Wisp1 mediates *Bmp3*-stimulated mesenchymal stem cell proliferation

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Abstract

Adipose tissue expansion, resulting from adipocyte hyperplasia and/or hypertrophy, is a hallmark of obesity. Adipocytes are derived from mesenchymal stem cells (MSCs) through adipogenesis, a process involving three key steps: proliferation, commitment and differentiation. Although studies have elaborated on the mechanisms regulating adipocyte commitment and differentiation, the factors that control MSC proliferation remain largely unknown. Previously, we demonstrated that bone morphogenetic protein 3 (*Bmp3*), the expression of which was upregulated in our rat model of hyperplastic visceral adiposity, potentially stimulated MSC proliferation. In the present study, we investigate the molecular target of *Bmp3*. We conducted DNA microarray analysis on MSCs treated with and without *Bmp3* and identified WNT1-inducible signaling pathway protein 1 (*Wisp1*) as a differentially expressed gene, whose expression was upregulated 3.7-fold by *Bmp3*. *Wisp1* is a proliferative agent in various non-adipose cell types and is implicated in adipogenesis. Therefore, we tested the hypothesis that *Wisp1* mediates *Bmp3* stimulation of MSC proliferation. We showed that *Bmp3* increased the expression of *Wisp1* as early as 3 h following *Bmp3* treatment in MSCs. Importantly, the upregulated *Wisp1* expression preceded *Bmp3*-induced MSC proliferation, as determined by [³H]-thymidine incorporation. Furthermore, treatment of MSCs with recombinant *Wisp1* led to a concentration-dependent increase in [³H]-thymidine incorporation with a maximal increase of 300%. In addition, siRNA-mediated knockdown of *Wisp1* expression attenuated *Bmp3*-induced MSC proliferation. Taken together, our present findings reveal *Wisp1* as a novel target of *Bmp3* and suggest that the *Bmp3*/*Wisp1* signaling pathway play a key role in MSC proliferation, and consequently adipogenesis.

Key Words

- adipogenesis
- proliferation
- mesenchymal stem cells
- *Wisp1*
- *Bmp3*

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Introduction

Obesity has reached epidemic proportions globally and is a major contributor to the global burden of chronic disease and disability (Dixon 2010, WHO 2015). These obesity-associated co-morbidities include insulin resistance, type-2 diabetes, strokes, hypertension, coronary artery disease, various cancers, chronic kidney disease, osteoarthritis, sleep apnea and premature death. At the

cellular level, obesity is characterized by excess adipose tissue expansion resulting from an increase in the adipocyte cell number or adipocyte cell size (Hirsch & Batchelor 1976, Shepherd *et al.* 1993). New adipocytes arise from a pre-existing population of undifferentiated progenitor cells, namely mesenchymal stem cells (MSCs), through adipogenesis, a process that involves three key

steps: proliferation, commitment and terminal differentiation (Moreno-Navarrete & Fernandez-Real 2012).

Adipocyte number is established during early life by various environmental factors (Spalding *et al.* 2008) as an adaptation to enhance future fat storage in order to face potential adverse environmental conditions in later life (Hales *et al.* 1997). In particular, low birth weight has been associated with increased adiposity in humans (Osmond & Barker 2000) and animal models (Sarr *et al.* 2012) during adulthood. We have previously shown that poor nutrition during early life, induced by maternal protein restriction (MPR) during pregnancy and lactation, leads to low birth weight and subsequent development of increased visceral adiposity in adult male rat offspring (Guan *et al.* 2005). This increased visceral adiposity was characterized by adipocyte hyperplasia. Indeed, we showed that adipocyte precursor cells derived from MPR offspring exhibited an accelerated rate of proliferation, and this persisted days after removal from their *in vivo* environment (Zhang *et al.* 2007). Based on these observations, we identified *Bmp3* (bone morphogenetic protein 3) as a key gene that was increased twofold in the adipose tissue of our MPR rat model of visceral adiposity (Guan *et al.* 2005). *Bmp3* expression was also upregulated in the adipose tissue of a diet-induced mouse model of obesity (Koza *et al.* 2006). As a member of the transforming growth factor beta (TGF β) superfamily, *Bmp3* was originally thought to act as a trophic factor for bone growth due to its high expression in bone tissue (Sampath *et al.* 1987), but subsequent gene-targeting studies revealed that *Bmp3* was anti-osteogenic (Bahamonde & Lyons 2001). We showed that *Bmp3* promoted MSC and preadipocyte proliferation via the TGF β /activin-signaling pathway (Stewart *et al.* 2010). However, the downstream molecular targets through which *Bmp3* stimulates MSC proliferation remain unknown. Therefore, the present study was designed to address this important question.

