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TGF-β1/WISP1/Integrin-α interaction mediates human chondrocytes dedifferentiation

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Abstract. – OBJECTIVE: To clarify the interaction between TGF-β1 and WISP1, and the effect of Integrin α5/V subunits on the WISP1 caused chondrocyte (CH) dedifferentiated phenotype.

PATIENTS AND METHODS: The knee joint cartilage from the trauma and osteoarthritis (OA) patients were collected. The patients of trauma group were confirmed to have no OA history. The protein level of WISP1, Integrin-α5/V, and type II/I collagen were analyzed by Western blotting. Besides, we isolated the CHs from the cartilage without OA and treated CHs with exogenic TGF-β1 and WISP1 protein. In addition to this, to regulate the α5 and αV subunits expression of CHs, we silenced two genes by siRNA transfection and upregulated them by exogenic protein supplement. Then, the CHs with different α5 and αV expression were treated with WISP1. To value the chondrogenic gene expression, we determined the type II collagen and SOX9 gene expression by immunofluorescence (IF) and RT-PCR, respectively. Meanwhile, the dedifferentiation markers of CH, type I collagen, and Runx2 expression was also analyzed. Cell proliferation was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.

RESULTS: The OA cartilage contains a higher level of type I collagen, WISP1, Integrin α5, and Integrin αV, but low type II collagen. The upregulation of TGF-β1 caused the increase of WISP1, as well as the high level of Integrin α5/V, and dedifferentiated gene. Besides, the upregulation of WISP1 also contributed to the TGF-β1 expression and CHs dedifferentiation. Apart from this, the silencing of the α5 subunit of Integrin aggravated the WISP1 induced CHs dedifferentiation, which was reversed by α5 upregulation. However, the αV subunit played an opposite role that mediated the WISP1-induced CHs dedifferentiation. Additionally, the interaction between TGF-β1 and WISP1 promoted the CHs proliferation, which was not affected by the Integrin-α5/V expression.

CONCLUSIONS: TGF-β1 and WISP1 interact to induce CHs dedifferentiation, which was mainly by the mediation of the Integrin-αV subunit. On the contrary, Integrin-α5 shows a protective effect during the WISP1 caused CHs dedifferentiation.

Key Words:
Chondrocyte differentiation, TGF-β1, WISP1, Integrin-α, Osteoarthritis.

Introduction

With the unhealthy lifestyle, overloaded work, and the aging of the human population, bone and degenerative joint diseases have become a high incidence disease, one of the representative conditions is osteoarthritis (OA)[1]. In recent years, with the deepening of people's understanding of OA, the focus of research mainly moves to articular cartilage, and it is believed that the degeneration and wear of cartilage is the key to lead to OA eventually. Chondrocytes (CHs) are terminally differentiated cells, so cartilage has poor self-repairing ability. Various injuries, inflammations, and degenerations can cause the dedifferentiation of CHs[2]. Morphologically, CHs regularly shift from round shape toward a more hypertrophic polygonal form, similar to the fibroblasts, which is recognized to be called "dedifferentiation" in the degenerative biology of CHs. Meanwhile, the chondrogenic phenotype is lost, accelerating the degradation and further calcification of the cartilage matrix. The dedifferentiated CHs enter the stage of proliferation and hypertrophy again and then apoptosis, leading to irreversible cartilage damage, which seriously aggravates the OA process[3].

CH dedifferentiation involves the regulation of multiple molecular signaling pathways, including Wnt, TGF-β, IHH/PTHrP, which bases on a variety of complex, interactive, and coordinated control network groups. Wnt1-inducible signaling pathway protein 1 (WISP1), also known as CCN4, is abundant in cysteine and belongs to one of the members of the CCN protein family[4]. WISP1 is a downstream target protein of the Wnt/β-catenin pathway

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and participates in the process of cartilage growth, chondrogenic differentiation, and chondrocyte dedifferentiation. The expression level of WISP1 is closely related to the severity of OA. Its abnormal activation promotes CHs to express a variety of dedifferentiation marker proteins, including matrix metalloproteinases (MMPs), Runx2, type I collagen, which results in the cartilage extracellular matrix (ECM) destruction[5]. Integrin, as a transmembrane glycoprotein on the cell membrane, widely exists on the cell surface, and its expression level is closely related to cell viability, cell adhesion, and cell phenotype. Integrin on the surface of CHs can transmit the signals in ECM to intracellular proteins, including the WISP-1[6], and regulates the growth, proliferation, and differentiation through multiple signaling pathways. Integrin is composed of α (120-185 kDa) and β (90-110 kDa) two subunits to form a heterodimer. The role of heterodimer Integrin composed of different subunits is different, mainly manifested in their specific binding sites and their functions in signaling pathways. It has been announced that Integrins regulates the development of OA. The Integrin-α1 deficiency changes CH cellularity, increases MMPs expression, suppresses proteoglycans production, and aggravates OA procedure[7]. Besides, Garciadiego-Cazares et al[8] elucidated that α5 subunits level decreases, and αV increases in the early stages of OA, which controls different CH phenotype.

