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Wnt/β-catenin signaling changes C2C12 myoblast proliferation and differentiation by inducing Id3 expression

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ABSTRACT

Canonical Wnt signaling plays important roles in regulating cell proliferation and differentiation. In this study, we report that inhibitor of differentiation (Id)3 is a Wnt-inducible gene in mouse C2C12 myoblasts. Wnt3a induced Id3 expression in a β-catenin-dependent manner. Bone morphogenetic protein (BMP) also potently induced Id3 expression. However, Wnt-induced Id3 expression occurred independent of the BMP/Smad pathway. Functional studies showed that Id3 depletion in C2C12 cells impaired Wnt3a-induced cell proliferation and alkaline phosphatase activity, an early marker of osteoblast cells. Id3 depletion elevated myogenin induction during myogenic differentiation and partially impaired Wnt3a suppressed myogenin expression in C2C12 cells. These results suggest that Id3 is an important Wnt/β-catenin induced gene in myoblast cell fate determination.

1. Introduction

Wnts are a family of growth factors controlling multiple biological processes such as embryogenesis, organogenesis and tumorigenesis [1–4]. In the presence of Wnt ligand, Wnt receptor frizzled (Fz) and its co-receptor low-density lipoprotein receptor-related protein-5 or 6 (LRP-5/6) recruit Axin and GSK3β to the plasma membrane, together with the scaffold protein Disheveled (Dvl) [5,6]. The membrane association of Axin and GSK3β disrupts the β-catenin destruction complex, resulting in accumulation of β-catenin in the nucleus, where it triggers target gene activation by displacing transcriptional repressors from DNA-bound LEF/TCF [7,8]. In recent years, canonical Wnt signaling pathway is identified to be crucial for bone formation and bone homeostasis. The mutations in LRP-5 profoundly affect skeletal development and result in low bone mass [9,10]. The Dickkopf-1 (Dkk-1) resistant LRP5V171 mutation leads to high bone density [11]. Conditional deletion of the β-catenin gene in osteoblasts leads to reduced bone-mass in vivo [12]. Canonical Wnt signaling is also reported to be involved in myogenic differentiation of mouse myoblast cells [13].

Id (inhibitor of DNA binding) proteins are helix–loop–helix (HLH) proteins, which lack basic region adjacent to the HLH domain that is essential for specific DNA binding in other bHLH proteins [14]. Id proteins repress bHLH proteins by binding and interfering with DNA interaction of HLH proteins. As direct target genes of bone morphogenetic proteins (BMPs), Id proteins regulate cell fate [15,16]. In this study, we found canonical Wnt/β-catenin signaling could induce Id3 expression independent of BMP/Smad signaling activation. Loss of function studies in C2C12 cells demonstrated that Id3 plays a pivotal role in canonical Wnt3a-induced cell fate determination.

2. Materials and methods

2.1. Reagent and plasmids

GAPDH antibody (Sigma), Id3 antibody (C-20, Santa Cruz), P-Smad1 (#9511 Cell signaling) and Smad1 (SC-7965 Santa Cruz), Myogenin (ab1835, Abcam); BMP response element (BRE)-Luc reporter, β-catenin WT/SY plasmids were previously described [17–19]. Dominant negative Lymphoid enhancer binding factor (LEF)-1 was cloned by polymerase chain reaction (PCR) into pcDNA3.1 vector.

2.2. Cell culture

Mouse myoblast C2C12, NIH3T3, C3H10T1/2, control and Wnt3a expressing L cells and HEK293T cells were maintained in growing Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified incubator with 5% CO_2. C2C12 myoblasts were seeded at a concentration of 0.35×10^6 cells per well in 6-well plates and cultured for 24 h to reach 100% confluence (day 0). To induce myogenic differentiation, cells were washed in PBS and cultured in low glucose DMEM supplemented with antibiotics and 2% heat-inactivated horse serum, referred to as differentiation medium (DM). All of

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the experiments were carried out from day 0 to day 6 after the induction of differentiation and cells were examined for myogenin expression. For all the Wnt3a conditioned medium (CM) treated experiment, L control CM was used as control treatment.

