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Jagged-1-mediated activation of notch signalling induces adipogenesis of adipose-derived stem cells

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Abstract

Objectives: Notch signalling plays an important role in many cell activities, involving proliferation, migration, differentiation and cell death. The aim of this study was to investigate effects of such signalling on adipogenesis of mouse adipose-derived stem cells (mASCs).

Materials and methods: Jagged1 (50 and 100 ng/ml) was added to mASCs to activate Notch signalling, 2 days before adipogenic induction. At 5 and 7 days after induction, oil red-O staining was performed to evaluate lipid accumulation. Then real-time PCR was performed to examine expression of Notch downstream genes (*Notch-1*, *-2*, *Hes-1* and *Hey-1*) and adipogenic transcription factor (PPAR-γ). Expressions of Hes-1 and PPAR-γ at protein level were confirmed by immunofluorescence staining.

Results: Our data indicated that Jagged1 promoted adipogenic differentiation of mASCs. Moreover, Jagged1 also increased expression of Notch downstream genes and PPAR-γ. Expressions of Hes-1 and PPAR-γ were found to be enhanced in Jagged1 pre-treated mASCs when compared to controls.

Discussion: The results led to the conclusion that activation of Notch signalling had stimulated adipogenesis of mASCs in the presence of adipogenic medium by promoting expression of PPAR-γ.

Introduction

Mesenchymal stem cells (MSCs) compose a group of adult stem cells that can differentiate into a variety of cell types, such as osteoblasts, chondrocytes, adipocytes, myocytes and beta-pancreatic islet cells. MSCs can be isolated from many organs and tissues including marrow, umbilical cord blood, adipose tissue, adult muscle and dental pulp of deciduous teeth (1–3). In recent years, research interest has grown rapidly on adipose tissue as a stem-cell source from which MSCs can be isolated. Because of the relatively high frequency of clonogenic cells and easy isolation from adipose tissue, adipose-derived stem cells (ASCs) are thought to hold promise for a wide range of therapeutic applications.

Notch signalling is a highly conserved pathway of multicellular organisms that orchestrates cell fate determination, including cell proliferation, migration, differentiation and cell death (4–6). Notch signalling is activated as a result of cell/cell contact through interactions of Notch receptors and their DSL (Delta and Serrate for Drososphila and LAG-2 of *Caenorhabditis elegans*) ligands. Mammals possess four Notch receptors: Notch1–Notch4 and five ligands: Dll1, Dll3, Dll4 (homologues of Delta) and Jagged1 & Jagged2 (homologues of Serrate) (4). The Notch receptor is activated when bound to members of the Delta and Jagged/Serrate families of ligands, leading to metalloprotease TNF-α converting enzyme and γ-secretase complex-dependent cleavage of the Notch intracellular domain (NICD) (7,8). NICD then translocates into the cell nucleus, where it interacts with the CSL family of transcriptional regulators and forms part of a Notch target gene-activating complex (9–11). Notch activation through NICD-CSL complex can in turn activate transcription of various target genes, including *Hes* (Hairy/ Enhancer of Split) (12,13), Hes-related repressor protein (HERP) (14, 15), nuclear factor-κB (NF-κB) (16) and peroxisome proliferator-activated receptor-γ (PPAR-γ) (17).

Notch-1 has been related to regulation of differentiation programmes in several vertebrate cell types such as in adipocytes (18), erythrocytes (19), myeloid cells (20), T cells (21,22), keratinocytes (23,24) and more.

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Moreover, constitutively activating Notch-1 induces expression of early differentiation markers and prevents expression of late differentiation markers. However, roles of Notch signalling in adipogenic differentiation of pre-adipocytes remain controversial. It has been argued that Notch is dispensable in cell fate specification of adipocytes (25). However, further studies indicate that activation of the Notch signalling pathway or expression of its target genes could either promote or inhibit differentiation of pre-adipocytes (18,26,27). The vital link between Notch signalling and adipogenesis is transcription factor PPAR-γ master of the complex process of adipogenic differentiation, involving many transcription regulators and extracellular signals (28). PPAR-γ has been suggested to interact with the Notch signalling network. For instance, Notch-1 is necessary for expression of PPAR-γ in murine preadipocytes (18). However, interactions between Notch signalling and PPAR-γ in ASCs has never been investigated.

In this study, we aimed to investigate effects of Notch signalling on adipogenesis of mouse adipose-derived mesenchymal stem cells. Between Jagged1 pre-treated groups and the control group, we compared (i) accumulation of lipid droplets; (ii) activation of the Notch signalling pathway and expression of PPAR-γ, after adipogenic induction; (iii) Hes-1 and PPAR-γ protein expression after adipogenic induction. Our data indicate that activation of Notch signalling enhanced adipogenesis of mouse adipose-derived mesenchymal stem cells by overexpression of PPAR-γ.