Materials and methods

Cell culture

The C3H10T1/2 MSC line was obtained from the American Type Culture Collection (Manassas, VA, USA). C3H10T1/2 stem cells were cultured in standard growth medium consisting of Minimal Essential Medium (MEM; Sigma–Aldrich Canada Ltd) and 10% fetal bovine serum (FBS; Sigma). All cultures were maintained in a humidified incubator at 37 °C and 5% CO₂. Growth medium was replaced every other day.

Genome-wide gene expression profiling: DNA microarray

C3H10T1/2 MSCs were treated with and without 100 ng/ml recombinant human *Bmp3* (R&D Systems, Minneapolis, MN, USA) for 6 h. At the end of treatment, total RNA was extracted from cultured cells using RNeasy Mini Kit (QIAGEN, Inc., Mississauga, ON, Canada) coupled with on-column DNase digestion with the RNase-Free DNase Set (QIAGEN) according to the manufacturer's instructions. A total of four arrays (GeneChip Mouse Genome 430 2.0 Array; Affymetrix, Santa Clara, CA, USA) were conducted using total RNA samples from two control and two *Bmp3*-treated sets of cells, as previously described (Guan *et al.* 2005). Briefly, microarrays were performed at the London Regional Genomics Centre (London, ON, Canada) following the standard procedures as outlined in the *Affymetrix GeneChip Expression Analysis Technical Manual*. The complete data set was submitted to the National Center for Biotechnology Information's Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>) database (acc. no. GSE73649). Expression values for the Affymetrix GeneChip data were globally normalized to a preset value and analyzed using GeneSpring version 10.0 Software (Silicon Genetics, Redwood City, CA, USA). Values for the mean expression level for each gene were calculated for the control and *Bmp3* treatment microarray data sets. Candidate genes were selected by a combination of statistical analysis ($P < 0.05$) and comparison analysis (displayed > 1.5 -fold increase or decrease from the control to *Bmp3* treatment).

Assessment of mRNA levels: real-time quantitative RT-PCR

The relative abundance of various mRNAs was assessed by a two-step real-time quantitative RT-PCR (qRT-PCR), as described previously (Rajakumar *et al.* 2015). Briefly, 1 μ g of total RNA was reverse-transcribed in a total volume of 20 μ l using the High Capacity cDNA Archive Kit (Applied Biosystems) following the manufacturer's instructions. For every RT reaction set, one RNA sample was set up without reverse-transcriptase enzyme to provide a negative control. Gene transcript levels of *Gapdh* (house-keeping gene), *Wisp1* (CCN; human gene nomenclature), *Cyr61* (CCN1), *Ctgf* (CCN2), *Nov* (CCN3), *Wisp2* (CCN5), and *Wisp3* (CCN6) were quantified separately by pre-designed and validated TaqMan Gene Expression Assays (Applied Biosystems; Table 1) following the manufacturer's instructions. Briefly, gene expression assays were performed with the TaqMan Gene Expression Master Mix

Table 1 TaqMan gene expression assays for various mouse genes.