2.3. Lentiviral transduction

Lentiviruses were produced by transfecting pLV-bc-CMV (for cDNA expression) plasmids together with helper plasmids pCMV-VSVG, pMDLg-RRE (gag/pol), and pRSV-REV into HEK293T human epidermal kidney cells. Cell supernatants were harvested 48 h after transfection and were either used to infect cells or stored at −80 °C. C2C12 were infected and maintained in puromycin medium as stable cells. Mouse Id3 shRNA (#1: TRCN0000071438; #2: TRCN 0000071439) MISSION® shRNA Lentiviral Transduction Particles were purchased from Sigma mission library, Inc (USA). PLKO.1 control shRNA was served as control.

2.4. Transient cell transfection and luciferase activity assay

Transient transfections and reporter assays were performed in triplicates as previously described [20]. In all reporter assays, a β-galactosidase expression plasmid was co-transfected and served as a control to correct for transfection efficiency. The experiments were performed in triplicates.

2.5. QRT-PCR (quantitative real-time–PCR)

Total RNA was isolated using NucleoSpin® RNA II kit (BIOKÉ, Netherlands) reagent. 1 μg RNA was reverse-transcribed using the RevertAid™ First Strand cDNA Synthesis Kits (Fermentas). Quantitative real-time PCR was accomplished with SYBR Green incorporation (Applied Bioscience) using a StepOne Plus real-time PCR system (Applied Bioscience). Results were normalized to those obtained with GAPDH. Primers used for QRT-PCR were:mGAPDH forward, 5′-AACTTTGGCATTGTGGAAGG-3′.mGAPDH reverse, 5′-ACACATTG GGGGTAGGAACA-3′.mAxin2 forward, 5′-GGTTCCGGCTATGTCTTT GC -3′.mAxin2 reverse, 5′-CAGTGCGTCGC TGGATAACTC -3′.mId1 forward, 5′-ACCCTGAACGGCGAGATCA-3′.mId1 reverse, 5′-TCGTC GGCTGGAACACAT-3′.mId3 forward, 5′-ACCTCCCGAACGCAGGTG CT-3′.mId3 reverse, 5′-ATGCCCTCAGGCTTCCGGCT-3′.

2.6. Western blotting

Western blotting was performed as previously described using standard techniques [21,22]. Antibodies used for immunoblotting are mentioned above.

2.7. Alkaline phosphatase (ALP) assay

Histochemical examination of alkaline phosphatase (ALP) activity in cells was performed as previously described using naphthol AS-MX phosphate (Sigma) and fast blue RR salt (Sigma) [23].

2.8. MTT cell viability assay

Cell viability was determined using the MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Promega, Madison, WI) as follows. Treatment medium was replaced with fresh treatment medium containing 20 μl/ml of the Cell Titer 96 Aqueous One Solution and incubated for 10 min at 37 °C after which optical density was measured at 490 nm using a microplate reader. The quantity of soluble formazan product, as measured by the amount of absorbance, was directly proportional to the number of viable cells.

3. Results

3.1. Wnt3a induces Id3 expression

Id1 and Id3 belong to helix–loop–helix protein family. Both Id1 and Id3 are direct target genes of BMP/Smad1/5/8 signaling

