ORIGINAL ARTICLE

MMP-2 and Notch signal pathway regulate migration of adipose-derived stem cells and chondrocytes in co-culture systems

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Abstract

Objectives: The crosstalk between chondrocytes and adipose-derived stem cells (ADSCs) could regulate the secretion of multiple growth factors. However, it is not clear how the paracrine action in co-culture systems affect cell migration. This study focused on the changes of cell migration of ADSCs and chondrocytes in co-culture conditions.

Materials and methods: Primary ADSCs and chondrocytes were isolated from Sprague-Dawley rat. Transwell co-culture systems, inoculated with ADSCs and chondrocytes, were established in vitro. The morphology of the cells was observed 7 days post-seeding by inverted phase-contrast microscope. Additionally, the cytoskeleton changes were investigated by immunofluorescence staining. To detect the abundance of Vinculin, we used immunofluorescence and Western blotting. Additionally, the expression level of *MMP-2*, *Hey1* and *Hes1* was examined to determine the mechanisms of co-culture-induced cell migration changes.

Results: The migration of ADSCs and chondrocytes in co-culture conditions significantly decreased compared with that in mono-culture groups, accompanied by the decrease of filopodia and the expression level of *MMP-2*.

Conclusions: The overall study showed that the migration of ADSCs and chondrocytes differs significantly depending on culture conditions. Moreover, the Notch signalling pathway may be involved in this process. Accordingly, by studying changes in migration caused by co-culture, we obtained new insight into the crosstalk between ADSCs and chondrocytes.

1 | INTRODUCTION

Over the past few decades, great improvements in tissue engineering have resulted from the use of co-culture systems.¹ The co-culture system was first established to explore intercellular communication in 1978.² Today, it is widely used for many purposes, such as to induce

cell differentiation,³ maintain cell function and viability, regulate cell proliferation,⁴ promote early embryonic development⁵ and increase metabolic yield.⁶ Cell co-culture could simulate cell communication and the formation of tissue structure, and therefore has great potential in tissue engineering and regenerative medicine.⁷

In the field of cartilage tissue engineering, the treatment of cartilage defects has always been a problem.⁸ Cartilage tissue is composed of collagen tissue, a small number of cells, and 60%-80%

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of water and other ingredients.^{9,10} Adult cartilage tissue has no blood vessels or nerves, resulting in limited self-healing ability.¹¹ Nowadays, a number of methods have been developed for cartilage regeneration.¹² Co-culture of other cell types (such as stem cells) with chondrocytes is considered a significant alternative because of the limited number of cells obtained from autologous cartilage.^{13,14} Our previous studies have shown that crosstalk between ADSCs and CH causes changes in the expression of BMP family genes and angiogenesis-related factors.¹¹ In co-cultured ADSCs, the mRNA level of transforming growth factor-beta1 (*TGF- β 1*), hypoxia-inducible factor-1a (*HIF-1a*) and vascular endothelial growth factor B (*VEGFB*) was significantly increased. In chondrocytes co-cultured with ADSCs, the expression of *Aggrecan* and *HIF-1a* gene was up-regulated.^{11,14} Transforming growth factor-beta1 is a crucial requirement for the chondrogenesis of stem cells, which could increase the expression of extracellular cartilage matrix such as collagen II and *Aggrecan*.¹⁵⁻¹⁷ Additionally, VEGF is considered as a survival and maturation factor in the process of cartilaginous template formation.¹⁸ In addition, we proved that the implantation of ADSCs and chondrocytes into *TGF- β 1*/scaffolds showed promising potential in vivo cartilage healing.

Cell migration is a basic cellular behaviour and plays key roles in embryonic development, angiogenesis, wound healing, immune responses, atherosclerosis and cancer metastasis.^{19,20} It is a multi-step process involving the extension of pseudopodia, new adhesion establishment and cell contraction.²¹⁻²³ However, few studies have focused on cell migration in co-culture conditions, despite its importance as a cellular behaviour.

The Notch signalling pathway is a highly conserved network that is essential for regulating cellular proliferation and differentiation during developmental process.²⁴⁻²⁶ It has been shown that Notch signalling also plays a role in cell migration.²⁷ However, the role of the Notch signalling pathway in cell migration in co-culture systems has not been fully understood. In mammals, there are five Notch ligands (*Delta-like [Dll]* 1, 3, 4, and *Jagged* 1, 2) and four Notch receptors (*Notch* 1-4). Interaction of Notch ligand and receptor of the adjacent cells facilitates the production and release of Notch signal proteins into the cytoplasm. The transcriptional activator complex of the Notch signalling pathway thus activates *HES*, *HEY*, *HERP* and other target genes.²⁸