Gene name	Assay ID
<i>Ctgf</i>	Mm01192931_g1
<i>Cyr61</i>	Mm00487498_m1
<i>Gapdh</i>	Mm99999915_g1
<i>Nov</i>	Mm00456855_m1
<i>Wisp1</i>	Mm00457574_m1
<i>Wisp2</i>	Mm00497471_m1
<i>Wisp3</i>	Mm01193670_m1
<i>Gapdh</i>	Mm99999915_g1

(Applied Biosystems; P/N #4369016) and the universal thermal cycling condition (2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C) on the ViiA 7 Real-Time PCR System (Applied Biosystems).

The relative amount of various gene specific mRNAs in each RNA sample was quantified by the comparative C_T method (also known as $\Delta\Delta C_T$ method) using the Applied Biosystems relative quantitation and analysis software according to the manufacturer's instructions. For each experiment, the amount of various gene specific mRNAs under different treatment conditions is expressed relative to the amount of transcript present in the untreated control.

Proliferation assay: [3 H]-thymidine incorporation

Proliferation capacity of C3H10T1/2 MSCs was assessed by measuring [3 H]-thymidine incorporation, as described previously (Stewart *et al.* 2010). Briefly, C3H10T1/2 cells were plated at low density (20–30% confluence) on 24-well plates and cultured in growth medium for 2–3 days until 50–60% confluence. Cells were growth-arrested in serum-free medium for 24 h and were then treated in the serum-free medium with 100 ng/ml of *Bmp3* for various times (3, 6, 12 and 24 h) or increasing concentrations of mouse recombinant *Wisp1* (0.1–2 μ g/ml; Cat #1680WS, R&D Systems) for 24 h. During the last 4 h of treatment, cells were pulsed labeled with [3 H]-thymidine (0.5 μ Ci/well; 75.2 Ci/mmol, PerkinElmer Life and Analytical Sciences, Woodbridge, ON, Canada). Cells were washed twice with ice-cold PBS, once with 5% trichloroacetic acid (TCA) and twice with 95% ethanol. Cells were then solubilized by the addition of 400 μ l of 0.5 M NaOH. The solubilized cell lysate (300 μ l) was added to 4 ml of scintillation fluid and the incorporation of [3 H]-thymidine into DNA was determined by scintillation counting. Results were expressed as a percentage of control.

Western blot analysis

Western blot analysis was used to determine WISP1 protein levels, as described previously (Stewart *et al.* 2010). Briefly, C3H10T1/2 stem cells were lysed in SDS Sample Buffer (62.5 mM Tris-HCl, pH 6.8, 2% w/v SDS, 10% v/v glycerol, 50 mM DTT and 0.01% w/v bromophenol blue). Equal concentrations of the whole cell lysates were subjected to a standard 10% SDS-PAGE. After electrophoresis, proteins were transferred to PVDF transfer membrane (Amersham Hybond-P, Cat# RPN303F) using a Bio-Rad Mini Transfer Apparatus. Non-specific antibody binding was blocked with 5% w/v milk in TTBS (0.1% v/v Tween-20 in TBS) for 1 h at room temperature. Membranes were then hybridized with primary antibody (WISP1 polyclonal antibody at 1:2000, CAT# PAB7404, Abnova, Taoyuan City, Taiwan; human GAPDH polyclonal antibody at 1:5000, Cat# IMG- 5567, Imgenex Corporation, San Diego, CA, USA) overnight at 4 °C. After three 10-min washes with TTBS, the membrane was incubated with the appropriate HRP-labeled secondary antibody, either anti-Goat (Cat# AP180P, Millipore, Etobicoke, ON, Canada, 1:10 000) or anti-rabbit (R&D Systems, Cat# HAF008, 1:1000) for 1 h at room temperature. Following another three 10-min washes in TTBS, proteins were detected by chemiluminescence (Western Lightning Plus-ECL, PerkinElmer Life and Analytical Sciences, Cat# NEL103001). The membrane was then exposed to X-ray film (Eastman Kodak, Rochester, NY, USA) for 1–60 min.