At present, TGF-β1 is another growth factor and known to promote cartilage formation, CH proliferation, and differentiation, which also interacts with WISP1[9] and affects the expression of Integrin-α5β1[10]. Zhang et al[11] reported that Integrin is involved in the regulation of CHs hypertrophy by the TGF-β/SMAD signaling pathway. Whether the different subunits of Integrin stimulated by WISP-1 feedbacks different effects on CHs needs further study. Therefore, in this study, we explored the impact of TGF-β1 and WISP1 in the dedifferentiation of CH, and the mediated role of the Integrin α5 and αV subunit. With TGFβ1/WISP/Integrin-α as the target, we hope to effectively slow down the progress of OA by inhibiting CH dedifferentiation.

Patients and Methods

Patient Tissue Collection

The source of the cartilage came from the patients with total knee replacement due to trauma or OA. Under sterile operation, 10 cases of cartilage specimens (5 from trauma, and 5 from OA) were harvested with a tax surgical blade from the distal femoral osteotomy. The size of the cartilage is about 0.8 cm × 0.8 cm × 0.5 cm and divided into two equal pieces. One was fixed with 4% formaldehyde, and the other one was stocked at -80°C. According to the Outerbridge grading method[12], cartilage from trauma patients did not degenerate, and cartilage from OA patients degenerated significantly. This investigation was approved by the Ethics Committee of Shandong Provincial Hospital. Signed written informed consent was obtained from all participants before the study.

CHs Isolation and Culture

We isolated the CHs from the non-degenerate cartilage. Briefly, cartilage was divided from the knee joint and then cut into fragments. After washed with phosphate-buffered saline (PBS), the fragments were digested in the solution containing Dispase II (2,4 U/mL, Sigma-Aldrich, St. Louis, MO, USA) and collagenase XI (1500 U/mL, Sigma-Aldrich, St. Louis, MO, USA) at 37°C overnight. We obtained the cell pellets after centrifuge and then re-suspended in Dulbecco's Modified Eagle's medium (DMEM/F12) growth medium containing 10% fetal bovine serum (FBS) and penicillin-streptomycin (1%, Gibco, Rockville, MD, USA). We treated CHs of the first generation with different drugs as follows: human TGF-β1 protein (ab271757, Abcam, Cambridge, MA, USA); human WISP1 protein (ab50041, Abcam, Cambridge, MA, USA); human Integrin-α5 protein (P08648, Geno Technology Inc., Singapore, Singapore); human Integrin-αV protein (H00003685-G01, Abnova, Taipei, Taiwan, China). To silence the Integrin-α5 or Integrin-αV gene expression, we also transfected the CHs with small interfering RNA (siRNA) (#106727; #106730; #RSS321228 as a negative control, Thermo Fisher Scientific, Waltham, MA, USA).

Transfection

The siRNA was purchased from Thermo Fisher Scientific (#106727; #106730; #RSS321228) (Waltham, MA, USA). CHs were seeded at 2×10^5 cells/ml in 6-well plate and transfected with siRNA in lipofectamine 2000 (10 μmol/mL; Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommended protocol. The medium was changed to WISP1 treatment after a 12-hour transfection.

Western Blotting (WB)

The type II collagen, type I collagen, WISP1, Integrin-α5 and Integrin-αV protein levels in the cartilage tissue was analyzed by WB methods.

The tissue moved from the patients was stocked at -80°C before isolation. Briefly, the cartilage was grinded, and the total protein was extracted using radioimmunoprecipitation assay (RIPA) lysis (Beyotime Inc., Shanghai, China). After the quantitative analysis and denaturation of protein, an equal amount of sample was separated by the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% non-fat milk and then incubated with the desired primary antibodies overnight at 4°C as following: type II collagen (1:800; Abcam, Cambridge, MA, USA), type I collagen (1:1000; Abcam, Cambridge, MA, USA), WISP1 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), Integrin-α5 (1:2000; Cell Signaling, Danvers, MA, USA), Integrin-αV (1:2000; Abcam, Cambridge, MA, USA) and β-Actin (1:3000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The next day, the membrane was washed with phosphate buffered saline-tween (PBST) and incubated with a secondary antibody at room temperature for one hour. In the last step, the membrane was washed and exposed using a chemiluminescent enhanced chemiluminescence (ECL) substrate (Thermo Fisher Scientific, Waltham, MA, USA). The protein expression was determined by the grey level of the protein bands.

Enzyme-Linked Immunosorbent Assay (ELISA) Assay

The protein level of TGF-β1, WISP1, Integrin-α5, and Integrin-αV in the non-treated and treated CHs was determined in the lysate of CHs by human ELISA kits (#MBS266143, # MBS2533358, #MBS2886168, #MBS705816, MyBioSource; Hangzhou, China) following the manufacturer's recommended protocol.

MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyl Tetrazolium Bromide) Assay

The proliferation in the non-treated and treated CHs was determined by the MTT assay. CHs were seeded in 96-well plates at a density of 5000/well in the growth medium. After indicated treatments, the MTT assay kit (Sigma-Aldrich, St. Louis, MO, USA) was added in each well following the manufacturer's recommended protocol. The blue formazan products in the cells were dissolved in 150 μl dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) and spectrophotometrically measured at a wavelength of 590 nm.

