TGF- β 1 Regulates Smooth Muscle Cells Differentiation from Adipose-Derived Stem Cells via the Wnt/ β -Catenin Pathway

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Research Article

Keywords: TGF- β 1, Pelvic floor dysfunction, Adipose-derived stem cells, Smooth muscle cells

Posted Date: September 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-765744/v1>

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Abstract

Background: Pelvic floor dysfunction (PFD) is a spectrum of disorders including stress urinary incontinence and pelvic organ prolapse. Transforming growth factor- β 1 (TGF- β 1) can induce mesenchymal stem cells (MSCs) to differentiate into smooth muscle cells (SMCs). SMCs derived from adipose-derived stem cells (ADSCs) can be used to repair damaged pelvic floor smooth muscle tissues, which is of great interest for clinical applications using stem cell therapy for PFD. The Wnt/ β -catenin pathway acts as a decisive factor in the fate of stem cells.

Methods and Results: In this study, we used medium containing TGF- β 1, TGF- β 1 inhibitor LY2109761, or Wnt/ β -catenin inhibitor KYA1797K, to induce ADSCs to differentiate into SMCs in vitro to explore the influence of TGF- β 1 on the myogenic differentiation of ADSCs via the Wnt/ β -catenin pathway. **Results:** 1) TGF- β 1 induces ADSC-derived SMCs to hyper-express the SMC markers including SMA- α , Desmin, Calponin, and SMMHC; 2) TGF- β 1 activates the Wnt/ β -catenin signaling pathway in ADSCs. After blocking TGF- β 1, the Wnt/ β -catenin pathway and myogenic differentiation in cells were inhibited; 3) the Wnt/ β -catenin pathway is involved in the differentiation of ADSCs into SMCs. After differentiation induction, the synchronized changes in the activation of Wnt/ β -catenin signaling and the expression of SMC-specific proteins showed a trend of simultaneous changes, and after the inhibition of the Wnt pathway, the adult muscle differentiation was significantly inhibited.

Conclusions: We established a simpler and more efficient method for inducing ADSCs to differentiate into SMCs using TGF- β 1 and demonstrated that the Wnt/ β -catenin signaling pathway is activated during this process.

Introduction

Pelvic floor dysfunction (PFD) refers to a range of disorders caused by abnormalities or dysfunction of muscles, ligaments, or nerves of the pelvic floor (Brandon et al.2009), and primarily includes stress urinary incontinence (SUI) and pelvic organ prolapse (POP). Recently, PFD incidence has increased, seriously impacting women's quality of life; according to a US study, SUI and POP affect 15%–17% and 3%–6% of women, respectively (Wilkins and Wu 2017). At present, surgery is the main clinical treatment to restore the anatomy and function of damaged pelvic floor muscle, but the postoperative complications are many, the recurrence rate is high, and the effect is not ideal. Therefore, tissue reconstruction using smooth muscle replacement therapy has become a key research direction.

Mesenchymal stem cells (MSCs) have been widely used in tissue engineering, stem cell transplantation, and immunotherapy (Wang et al.2017). Adipose-derived stem cells (ADSCs), a type of MSC, have been widely studied since their discovery because of their advantages: convenient extraction of large quantities, easy in vitro expansion, good genetic stability, low immunogenicity, and multi-directional differentiation potential (Zuk et al.2001; Kolaparthi et al.2015; Levi et al.2013). Previous studies have demonstrated that ADSCs can differentiate into SMCs under certain conditions (Salemi et al.2015;

Rodríguez et al.2006). Hence, using ADSCs as seed cells to engineer smooth muscle tissue for PFD treatment has great potential.