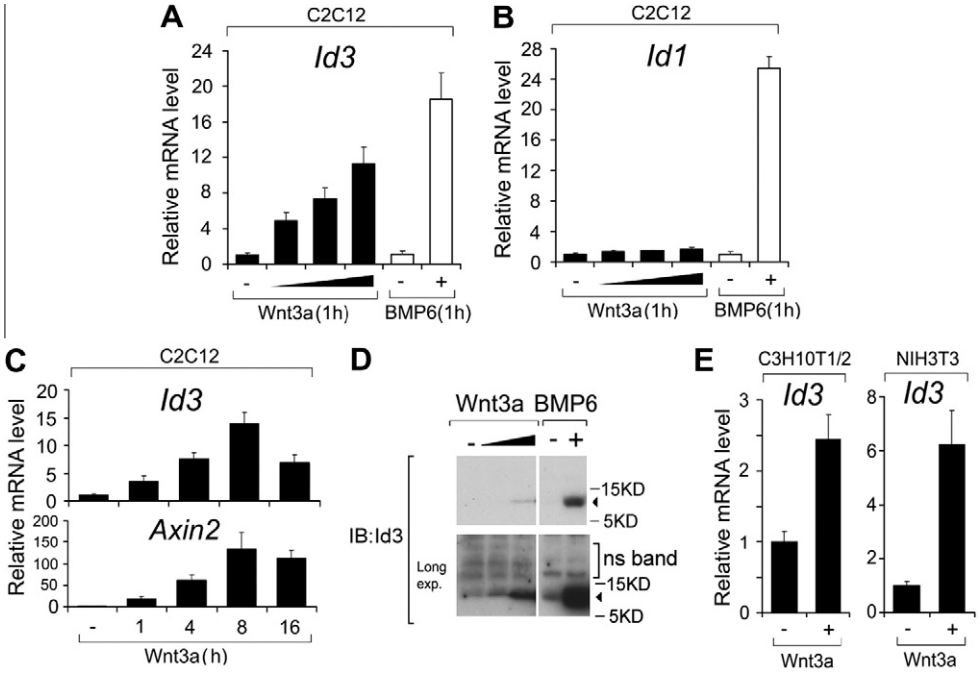

Fig. 1. Wnt3a induces Id3 expression. (A and B) C2C12 cells were stimulated with increasing doses of Wnt3a conditional medium (CM). One hour after stimulation, Id3 and Id1 expression was measured by q-PCR. Cells with BMP6 (50 ng/ml) treatment were employed as positive control. (C) time-course induction of Id3 and Axin2 by Wnt3a CM is analyzed by q-PCR in C2C12 cells. (D) Western blot showing endogenous Id3 expression induced by 1 h treatment of Wn3a CM (1:6 or 1:3) or BMP6 (50 ng/ml) in C2C12 cells. (E) q-PCR analysis of Id3 expression in NIH3T3 and C3H10T1/2 cells treated with or without Wn3a CM (1:3).

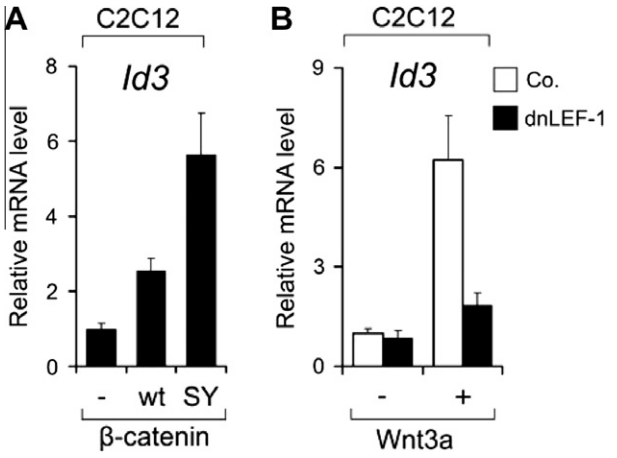

Fig. 2. Canonical Wnt/β-catenin pathway is required for Wnt3a induced Id3 expression in C2C12 cells. (A) C2C12 cells were infected with control lentivirus (−) or lentivirus expressing β-catenin wild type (WT) or constitutively active form of β-catenin (SY). Id3 expression was measured by q-PCR. (B) Wnt3a CM induced Id3 expression was analyzed in control or Dominant Negative LEF-1 (dnLEF-1) expressing C2C12 cells.

pathway. In C2C12 cells, transient stimulation of Wnt3a induced Id3 gene expression in a dose dependent manner (Fig. 1A), whereas the Id1 expression was not affected (Fig. 1B). The time course experiments showed that Id3 induction by Wnt3a was similar to Axin2, the direct downstream target gene of canonical Wnt/β-catenin signaling pathway (Fig. 1C). Western blotting also showed Wnt3a induced Id3 protein expression in C2C12 cells although it was not that strong as compared to BMP-induced Id3 expression (Fig. 1D). Similar observation was obtained in NIH3T3 and C3H10T1/2 cells (Fig. 1E).

3.2. Wnt3a induces Id3 expression via canonical Wnt/β-catenin pathway

The data presented above suggests the canonical Wnt/β-catenin pathway may mediate Id3 induction by Wnt3a. To verify this, we infected C2C12 cells with lentivirus expressing wild type β-catenin (WT) or constitutively active β-catenin (S33Y). Q-PCR analysis showed Id3 expression was elevated in β-catenin-stably expressed C2C12 cells (Fig. 2A). Furthermore, we found Wnt3a was unable to induce Id3 expression in the cells transfected with dominant negative form of LEF1 that lacks N-terminal β-catenin binding domain (Fig. 2B). This observation is consistent with the fact that Id3 promoter region has TCF/LEF binding site (see Section 4). Taken together, these results indicate that β-catenin mediated transcription is required for Id3 induction by Wnt3a.