Immunofluorescence (IF)

The protein level of type II collagen and type I collagen in the non-treated and treated CHs was determined using IF staining. The CHs were seeded on the coverslip at the density of 10^4/well in the 12 well-plate. Before staining, the cells on the coverslip were washed with PBS, fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton-X at room temperature. Then, the coverslip was treated with 5% bovine serum albumin (BSA) (Gibco, Rockville, MD, USA) at room temperature for one hour. Following, we incubated the coverslip with primary antibodies: type II collagen and type I collagen (1:200, Abcam, Cambridge, MA, USA) at 4°C overnight. After incubated with Alexa Fluor568 secondary antibody (Invitrogen, Carlsbad, CA, USA) and 4',6-diamidino-2-phenylindole (DAPI) in the dark for one hour, the protein expression was determined by the intensity of the fluorescence.

Real Time-PCR Analysis (RT-PCR)

We determined the relative gene expression in the mRNA level with RT-PCR. Briefly, total RNA in the non-treated and treated CHs was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommended protocol. RNA was continuously reverse-transcribed into cDNA by a reverse transcription kit (Invitrogen, Carlsbad, CA, USA). The mRNA expression of Integrin-α5, Integrin-αV, SOX9, and Runx2 was determined by RT-PCR analysis using SYBR Green Master (TOYOBO, Osaka, Japan) and achieved by the normalization of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA level calculated following the $2^{-\Delta\Delta Ct}$ method. The primers are as follows: SOX-9 Forward (F) 5′-AGCGAACGCACATCAAGAC-3′, Reverse (R) 5′-CTGTAGGCGATCTGTTGGGG-3′; Runx-2 (F) 5′-TGGTTACTGTCATGGCGGGTA-3′; (R) 5′-TCTCAGATCGTTGAACCTTGCTA-3′; Integrin-α5 (F) 5′-GGCTTCAACTTAGACGCGGAG-3′, (R) 5′-TGGCTGGTATTAGCCTTGGGT-3′; Integrin-αV (F) 5′-ATCTGTGAGGTCGAAACAGGA-3′, (R) TGGAGCATACTCAACAGTCTTTG-3′; GAPDH (F) 5′-ACAACTTTGGTATCGTGGAAGG-3′, (R) 5′-GCCATCACGCCACAGTTTC-3′.

Statistical Analysis

The fluorescence intensity and the grey level of the WB bands were analyzed using ImageJ software. All the data were analyzed with GraphPad 5.0 software (La Jolla, CA, USA) and expressed as mean ± standard deviation (SD). The *t*-test was

used for analyzing measurement data. Differences between two groups were analyzed by using the Student's *t*-test. A comparison between multiple groups was made using a one-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). *p-value* <0.05 showed a statistical significance.

Results

WISP1 and Integrin-α Alterations in OA Cartilage

To evaluate the changes of WISP1, Integrin-α1, and Integrin-αV in the progress of human OA. We isolated the protein of the cartilage from the knee joint with or without OA. WB showed that type II collagen decreased, and the type I collagen increased obviously in the OA condition (Figure 1A, 1B). In addition to this, the WISP1 protein level was significantly raised in the OA cartilage compared to the non-degenerated group. Both Integrin-α1 and αV protein were also highly expressed in the OA condition, which was little contained in control.

Exogenic TGF-β1 and WISP1 Supplement Promoted CHs Dedifferentiation

Since the endogenous production of WISP1 and Integrin-α1/αV was increased in the OA cartilage, we continuously treated CHs with the exogenic TGF-β1 and WISP1 protein to explore their effects on Integrin-α1/αV and the CH phenotype. The CHs were seeded at the density of 10^4 cells/well in the 12-well plate and treated with TGF-β1 (100 pg/mL) or WISP1 (2 μg/mL) for three days. After treatment, the CHs were harvested, and we analyzed the TGF-β1 and WISP1 protein content in the CHs using the ELISA method. The results showed that not only TGF-β1 treatment upregulated the cellular TGF-β1 expression but also the WISP1, not significantly as TGF-β1, a supplement could increase the cellular TGF-β1 level compared to the control (Figure 2A). Similarly, both WISP1 and TGF-β1 stimulation could trigger the WISP1 expression in the CHs compared to the control. These results indicated that there is a crosstalk between the TGF-β1 and WISP1 in the CHs (Figure 2B). The CHs proliferation was increased after the treatment of TGF-β1 and WISP1 compared to the control (Figure 2C). Besides, we analyzed the mRNA expression of the Integrin-α1/αV, SOX9, Runx2 by RT-PCR. Compared to the control, TGF-β1 and WISP1 induced the upregulation of Integrin-α1/αV mRNA level, which was more significant in the αV (Figure 2D). Furthermore, the chondrogenic gene SOX9 mRNA expression was reduced under the stimulation of TGF-β1 and WISP1. On the contrary, hypertrophic gene Runx2 raised, resulting from TGF-β1 and WISP1 treatment (Figure 2E). With the progress of the CH dedifferentiation, the ratio of type II collagen and type I collagen changes. As shown in Figure 2F and G, type II collagen protein expression is markedly