The siRNA-mediated knockdown of *Wisp1* expression

To provide direct evidence for the involvement of *Wisp1* in mediating the effects of *Bmp3* on MSC proliferation, a siRNA-mediated knockdown approach was utilized (Guan *et al.* 2013). Briefly, C3H10T1/2 MSCs were plated on 24-well plates and cultured under standard conditions for 48 h. Cells were then transfected 100 nM of the Silencer Select Pre-designed siRNA-targeting mouse *Wisp1* (CAT# S76051; Ambion) in Opti-MEM I medium (Invitrogen) containing 2 μ l/well of Lipofectamine 2000 (Invitrogen), following the manufacturer's instructions. Cells were also transfected in an identical manner with 100 nM of the negative control #1 siRNA (Cat# 4624; Ambion) or with the transfection agent alone to serve as controls. At 48 h post-transfection, cells were collected for western blot analysis. Alternatively, 12 h after transfection, cells were treated for 48 h with 100 ng/ml *Bmp3*. At the end of treatment, proliferation capacity was determined by [3 H]-thymidine incorporation, as described above.

Statistical analysis

Results are presented as mean $\pm$ S.E.M. of four independent experiments each performed in triplicate. Data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* test, or Student's *t*-test as indicated. Significance was set at $P < 0.05$.

Results

Effects of Bmp3 on MSC transcriptome

As a first step in identifying the downstream factors that are responsible for mediating Bmp3-induced MSC proliferation, we conducted genome-wide gene expression profiling and identified 134 and 164 genes that were up- and downregulated by Bmp3 respectively (data not shown). Among the significantly upregulated genes, WNT-inducible secreted protein 1 (*Wisp1/CCN4*), whose expression was upregulated 3.7-fold by Bmp3 (Fig. 1A), was of

Figure 1

Effects of Bmp3 on transcriptome and *Wisp1* expression. C3H10T1/2 mesenchymal stem cells were treated with 100 ng/ml recombinant Bmp3 for 6 h. At the end of treatment, total RNA was isolated and subjected to standard DNA microarray analysis using Affymetrix Gene Expression Arrays. *Wisp1* was one of the most upregulated genes, the expression of which was increased 3.7-fold by Bmp3 (A). The same RNA samples were then subjected to qRT-PCR using pre-designed and validated TaqMan Gene Expression Assays to verify the increased expression of *Wisp1* as revealed by DNA microarray (B; *** $P < 0.001$ vs control). Alternatively, at the end of Bmp3 treatment, cells were lysed and levels of WISP1 protein were determined by western blotting (C; *** $P < 0.001$ vs control).

particular interest, because it has been previously shown to promote proliferation of cardiac fibroblasts (Venkatachalam *et al.* 2009), lung epithelial cells (Königshoff *et al.* 2009) and osteogenic cells (Inkson *et al.* 2009). Consequently, we selected *Wisp1* as a potential candidate gene and verified the magnitude of the increase in levels of *Wisp1* mRNA following Bmp3 treatment with qRT-PCR (Fig. 1B; $P < 0.001$). Importantly, we showed that there was a corresponding increase in levels of WISP1 protein (Fig. 1C; $P < 0.001$).

Effects of Bmp3 on the expression of CCN family genes

Wisp1 belongs to the CCN family genes, which include *Cyr61* (CCN1), *Ctgf* (CCN2), *Nov* (CCN3), *Wisp2* (CCN5), and *Wisp3* (CCN6) (mouse and human gene nomenclatures, respectively). To establish whether the effects of Bmp3 were specific to *Wisp1*, we determined mRNA levels of all six CCN family genes by qRT-PCR. We showed that mRNA levels of *Cyr61*, *Ctgf* and *Wisp1* were increased by 1.7-, 6.5- and 4.1-fold following Bmp3 treatment respectively (Fig. 2). By contrast, the mRNA level of *Nov*, *Wisp2* and *Wisp3* were unchanged following Bmp3 treatment (Fig. 2).