3.3. Wnt3a does not activate BMP/Smad pathway

Id3 expression was additively induced by Wnt3a and BMP in C2C12 cells (Fig. 3A). It has been reported that BMP4 and BMP6 are induced by Wnt signaling in cancer cells [24,25]. To test whether Wnt3a induced Id3 expression occurs via BMP signaling activation, we examined whether Wnt3a can activate BMP selective Smad1 phosphorylation and Smad1-dependent transcriptional reporter activity in C2C12 cells. As shown in Fig. 3B, Wnt3a treatment did not potentiate BMP2-induced phosphorylated Smad1. By using BMP/Smad responsive transcriptional reporter BRE-Luc, we found Wnt3a did not induce BRE-Luc activity, although BMP6 strongly induced it (Fig. 3C). In addition, in BRE-Luc stably expressed C2C12 cells, Wnt3a had no significant effects on BMP2 or BMP6 induced BRE-Luc activity (Fig. 3D). Taken together, Wnt3a-induced Id3 expression is not mediated via activation of BMP/Smad pathway.

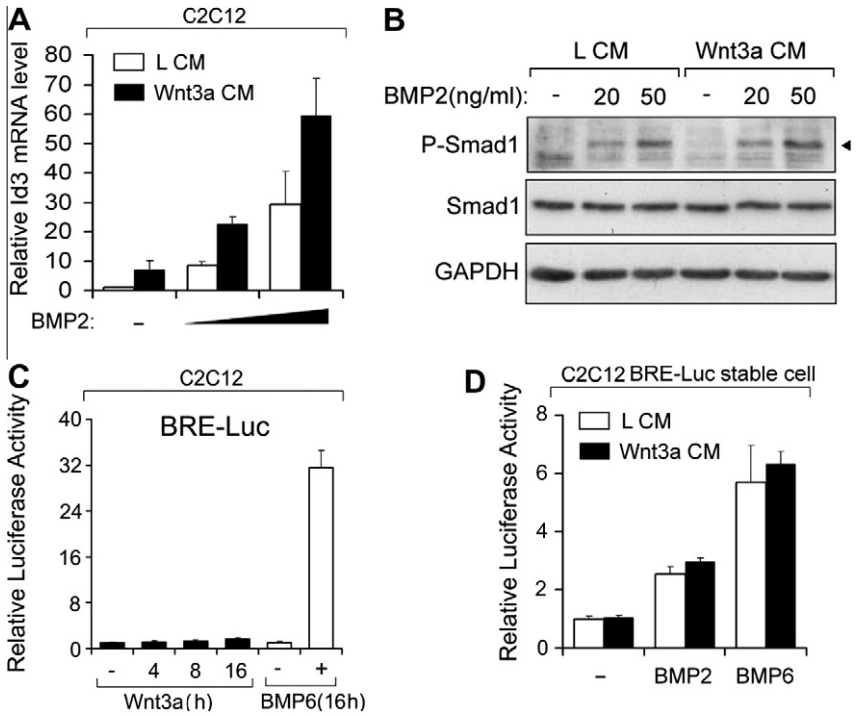

Fig. 3. Wnt3a induced Id3 expression is not through activation of BMP pathway. (A) C2C12 cells were stimulated with BMP2 (low dose: 20 ng/ml; high dose: 200 ng/ml) and Wnt3a CM as indicated for 10 h. Id3 expression was analyzed by q-PCR. (B) Western blot analysis of BMP pathway activation in C2C12 cells with 1 h treatment of BMP2 and Wnt3a CM as indicated. (C) BRE-Luc transfected C2C12 cells were stimulated with Wnt3a CM. as indicated. Cells were harvested for luciferase assay. (D) BRE-Luc stable expressed C2C12 cells were stimulated with Wnt3a CM in combination with BMP2 (50 ng/ml) or BMP6 (50 ng/ml) for 10 h. Cells were harvested for luciferase assay.