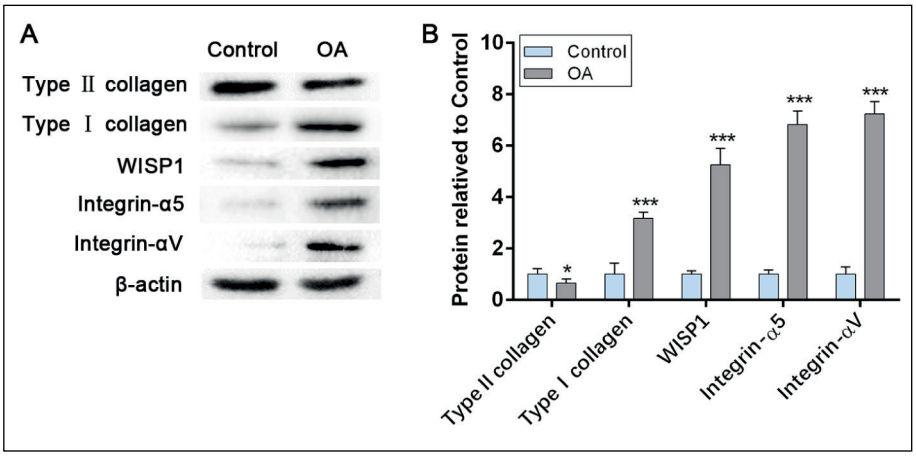

Figure 1. WISP1 and Integrin-α alterations in OA cartilage. Cartilage from trauma patients without OA was grouped as control. **A**, The protein expression of type II collagen, type I collagen, WISP1, Integrin-α5, Integrin-αV, and β-actin were analyzed by WB. **B**, The quantification analysis of the gray value. The values are mean ± SD of three independent experiments. *p<0.05, ***p<0.001 compared to the control.

suppressed under the treatment of TGF-β1 and WISP1. Meanwhile, the type I collagen changes highly increased compared to the control. Therefore, the crosstalk between TGF-β1 and WISP1 promoted the CHs dedifferentiation and proliferation, accompanying with an upregulation of Integrin-α1/αV.

Integrin-α5 and αV Deficiency Weakened WISP1 Induced CHs Dedifferentiation

To clarify whether the existence of α5 and αV subunit of Integrin mediate the effect of WISP1 on CHs, we silenced the α5 and αV subunit and then treated with WISP1 (2 μg/mL) for three days. The