Effects of Bmp3 on Wisp1 expression and MSC proliferation

Given that *Wisp1* was upregulated following Bmp3 treatment and is a known pro-survival factor in various non-adipose cell types (Inkson *et al.* 2009, Königshoff *et al.* 2009, Venkatachalam *et al.* 2009, Schlegelmilch *et al.* 2014), coupled with previous findings that human adipocyte differentiation was associated with elevated *Wisp1* expression (Murahovschi *et al.* 2015), we examined the involvement of *Wisp1* in mediating Bmp3-induced MSC proliferation. We first determined if Bmp3-induced upregulation of *Wisp1* expression preceded Bmp3-stimulated cell proliferation by conducting a time-dependent experiment. We showed that Bmp3 increased *Wisp1* mRNA as early as 3 h, while an increase in [3 H]-thymidine incorporation was not observed until 12 h post-treatment (Fig. 3A and B).

Effects of Wisp1 on MSC proliferation

To determine if *Wisp1* stimulates MSC proliferation, we treated C3H10T1/2 MSCs with various concentrations of recombinant *Wisp1* and assessed cell proliferation by standard [3 H]-thymidine incorporation assays. We showed that *Wisp1* increased [3 H]-thymidine incorporation in a

Figure 2

Effects of *Bmp3* on the expression of *CCN* family genes. C3H10T1/2 mesenchymal stem cells were treated with 100 ng/ml recombinant *Bmp3* for 6 h. At the end of treatment, total RNA was isolated, and individual mRNA levels were determined by qRT-PCR using pre-designed and validated TaqMan Gene Expression Assays. Each bar represents mean $\pm$ S.E.M. of four independent experiments each performed in triplicate (* P <0.05, ** P <0.01 and *** P <0.001 vs control). ND, not detectable.

concentration-dependent manner, with a maximal effect at 1 μ g/ml, and to a similar degree as *Bmp3* (>3-fold increase; Fig. 4).

Effects of siRNA-mediated knockdown of *Wisp1* expression on *Bmp3* stimulation of MSC proliferation

To provide direct evidence for the involvement of *Wisp1* in mediating *Bmp3*-induced MSC proliferation, we transfected C3H10T1/2 MSCs with siRNA specific for mouse *Wisp1* and examined the effect of *Bmp3* on [3 H]-thymidine incorporation. As shown in Fig. 5, transfection of the cells with *Wisp1*-specific siRNA resulted in an 80% reduction in WISP1 protein levels. Furthermore, siRNA-mediated knockdown of *Wisp1* expression significantly attenuated the stimulatory effect of *Bmp3* on [3 H]-thymidine incorporation (from >350 to 150% of control) (Fig. 5C).

Discussion

In the present study, we demonstrate that i) *Bmp3* induces dynamic changes in C3H10T1/2 MSC transcriptome, including a significant increase in the expression of *Wisp1*, a key pro-survival factor previously identified in non-adipose cells (Venkatachalam *et al.* 2009, Schlegelmilch *et al.* 2014); ii) *Bmp3*-induced upregulation of *Wisp1* expression proceeds *Bmp3*-stimulated MSC proliferation; iii) recombinant *Wisp1* potently stimulates MSC proliferation; and iv) siRNA-mediated knockdown

of *Wisp1* expression significantly attenuates the stimulatory effect of *Bmp3* on MSC proliferation. Taken together, these findings reveal *Wisp1* as a novel target of *Bmp3*, and demonstrate that the *Bmp3*/*Wisp1* signaling pathway plays an important role in MSC proliferation. However, the physiological significance of these findings *in vivo* remains to be determined.