To improve our understanding of cell migration in co-culture systems, we examined the migration of adipose-derived stem cells (ADSCs) and chondrocytes (CHs) in mono-culture and co-culture conditions. We used the xCELLigence DP RTCA instrument (Roche Diagnostics, Basel, Switzerland) as a non-invasive and label-free method to detect cell migration. The xCELLigence system conducts real-time monitoring of cell proliferation, migration and invasion via a culture plate with interdigitated gold microelectrodes. The activity of the cells on the plate causes a change in electrical impedance and is detected by the RTCA station.^{29,30} Moreover, to explore the mechanisms underlying changes in cell migration, we also examined the abundance of filopodia, the secretion of *MMP-2*, the expression of Vinculin and the involvement of the Notch signalling pathway.

2 | MATERIALS AND METHODS

2.1 | Cell isolation and culture

Adipose-derived stem cells were isolated from subcutaneous adipose tissue of 7-day female SD rats. Minced fat was digested with collagenase I for 60 minutes. Then it was neutralized with 10% foetal bovine serum (FBS)-supplemented low-glucose DMEM. The suspension was centrifuged at 200 g for 5 minutes. Subsequently, removed supernatant and ADSCs were resuspended by adding 10% FBS low-glucose DMEM. Following, ADSCs were inoculated in T25 flasks and cultured at 37°C in a 5% CO₂ incubator and passed through to passage 3 to obtain purified ADSCs.

Chondrocytes were isolated from the knee cartilage of 3- to 5-day SD rats. Briefly, the cartilage was cut into small pieces. They were pre-treated with 0.25% trypsin for 30 minutes and then washed three times with phosphate-buffered saline (PBS) to remove trypsin. Subsequently, the cartilage tablets were digested with collagenase type II (0.1%) for 2-3 hours in a 37° water bath. After 5-minute centrifugation at 200 g, the mixed cartilage and chondrocytes were suspended in low-glucose DMEM with 10% FBS. Chondrocytes were then inoculated in T25 flasks and cultured at 37°C in a 5% CO₂ incubator and passed through passage 3 to obtain purified CHs.

2.2 | Co-culture of ADSCs and chondrocytes

In order to detect the migration of ADSCs and chondrocytes under different culture conditions, we co-cultured ADSCs and chondrocytes with transwell (0.4 mm). The ADSCs were seeded on a six-well plate (85%-95% concentration) and chondrocytes in the transwell chamber similarly, chondrocytes in six-well plate and ADSCs in transwell chamber were also used for detection. After 24 hours of equilibration, the medium was exchanged with 2% FBS low-glucose DMEM. After 12 hours of starvation, the medium was replaced with fresh 1% FBS low-glucose DMEM and transwell chamber and plates were placed together.

2.3 | Immunofluorescent staining

We investigated the cytoskeleton morphology and Vinculin protein expression of ADSCs and chondrocytes by immunofluorescent staining. Briefly, the cells were washed three times with PBS and then fixed with 4% paraformaldehyde. The samples were permeabilized with Triton X-100. After blocking for 1 hour with goat serum at 37°C, the samples were incubated with primary antibody solution (against Vinculin, ab73412; Abcam, Cambridge, UK) overnight. Subsequently, the cells were treated with anti-rabbit secondary antibody at 37°C for 1 hour. Next, the samples were treated with rhodamine phalloidin and 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) to observe the F-actin and nucleus of the cells. Immunofluorescence micrographs were obtained using a microscope.

2.4 | Wound healing assay

We detected horizontal migration of cultured ADSCs and chondrocytes using wound healing assay. In brief, ADSCs and chondrocytes were seeded at 1×10^4 cells per cm^2 (six-well plates, Corning, NY, USA) at 37°C under 5% CO_2 . After 24 hours of equilibration, the medium was exchanged with 2% FBS low-glucose DMEM. After 12 hours of starvation, the medium was replaced with fresh 1% FBS low-glucose DMEM. A sterile pipette tip was used to scratch the adherent cells to form a wound. The cell debris was washed with PBS. Finally, cells were exposed to 1% FBS low-glucose DMEM and photographed at 0 and 24 hours after incubation. The wound areas were measured using Image J. (NIH, Bethesda, MD, USA).

2.5 | Real-time cell analysis of cell migration (RTCA)

Real-time cell analysis (RTCA) of cell migration was investigated using xCELLigence DP RTCA instrument. Firstly, fresh low-glucose DMEM containing 1% FBS was added to the lower chamber and upper chamber. Then the solution was equilibrated in the incubator for 1 hour to establish a background reading. Subsequently, 6×10^5 cells/well were inoculated on the chamber with porous membrane. The migrating cells migrate to the lower side of the membrane, where they will be detected by the electrode. The cell migration plate was placed on the RTCA station and the cell index was measured every 15 minutes with RTCA software.