The Wnt/ β -catenin pathway, which coordinates many biological processes, such as cellular localization, cell proliferation, differentiation, and apoptosis, and tissue-specific homeostasis, is a signaling pathway controlling embryonic development and adult homeostasis (Teo et al.2010; Petersen et al.2009). β -catenin is an endocytic signal transmitter in the Wnt pathway, and activated β -catenin induces myogenic differentiation of rat MSCs (Shang et al.2007). Transforming growth factor (TGF)- β family proteins can redirect differentiation of either fully differentiated or uncommitted cells along a specific spectrum (Derynck et al.2007). Multiple studies have found that the addition of TGF- β 1 to culture medium induces myogenic differentiation of MSCs (Wang et al.2010; Chen et al.2004).

Although previous reports have demonstrated the capability of ADCSs to differentiate into SMCs, the methods are complicated, and an abundance of target cells is difficult to obtain quickly. The underlying mechanism to effectively induce ADCSs to differentiate into SMCs remains poorly understood. Therefore, this study aimed to demonstrate a relatively simple method using TGF- β 1 to induce ADCS differentiation into SMCs, allowing a harvest of a bulk of cells. Additionally, to further refine the induction protocol from adipose stem cells to smooth muscle cells, we explored whether specific activation of the Wnt/ β -catenin signaling pathway could modulate myogenic differentiation.

Materials And Methods

This study was approved by the ethics committee of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University (Wenzhou, China).

ADSC isolation culture and identification

Adipose tissue was obtained from patients undergoing liposuction and digested with an equal volume of type I collagenase (GIBCO, USA) on a shaker at 37 °C for 45 min. Undigested tissue was removed with 100-mesh and 200-mesh screens; the remaining tissue was centrifuged at 1200 rpm for 5 min, and then the final pellet resuspended in complete medium (CM, Cyagen, USA). The cells were seeded at 1×10^4 /mL density in culture flasks and incubated at 5% CO₂ at 37 °C. The cells were passaged when they reached 80%–90% confluence, and third- to fifth-generation cells were used for subsequent experiments. The CM was changed every three days. Differentiation of ADSCs was induced using Human Adipose-derived Stem Cell Osteogenic Differentiation Medium (Cyagen), Chondrogenic Differentiation Medium (Cyagen), Adipogenic Differentiation Medium (Cyagen) and detected by alizarin red, alizarin blue, and oil red O staining.

Flow cytometry

Third generation ADSCs were washed three times with phosphate buffer saline (PBS), adjusted to $1 \times 10^6/\text{mL}$, and incubated with PE anti-CD29 (Biolegend, USA), PE anti-CD31 (Biolegend), PE anti-CD44 (Biolegend), PE anti-CD45, PE (Multi Sciences, China) and anti-CD90 (Biolegend). PE-coated non-specific mouse IgG1 (Multi Sciences) was used as a negative control. Samples were processed with a flow cytometer, and the data were analyzed with FlowJo v10 software.

Induction of differentiation of ADSCs into SMCs and Wnt/ β -catenin pathway inhibition assay

Third generation ADSCs were inoculated into 6-well plates and reached 80% confluence. Cells were then incubated in SMC-induction medium (5 ng/mL TGF- β 1 (PeproTech, USA) in CM). Cells were cultured in the induction medium over 12 days, and the medium was changed every 2 days. The evolution of cells during induction was observed under the microscope. Proteins were extracted every three days (12 days in total) and stored at -80°C , and the protein levels of the different time-period groups were compared by western blotting.

The Wnt pathway was analyzed using TGF- β 1 inhibitor LY2109761 (MCE, USA) or Wnt/ β -catenin pathway inhibitor KYA1797K (MCE, USA). Briefly, at 80% confluence of the cells, the medium was replaced with SMC-induction medium supplemented with LY2109761 (10 μM) or KYA1797K (10 μM). An equal volume of DMSO (Solarbio, China) was added to the control group. Ten days after incubation, the protein levels were compared with those of the control and blank groups by western blotting.