3.4. Id3 mediated Wnt3a-induced cell proliferation and alkaline phosphatase activity in C2C12 cells

Canonical Wnt pathway is important in multiple cellular processes. In C2C12 cells, Wnt3a can induce both cell proliferation and convert myoblast differentiation into osteoblast-like cells. ALP is an early marker for oseoblast differentiation. We next tested whether Id3 is involved in these responses. By using two independent Id3 shRNAs, we constructed C2C12 stable cell lines with selective Id3 depletion (Fig. 4A). As measured by cell counting and cell viability assay, Wnt3a-induced cell proliferation was impaired upon Id3 depletion (Fig. 4 B and C). Moreover, Wnt3a-induced ALP activity was severely blocked in Id3-depleted cells (Fig. 4 D and E). Next, we checked muscular differentiation of C2C12 stable cells. As shown in Fig. 4F and G, Id3 depletion elevated myogenin induction during myogenic differentiation and partially inhibited Wnt3a-induced suppression of myogenin expression in C2C12 cells. Taken together, Id3 mediated Wnt3a-induced C2C12 cell proliferation and conversion of myoblast into osteoblast-like cells.

4. Discussion

In this story, we showed that expression of Id3 can be induced by canonical Wnt signaling in C2C12 cells. Q-PCR and Western blot analysis demonstrated that Id3 mRNA level and protein is promoted by Wnt3a stimulation. Although previous studies reported that the BMP signaling can be increased by Wnt stimulation [26],

