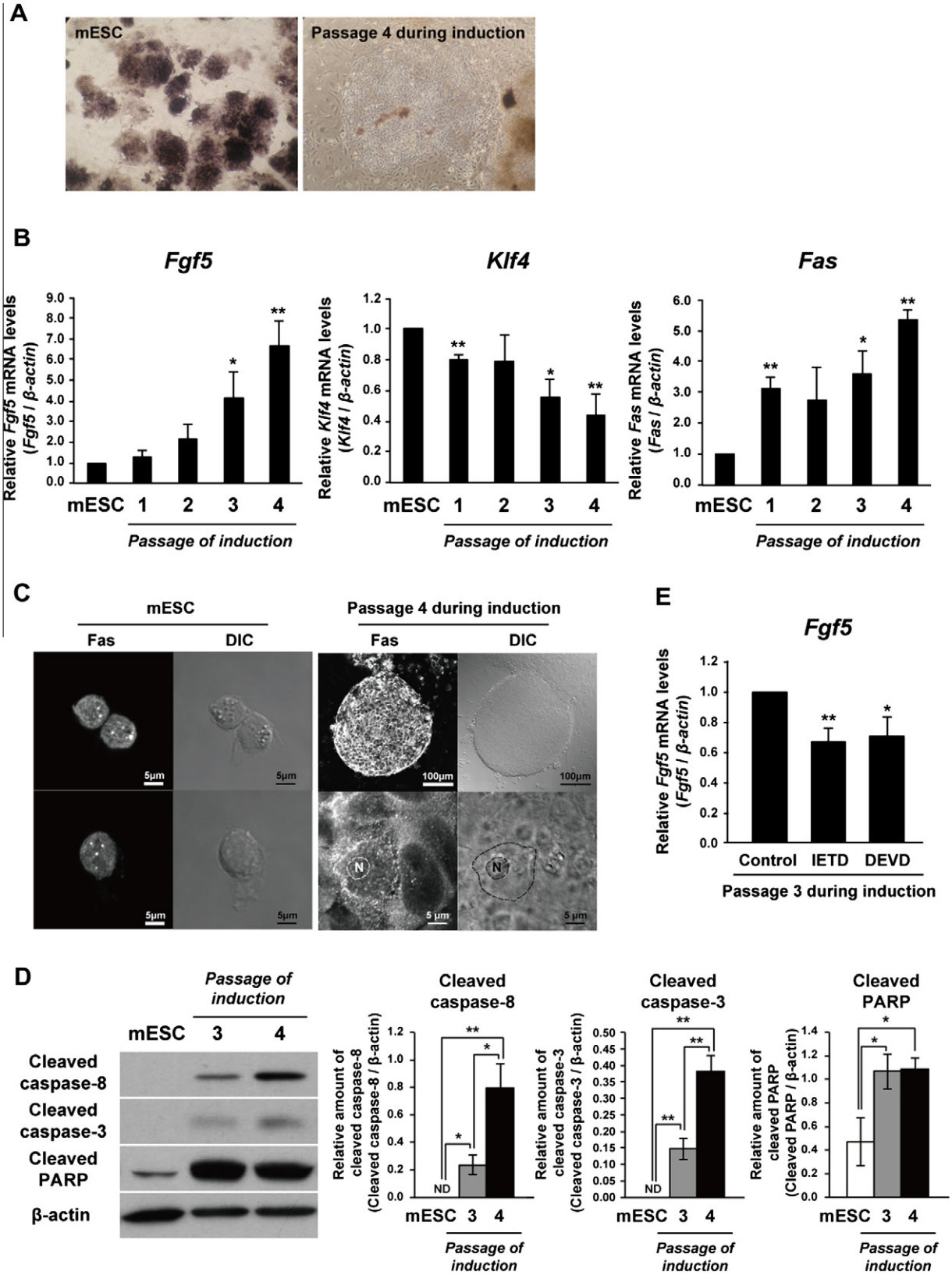

Fig. 1. Fas signaling is activated during induction to mEpiSCLCs. (A) Bright-field images of cells for stained for alkaline phosphatase before (mESC) and at passage 4 during induction. Magnification at ×20. (B) Real-time PCR analysis of the genes, *Fgf5* (an epiblast marker), *Klf4* (a mESC marker), and *Fas*, during induction from mESC to mEpiSCLC at progressive passages: from mESC up to passage 4. The results are shown after normalization against the values obtained with mESC (value = 1). Error bars indicate s.e.m. ($n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison to mESC). (C) Immunocytochemical staining using anti-Fas antibody at passage 4 during induction. Representative confocal images from two independent experiments are shown. N, nucleus. Scale bar, 100 μm or 5 μm. (D) Western blot analyses for cleaved caspase-8, -3, and PARP during induction from mESC to mEpiSCLC at progressive passages: from mESC up to passage 4. The histograms show mean densitometric