Immunofluorescence

To authenticate that SMCs can be differentiated from ADSCs, immunofluorescence staining of intracellular molecules was performed. After fixation in 4% paraformaldehyde for 15 min, permeabilization with 0.5% Triton X-100 (dissolved in PBS) for 20 min, and blocking with 10% FBS for 30 min at 37°C , the cells were incubated with antibodies including mouse anti-desmin (1:100, Abcam, USA), mouse anti- α -SMA (1:100, Abcam), rabbit anti-SMMHC (1:100, Affinity, USA), or rabbit anti-calponin (1:100, Affinity) at 4°C overnight and then incubated with immunofluorescence-labeled secondary antibodies (Affinity) for 2 h at 37°C , protected from light. Nuclei were reverse dyed with DAPI (Beyotime Biotechnology, China). We observed protein expression under a fluorescence microscope.

Western blotting

Total protein was harvested from the cells using RIPA (Beyotime Biotechnology). Equivalent proteins were separated by 6%–10% SDS-PAGE and transfected onto PVDF membranes. The protein-transfected PVDF membranes were blocked with TBST (Tris buffered saline with Tween) containing 5% skim milk for 90 min, then incubated with primary antibody on a shaker at 4°C overnight, followed by incubation with

horseradish peroxidase-conjugated anti-rabbit IgG (1:5000, Solarbio) for 45 min. The primary antibodies were rabbit monoclonal β -catenin (1:10000, Abcam), calponin (1:2000, Affinity), α -SMA (1:500, Affinity), desmin (1:500, Affinity), SMMHC (1:1000, Affinity), or GAPDH (1:3000, Affinity). Protein expression was assessed using ECL reagent (Affinity). Quorum relative protein expression intensity was determined by optical density using ImageJ software.

Statistical analysis

All data are represented as mean $\pm$ standard deviation (SD) from three independently conducted experiments (unless otherwise noted). Differences between the experimental and control groups were determined using one-way ANOVA. $P < 0.05$ indicated statistical significance. Statistical tests were performed by utilizing the GraphPad Prism 8 software.

Results

Culture and identification of ADSCs

Alizarin red, alizarin blue, and oil red O staining experiments showed that ADSCs could be inductively differentiated into osteoblasts, adipose cells, and chondroblasts (Figure 1(b–d)). In addition, the results by flow cytometry showed that ADSCs were significantly high in expression of CD29, CD90, and CD44, whereas CD31 and CD45 were negative, indicating that our extracts from adipose tissue were pure ADSCs without contamination by endothelial cells and leukocytes (Figure 1(e)).

ADSCs produce a smooth muscle layer-like phenotype after TGF- β 1 induction

Cells were sampled during induction to observe their morphology. Uninduced ADSCs appeared as fibroblast-like spindle cells. After induction, the cells became slightly longer and flatter than before and grew in a "peak and valley" pattern (Figure 3(a)). The uninduced ADSCs already expressed α -SMA (Figure 2 (5)), none stained positively for the SMC markers desmin, calponin, and SMMHC, and the expression of α -SMA, desmin, calponin, and SMMHC were all significantly increased after 1 week of induction culture (Figure 2).

Wnt/ β -catenin participates in regulating differentiation of ADSCs into SMCs

To investigate whether the Wnt/ β -catenin signal is involved in smooth muscle cell differentiation from ADSCs, we used western blotting to detect the levels of β -catenin, and SMC-specific proteins. The expression of β -catenin increased significantly from day 3 forward and remained at a consistent level.

Levels of α -SMA and desmin began increasing significantly on day 3 and remained high, while expression of mid- and late-stage SMC-specific proteins calponin and SMMHC occurred from days 6–9 (Figure 3b). Therefore, the expression pattern of Wnt/ β -catenin pathway proteins during differentiation corresponded to the SMC-specific markers. The above findings showed that TGF- β 1 contributed to the differentiation of ADSCs into SMCs via the Wnt/ β -catenin pathway.