Materials and methods

Isolation and culture of mASCs

Three-week-old Kunming mice from the Sichuan University Animal Center were used in this study, in accordance with the International Guiding Principles for Animal Research (1985). All surgical procedures were performed under approved anaesthetic methods using Nembutal at 35 mg/kg. Inguinal fat pads were dissected from the mice, chopped and washed extensively with sterile PBS to remove contaminating debris. Then they were incubated in 0.075% type 1 collagenase (Sigma-Aldrich, St. Louis, MO, USA) for 60 min at 37 °C with agitation. Cells released from adipose tissues were filtered and collected by centrifugation at 1200 ***g*** for 10 min. Resulting pellets were resuspended, washed three times in medium and cells were seeded in tissue culture-treated flasks in basic medium (α-MEM plus 10% FBS). Cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C and mASCs were passaged three times prior to all assays.

Jagged1 treatment, adipogenic induction and oil red-O staining

Fourth passage mASCs were seeded into six-well plates at 1×10^5 cells/well. Once 60% confluency was reached, all wells were divided into three experimental groups: one control and two Jagged1 pre-treated groups, with at least three parallel wells in each group. Recombinant Rat Jagged 1 Fc Chimera (R&D Systems, Minneapolis, MN, USA) was dissolved in sterile PBS to obtain stock solution of 200 μg/ml, which was then diluted with basic medium to desired concentrations. Cells cultured in basic medium alone was set as control. In Jagged1 groups, mASCs were incubated in Jagged1 solution in gradient concentrations (50 and 100 ng/ml) for 2 days. Cells in control and Jagged1 pre-treated groups were then cultured in adipogenic differentiation medium containing α-MEM, 10% FBS, dexamethasone (1 μM), insulin (10 μM), indomethacin (200 μM) and 3-isobutyl-1-methylxanthine (0.5 mM) (Sigma-Aldrich, Oakville, ON, Canada). Lipid droplets of differentiated mASCs were analysed using oil red-O (ORO) staining as follows: Cells in each well were fixed in 10% paraformaldehyde solution for 20 min, washed in PBS and stained in ORO (Amresco, Solon, OH, USA) solution (in 60% isopropanol) for 20 min. To quantify adipogenic progress, 10 random microscopic fields (amplification time: 10×10) were observed using an Olympus IX 710 microscope (Olympus, Tokyo, Japan) for each well. Images were captured for each field and image analysis was carried out using Image-Pro Plus 6.0.0.260 (Media Cybernetics, Silver Spring, MD, USA). After microscopic study, quantification of lipid accumulation was measured by ORO staining extraction assay. Four hundred microlitre extraction solution (100% isopropanol) was added to each well, gently mixed for 15 min, then extract was transferred to a 96-well plate; absorbance was recorded at 510 nm using a Varioskan Flash spectral scanning multimode reader (Thermo Scientific, Waltham, MA, USA).

Extraction of total RNA, RT-PCR and real-time PCR

We assessed expression of *Notch-1*, *Hes-1*, *Hey-1*, *PPAR-γ1*, *-γ2* at transcriptional levels by real-time PCR. Initially, total RNA was extracted from fresh cells using Simply P total RNA extraction kit (BioFlux, Hangzhou, China) and was reverse transcribed into cDNA using a PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio, Tokyo, Japan) according to manufacturer's instructions. Total RNA and cDNA of each sample were examined using agarose electrophoresis according to the protocol outlined in Molecular Cloning: A Laboratory Manual (2001, 3rd edition). To establish the standard

curve for a certain gene, cDNA samples were amplified using an RT-PCR kit (Tiangen, China) with primers as displayed in Table 1. Expression of certain genes was then quantified with real-time PCR, utilizing SYBR® Premix ExTaq™(Perfect Real Time) kit (Takara, Japan). Reactions were carried out on an ABI 7300 system (ABI, Foster City, CA, USA). For each reaction, a melting curve was generated to test primer dimmer formation and false priming. Then relative expression of mRNA levels was carried out by means of the double standard curve method. GAPDH was used for normalization of real-time PCR results.