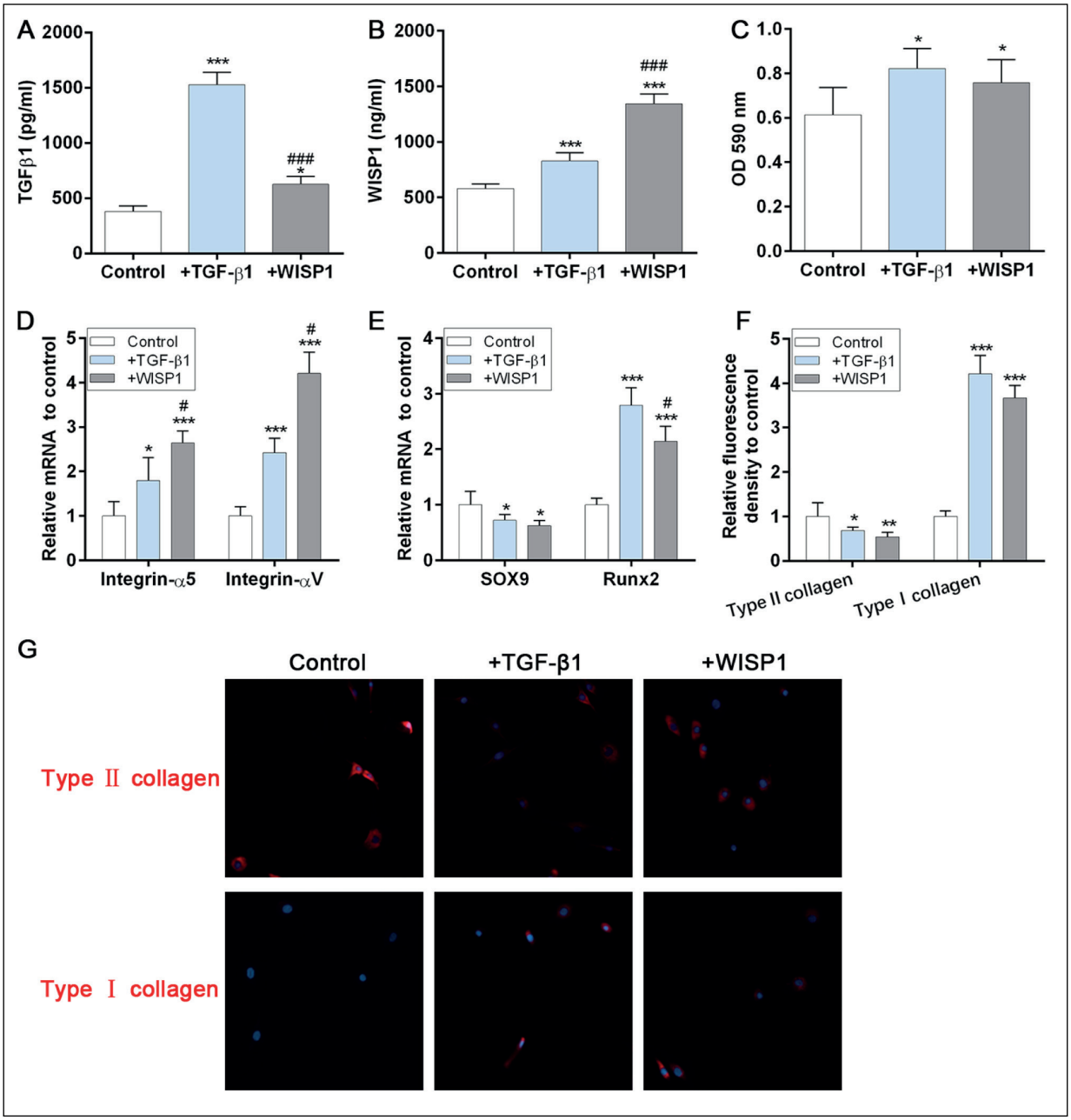

Figure 2. Exogenic TGF-β1 and WISP1 supplement promoted CHs dedifferentiation. CHs isolated from the trauma cartilage ware treated with TGF-β1 and WISP1 protein for three days. **A**, TGF-β1 and (**B**) WISP1 content in the CHs after treatment were analyzed by ELISA. **C**, CHs proliferation was determined by MTT assay. **D**, Integrin-α5, Integrin-αV, **E**, SOX9, and Runx2 mRNA expression were measured by RT-PCR. **F**, The quantification analysis of the intensity of type II and I collagen. **G,** IF staining of type II and I collagen (red) and nucleus (blue) (magnification: 400×). The values are mean ± SD of three independent experiments. *p<0.05, **p<0.01, ***p<0.001 compared to the control; $^{\#}p$<0.05, $^{\#\#\#}p$<0.001 compared to the TGF-β1 group.

null-siRNA was transfected as a negative control. After treatment, we analyzed the cellular Integrin-α5 and αV using ELISA assay. As shown in Figure 3A and 3B, the silenced efficacy was more than 70 %. In the negative transfected group, WISP1 was effective in triggering the expression of α5 and αV subunit. However, compared to the control, the proliferation was promoted by WISP1, even the Integrin was silenced (Figure 3C). As a result before, WISP1 suppressed the SOX9 mRNA expression and promoted the Runx2 expression. Of note, the silencing of the α5 subunit aggravated the downregulation of SOX9 and the upregulation of Runx2 compared to the negative silenced group. However, no significance was noticed after silencing αV compared to the negative silenced group (Figure 3D, 3E). Apart from this, α5 blocking aggravated the reduction of type II collagen and increased type I collagen compared to the negative control. Besides, the αV deficiency alleviated45 the type I collagen protein expression caused by WISP1 treatment (Figure 3F, 3G, 3H).

Different effect of Integrin-α5 and ĐV on the Medication of WISP1 Treated CHs

Silencing of Integrin-α5 aggravated the dedifferentiation of CHs, whereas, the silencing of Integrin-αV weakened WISP1 induced type I collagen expression. When considering the different effect that α1 and αV involving in the WISP1 treatment, we pretreated the CHs with Integrin-α1 (10 μg/mL) and Integrin-αV (10 μg/mL) protein for one day to overexpress α1 and αV subunit. Following, the Integrin pretreated CHs were