Inhibition of Wnt/ β -catenin blocked differentiation of ADSCs into SMCs

To explore whether Wnt/ β -catenin is essential for the differentiation of ADSCs into SMCs, we measured the levels of the key Wnt pathway marker, β -catenin, in the presence of the TGF- β 1 inhibitor LY2109761 or the Wnt/ β -catenin-specific inhibitor KYA1797K. We also determined the specific proteins associated with SMCs, including α -SMA, calponin, desmin, and SMMHC. Pharmacological inhibition of TGF- β 1 and Wnt pathways significantly downregulated β -catenin expression, triggering a further decrease in SMC-specific protein levels (Figure 4), compared to non-inhibitor-treated controls.

As shown in Figure 5, we hypothesize that TGF- β 1 activates the Wnt pathway, triggers the accumulation of β -catenin in the cytoplasm that transfers to the nucleus, and promotes the differentiation of ADSCs to SMCs, while the addition of pathway inhibitors reduces this differentiation.

Discussion

In this study, we directly compared the expression of β -catenin and SMC marker proteins between undifferentiated ADSCs and TGF- β 1-induced ADSCs. We also compared the expression of β -catenin and SMC marker proteins after addition of LY2109761 or KYA1797K, and found that TGF- β 1 can specifically stimulate the differentiation of ADSCs into SMCs by activating the Wnt/ β -catenin signaling pathway. We successfully established ADSC-derived SMCs in vitro, which may be used in reconstruction of pelvic floor muscles.

TGF- β 1 is a multifunctional protein, which is engaged in a diverse range of biological processes, including cell growth, differentiation, and migration (Derynck et al.2007). It has been demonstrated that TGF- β 1 can induce the differentiation of MSCs into SMCs (Kurpinski et al.2010; Narita et al.2008; Wang et al.2010). Previously, it has been established that TGF- β 1 enhances the gene transcription and protein level of myocardin in ADSCs. Myocardin acts like a cofactor for blood serum response factor and has a significant effect on the performance of SMC-specific genes (Chen et al.2002; Du et al.2003; Wang et al.2003).

The Wnt/ β -catenin signaling pathway is highly evolutionarily conserved and is involved in the control of cell proliferation, differentiation, apoptosis, and cellular localization during embryonic development(Petersen and Reddien 2009). Recently, it has been indicated that the Wnt/ β -catenin pathway plays a key part in MSC differentiation. For example, Sun et al. found that IL-1 β promotes angiogenesis in

human lung-derived MSCs through upregulation of β -catenin (Sun et al.2016). In addition, studies on pulmonary fibrosis have shown that Wnt/ β -catenin signaling can induce fibroblast differentiation from MSCs (Sun et al.2014). However, several investigations have suggested that downregulation of the Wnt/ β -catenin pathway can be critical to MSC differentiation into mature osteoblasts (van der Horst et al.2005). Our experimental studies revealed that activation of Wnt/ β -catenin pathway facilitated the differentiation of SMCs from ADSCs.

Measurements of SMC protein expression in our study partly confirmed other results that investigated the differentiation of human MSCs into SMCs (Dahal et al.2020; Abolhasani et al.2020). SMC markers regulate or are directly involved in the contraction of SMCs. For example, α -SMA, a key SMC early differentiation marker (Beamish et al.2010), is involved in vasoconstriction and blood pressure homeostasis (Xu et al.2019), and inhibition of α -SMA leads to decreased cellular motility and myofibroblast contraction (Rockey et al.2013) during wound healing in vivo. Desmin, a general myogenic marker, is a key subunit of the intermediate filament in cardiac, skeletal, and smooth muscles (Paulin et al.2004). Calponin is an intermediate marker that combines with actin to regulate SMC contraction(Andersson et al.2004), and SMMHC, the most well-established SMC marker, acts as an ATPase and exerts force along actin filaments(Babu et al.2004).