Immunofluorescence staining of Hes-1 and PPAR-γ

To demonstrate distribution of Hes-1 and PPAR-γ proteins, cells were seeded on glass coverslips for immunofluorescence (IF) staining. Before staining, cells were treated with 100 ng/ml Jagged1 in basic medium, for 2 days. Controls were cells cultured in basic medium. Cells were next induced to adipocytes in DM for 6 days. They were then washed briefly in PBS, fixed in cold paraformaldehyde for 20 min at room temperature, then blocked in 0.5% bovine serum albumin for 20 min. Coverslips were subsequently incubated overnight at 4 °C in rabbit polyclonal antibody against Hes-1 (1:100) (Abcam, Cambridge, UK), rabbit polyclonal antibody against PPAR-γ (1:100) (Abcam) and rabbit polyclonal antibody against PPAR-γ with phosphoserine at residue 84 (1:100) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Sequentially, slides were incubated with secondary antibodies conjugated to fluorescein isothiocyanate (Invitrogen, Carlsbad, CA, USA) for 1 h at room temperature, and nuclei were counterstained with DAPI (Molecular Probes, Eugene, OR, USA) for 5 min. After being rinsed in PBS, cells were observed and photomicrographs were taken using the Olympus IX 710 microscope. Images were analysed using Image-Pro Plus 6.0.0.260 and integral optical density (IOD) was measured to evaluate Hes-1 and PPAR-γ concentration.

Table 1. Primer Sequences of target genes and GAPDH for real-time PCR assay

Genes	NM	Primer Sequence (5′–3′)
Hes-1	008235.2	F: GCCAATTTGCCTTTCTCATC R: AGCCACTGGAAGGTGACACT
Hey-1	010423.2	F: AATGGCCACGGGAACGCTGG R: CACCACGGGAAGCACCGGTC
Notch-1	008714.3	F: AACGTGGTCTTCAAGCGTGAT R:AGCTCTCCACACGGTTCATC
Notch-2	010928.2	F: TTGTGTGCCGCAGGGCATGT R: AGCCGACTTGGCAGGTGCAC
PPAR-γ1	001127330.1	F: CCAACTTCGGAATCAGCTCT R: CAACCATTGGGTCAGCTCTT
PPAR-γ2	011146.3	F: TGCACTGCCTATGAGCACTT R: TGATGTCAAAGGAATGCGAG
GAPDH	017008	F: TATGACTCTACCCACGGCAAGT R: ATACTCAGCACCAGCATCACC

Data analysis

All experiments were repeated a minimum of three times and representative data are presented as mean ± SD. ANOVA was used to analyse differences within groups in all assays. To specify significant difference between experimental groups and controls, the Dunnett *t*-test was conducted. To determine effectiveness of different Jagged1 concentrations, data were also analysed using the LSD *t*-test. *P*-values <0.05 were considered significantly different in all *t*-tests.

Results

Jagged1 promoted accumulation of lipid droplets

After 2 days treatment with Jagged1 (50 and 100 ng/ml), mASCs were cultured in 24-well plates in insulin-containing cocktail DM. After 5 and 7 days differentiation, oil red-O staining had been performed to analyse adipogenesis (Fig. 1a). At early time points, morphological transformation of mASCs was observed. Cells were found to have become more spherical in shape. Next to this, large numbers of small lipid droplets appeared in the cells, throughout the cytoplasm. Oil red-O positively stained lipid vacuoles, strongly suggesting that Jagged1 had significantly advanced differentiation and the process of adipogenesis (Fig. 1b). Furthermore, oil red-O spectrophotometer quantification was evaluated for each group (Fig. 1c). Lipid droplets in the 100 ng/ml Jagged1 groups were significantly more concentrated than in controls on days 5 and 7, respectively.

Jagged1 promoted Hes-1, Hey-1, Notch-1, 2 and PPAR-γ transcription after adipogenic induction

After 5 and 7 days induction of differentiation, Hes-1, Hey-1, Notch-1, -2 and PPAR-γ1, -γ2 mRNAs were detected using real-time PCR (Fig. 2). mRNA level of Hes-1 in 100 ng/ml Jagged1 groups was significantly higher than in 50 ng/ml groups and control groups at each time point, and on day 7 was significantly higher than on day 5 in 100 ng/ml Jagged1 groups. However, transcript levels of the gene coding for Hey-1 were similar to that of the control in Jagged1 groups on day 5. mRNA levels were found to be higher compared to