Although accelerated adipogenesis is a hallmark of obesity, the precise molecular mechanisms and factors that govern adipogenesis are incompletely understood. Adipogenesis begins with the commitment of MSCs to the adipocyte lineage, followed by terminal differentiation of preadipocytes to lipid-filled mature adipocytes (Rosen & MacDougald 2006, James 2013). Prior to these stages

Figure 3

Time-dependent effects of *Bmp3* on *Wisp1* expression and cell proliferation. C3H10T1/2 mesenchymal stem cells were treated with 100 ng/ml recombinant *Bmp3* for various times. At the end of treatment, total RNA was isolated, and levels of *Wisp1* mRNA (A) were determined by qRT-PCR using pre-designed and validated TaqMan Gene Expression Assays. Alternatively, cell proliferation (B) was assessed by a standard [3 H]-thymidine incorporation assay, as described in the section 'Materials and methods'. Each point represents mean $\pm$ S.E.M. of four independent experiments each performed in triplicate (* P <0.05, ** P <0.01 and *** P <0.001 vs control).

Figure 4

Effects of *Wisp1* on cell proliferation. C3H10T1/2 mesenchymal stem cells were treated with increasing concentrations of recombinant *Wisp1* for 24 h. At the end of treatment, cell proliferation was assessed by a standard [³H]-thymidine incorporation assay, as described in the section 'Materials and methods'. Each point represents mean $\pm$ S.E.M. of four independent experiments each performed in triplicate (** $P < 0.01$ and *** $P < 0.001$ vs control).

however, MSCs undergo proliferation, a process that is critical but poorly understood (Rosen & MacDougald 2006, Moreno-Navarrete & Fernandez-Real 2012). We previously showed that *Bmp3* stimulated MSC proliferation via the TGF- β /activin-signaling pathway (Stewart *et al.* 2010). In this study, we sought to identify the downstream molecular targets that mediate *Bmp3*-induced MSC proliferation. Using DNA microarray, we first identified genes that were differentially regulated by *Bmp3*. Prominently among the upregulated genes was *Wisp1*, the increased expression of which is known to be associated with human adipocyte differentiation (Murahovschi *et al.* 2015). *Wisp1* is also a pro-survival factor, because it stimulates proliferation of several non-adipose cell types, including cardiac fibroblasts (Venkatachalam *et al.* 2009), lung epithelial cells (Königshoff *et al.* 2009), osteogenic cells (Inkson *et al.* 2009). Furthermore, reduced expression of *Wisp1* is associated with enhanced apoptosis in cultured human mesenchymal stromal cells (Schlegelmilch *et al.* 2014). Therefore, we chose *Wisp1* as a candidate gene for this study. We first confirmed the upregulated expression of *Wisp1* by *Bmp3* with qRT-PCR and western blotting. We then determined if the effects of *Bmp3* were specific to *Wisp1* or the entire CCN family of genes. Our results indicated that *Bmp3* discretely upregulated the expression of two other CCN family genes, *Cyr61* and *Ctgf*, while expression of the remaining members of CCN family was

unchanged. To the best of our knowledge, this is the first study to identify certain members of the CCN family as downstream targets of *Bmp3*. Although *Cyr61* has been shown to play a crucial role in cutaneous wound healing (Jun & Lau 2010), while *Ctgf* is a known mediator of organ fibrosis (Chen & Lau 2009), the functional significance of *Bmp3*-induced upregulation of *Cyr61* and *Ctgf* awaits future exploration.

Our next step was to examine the involvement of *Wisp1* in mediating *Bmp3*-induced MSC proliferation.

Figure 5

Effects of siRNA-mediated knockdown of *Wisp1* expression on *Bmp3* stimulation of cell proliferation. C3H10T1/2 mesenchymal stem cells were plated on 24-well plates and cultured under standard conditions for 48 h. Cells were then transfected with scrambled siRNA or siRNA specific for mouse *Wisp1*, as described in the section 'Materials and methods'. Forty-eight hours after transfection, levels of WISP1 protein were determined by western blotting (A and B). Alternatively, cells were treated with 100 ng/ml recombinant *Bmp3* for 24 h, and the rate of proliferation was assessed by a standard [³H]-thymidine incorporation assay (C), as described in the section 'Materials and methods'. Each point represents mean $\pm$ S.E.M. of four independent experiments each performed in triplicate (* $P < 0.05$ and *** $P < 0.001$ vs control).