In recent years, ADSCs have gained wide application in reconstructive and plastic surgery. ADSCs have multi-directional differentiation potential and can be artificially induced into smooth muscle cells, bone cells, chondrocytes, and adipocytes (Rodríguez et al.2006; van Zoelen et al.2016; Dzobo et al.2016; Chen et al.2020). ADSCs have significant advantages, such as less invasive collection, high proliferative capacity, excellent regenerative ability, and multiplicity of differentiation potential, as well as, extremely low immunogenicity, making them particularly suitable for tissue engineering and gene therapy. In addition, many studies have shown that ADSCs can differentiate into SMCs, which have promising applications in the treatment of diseases and injuries of muscle tissues and organs such as the blood vessels, bladder, and ureters(Lin et al.2020; Pokrywczynska et al.2018; Zhao et al.2016). The immunofluorescence and western blotting data showed increased expression in the major Wnt/ β -catenin signaling proteins and SMC developmental markers after 12 days of induction, which to some extent confirmed the previous study(Jiang et al.2019). Adding TGF- β 1 and Wnt/ β -catenin pathway inhibitors to the SMC-induction medium significantly suppressed activation of β -catenin, as well as lowered protein levels of SMC marker at later stages, further confirming the association between TGF- β 1 and the Wnt/ β -catenin pathway in the differentiation of ADSCs to SMCs.

Conclusion

In summary, we established a simpler and more efficient method for inducing ADSCs to differentiate into SMCs using TGF- β 1. Additionally, we demonstrated that the Wnt/ β -catenin signaling pathway is activated during this process. This method is more convenient and efficient for obtaining SMCs on a large-scale and provides a potential resource for pelvic floor muscle reconstruction in treatment of PFD.

Declarations

Funding

This study was supported by the National Natural Science Foundation of China (81971850) and the Municipal Science and Technology Bureau Foundation of Wenzhou (Y2020085, Y20190017, Y20180148).

Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Author contributions

LM and LL conceived and designed the study, and critically revised the manuscript. XL performed the experiments, analyzed the data and drafted the manuscript. HC, ZZ, TL, QW, YH and JW participated in study design, study implementation and manuscript revision. All authors read and approved the final manuscript.

Ethical approval

This study was approved by the ethics committee of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University (Wenzhou, China).

References

1. Brandon CJ, Lewicky-Gaupp C, Larson KA, Delancey JO (2009) Anatomy of the perineal membrane as seen in magnetic resonance images of nulliparous women. *Am J Obstet Gynecol* 200:10.1016/j.ajog.2009.03.004