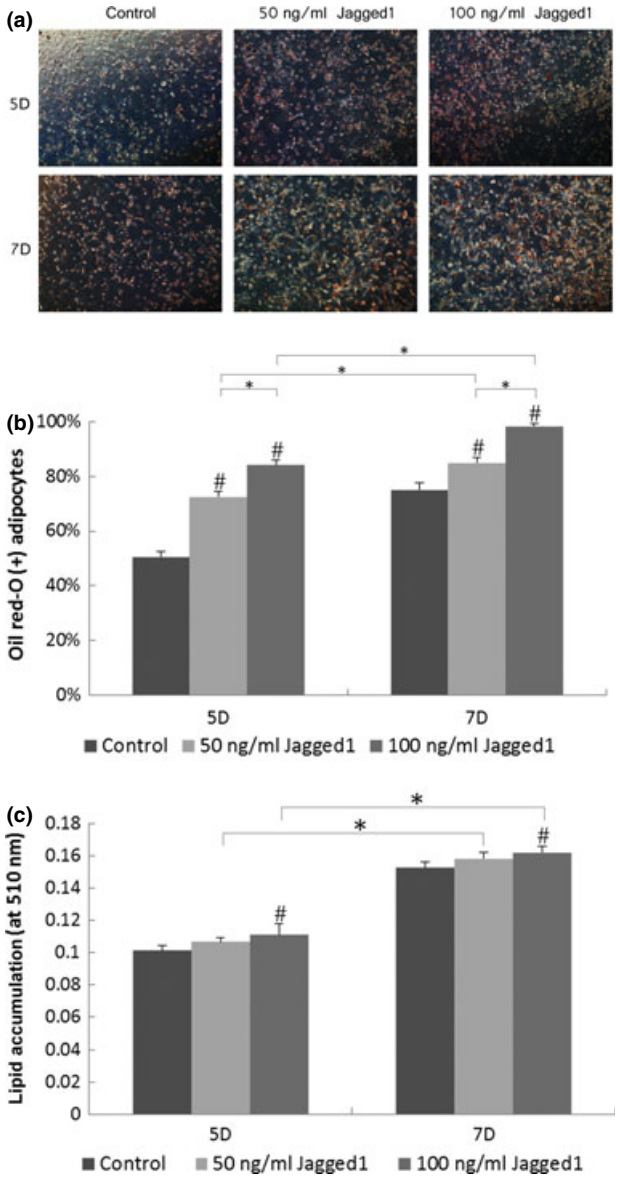

Figure 1. Jagged1 promotes lipid accumulation in ASCs. (a) After 5 and 7 days in differentiation medium, oil red-O staining was carried out to investigate adipogenic differentiation of ASCs. (b) Oil red-O positively stained adipocytes in each group suggest that Jagged1 advanced the process of adipogenesis. (c) Oil red-O spectrophotometer quantification was performed for each group. The data demonstrate that lipid accumulation of the100 ng/ml Jagged1 group, was significantly higher than that of the control group, at each time point, as indicated by #. Jagged1 pre-treatment also promoted adipogenesis time dependently as indicated by *. Both # and * represent $P < 0.05$.

controls and with significant difference between the two Jagged1 groups on day 7. Also, it was significantly higher than on day 5 in both Jagged1-treated groups. Notch-1 transcript levels in 100 ng/ml Jagged1 groups was significantly higher than the 50 ng/ml Jagged1 and control groups at each time point, and on day 7, it was significantly higher than on day 5 in both Jagged1-treated groups. Transcript levels of the gene coding for Notch-2 were similar to those of controls in both Jagged1 groups on day 5. On day 7, however, in the 100 ng/ml Jagged1 groups, it was significantly higher than in 50 ng/ml Jagged1 and control groups, and also higher than in 100 ng/ml Jagged1 groups on day 5 respectively. PPAR-γ1 transcript levels in 100 ng/ml Jagged1 groups were found to be significantly higher than in 50 ng/ml Jagged1 and control groups at each time point. On day 5, mRNA level of PPAR-γ2 in both Jagged1 groups was significantly higher than in control groups, but without significant difference between the two treated groups. In the 100 ng/ml Jagged1 groups, it was significantly higher than in 50 ng/ml Jagged1 and control groups on day 7, and also higher than the 100 ng/ml Jagged1 groups on day 5.

Jagged1 promoted Hes-1 and PPAR-γ protein expression after 6 days adipogenic induction

PPAR-γ is commonly activated by dephosphorylation in cell cytoplasm. Dephosphorylated PPAR-γ moves into nuclei and functions as an intranuclear transcriptional factor. After 6 days differentiation, IF was performed with anti-Hes-1 (Fig. 3a), anti-PPAR-γ (Fig. 3b) anti-ph-PPAR-γ (Fig. 3c) antibodies. We observed that Hes-1 was expression was significantly higher in Jagged1-treated groups (2.86 ± 0.05%) than in controls (2.23 ± 0.06%). PPAR-γ was found to be condensed in nuclei, with significantly higher IOD in Jagged1-treated groups (2.97 ± 0.08%) than in controls (2.25 ± 0.14%). ph-PPAR-γ was also found to be higher in Jagged1 (1.54 ± 0.07%) than control groups (1.20 ± 0.08%)(Fig. 3d).

Discussion

In this study, we have demonstrated that Jagged-1 increased adipogenic differentiation of ASCs by activating Notch signalling. Addition of Jagged-1 influenced cell distribution of multiple Notch receptors and activated Notch signalling. After Jagged-1-mediated activation of Notch signalling, not only were Notch receptor levels increased during the differentiation process, but triggering of ASCs terminal adipogenic induction and accumulation of lipid droplets were found.