readings±s.e.m. ($n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$). ND, not detected. (E) Real-time PCR analysis of *Fgf5* (an epiblast marker) in the cells treated with IETD or DEVD at passage 3. The results are shown after normalization against the values obtained with control cells (value = 1). Error bars indicate s.e.m. ($n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison to control cells).

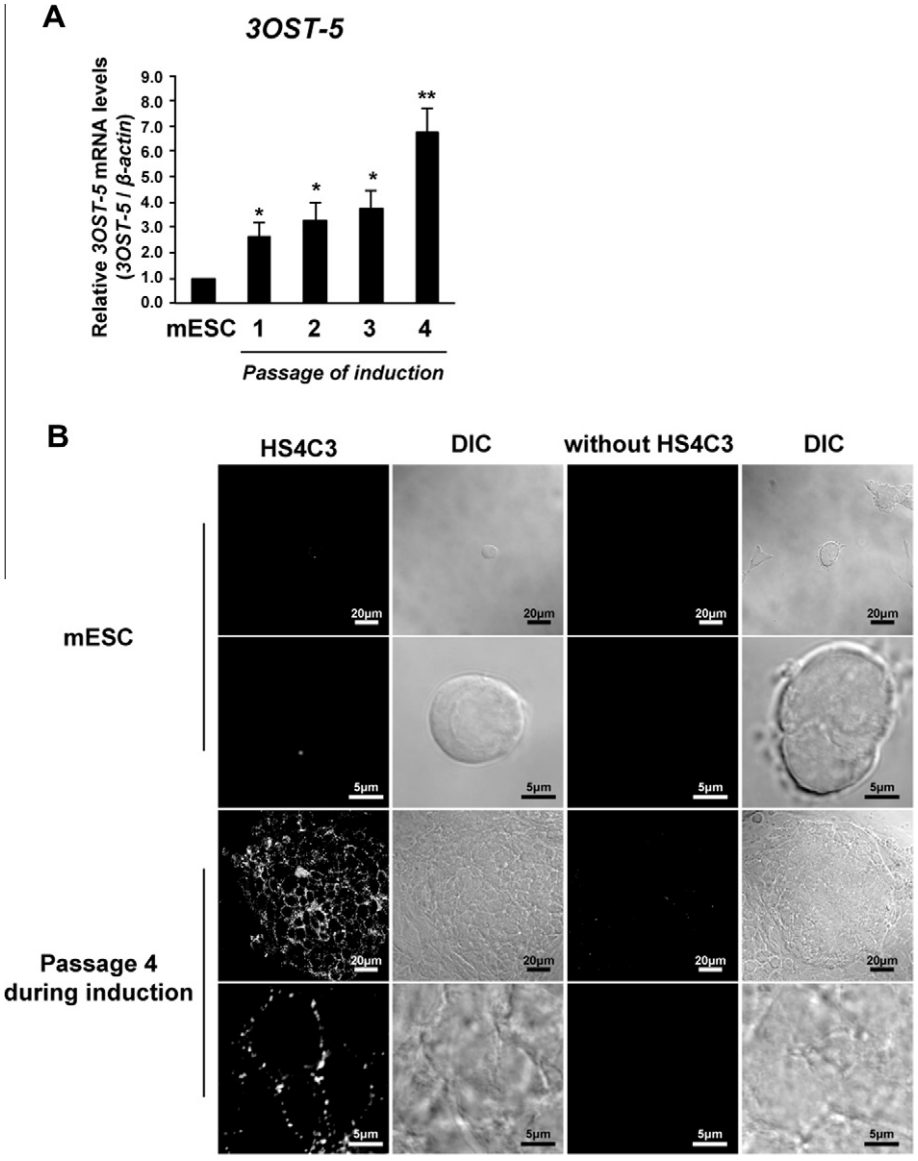

Fig. 2. HS4C3-binding epitope is increased during induction to mEpiSCLCs. (A) Real-time PCR analysis of *3OST-5* (HS4C3-binding epitope synthesis gene) during induction from mESC to mEpiSCLC at progressive passages: from mESC up to passage 4. The results are shown after normalization against the values obtained with mESC (value = 1). Error bars indicate s.e.m. ($n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison to mESC). (B) Immunocytochemical staining with or without HS4C3 at passage 4 during induction. Representative confocal images from two independent experiments are shown. Scale bar, 20 μm or 5 μm.