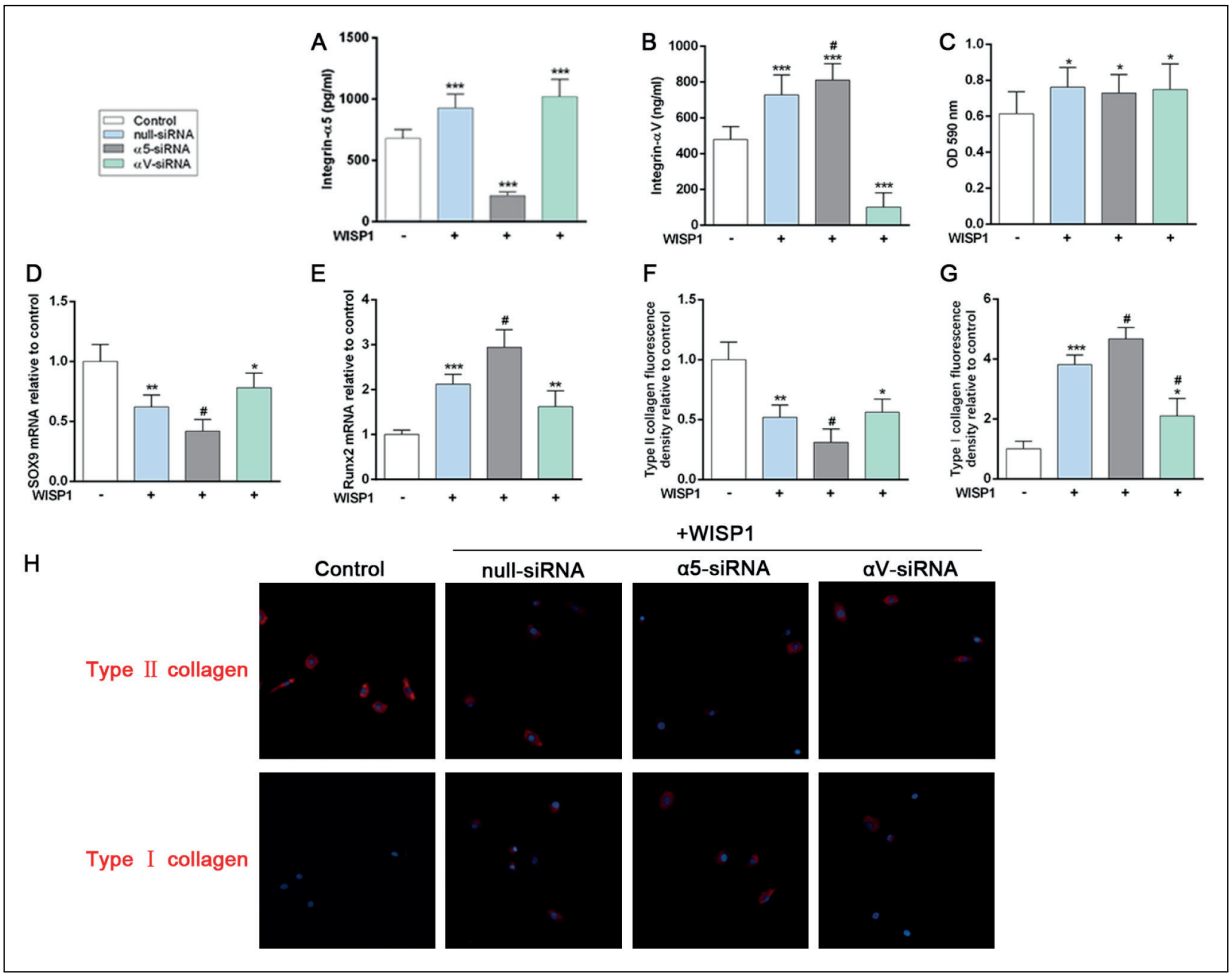

Figure 3. α5 and αV deficiency weakened WISP1 induced CHs dedifferentiation. CHs were transfected with the siRNA that coding null, α5, or αV, and then treated with WISP1 for three days. **A**, Integrin-α5 and **B**, Integrin-αV content in the CHs after treatment were analyzed by ELISA. **C,** CHs proliferation was determined by MTT assay. **D**, SOX9, **E**, Runx2 mRNA expression was measured by RT-PCR. The quantification analysis of the intensity of **F**, type II collagen, **G**, type I collagen. **H**, IF staining of type II and I collagen (red) and nucleus (blue) (magnification: 400×). The values are mean ± SD of three independent experiments. *p<0.05, **p<0.01, ***p<0.001 compared to the control; $^{\#}p$<0.05 compared to the null-siRNA group.

cultured with or without WISP1 protein (2 μg/mL) for another three days. As shown in Figure 4A and 4B, the cellular α1 and αV protein were increased after exogenic Integrin-α1 and Integrin-αV protein stimulation, which was more significant with the addition of WISP1. Without WISP1 stimulation, the overexpression of cellular α1 and αV protein did not promote the proliferation of CHs. However, the CHs proliferation was increased again with the presence of WISP1 (Figure 4C). Apart from this, simply increase of α1 or αV did not affect the SOX9 and Runx2 expression compared to the control. However, the SOX9 mRNA expression was increased under the combination of WISP1 and Integrin-α1 and decreased under the combination of WISP1 and Integrin-αV (Figure 4D). Compared to the control, the Runx2 level of αV overexpressed CHs was significantly increased after pretreated with WISP1 (Figure 4E). Finally, the result of the IF staining also suggested that the overexpression of α1 or αV subunit made no difference in the type II collagen and typed I collagen expression. However, the α1-overexpression contributed to the WISP1-induced type II collagen production, which was suppressed in Figure 3F. Additionally, the α1-overexpression also reversed the WISP1-induced type I collagen upregulation in Figure 3G. Compared to the control, α5-overexpression aggravated the dedifferentiation of CHs with the reduction of type II collagen and the increase of type I collagen (Figure 4F, 4G, 4H).