2. Wilkins MF, Wu JM (2017) Lifetime risk of surgery for stress urinary incontinence or pelvic organ prolapse. *Minerva Ginecol* 69:10.23736/S0026-4784.16.04011-9
3. Wang X, Wang Y, Gou W, Lu Q, Peng J, Lu S (2013) Role of mesenchymal stem cells in bone regeneration and fracture repair: a review. *Int Orthop* 37:10.1007/s00264-013-2059-2
4. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 7:10.1089/107632701300062859
5. Kolaparthi LK, Sanivarapu S, Moogla S, Kutcham RS (2015) Adipose tissue - adequate, accessible regenerative material. *Int J Stem Cells* 8:10.15283/ijsc.2015.8.2.121
6. Levi B, Glotzbach JP, Sorkin M, Hyun J, Januszyk M, Wan DC, Li S, Nelson Longaker ER MT and Gurtner GC (2013) Molecular analysis and differentiation capacity of adipose-derived stem cells from lymphedema tissue. *Plast Reconstr Surg* 132:10.1097/PRS.0b013e31829ace13
7. Salemi S, Tremp M, Plock JA, Andersson KE, Gobet R, Sulser T, Eberli D (2015) Differentiated adipose-derived stem cells for bladder bioengineering. *Scand J Urol* 49:10.3109/21681805.2015.1004642
8. Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ. (2006) Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. *Proc Natl Acad Sci U S A* 103:10.1073/pnas.0604850103
9. Teo JL, Kahn M (2010) The Wnt signaling pathway in cellular proliferation and differentiation: a tale of two coactivators. *Adv Drug Deliv Rev* 62:10.1016/j.addr.2010.09.012
10. Petersen CP, Reddien PW (2009) Wnt signaling and the polarity of the primary body axis. *Cell* 139:10.1016/j.cell.2009.11.035. PMID: 20005801
11. Shang Y, Zhang C, Wang S, Xiong F, Zhao C, Peng F, Feng S, Yu M, Li M, Zhang Y (2007) Activated beta-catenin induces myogenesis and inhibits adipogenesis in BM-derived mesenchymal stromal cells. *Cytotherapy* 9:10.1080/14653240701508437
12. Derynck R, Akhurst RJ (2007) Differentiation plasticity regulated by TGF-beta family proteins in development and disease. *Nat Cell Biol* 9:10.1038/ncb434
13. Wang C, Yin S, Cen L, Liu Q, Liu W, Cao Y, Cui L (2010) Differentiation of adipose-derived stem cells into contractile smooth muscle cells induced by transforming growth factor-beta1 and bone morphogenetic protein-4. *Tissue Eng Part A* 16:10.1089/ten.TEA.2009.0303
14. Chen S, Lechleider RJ (2004) Transforming growth factor-beta-induced differentiation of smooth muscle from a neural crest stem cell line. *Circ Res* 94:10.1161/01.RES.0000126897.41658.81
15. Derynck R, Akhurst RJ (2007) Differentiation plasticity regulated by TGF-beta family proteins in development and disease. *Nat Cell Biol* 9:10.1038/ncb434
16. Kurpinski K, Lam H, Chu J, Wang A, Kim A, Tsay E, Agrawal S, Schaffer DV, Li S (2010) Transforming growth factor-beta and notch signaling mediate stem cell differentiation into smooth muscle cells. *Stem Cells* 28:10.1002/stem.319. PMID: 20146266