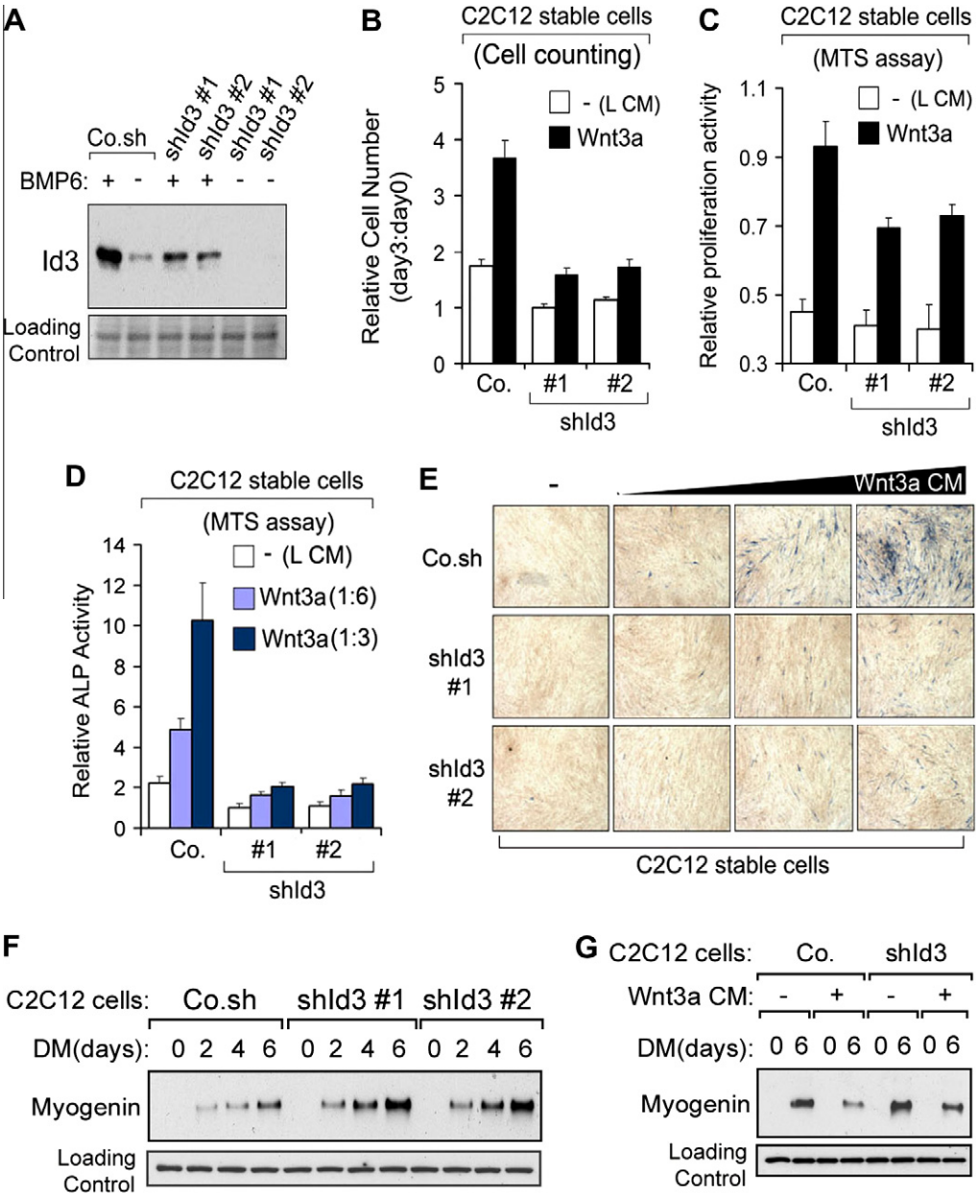

Fig. 4. Id3 depletion impaired Wnt3a induced cellular proliferation and ALP activity in C2C12 cells. (A) Western blot of Id3 expression in Id3 depleted C2C12 cells. 1 h BMP6 (50 ng/ml) was employed as positive control. (B and C) C2C12 cells were cultured in growing medium (5% FBS in L control CM or Wnt3a CM). Cell number was counted at day 0 and day 3 (B); MTS activity was measured at day 0 and day 3 (C). (D and E) C2C12 cells stably expressing Id3 shRNA #1, #2 was analyzed for Wnt3a-induced ALP activity (D) the histochemically stained cell was shown in (E). Data show the mean and SD of triplicates. Co.sh, control non-targeting shRNA. (F), C2C12 stable cells were culture in differentiation medium (DM) for 0–6 days as indicated, cells were harvested for anti-myogenin immunoblotting. (G) Control and Id3 depleted C2C12 cells were cultured in differentiation medium (DM) with or without Wnt3a treatment for 6 days, cells were harvested for anti-myogenin immunoblotting.

the evidence that Id1 expression was not induced suggests that BMP/Smad is not likely to be involved in the Id3 induction by Wnt. To confirm this, experiments were performed to examine BMP signaling in C2C12 cells. Both BMP specific reporter and BMP induced Smad phosphorylation was barely affected by Wnt3a stimulation. To further understand the mechanism underlying Wnt-induced Id3 expression, we infected C2C12 cell with lentivirus expressing β-catenin, the essential factor of canonical Wnt signaling. Expression of Id3 was promoted in β-catenin ectopic expressed cells and was even higher in cells expressing stabilized β-catenin, indicating β-catenin mediated transcription is involved in Id3 induction by Wnt. To further consolidate this result, we specifically blocked β-catenin mediated transcription by expressing dnLEF-1 in C2C12 cells. This indeed impaired Wnt3a induced Id3 expression. These results together indicate β-catenin mediated transcriptional induction of Id3 by Wnt. Since TCF/LEF binding site (5′-CTTTGAA-3′) is relatively conserved. A sequences alignment uncovered high similar motif in Id3 promoter region (−300 bp prior to transcriptional star site) but not in corresponding region of Id1 promoter, suggesting potential regulatory machinery. Future work is required to ascertain the binding to TCF/LEF transcriptional factors to this conserved site.

We next examined the functional meaning underlying. Study through loss of function showed Id3 is required for canonical Wnt signaling promoted C2C12 cell proliferation. In osteoblast differentiation assay, Id3 was required for Wnt3a-induced ALP activity, the early marker for osteoblasts. We also observed Id3 antagonized skeletal muscle directed differentiation of myoblast C2C12 cells.

We observed an additive stimulatory effect on Id3 expression by co-stimulation with BMP and Wnt. Previously, Wnt and BMP were shown to act synergistically to promote osteoblast differentiation in vitro and in vivo [27,28]. Whether Id3 plays a role new bone formation downstream of Wnt and BMP awaits further investigations.

Whereas the induction of ALP by Wnt3a is independent of BMP signaling, BMP –induced ALP in C2C12 cells relies at least in part on Wnt/β-catenin signaling and is associated with increased Wnt expression [29]. Consistent with this finding, we found that Id3 depletion reduced ALP activity that was initiated by BMP (data not shown). Id1 has been shown to activate β-catenin in Akt-dependent manner [30]. It will be interesting to examine whether Id3 can do the same.

In conclusion, our results demonstrate that upon Wnt3a stimulation of C2C12 cells, β-catenin signaling is activated and mediates the induction of Id3 expression. Wnt-induced Id3 expression plays a critical role in promoting cell proliferation and the conversion of myoblasts into osteoblast-like cells. We provide a new mechanism by which canonical Wnt signaling regulates cell proliferation and differentiation.