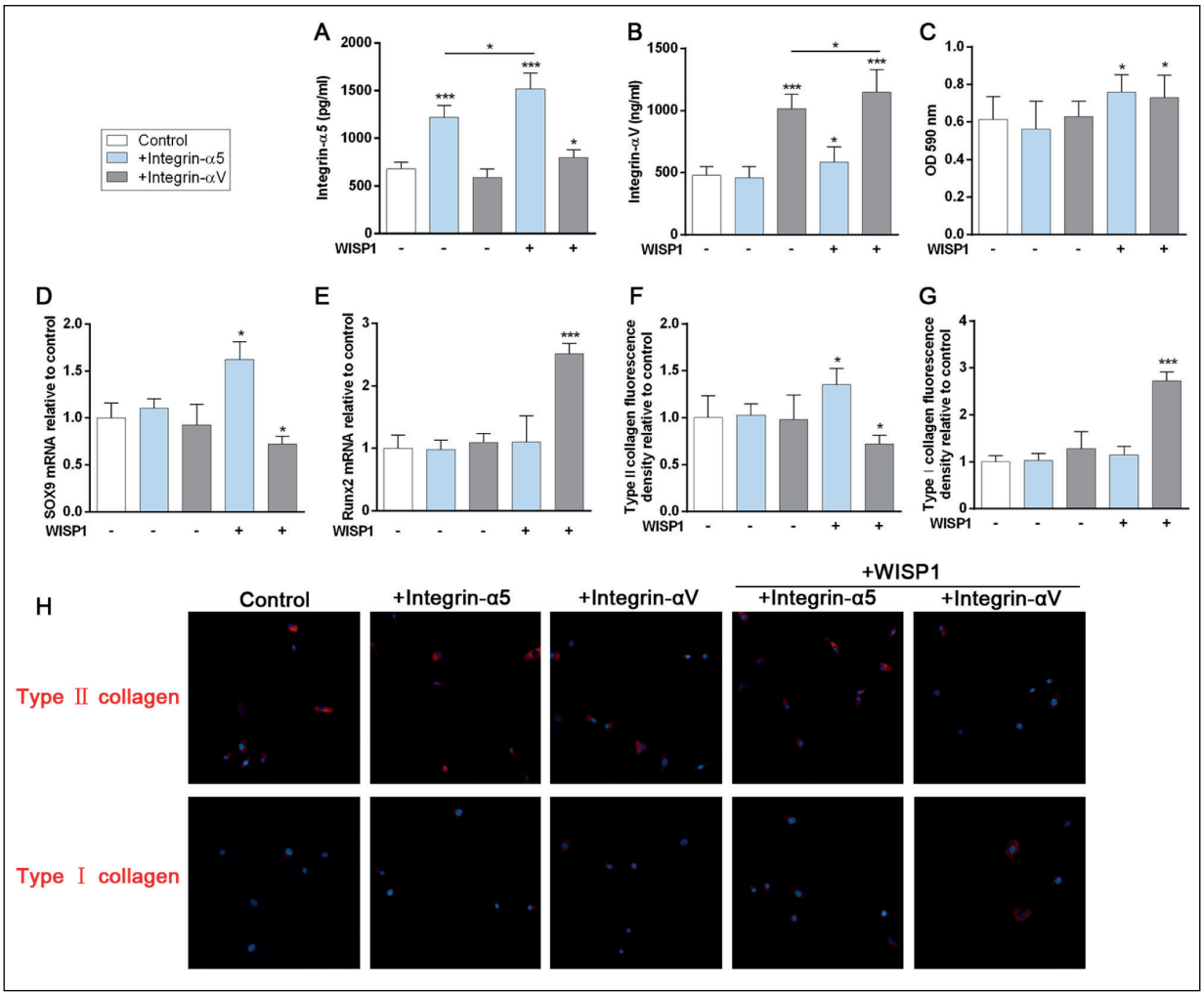

Figure 4. Different effects of Integrin-α5 and αV on the medication of WISP1 treated CHs. CHs were pretreated with Integrin-α5 or αV and then treated with WISP1 for three days. **A**, Integrin-α5 and **B**, Integrin-αV content in the CHs after treatment were analyzed by ELISA. **C,** CHs proliferation was determined by MTT assay. **D**, SOX9 (**E**) Runx2 mRNA expression was measured by RT-PCR. The quantification analysis of the intensity of **F**, type II collagen, **G**, type I collagen. **H**, IF staining of type II and I collagen (red) and nucleus (blue) (magnification: 400×). The values are mean ± SD of three independent experiments. **p*<0.05, ****p*<0.001 compared to the control.

Discussion

In this study, we explored the α5 and αV subunits of the Integrin family and correlated them with the TGF-β1 and WISP1 induced CHs dedifferentiation. Excessive activation of TGF-β signaling will cause the loss of CHs differentiation phenotype, and a cellular behavior similar to that of terminal hypertrophic CHs during growth plate development, which ultimately promotes and exacerbates the process of OA[13,14]. WISP1 involves in the development of subchondral bone, and it highly expressed the CHs of OA patients[15]. In addition, WISP1 also plays an essential role in the dynamic balance of bone formation and bone resorption[16]. It can promote the differentiation of bone precursor cells into osteoblasts, which is conducive to the regeneration and repair of bone after a fracture. Conversely, the loss of WISP can lead to a reduction in bone minerals[17]. WISP1 overexpression promotes the chondrogenesis of hBMSCs via the mediation of TGF-β3 related Smad2/3 phosphorylation[18]. The interaction between WISP1 and TGF-β1 enhanced the fibrosis in rat tubular epithelial cells[19]. However, whether the interplay between them plays a role in the CHs dedifferentiation remains unknown. The impact of the TGF-β family on cartilage is currently controversial. We found the TGF-β1 treatment could promote WISP1 expression, and WISP1 also upregulated the TGF-β1. The crosstalk between them promoted the CHs proliferation, which was independent of Integrin.