2.6 | Gelatin zymography

The activity of MMP-2 from the supernatant was determined by gelatin zymography. Briefly, the samples were electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, containing 0.05% gelatin). Followed by incubation in 2.5% Triton X-100 for 0.5 hour for three times, the samples were incubated in proteolytic buffer (50 mmol/L CaCl_2 , 0.5 mol/L NaCl, 50 mmol/L Tris, pH 7.8) at 37°C for 12–16 hours. Finally, the gel was stained with coomassie brilliant blue for 1 hour. After decolorization, the strength of the bands is proportional to the activity of MMP-2. The optical density of the bands was analysed by gel image analysis system Image-Pro Plus (Media Cybernetics, Silver Spring, USA) and the statistical analysis of the data was performed.

2.7 | Quantitative real-time PCR (qPCR)

Briefly, *MMP-2*, *Hey1* and *Hes1* mRNA level was detected using qPCR. Total mRNA was extracted from ADSCs and chondrocytes using TRIzol (Sigma, St. Louis, MO, USA). After treatment with DNase I, the samples were reverse transcribed using the first-strand cDNA synthesis kit (Fermentas, Burlington, Canada) to generate cDNA. The selected primers are listed in Table 1. The efficiency of PCR experiments was normalized by the amplification of GAPDH and amplified the target mRNA with SYBR Green I PCR Master Mix. The reaction was carried out using a ABI 7300 thermal cycler (Applied Biosystems,

TABLE 1 Sequences of forward and reverse primers of selected genes designed for qPCR

mRNA	Gene ID	Primer pairs	
GAPDH	24383	Forward	ACAGCAACAGGGTGGTGGAC
		Reverse	TTTGAGGGTGCAGCGAACTT
MMP-2	81686	Forward	TGGTGTGGCACCACCGAGGA
		Reverse	CCTTGCCATCGCTTCGGCCA
Hey1	155437	Forward	ACATCACCCACACATCGCAC
		Reverse	GAGAGGTGGAAGGCGAGAGTA
Hes1	29577	Forward	GCTGGAGAGGCTGCCAAGGT
		Reverse	GGTCCCCTGTTGCTGGTGT

Foster, CA, USA) and the procedure was as follows: denaturation at 95°C for 30 seconds, followed by denaturation at 95°C for 5 seconds and 40°C at for 40 cycles.

2.8 | Western blot analysis

The expression level of Vinculin, Hey1 and Hes1 protein in ADSCs and chondrocytes was detected by Western blot analysis. Proteins samples were extracted at 1, 2, 3 and 5 days using a Total Protein Extraction Kit (KeyGen, Nanjing, China). Then, the lysate was shaken at 4°C for 1 minute for three times. Subsequently, protein samples and protein loading buffer (5X) were mixed and boiled in boiling water bath for 3–5 minutes to fully denature the protein. The samples were then separated by SDS-PAGE on a polyacrylamide gel at 300 V for 30 minutes. Subsequently, the isolated protein was transferred to a polyvinylidene fluoride film and blocked in skim milk at room temperature for 1 hour. The primary antibody to GAPDH, Vinculin-specific antibody (Abcam), Hey1 (Abcam) or Hes1 (Abcam) were added and incubated at 4°C overnight. The samples were then incubated at room temperature with a secondary antibody for 1 hour. The film was washed three times with TBST (Tris buffered saline + Tween). The film was soaked in the luminescent solution and then exposed.

2.9 | Statistical analysis

All experiments were performed in triplicate and repeated at least three times. Statistical analysis was conducted using SPSS 16.0 (IBM, Armonk, NY, USA). Comparisons between the samples were performed with independent sample *t* test analysis. Differences were considered significant if the two-tailed *P*-value was less than 0.05.

3 | RESULTS

3.1 | Morphological features of ADSCs and chondrocytes in mono- and co-culturing conditions

The cell morphology of ADSCs and chondrocytes was observed with phase-contrast microscopy (Figure 1A). It showed that low

FIGURE 1 Cell morphologies and cell behaviours of adipose-derived stem cells (ADSCs) and chondrocytes. A, Schematic illustration of the transwell co-culture systems. Cell morphologies of ADSCs and chondrocytes after 7 days of mono- and co-culture are shown in phase-contrast. B, Fluorescent images of nucleus (blue) and cytoskeleton (green) at day 5 post-seeding.

concentrations of serum in the culture medium maintained normal cell viability and cell integrity. Phalloidin staining was used to investigate the distribution of F-actin. The immunofluorescence images show that cells in mono-culture groups employed more filopodia than that in co-culture groups, suggesting a more significant cell migration in mono-culture groups (Figure 1B).