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References

[1] C.Y. Logan, R. Nusse, The Wnt signaling pathway in development and disease, Annu. Rev. Cell Dev. Biol. 20 (2004) 781–810.
[2] T. Reya, H. Clevers, Wnt signalling in stem cells and cancer, Nature 434 (2005) 843–850.
[3] R.T. Moon, A.D. Kohn, G.V. De Ferrari, A. Kaykas, WNT and beta-catenin signalling: diseases and therapies, Nat. Rev. Genet. 5 (2004) 691–701.
[4] B.T. MacDonald, K. Tamai, X. He, Wnt/beta-catenin signaling: components, mechanisms, and diseases, Dev. Cell 17 (2009) 9–26.
[5] X. Zeng, K. Tamai, B. Doble, S. Li, H. Huang, R. Habas, H. Okamura, J. Woodgett, X. He, A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation, Nature 438 (2005) 873–877.
[6] J. Bilic, Y.L. Huang, G. Davidson, T. Zimmermann, C.M. Cruciat, M. Bienz, C. Niehrs, Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation, Science 316 (2007) 1619–1622.
[7] J. Behrens, J.P. von Kries, M. Kuhl, L. Bruhn, D. Wedlich, R. Grosschedl, W. Birchmeier, Functional interaction of beta-catenin with the transcription factor LEF-1, Nature 382 (1996) 638–642.
[8] E. Brunner, O. Peter, L. Schweizer, K. Basler, Pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the wingless signal in Drosophila, Nature 385 (1997) 829–833.
[9] Y. Gong, R.B. Slee, N. Fukai, G. Rawadi, S. Roman-Roman, A.M. Reginato, H. Wang, T. Cundy, F.H. Glorieux, D. Lev, M. Zacharin, K. Oexle, J. Marcelino, W. Suwairi, S. Heeger, G. Sabatakos, S. Apte, W.N. Adkins, J. Allgrove, M. Arslan-Kirchner, J.A. Batch, P. Beighton, G.C. Black, R.G. Boles, L.M. Boon, C. Borrone, H.G. Brunner, G.F. Carle, B. Dallapiccola, A. De Paepe, B. Floege, M.L. Halfhide, B. Hall, R.C. Hennekam, T. Hirose, A. Jans, H. Juppner, C.A. Kim, K. Keppler-Noreuil, A. Kohlschuetter, D. LaCombe, M. Lambert, E. Lemyre, T. Letteboer, L. Peltonen, R.S. Ramesar, M. Romanengo, H. Somer, E. Steichen-Gersdorf, B. Steinmann, B. Sullivan, A. Superti-Furga, W. Swoboda, M.J. van den Boogaard, W. Van Hul, M. Vikkula, M. Votruba, B. Zabel, T. Garcia, R. Baron, B.R. Olsen, M.L. Warman, LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development, Cell 107 (2001) 513–523.
[10] R.D. Little, J.P. Carulli, R.G. Del Mastro, J. Dupuis, M. Osborne, C. Folz, S.P. Manning, P.M. Swain, S.C. Zhao, B. Eustace, M.M. Lappe, L. Spitzer, S. Zweier, K. Braunschweiger, Y. Benchekroun, X. Hu, R. Adair, L. Chee, M.G. FitzGerald, C. Tulig, A. Caruso, N. Tzellas, A. Bawa, B. Franklin, S. McGuire, X. Nogues, G. Gong, K.M. Allen, A. Anisowicz, A.J. Morales, P.T. Lomedico, S.M. Recker, P. Van Eerdewegh, R.R. Recker, M.L. Johnson, A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait, Am. J. Hum. Genet. 70 (2002) 11–19.
[11] L.M. Boyden, J. Mao, J. Belsky, L. Mitzner, A. Farhi, M.A. Mitnick, D. Wu, K. Insogna, R.P. Lifton, High bone density due to a mutation in LDL-receptor-related protein 5, N. Engl. J. Med. 346 (2002) 1513–1521.
[12] D.A. Glass 2nd, P. Bialek, J.D. Ahn, M. Starbuck, M.S. Patel, H. Clevers, M.M. Taketo, F. Long, A.P. McMahon, R.A. Lang, G. Karsenty, Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation, Dev. Cell 8 (2005) 751–764.
[13] S. Tanaka, K. Terada, T. Nohno, Canonical Wnt signaling is involved in switching from cell proliferation to myogenic differentiation of mouse myoblast cells, J. Mol. Signal. 6 12