focused on the cells in early transition state during induction from mESC to mEpiSCLC at progressive passages: from mESC up to passage 4. The cells at passage 4 were no longer positive for alkaline phosphatase activity (Fig. 1A). The expression of *Fgf5*, an epiblast marker, was increased and *Klf4*, a mESC marker, was decreased from mESC up to passage 4 (Fig. 1B). Considering that apoptosis occurs during mESC differentiation [24,25], we expected Fas signaling to be up-regulated during the transition from mESC to mEpiSCLC. The expression of *Fas* mRNA and Fas protein increased from mESC up to passage 4 (Fig. 1B and C). Furthermore, confocal slice images showed that Fas was localized on and beneath the cell surface of the cells at passage 4, but not on and beneath that of mESCs (Fig. 1C). It has been reported that Fas is translocated from intracellular pools to the cell surface and Fas signaling is activated simply by the redistribution of Fas into lipid rafts on the cell surface [26,27]. Fas signaling subsequently activates caspase-8 and caspase-3 in turn. Thus we performed Western blot analysis and showed that caspase-8 and caspase-3 were activated at passage 3 and 4 (Fig. 1D). Caspase-3 can cleave several target proteins, one of which is poly (ADP-ribose) polymerase (PARP), and consistent with its activation cleaved PARP was increased at passage 3 and 4 (Fig. 1D). These results demonstrate that Fas signaling was activated during induction to mEpiSCLCs. Therefore, we expected that Fas signaling might be required for the transition from the naïve to the primed state. To investigate this possibility, we used inhibitors of caspase-8 and caspase-3, IETD and DEVD, respectively, and treated mESC continuously from 2 days before induction up to passage 3. Treatment with IETD and DEVD led to a reduction in the expression of *Fgf5* (Fig. 1E). This clearly shows that Fas signaling was necessary for the transition from the naïve to the primed state.

3.2. HS4C3-binding epitope is increased during induction to mEpiSCLCs

We previously reported that the HS4C3-binding epitope, which includes the 3-*O* HS structure, was increased, and activates Fas signaling during the differentiation of mESCs [20]. However, the role