Notch signalling involves a complex network of processes and plays a critical role in regulation of differentiation and proliferation of stem cells (4,6). γ-secretase targets a range of signalling pathways and has important effects on regulating Notch signalling, which in turn is involved in developmental decisions in a diverse range of progenitor cells (29). Upon receptor engagement,

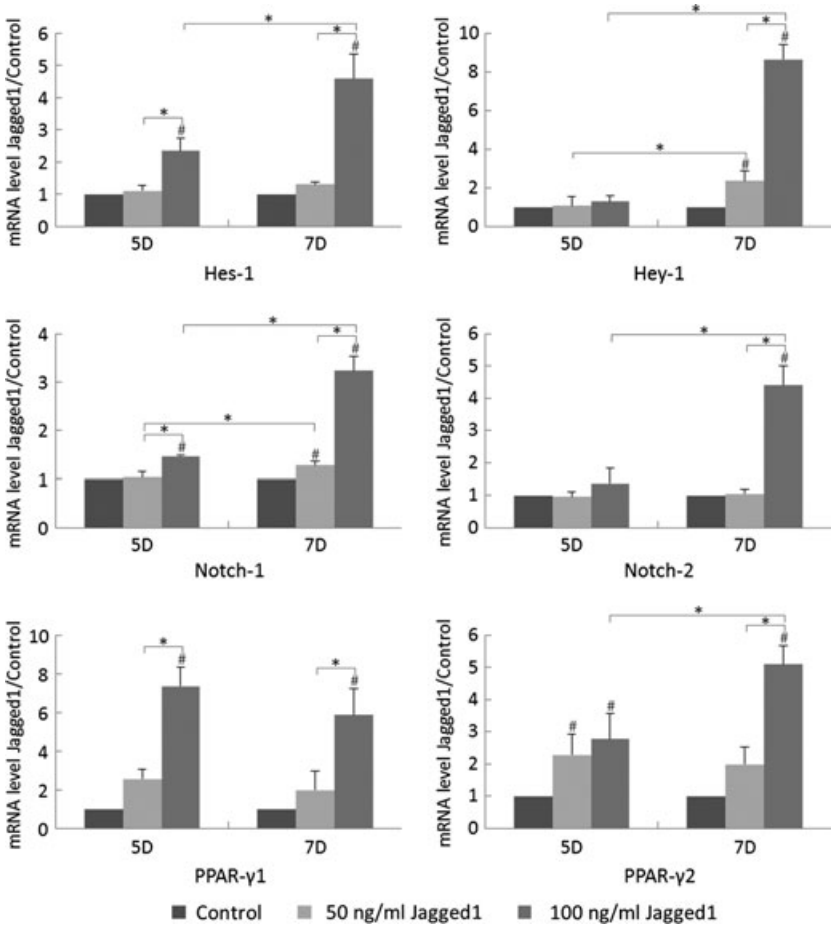

Figure 2. Jagged 1 upregulated PPAR-γ expression by activating Notch signalling. At days 5 and 7 of adipogenic induction, Jagged1 pre-treated group showed higher expressions of Hes-1, Hey-1, Notch-1, -2 and PPAR-γ1, PPAR-γ2. #,*Significantly different from control and specified Jagged1 group, respectively ($P < 0.05$ by #Dunnett *t*-test and *LSD *t*-test).

cleavage and nuclear redistribution of intracellular domains of Notch receptors, is thought to convert the ubiquitous transcription factor CSL, from a repressor to be an activator, through dissociation of nuclear co-repressors and histone deacetylase 1 (30) and recruitment of co-activators (10) and histone acetyltransferases (31). This triggers a cascade of transcriptional regulation that ultimately affects cell fate. Notch, a transmembrane receptor member of the homeotic epidermal growth factor-like family of proteins, participates in cell/cell signalling to control cell fate during development. Activated Notch-1 constructs lacking the extracellular region prevent differentiation of several types mammalian cell *in vitro*. This effect, however, bypasses normal mechanisms of cell/cell interactions in which Notch-1 participates. *Notch-1* is a target gene of Notch signalling. Expression and function of Notch-1 are necessary conditions for adipogenesis, and it can induce PPAR-γ expression, which plays a pivotal role during adipogenic differentiation (18). Our real-time PCR data show that Notch 1 and 2 may be involved in this process, possibly in co-operation. This suggests that sequential activation of different Notch receptors may co-ordinate the ASCs differentiation programme.