[14] J.D. Norton, R.W. Deed, G. Craggs, F. Sablitzky, Id helix–loop–helix proteins in cell growth and differentiation, Trends Cell Biol. 8 (1998) 58–65.
[15] A. Hollnagel, V. Oehlmann, J. Heymer, U. Ruther, A. Nordheim, Id genes are direct targets of bone morphogenetic protein induction in embryonic stem cells, J. Biol. Chem. 274 (1999) 19838–19845.
[16] Z. Zebedee, E. Hara, Id proteins in cell cycle control and cellular senescence, Oncogene 20 (2001) 8317–8325.
[17] O. Korchynskyi, P. ten Dijke, Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter, J. Biol. Chem. 277 (2002) 4883–4891.
[18] X. Gao, J. Wen, L. Zhang, X. Li, Y. Ning, A. Meng, Y.G. Chen, Dapper1 is a nucleocytoplasmic shuttling protein that negatively modulates Wnt signaling in the nucleus, J. Biol. Chem. 283 (2008) 35679–35688.
[19] F. Zhou, L. Zhang, K. Gong, G. Lu, B. Sheng, A. Wang, N. Zhao, X. Zhang, Y. Gong, LEF-1 activates the transcription of E2F1, Biochem. Biophys. Res. Commun. 365 (2008) 149–153.
[20] L. Zhang, X. Gao, J. Wen, Y. Ning, Y.G. Chen, Dapper 1 antagonizes Wnt signaling by promoting dishevelled degradation, J. Biol. Chem. 281 (2006) 8607–8612.
[21] L. Zhang, F. Zhou, T. van Laar, J. Zhang, H. van Dam, P. Ten Dijke, Fas-associated factor 1 antagonizes Wnt signaling by promoting beta-catenin degradation, Mol. Biol. Cell 22 1617–24
[22] F. Zhou, L. Zhang, T. van Laar, H. van Dam, P. Ten Dijke, GSK3beta inactivation induces apoptosis of leukemia cells by repressing the function of c-Myb. Mol. Biol. Cell 22 3533–40
[23] M. van Dinther, N. Visser, D.J. de Gorter, J. Doorn, M.J. Goumans, J. de Boer, P. ten Dijke, ALK2 R206H mutation linked to fibrodysplasia ossificans progressiva confers constitutive activity to the BMP type I receptor and sensitizes mesenchymal cells to BMP-induced osteoblast differentiation and bone formation, J. Bone Miner. Res. 25 1208–15
[24] J.S. Kim, H. Crooks, T. Dracheva, T.G. Nishanian, B. Singh, J. Jen, T. Waldman, Oncogenic beta-catenin is required for bone morphogenetic protein 4 expression in human cancer cells, Cancer Res. 62 (2002) 2744–2748.
[25] J. Dai, C.L. Hall, J. Escara-Wilke, A. Mizokami, J.M. Keller, E.T. Keller, Prostate cancer induces bone metastasis through Wnt-induced bone morphogenetic protein-dependent and independent mechanisms, Cancer Res. 68 (2008) 5785–5794.
[26] L.C. Fuentealba, E. Eivers, A. Ikeda, C. Hurtado, H. Kuroda, E.M. Pera, E.M. De Robertis, Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal, Cell 131 (2007) 980–993.
[27] G. Mbalaviele, S. Sheikh, J.P. Stains, V.S. Salazar, S.L. Cheng, D. Chen, R. Civitelli, Beta-catenin and BMP-2 synergize to promote osteoblast differentiation and new bone formation, J. Cell Biochem. 94 (2005) 403–418.

[28] Y. Chen, H.C. Whetstone, A. Youn, P. Nadesan, E.C. Chow, A.C. Lin, B.A. Alman, Beta-catenin signaling pathway is crucial for bone morphogenetic protein 2 to induce new bone formation, J. Biol. Chem. 282 (2007) 526–533.
[29] G. Rawadi, B. Vayssiere, F. Dunn, R. Baron, S. Roman-Roman, BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop, J. Bone Miner. Res. 18 (2003) 1842–1853.
[30] J.Y. Lee, M.B. Kang, S.H. Jang, T. Qian, H.J. Kim, C.H. Kim, Y. Kim, G. Kong, Id-1 activates Akt-mediated Wnt signaling and p27(Kip1) phosphorylation through PTEN inhibition, Oncogene 28 (2009) 824–831.