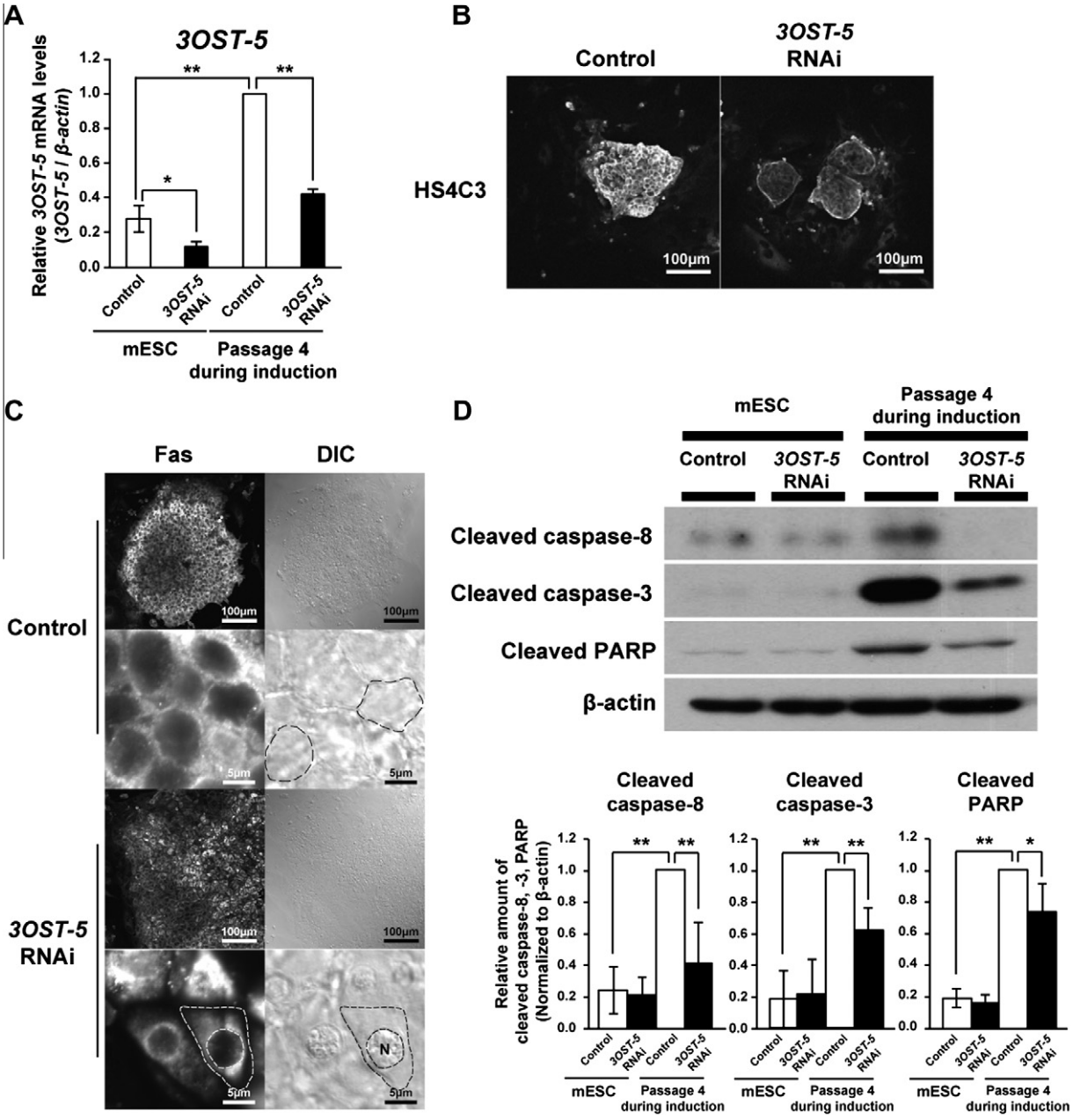

Fig. 3. Decrease in the HS4C3-binding epitope inhibits Fas signaling during induction to mEpiSCLCs. (A) Real-time PCR analysis of *3OST-5* (HS4C3-binding epitope synthesis gene) in *3OST-5* KD mESCs and *3OST-5* KD cells at passage 4. The results are shown after normalization against the values obtained with control cells at passage 4 (value = 1). The values shown are the means ± s.e.m. ($n = 5$ or 6, $^{*}p < 0.05$, $^{**}p < 0.01$). (B) Immunocytochemical staining with HS4C3 in *3OST-5* KD cells at passage 4. Representative confocal images from two independent experiments are shown. Scale bar, 100 μm. (C) Immunocytochemical staining with anti-Fas antibody in *3OST-5* KD cells at passage 4. Representative confocal images from two independent experiments are shown. N, nucleus. Scale bar, 100 μm or 5 μm. (D) Western blot analyses for cleaved caspase-8, -3, and PARP in *3OST-5* KD cells at passage 4. Representative results are shown. The histograms show mean densitometric readings ± s.e.m. ($n = 3$–5, $^{*}p < 0.05$, $^{**}p < 0.01$) after normalization against the values obtained with control cells at passage 4 (value = 1).

of the HS4C3-binding epitope and Fas signaling in the transition from the naïve to the primed state is still unknown. The expression pattern analyzed by real-time PCR showed up-regulation of *3OST-5*, the HS4C3-binding epitope synthesis gene, during induction from mESC to mEpiSCLC at progressive passages: from mESC up to passage 4 (Fig. 2A). Furthermore, we observed an increase in the HS4C3-binding epitope detected as dots on the surface of the cells at passage 4 (Fig. 2B). This suggests that the HS4C3-binding epitope is necessary for the transition from the naïve to the primed state via Fas signaling.