By conducting a time-dependent experiment, we determined that *Bmp3* upregulated the expression of *Wisp1* before stimulating cell proliferation, suggesting the involvement of *Wisp1* in mediating BMP3 stimulation of MSC proliferation. To provide direct evidence in support of this contention, we showed that treatment with recombinant *Wisp1* led to a concentration-dependent increase in MSC proliferation. This is consistent with the findings of two previous studies showing that *Wisp1* was a proliferative factor in human MSCs, aiding in bone development (Thorfve *et al.* 2014) and fracture repair (French *et al.* 2004).

To provide additional evidence for the involvement of *Wisp1* in mediating *Bmp3*-induced MSC proliferation, we used a loss-of-gene function approach. We found that siRNA-mediated knockdown of *Wisp1* expression significantly attenuated the stimulatory effect of *Bmp3* on MSC proliferation, indicating that *Wisp1* is a key mediator of *Bmp3* stimulation of MSC proliferation. Given that the knockdown of *Wisp1* expression did not completely block *Bmp3*-induced MSC proliferation, it is possible that factors other than *Wisp1* may also be involved in mediating the effects of *Bmp3*. It is also probable that the incomplete knockdown of *Wisp1* expression may account for the incomplete blockade of *Bmp3*-induced MSC proliferation. It is interesting to note that the stimulatory effects of *Wisp1* on vascular smooth muscle cell proliferation was attenuated but not prevented by siRNA-mediated knockdown of *Wisp1* expression (Liu *et al.* 2013). Obviously, future studies will be required to explore these possibilities.

In the past several decades, *Wisp1* has emerged as a prominent pro-survivor and proliferative factor (Venkatachalam *et al.* 2009, Maiese 2014). Importantly, aberrant function or dysregulation of the *Wisp1* signaling is associated with a variety of disorders, including musculoskeletal, vascular and neurodegenerative (Maiese 2014), as well as various cancers (Gurbuz & Chiquet-Ehrismann 2015), including breast (Chiang *et al.* 2015), prostate (Ono *et al.* 2013) and gastrointestinal (White *et al.* 2012). In the present study, we identify *Wisp1* as a novel mediator of *Bmp3*-induced MSC proliferation, a key but poorly understood component of adipogenesis. We recognize that MSCs can give rise to four distinct cell types, including adipocytes, chondrocytes, myocytes and osteocytes (Rosen & MacDougald 2006). As such, it is possible that increased MSC proliferation may have implications for the commitment and differentiation of these pluripotent MSCs to any of the four diverse cell types. However, we

have provided evidence to support the contention that stimulation of MSC proliferation by the *Bmp3*/*Wisp1* signaling pathway is a mechanism important to adipogenesis. We previously demonstrated that *Bmp3* was upregulated in the adipose tissue of an experimental rat model of visceral adiposity and promoted proliferation of both MSCs and 3T3-L1 preadipocytes (Stewart *et al.* 2010). In the present study, we show that *Bmp3* potently stimulates the expression of *Wisp1*, which precedes *Bmp3*-induced MSC proliferation. We also demonstrate that *Wisp1* stimulates MSC proliferation to the same degree as *Bmp3*. Our contention is also corroborated by a recent study that showed that the expression of *Wisp1* was associated with adipocyte differentiation and was elevated in the visceral adipose tissue of obese humans (Murahovschi *et al.* 2015). However, the question of whether *Wisp1* stimulates adipogenic differentiation of MSCs as well as the receptor and the signaling pathway that mediates the effects of *Wisp1* remain to be determined. In conclusion, our present findings reveal that the *Bmp3*/*Wisp1* signaling pathway plays a key role in MSC proliferation and may also have important implications for adipogenesis and the pathogenesis of obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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