Conflicting findings have been reported concerning roles of Notch signalling in terminal differentiation of pre-adipocytes. Current data suggest that activation of Notch signalling could either inhibit or promote adipogenesis of stem cells. The process of adipogenesis involves downregulation of the gene encoding Hes-1, which is now considered to function both as a repressor or else as an activator. However, promoter analyses of upregulated and downregulated genes in 3T3 pre-adipocytes have indicated that Notch most likely blocks adipogenesis by induction of Hes-1 homodimers, which repress transcription of key target genes (27). It establishes a new target downstream of the Notch–Hes-1 pathway and suggests a dual role for Hes-1 in adipocyte development (32). Nevertheless, findings in our study suggest that Hes-1 transcription in mASCs was promoted after 2 days Jagged1 treatment during subsequent adipocyte differentiation, for at least 5 days. IF assay also demonstrated that Hes-1 protein expression is significantly increased in Jagged1 groups, and it may be that there are further pathways over Notch–Hes-1. Hey-1 is also promoted by Jagged1, but not as dramatically as Hes-1, specially during early differentiation (5 days).

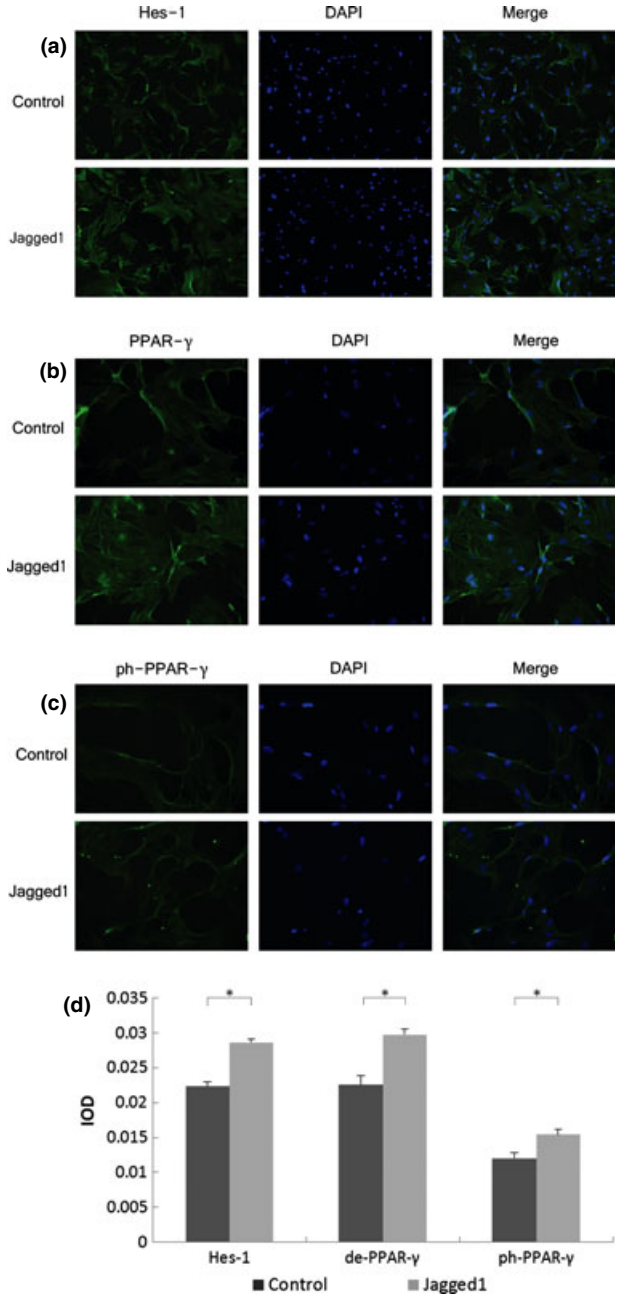

Figure 3. Immunofluorescence staining revealed expression of Hes-1 and PPAR-γ in differentiated mASCs. (a–c) At day 6 of adipogenic differentiation, expression of Hes-1, de-PPAR-γ and ph-PPAR-γ in the Jagged1 pre-treated group and controls was examined. (d) IOD of Hes-1, de-PPAR-γ and ph-PPAR-γ was significantly higher in the Jagged 1 group than in controls. $*P < 0.05$. Magnification ×400.