3.3. Decrease in the HS4C3-binding epitope inhibits Fas signaling during induction to mEpiSCLCs

In order to examine whether the HS4C3-binding epitope is required for Fas signaling during induction to mEpiSCLCs, we performed stable knockdown (KD) of *3OST-5*, which synthesizes the HS4C3-binding epitope, by RNAi in mESCs, and carried out induction from mESCs to mEpiSCLCs. The expression of *3OST-5* was increased at passage 4 during induction both in control and KD cells (Fig. 3A). However, in stable *3OST-5* KD cells at passage 4,

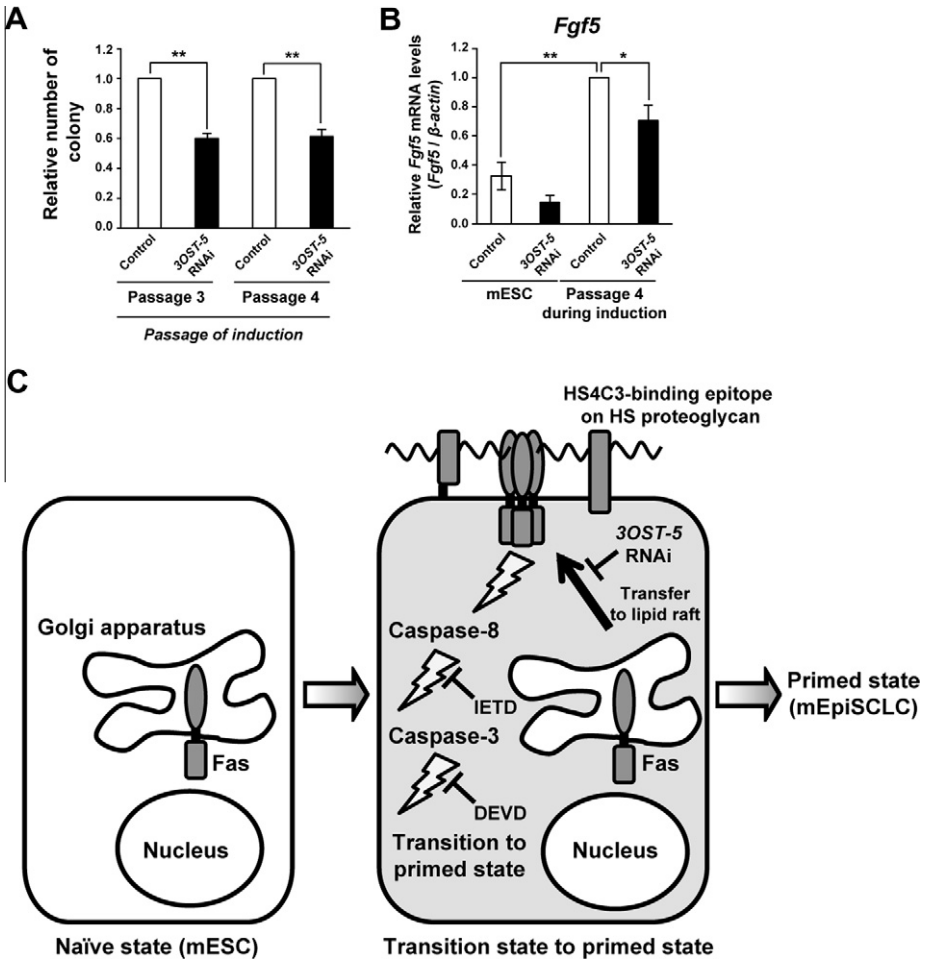

Fig. 4. Decrease of the HS4C3-binding epitope inhibits the transition to mEpiSCLCs. (A) Measurement of number of colonies in *3OST-5* KD cells at passage 3 and 4. The results are shown after normalization against the values obtained with control cells (value = 1). The values shown are the means ± s.e.m. (n = 6 or 12, $^{**}p < 0.01$). (B) Real-time PCR analysis of *Fgf5* (an epiblast marker) in *3OST-5* KD mESCs and *3OST-5* KD cells at passage 4. The results are shown after normalization against the values obtained with control cells at passage 4 (value = 1). The values shown are the means ± s.e.m. (n = 5 or 6, $^{*}p < 0.05$, $^{**}p < 0.01$). (C) Scheme for the transition to the primed state. In the naïve state (mESC), Fas signaling is silent because of the intracellular localization of Fas. In the transition state to the primed state, the expression of *Fas* is increased, Fas localizes on and beneath the cell surface, and Fas signaling is activated via the HS4C3-binding epitope by the transfer of Fas to lipid rafts as reported [20]. Fas signaling via the HS4C3-binding epitope is required for the transition from the naïve to the primed state.

the expression of *3OST-5* and the HS4C3-binding epitope decreased compared to control cells at passage 4 (Fig. 3A and B).