17. Narita Y, Yamawaki A, Kagami H, Ueda M, Ueda Y (2008) Effects of transforming growth factor-beta 1 and ascorbic acid on differentiation of human bone-marrow-derived mesenchymal stem cells into smooth muscle cell lineage. *Cell Tissue Res* 333:10.1007/s00441-008-0654- 0
18. Wang C, Yin S, Cen L, Liu Q, Liu W, Cao Y, Cui L (2010) Differentiation of adipose-derived stem cells into contractile smooth muscle cells induced by transforming growth factor-beta1 and bone morphogenetic protein-4. *Tissue Eng Part A* 16:10.1089/ten.TEA.2009.0303
19. Chen J, Kitchen CM, Streb JW, Miano JM (2002) Myocardin: a component of a molecular switch for smooth muscle differentiation. *J Mol Cell Cardiol* 34:10.1006/jmcc.2002.2086
20. Du KL, Ip HS, Li J, Chen M, Dandre F, Yu W, Lu MM, Owens GK, Pamacek MS (2003) Myocardin is a critical serum response factor cofactor in the transcriptional program regulating smooth muscle cell differentiation. *Mol Cell Biol* 23: 10.1128/mcb.23.7.2425-2437.2003
21. Wang Z, Wang DZ, Pipes GC, Olson EN (2003) Myocardin is a master regulator of smooth muscle gene expression. *Proc Natl Acad Sci USA* 100:10.1073/pnas.1232341100
22. Petersen CP, Reddien PW (2009) Wnt signaling and the polarity of the primary body axis. *Cell* 139:10.1016/j.cell.2009.11.035
23. Sun J, Chen J, Cao J, Li T, Zhuang S, Jiang X (2016) IL-1 β stimulated β -catenin up-regulation promotes angiogenesis in human lung-derived mesenchymal stromal cells through a NF- κ B-dependent microRNA-433 induction. *Oncotarget* 7:10.18632/oncotarget.10683
24. Sun Z, Wang C, Shi C, Sun F, Xu X, Qian W, Nie S, Han X (2014) Activated Wnt signaling induces myofibroblast differentiation of mesenchymal stem cells, contributing to pulmonary fibrosis. *Int J Mol Med* 33:10.3892/ijmm.2014.1672
25. van der Horst G, van der Werf SM, Farih-Sips H, van Bezooijen RL, Löwik CW, Karperien M (2005) Downregulation of Wnt signaling by increased expression of Dickkopf-1 and - 2 is a prerequisite for late-stage osteoblast differentiation of KS483 cells. *J Bone Miner Res* 20:10.1359/JBMR.050614
26. Dahal S, Swaminathan G, Carney S, Broekelmann T, Mecham R, Ramamurthi A (2020) Pro-elastogenic effects of mesenchymal stem cell derived smooth muscle cells in a 3D collagenous milieu. *Acta Biomater* 105:10.1016/j.actbio.2020.01.030
27. Abolhasani S, Rajabibazl M, Khani MM, Parandakh A, Hoseinpoor R (2020) The cooperative effects of micro-grooved topography and TGF- β 1 on the vascular smooth muscle cell contractile protein expression of the mesenchymal stem cells. *Differentiation* 115:10.1016/j.diff.2020.06.003
28. Beamish JA, He P, Kottke-Marchant K, Marchant RE (2010) Molecular regulation of contractile smooth muscle cell phenotype: implications for vascular tissue engineering. *Tissue Eng Part B* 16:10.1089/ten.TEB.2009.0630
29. Xu Z, Zhang L, Yu Q, Zhang Y, Yan L, Chen ZJ (2019) The estrogen- regulated lncRNA H19/miR-216a-5p axis alters stromal cell invasion and migration via ACTA2 in endometriosis. *Mol Hum Reprod* 25:10.1093/molehr/gaz040
30. Rockey DC, Weymouth N, Shi Z (2013) Smooth muscle α actin (Acta2) and myofibroblast function during hepatic wound healing. *PLoS One* 8:10.1371/journal.pone.0077166

31. Paulin D, Li Z (2004) Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. *Exp Cell Res* 301:10.1016/j.yexcr.2004.08.004
32. Andersson KE, Arner A (2004) Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol Rev* 84:10.1152/physrev.00038.2003
33. Babu GJ, Warshaw DM and Periasamy M. (2000). Smooth muscle myosin heavy chain isoforms and their role in muscle physiology. *Microsc Res Tech* 50:10.1002/1097-0029(20000915)50:6 < 532::AID-JEMT10 > 3.0.CO;2-E.
34. Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ (2006) Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. *Proc Natl Acad Sci U S A* 103:10.1073/pnas.0604850103
35. van Zoelen EJ, Duarte I, Hendriks JM, van der Woning SP (2016) TGFβ- induced switch from adipogenic to osteogenic differentiation of human mesenchymal stem cells: identification of drug targets for prevention of fat cell differentiation. *Stem Cell Res Ther* 7:10.1186/s13287-016-0375-3
36. Dzobo K, Turnley T, Wishart A, Rowe A, Kallmeyer K, van Vollenstee FA, Thomford NE, Dandara C, Chopera D, Pepper MS, Parker MI (2016) Fibroblast-derived extracellular matrix induces chondrogenic differentiation in human adipose-derived mesenchymal stromal/stem cells in vitro. *Int J Mol Sci* 17:10.3390/ijms17081259
37. Chen Y, Li K, Zhang X, Chen J, Li M, Liu L (2020) The novel long noncoding RNA lncRNA-Adi regulates adipogenesis. *Stem Cells Transl Med* 9:10.1002/sctm.19–0438
38. Lin J, Zhu Q, Huang J, Cai R, Kuang Y (2020) Hypoxia promotes vascular smooth muscle cell (VSMC) differentiation of adipose-derived stem cell (ADSC) by regulating Mettl3 and paracrine factors. *Stem Cells Int* 2020:10.1155/2020/2830565
39. Pokrywczynska M, Jundzill A, Rasmus M, Adamowicz J, Balcerczyk D, Buhl M, Warda K, Buchholz L, Gagat M, Grzanka D, Drewa T (2018) Understanding the role of mesenchymal stem cells in urinary bladder regeneration-a preclinical study on a porcine model. *Stem Cell Res Ther* 9:10.1186/s13287-018-1070-3
40. Zhao Z, Yu H, Fan C, Kong Q, Liu D, Meng L (2016) Differentiate into urothelium and smooth muscle cells from adipose tissue-derived stem cells for ureter reconstruction in a rabbit model. *Am J Transl Res* 8:3757–3768
41. Jiang W, Wang D, Alraies A, Liu Q, Zhu B, Sloan AJ, Ni L, Song B (2019) Wnt-GSK3β/β-catenin regulates the differentiation of dental pulp stem cells into bladder smooth muscle cells. *Stem Cells Int* 2019:10.1155/2019/8907570