Activation of Notch signalling by Jagged1 promotes formation of adipocytes and upregulates expression of important adipocyte-specific marker genes. PPAR-γ is an important transcription factor for normal adipogenic differentiation. Increased adipogenic formation seen in Jagged1-treated mASCs may thus be due to the observed increase in PPAR-γ expression in these samples. PPAR-γ is the most important player in transcriptional control of adipogenesis (33–35). When activated by its ligands, PPAR-γ alone can drive the complete adipogenic process. It has been reported that expression of PPAR-γ is turned on early in adipogenesis, following expression of transcription factors CÆBP-β and CÆBP-δ, leading to increased expression of CÆBP-α (36,37). PPAR-γ expression and activation are key control components for driving cells to the adipocyte phenotype. PPAR-γ is expressed preferentially in adipose tissue, and its expression increases during the process of adipogenesis. It seems that Notch-1 is linked to expression of *PPAR-γ* gene by hormonal induction of adipocyte differentiation. Our data demonstrate that transcription of *PPAR-γ* was induced dose-dependently by Jagged1. During MSC differentiation, PPAR-γ is activated by a process of dephosphorylation; it then translocates to cell nuclei where it binds to target DNA sequences and exerts its effects (38). Results of IF analysis from the current study suggest that promotion of de-PPAR-γ in Jagged1 groups may due to activation of the PPAR-γ dephosphorylation-related signalling pathway by Jagged1 or increasing translation of PPAR-γ mRNA. Indeed, ph-PPAR-γ was also promoted by Jagged1 in our study. This indicates that a mechanism involving activation of PPAR-γ dephosphorylation may be triggered by Jagged1. Therefore, increased de-PPAR-γ in nuclei provides a reasonable explanation for promoted adipogenesis processes in Jagged1 cases.

In conclusion, we demonstrate that activation of Notch signalling by Jagged1 enhanced adipogenesis of mASCs, with induction of PPAR-γ. Upregulation of PPAR-γ is triggered by soluble Notch ligands through a novel signalling mechanism.

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References

1 Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH (2004) Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood* **103**, 1669–1675.