Confocal slice images of *3OST-5* KD cells at passage 4 showed that Fas was located in the intracellular Golgi compartment and around the nucleus but not on the cell surface as observed in control cells (Fig. 3C). Furthermore, cleaved caspase-8, -3, and PARP were decreased in *3OST-5* KD cells at passage 4 as compared to control cells (Fig. 3D). These data clearly demonstrate that the HS4C3-binding epitope regulates Fas signaling during the transition from the naïve to the primed state.

3.4. Decrease of the HS4C3-binding epitope inhibits the transition to mEpiSCLCs

At passage 3 and 4, we observed a decreased number of colonies from *3OST-5* KD cells (Fig. 4A). This suggests that the HS4C3-binding epitope is required for proliferation and survival during the transition to mEpiSCLCs. Next, we examined the expression of *Fgf5* (an epiblast marker). In *3OST-5* KD cells at passage 4, the expression of *Fgf5* was reduced as compared to control cells. This showed that *3OST-5* KD cells could not be induced normally to mEpiSCLCs (Fig. 4B).

4. Discussion

Herein, we report for the first time that Fas signaling via the HS4C3-binding epitope contributes to the transition from the naïve to the primed state. From our results, we propose the following novel mechanism (Fig. 4C). In the naïve state (mESC), Fas and the HS4C3-binding epitope exhibit low expression, Fas remains intracellular, and Fas signaling is in the inactivated state (Fig. 1B–D and [20]). In the transition state to the primed state, the expression of *Fas* is up-regulated, Fas localizes on and beneath the cell surface, and Fas signaling is activated by transfer of Fas to lipid rafts on the cell surface (Figs. 1B–D and 3C and D). The transfer of Fas to lipid rafts is controlled by the HS4C3-binding epitope [20]. Therefore, we propose the novel mechanism whereby Fas signaling via the HS4C3-binding epitope is one of the essential signaling mechanisms involved in the transition from the naïve to the primed state.

Recently, consistent with our results, it has been described that caspase-3 is activated during induction from mESCs to mEpiSCLCs, although the mechanisms upstream of activated caspase-3 were not mentioned [28]. In this report, we show that Fas signaling is upstream of this activation. We have also reported the relationship between Fas signaling and activation of caspase-3 during differentiation of mESCs, for example, during differentiation to primitive

endoderm [20]. Considering these results, Fas signaling seems to play an important role in many types of differentiation.

It has not yet been reported what molecule is degraded by activated caspase-3 during the transition from the naïve to the primed state. Experiments are in progress to identify these activated caspase-3 targets during this transition. Although many researchers have tried the shift to hESC and hiPSC in the naïve state from those in the primed state [29–33], the methods are not yet established. Considering our findings, the inhibition of Fas signaling may help to develop novel methodology that induces production of hESC and hiPSC in the naïve state from those in the primed state.

Acknowledgments

We thank Dr. Seiji Hitoshi for the kind gift of experimental material. Our research was partially supported by the fund from MEXT, the Matching Fund for Private Universities, S0901015, 2009–2014.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.12.005.