Figures

Figure 1

The cultivation of ADSCs and the identification of their differentiation ability. ADSCs were cultured to the third generation (a), and Alizarin Red staining (b), Oil Red O staining (c) and Alizarin Blue staining (d) were used to evaluate the differentiation of ADSCs into osteoblasts, adipose cells and chondroblasts. Flow cytometry analysis of molecular surface antigen markers (e) showed that CD31 and CD45 were

negative, CD29, CD44, and CD90 were positive, and PE-bound non-specific mouse IgG1 was used as a negative control.

Figure 2

ADSCs were induced to produce SMC marker proteins, calponin (2- 4), desmin (6-8), SMMHC (10-12) and α -SMA (14-16) positive expression, and non-induced ADSCs were used as negative controls (1,5,9,13), the

cells express a little α -SMA before induction (5), and the rest of the proteins are negative (1,9,13). The nucleus was stained with DAPI.

(a)

(b)

Figure 3

The role of Wnt/ β -catenin in ADSCs SMC differentiation. After 14 days of culture, the cells were observed under a microscope. Compared with the non-induced cells (a-DEF), the cells were still spindle-shaped after TGF- β 1 induction, but they were slightly longer and flatter, showing "peak-to-valley" growth (a-ABC).

Western blotting detects the expression levels of β -catenin protein in the experimental group and the control group, and simultaneously detects the protein expression levels of α -SMA, desmin, SMMHC, and calponin (b). GAPDH was used as an internal reference. Use ImageJ software to analyze the band intensity. All results represent the mean $\pm$ standard deviation of three independent experiments (n = 3). With one-way analysis of variance test, there is a significant difference compared with the 0d group (*p < 0.05).

Figure 4

Blocking TGF- β 1/Wnt/ β -catenin inhibits the differentiation of ADSCs into SMC. TGF- β 1 and Wnt/ β -catenin inhibitor drugs were added respectively. After 10 days of induction, the level of β -catenin, a key protein of the Wnt pathway, decreased, and the expression of α -SMA, desmin, calponin, and SMMHC at the protein level decreased. All results represent the mean $\pm$ standard deviation of three independent experiments (n = 3). *: There is a significant difference between the TGF- β 1 group and the blank group, **: There is a significant difference between the TGF- β 1 group and the addition of inhibitory drugs, p < 0.05.

Figure 5

The Wnt /β-catenin pathway can be activated by TGF-β1, and β- catenin accumulates in the cytoplasm and transfers to the nucleus, thereby promoting the differentiation of ADSCs and producing SMC-related proteins. β- catenin inhibitor LY2109761 and Wnt / β-catenin pathway inhibitor KYA1797K block Wnt / β-catenin signaling and inhibit differentiation.

Supplementary Files

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