2 Kern S, Eichler H, Stoeve J, Kluter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* **24**, 1294–1301.
3 Phinney DG, Kopen G, Isaacson RL, Prockop DJ (1999) Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation. *J. Cell. Biochem.* **72**, 570–585.
4 Miele L, Osborne B (1999) Arbiter of differentiation and death: Notch signaling meets apoptosis. *J. Cell. Physiol.* **181**, 393–409.
5 Li L, Milner LA, Deng Y, Iwata M, Banta A, Graf L *et al.* (1998) The human homolog of rat Jagged1 expressed by marrow stroma inhibits differentiation of 32D cells through interaction with Notch1. *Immunity* **8**, 43–55.
6 Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. *Science* **284**, 770–776.
7 Brou C, Logeat F, Gupta N, Bessia C, LeBail O, Doedens JR *et al.* (2000) A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol. Cell* **5**, 207–216.
8 Mumm JS, Schroeter EH, Saxena MT, Griesemer A, Tian X, Pan DJ *et al.* (2000) A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. *Mol. Cell* **5**, 197–206.
9 Aster JC, Robertson ES, Hasserjian RP, Turner JR, Kieff E, Sklar J (1997) Oncogenic forms of NOTCH1 lacking either the primary binding site for RBP-Jkappa or nuclear localization sequences retain the ability to associate with RBP-Jkappa and activate transcription. *J. Biol. Chem.* **272**, 11336–11343.
10 Zhou S, Fujimuro M, Hsieh JJ, Chen L, Miyamoto A, Weinmaster G *et al.* (2000) SKIP, a CBF1-associated protein, interacts with the ankyrin repeat domain of NotchIC to facilitate NotchIC function. *Mol. Cell. Biol.* **20**, 2400–2410.
11 Wu L, Aster JC, Blacklow SC, Lake R, Artavanis-Tsakonas S, Griffin JD (2000) MAML1, a human homologue of Drosophila mastermind, is a transcriptional co-activator for NOTCH receptors. *Nat. Genet.* **26**, 484–489.
12 Wu L, Griffin JD (2004) Modulation of Notch signaling by mastermind-like (MAML) transcriptional co-activators and their involvement in tumorigenesis. *Semin. Cancer Biol.* **14**, 348–356.
13 Grottkau BE, Chen XR, Friedrich CC, Yang XM, Jing W, Wu Y *et al.* (2009) DAPT enhances the apoptosis of human tongue carcinoma cells. *Int. J. Oral Sci.* **1**, 81–89.
14 Iso T, Chung G, Hamamori Y, Kedes L (2002) HERP1 is a cell type-specific primary target of Notch. *J. Biol. Chem.* **277**, 6598–6607.
15 Iso T, Kedes L, Hamamori Y (2003) HES and HERP families: multiple effectors of the Notch signaling pathway. *J. Cell. Physiol.* **194**, 237–255.
16 Ohazama A, Hu Y, Schmidt-Ullrich R, Cao Y, Scheidereit C, Karin M *et al.* (2004) A dual role for Ikk alpha in tooth development. *Dev. Cell* **6**, 219–227.
17 Nickoloff BJ, Qin JZ, Chaturvedi V, Denning MF, Bonish B, Miele L (2002) Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-kappaB and PPARgamma. *Cell Death Differ.* **9**, 842–855.
18 Garces C, Ruiz-Hidalgo MJ, Font de Mora J, Park C, Miele L, Goldstein J *et al.* (1997) Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis. *J. Biol. Chem.* **272**, 29729–29734.
19 Shelly LL, Fuchs C, Miele L (1999) Notch-1 inhibits apoptosis in murine erythroleukemia cells and is necessary for differentiation induced by hybrid polar compounds. *J. Cell. Biochem.* **73**, 164–175.
20 Milner LA, Bigas A (1999) Notch as a mediator of cell fate determination in hematopoiesis: evidence and speculation. *Blood* **93**, 2431–2448.
21 Yasutomo K, Doyle C, Miele L, Fuchs C, Germain RN (2000) The duration of antigen receptor signalling determines CD4+ versus CD8+ T-cell lineage fate. *Nature* **404**, 506–510.
22 Pui JC, Allman D, Xu L, DeRocco S, Karnell FG, Bakkour S *et al.* (1999) Notch1 expression in early lymphopoiesis influences B versus T lineage determination. *Immunity* **11**, 299–308.
23 Rangarajan A, Talora C, Okuyama R, Nicolas M, Mammucari C, Oh H *et al.* (2001) Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO J.* **20**, 3427–3436.
24 Lowell S, Jones P, Le Roux I, Dunne J, Watt FM (2000) Stimulation of human epidermal differentiation by delta-notch signalling at the boundaries of stem-cell clusters. *Curr. Biol.* **10**, 491–500.
25 Nichols AM, Pan Y, Herreman A, Hadland BK, De Strooper B, Kopan R *et al.* (2004) Notch pathway is dispensable for adipocyte specification. *Genesis* **40**, 40–44.
26 Ross DA, Rao PK, Kadesch T (2004) Dual roles for the Notch target gene Hes-1 in the differentiation of 3T3-L1 preadipocytes. *Mol. Cell. Biol.* **24**, 3505–3513.
27 Ross DA, Hannenhalli S, Tobias JW, Cooch N, Shiekhattar R, Kadesch T (2006) Functional analysis of Hes-1 in preadipocytes. *Mol. Endocrinol.* **20**, 698–705.
28 Rosen ED, MacDougald OA (2006) Adipocyte differentiation from the inside out. *Nat. Rev. Mol. Cell Biol.* **7**, 885–896.
29 Sarmento LM, Huang H, Limon A, Gordon W, Fernandes J, Tavares MJ *et al.* (2005) Notch1 modulates timing of G1-S progression by inducing SKP2 transcription and p27 Kip1 degradation. *J. Exp. Med.* **202**, 157–168.
30 Kao HY, Ordentlich P, Koyano-Nakagawa N, Tang Z, Downes M, Kintner CR *et al.* (1998) A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. *Genes Dev.* **12**, 2269–2277.
31 Kurooka H, Honjo T (2000) Functional interaction between the mouse notch1 intracellular region and histone acetyltransferases PCAF and GCN5. *J. Biol. Chem.* **275**, 17211–17220.
32 Huang Y, Yang X, Wu Y, Jing W, Cai X, Tang W *et al.* (2010) Gamma-secretase inhibitor induces adipogenesis of adipose-derived stem cells by regulation of Notch and PPAR-gamma. *Cell Prolif.* **43**, 147–156.
33 Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B (2004) Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. *Aging Cell* **3**, 379–389.
34 Fink T, Abildtrup L, Fogd K, Abdallah BM, Kassem M, Ebbesen P *et al.* (2004) Induction of adipocyte-like phenotype in human mesenchymal stem cells by hypoxia. *Stem Cells* **22**, 1346–1355.
35 Schadinger SE, Bucher NL, Schreiber BM, Farmer SR (2005) PPARgamma2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. *Am. J. Physiol. Endocrinol. Metab.* **288**, E1195–E1205.
36 Burgermeister E, Schnoebelen A, Flament A, Benz J, Stihle M, Gsell B *et al.* (2006) A novel partial agonist of peroxisome proliferator-activated receptor-gamma (PPARgamma) recruits PPAR-gamma-coactivator-1alpha, prevents triglyceride accumulation, and potentiates insulin signaling in vitro. *Mol. Endocrinol.* **20**, 809–830.
37 Astudillo P, Rios S, Pastenes L, Pino AM, Rodriguez JP (2008) Increased adipogenesis of osteoporotic human-mesenchymal stem cells (MSCs) characterizes by impaired leptin action. *J. Cell. Biochem.* **103**, 1054–1065.
38 Wu L, Cai X, Dong H, Jing W, Huang Y, Yang X *et al.* (2010) Serum regulates adipogenesis of mesenchymal stem cells via MEK/ERK-dependent PPARgamma expression and phosphorylation. *J. Cell Mol. Med.* **14**, 922–932.