References

[1] M.J. Evans, M.H. Kaufman, Establishment in culture of pluripotential cells from mouse embryos, Nature 292 (1981) 154–156.
[2] G.R. Martin, Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells, Proc. Natl. Acad. Sci. USA 78 (1981) 7634–7638.
[3] Y. Suda, M. Suzuki, Y. Ikawa, S. Aizawa, Mouse embryonic stem cells exhibit indefinite proliferative potential, J. Cell Physiol. 133 (1987) 197–201.
[4] R.S. Beddington, E.J. Robertson, An assessment of the developmental potential of embryonic stem cells in the midgestation mouse embryo, Development 105 (1989) 733–737.
[5] A. Bradley, M. Evans, M.H. Kaufman, E. Robertson, Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines, Nature 309 (1984) 255–256.
[6] J. Nichols, A. Smith, Naive and primed pluripotent states, Cell Stem Cell 4 (2009) 487–492.
[7] Q.L. Ying, J. Wray, J. Nichols, L. Batlle-Morera, B. Doble, J. Woodgett, P. Cohen, A. Smith, The ground state of embryonic stem cell self-renewal, Nature 453 (2008) 519–523.
[8] I.G. Brons, L.E. Smithers, M.W. Trotter, P. Rugg-Gunn, B. Sun, S.M. Chuva de Sousa Lopes, S.K. Howlett, A. Clarkson, L. Ahrlund-Richter, R.A. Pedersen, L. Vallier, Derivation of pluripotent epiblast stem cells from mammalian embryos, Nature 448 (2007) 191–195.
[9] P.J. Tesar, J.G. Chenoweth, F.A. Brook, T.J. Davies, E.P. Evans, D.L. Mack, R.L. Gardner, R.D. McKay, New cell lines from mouse epiblast share defining features with human embryonic stem cells, Nature 448 (2007) 196–199.
[10] M. Ohgushi, M. Matsumura, M. Eiraku, K. Murakami, T. Aramaki, A. Nishiyama, K. Muguruma, T. Nakano, H. Suga, M. Ueno, T. Ishizaki, H. Suemori, S. Narumiya, H. Niwa, Y. Sasai, Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells, Cell Stem Cell 7 (2010) 225–239.
[11] G. Chen, Z. Hou, D.R. Gulbranson, J.A. Thomson, Actin–myosin contractility is responsible for the reduced viability of dissociated human embryonic stem cells, Cell Stem Cell 7 (2010) 240–248.
[12] N. Sasaki, K. Okishio, K. Ui-Tei, K. Saigo, A. Kinoshita-Toyoda, H. Toyoda, T. Nishimura, Y. Suda, M. Hayasaka, K. Hanaoka, S. Hitoshi, K. Ikenaka, S. Nishihara, Heparan sulfate regulates self-renewal and pluripotency of embryonic stem cells, J. Biol. Chem. 283 (2008) 3594–3606.
[13] F. Lanner, K.L. Lee, M. Sohl, K. Holmborn, H. Yang, J. Wilbertz, L. Poellinger, J. Rossant, F. Farnebo, Heparan sulfation-dependent fibroblast growth factor signaling maintains embryonic stem cells primed for differentiation in a heterogeneous state, Stem Cells 28 (2010) 191–200.
[14] M. Forsberg, K. Holmborn, S. Kundu, A. Dagälv, L. Kjellén, K. Forsberg-Nilsson, Undersulfation of heparan sulfate restricts differentiation potential of mouse embryonic stem cells, J. Biol. Chem. 287 (2012) 10853–10862.
[15] D.C. Kraushaar, Y. Yamaguchi, L. Wang, Heparan sulfate is required for embryonic stem cells to exit from self-renewal, J. Biol. Chem. 285 (2010) 5907–5916.
[16] R.J. Holley, C.E. Pickford, G. Rushton, G. Lacaud, J.T. Gallagher, V. Kouskoff, C.L. Merry, Influencing hematopoietic differentiation of mouse embryonic stem cells using soluble heparin and heparan sulfate saccharides, J. Biol. Chem. 286 (2011) 6241–6252.
[17] N. Sasaki, T. Hirano, T. Ichimiya, M. Wakao, K. Hirano, A. Kinoshita-Toyoda, H. Toyoda, Y. Suda, S. Nishihara, The 3′-phosphoadenosine 5′-phosphosulfate transporters, PAPST1 and 2, contribute to the maintenance and differentiation of mouse embryonic stem cells, PLoS One 4 (2009) e8262.