Integrin is a transmembrane glycoprotein on the surface of CHs, and consists of two subunits α and β to form a heterodimer[20]. It can recognize many proteins in ECM through specific ligands, especially fibronectin, type II, and type VI collagen, and can also sense the corresponding mechanical force as a mechanical sensor[21]. Integrin transmits signals in CHs using ECM-Integrin-Cell, which depends on different members of the Integrin family with different specificities[22]. Loeser et al[23] also found in the study that the main Integrin types on the surface of normal CHs include α1β1, α3β1, α5β1, α10β1, αVβ3 and αVβ5, while the main types of OA patients are α2β1, α4β1 and α6β1. This not only shows that Integrin is closely related to the processes of CHs survival, proliferation, dedifferentiation, and matrix remodeling but also suggests that most of the stimulation signals that promote or inhibit dedifferentiation can be regulated through the ECM-Integrin-Cell manners[24,25]. Accompanied by the morphological shift of dedifferentiated CHs, specific genes expression synthesis profile is changed as well. In the early CH formation stage, the transcription factor Sox9 is upregulated, which supports the deposition of type II collagen and aggrecan; in the dedifferentiated CH hypertrophy stage, the volume of CH increases, and the terminal differentiation marker osteogenic transcription factor Runx2, and type I collagen highly accumulate[26,27].

As the degree of CHs dedifferentiation increases, the expression level of ECM-related genes decreases significantly, and Integrin expression gradually increases. But not all members of the Integrin family showed a consistent response to CH dedifferentiation. Dedifferentiation of CH will cause the expression of Integrin-α5 to decrease, while the expression of α1, α2, and α3 increase. Growth differentiation factor 5 (GDF-5) can induce the expression of α5 subunit, thus maintaining the chondrogenic phenotype and resisting dedifferentiation. However, the expression of Integrin-αV in hypertrophic CHs was reversed. Bone morphogenetic protein-7 (BMP-7) can induce the expression of αV and increase the degree of dedifferentiation of hypertrophic CHs[8]. Integrin-αVβ1 promotes endochondral ossification-recruiting blood vessels[28], resulting in the osteoblast formation. The Integrin-α5β1 plays a particular role during the establishment of skeletal elements, silencing α5β1 function during skeletal development, causing ectopic joint formation, suggesting that α5β1 subunit coordinate the joint formation and cartilage differentiation in appendicular skeleton[29]. Integrin can mediate CH to respond to external environmental stimuli and regulate various cell functions. However, in our study, independent Integrin does not have a clear tendency to promote or inhibit dedifferentiation, but external WISP1 stimulus that induces dedifferentiation to enter the cell through a specific Integrin subunit, regulating the phenotype of CH dedifferentiation. From the data *in vitro*, we confirmed the WISP1 could upregulate the Integrin-α5/V expression of CHs, which might be a reason why the Integrin-α5/V was also highly expressed in the OA cartilage. With the accumulation of WISP1 in the ECM of OA cartilage, the Integrin-α5/V expression was passively upregulated.

We silenced Integrin-α5, and it aggravated the WISP1 triggered the reduction of SOX9, type II collagen, and the increase of type I collagen, Runx2. On the contrary, overexpressed Integrin-α5 partly reversed these adverse effects caused by WISP1. Besides, Integrin-αV silencing weak-

ened the WISP1 induced type I collagen, and Integrin-αV overexpression combining with WISP1 promoted the expression of the dedifferentiated markers. These data suggest that WISP1 participates in the CH dedifferentiation associating with the mediation of αV Integrin, meanwhile the α5 might play a protective role in the maintenance of the chondrogenic phenotype, which is apparently contradictory to αV Integrin. Under the stimulation of WISP1, α5 and αV were simultaneously increased. However, WISP1 overall promotes the CHs dedifferentiation, which may be related to the upregulation of αV Integrin being stronger than α5. Although Integrin contains many other subunits, we have at least clarified the role of α5 and αV in the process of CHs dedifferentiation.

Conclusions

Finally, Integrin may be an important link to mediate the phenomenon of CHs dedifferentiation, of which α5 acts as a protective factor and αV plays the opposite role. Upregulation of Integrin-α5 might alleviate or even reverse the TGF-β1/WISP1 induced dedifferentiation CHs. Beyond that, silencing the αV subunit could also weaken the terrible effects caused by TGF-β1/WISP1. Any distortion in the TGF-β1/WISP1/Integrin-α signaling leads to the different results of CHs phenotype, that control the development of OA. Enhanced dedifferentiated (hypertrophic/fibroblast-like) phenotypes of CHs normally exist in OA cartilage. The plasticity of CHs is well reflected in both normal conditions and OA course, which can be biological intervened. As the receptors for signal recognition widely exists on the surface of CH, Integrin can be further used as a foothold to deeply study the mechanism of mediating other signaling pathways to affect CHs dedifferentiation and provide new strategies for the treatment of OA.

Conflict of Interests

The authors declare that they have no conflict of interest.

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