[18] A.V. Nairn, A. Kinoshita-Toyoda, H. Toyoda, J. Xie, K. Harris, S. Dalton, M. Kulik, J.M. Pierce, T. Toida, K.W. Moremen, R.J. Linhardt, Glycomics of proteoglycan biosynthesis in murine embryonic stem cell differentiation, J. Proteome Res. 6 (2007) 4374–4387.
[19] R.J. Baldwin, G.B. ten Dam, T.H. van Kuppevelt, G. Lacaud, J.T. Gallagher, V. Kouskoff, C.L. Merry, A developmentally regulated heparan sulfate epitope defines a subpopulation with increased blood potential during mesodermal differentiation, Stem Cells 26 (2008) 3108–3118.
[20] K. Hirano, N. Sasaki, T. Ichimiya, T. Miura, T.H. Van Kuppevelt, S. Nishihara, 3-*O*-sulfated heparan sulfate recognized by the antibody HS4C3 contributes to the differentiation of mouse embryonic stem cells via Fas signaling, PLoS One 7 (2012) e43440.
[21] A. Nagy, J. Rossant, R. Nagy, W. Abramow-Newerly, J.C. Roder, Derivation of completely cell culture-derived mice from early-passage embryonic stem cells, Proc. Natl. Acad. Sci. USA 90 (1993) 8424–8428.
[22] G. Guo, J. Yang, J. Nichols, J.S. Hall, I. Eyres, W. Mansfield, A. Smith, Klf4 reverts developmentally programmed restriction of ground state pluripotency, Development 136 (2009) 1063–1069.
[23] N.C. Smits, J.F. Lensen, T.J. Wijnhoven, G.B. Ten Dam, G.J. Jenniskens, T.H. van Kuppevelt, Phage display-derived human antibodies against specific glycosaminoglycan epitopes, Methods Enzymol. 416 (2006) 61–87.
[24] D. Duval, M. Malaisé, B. Reinhardt, C. Kedinger, H. Boeuf, A p38 inhibitor allows to dissociate differentiation and apoptotic processes triggered upon LIF withdrawal in mouse embryonic stem cells, Cell Death Differ. 11 (2004) 331–341.
[25] J. Fujita, A.M. Crane, M.K. Souza, M. Dejosez, M. Kyba, R.A. Flavell, J.A. Thomson, T.P. Zwaka, Caspase activity mediates the differentiation of embryonic stem cells, Cell Stem Cell 2 (2008) 595–601.
[26] S. Schütze, V. Tchikov, W. Schneider-Brachert, Regulation of TNFR1 and CD95 signalling by receptor compartmentalization, Nat. Rev. Mol. Cell Biol. 9 (2008) 655–662.
[27] V.N. Ivanov, P. Lopez Bergami, G. Maulit, T.A. Sato, D. Sassoon, Z. Ronai, FAP-1 association with Fas (Apo-1) inhibits Fas expression on the cell surface, Mol. Cell Biol. 23 (2003) 3623–3635.
[28] M.Y. Turco, L. Furia, A. Dietze, L. Fernandez Diaz, S. Ronzoni, A. Sciullo, A. Simeone, D. Constam, M. Faretta, L. Lanfrancone, Cellular heterogeneity during embryonic stem cell differentiation to epiblast stem cells is revealed by the ShcD/RaLP adaptor protein, Stem Cells 30 (2012) 2423–2436.
[29] N. Sasaki, M. Shinomi, K. Hirano, K. Ui-Tei, S. Nishihara, LacdiNAc (GalNAcβ1-4GlcNAc) contributes to self-renewal of mouse embryonic stem cells by regulating leukemia inhibitory factor/STAT3 signaling, Stem Cells 29 (2011) 641–650.
[30] J. Hanna, A.W. Cheng, K. Saha, J. Kim, C.J. Lengner, F. Soldner, J.P. Cassady, J. Muffat, B.W. Carey, R. Jaenisch, Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs, Proc. Natl. Acad. Sci. USA 107 (2010) 9222–9227.
[31] Y. Xu, X. Zhu, H.S. Hahm, W. Wei, E. Hao, A. Hayek, S. Ding, Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules, Proc. Natl. Acad. Sci. U S A 107 (2010) 8129–8134.
[32] H. Zhou, W. Li, S. Zhu, J.Y. Joo, J.T. Do, W. Xiong, J.B. Kim, K. Zhang, H.R. Schöler, S. Ding, Conversion of mouse epiblast stem cells to an earlier pluripotency state by small molecules, J. Biol. Chem. 285 (2010) 29676–29680.
[33] B. Greber, G. Wu, C. Bernemann, J.Y. Joo, D.W. Han, K. Ko, N. Tapia, D. Sabour, J. Sterneckert, P. Tesar, H.R. Schöler, Conserved and divergent roles of FGF signaling in mouse epiblast stem cells and human embryonic stem cells, Cell Stem Cell 6 (2010) 215–226.