3.2 | The migration of ADSCs and chondrocytes in bidirectional wound healing assay

In order to explore the changes in horizontal migration of ADSCs and chondrocytes in mono-culture and co-culture systems, *in vitro* wound healing assays were employed. As shown in Figure 2A, ADSCs and chondrocytes migrated to the wound area in 24 hours. Statistical analysis was performed by calculating the wound area of each group with Image J (NIH, Bethesda, MD, USA) and the results showed that the wound area of ADSCs and chondrocytes under mono-culture conditions was significantly smaller than co-culture (Figure 2B).

3.3 | Real-time cell analysis (RTCA) of motility of ADSCs and chondrocytes in mono- and co-culturing conditions

To further confirm vertical migration of the two cell types in mono- and co-culture systems, we used RTCA, an instrument that employed real-time cell monitoring, to accurately determine the kinetics of migration activity in a given population of cells. Figure 3A,B showed differences in migration index of ADSCs and

chondrocytes during the 36-hour test period. The reactions of chondrocytes migration to co-culture condition were more intense than that of ADSCs. Statistical analysis of RTCA results revealed that cell migration was significantly stronger than co-culture conditions in chondrocytes and ADSCs in mono-culture in 12, 24 and 36 hours (Figure 3C,D). The results of RTCA are consistent with previous results in the wound healing assays. Therefore, it could be summarized that migration of ADSCs and chondrocytes in a mono-culture condition is significantly stronger than that in co-culture systems.

3.4 | Focal adhesion differs in mono- and co-culturing conditions

Vinculin, an adhesion protein of 117 kDa, is thought to transmit forces between the extracellular matrix (ECM) and the actin cytoskeleton.³¹ Studies have shown that Vinculin could restrain the cellular migration velocity.^{32,33} Thus, to detect the variation of Vinculin expression in ADSCs and chondrocytes, we used immunofluorescence staining and Western blotting. Fluorescence microscopy imaging of the nucleus (blue) and Vinculin (red) was employed at day 3 post-seeding (Figure 4A). Compared with the mono-culture group, the Vinculin expression in the co-culture group showed a significant increase in Vinculin expression in co-culture groups. To further validate these results, Vinculin was detected by Western blot at 1, 2, 3 and days post-seeding. Similarly, the results of Western blotting showed that Vinculin expression increased gradually over time. The expression level in co-culture group was higher than that of mono-culture group (Figure 4B).

FIGURE 2 The migration of adipose-derived stem cells (ADSCs) and chondrocytes by wound healing assay. A, The effects of culture conditions on migration of ADSCs and chondrocytes detected by wound healing assay. B, Histogram representation of cell number in the wound area at 0 and 24 h in mono- and co-culture conditions. Data are presented as mean $\pm$ SD ($n = 3$). Statistical analysis: $*P < .05$

3.5 | Secretion of MMP-2 in ADSCs and chondrocytes was influenced by co-culturing conditions

To examine the activity of MMP-2, we used gelatin zymography. Gelatin zymography was used to determine the activity of degrading denatured collagen I of gelatinase. It is a useful tool to detect and analyse the gelatinases expressed in different cell types qualitatively.³⁴ The samples were electrophoresed in SDS polyacrylamide gel impregnation with gelatin and subjected to a series of treatments to renature the enzymes and degrade the gelatin. Finally, the gel was stained with coomassie brilliant blue. After decolorization, the strength of the bands is proportional to the activity of MMP-2. It can be summarized from the Figure 5A that in the two cell types, MMP-2 activity was gradually increased in the 1, 2, 3, 5 and 7 days. Moreover, statistical analysis of the results in Figure 5A showed that MMP-2 in mono-culture groups was gradually higher than that in co-culture groups (Figure 5B). The expression of MMP-2 gene was examined by qPCR. For ADSCs, relative fold changes of the MMP-2 gene expression in mono-culture group and co-culture

group were 1 and 0.97 on the second day respectively. On the fifth day, the relative mRNA level fold changes of MMP-2 were 1.08 times and 0.71 times in mono-culture and co-culture groups respectively. For chondrocytes, the mRNA level of MMP-2 employed the same trend with ADSCs. MMP-2 gene expression was attenuated to 0.713 and 0.34 times in the co-culture group in day 2 and day 5, compared with the single culture group (Figure 5C). Taken together, for the two cell types, the expression of MMP-2 gene in co-culture group was down-regulated, and the differences was statistically significant.

3.6 | Role of the Notch pathway in cell migration in mono- and co-culture systems

To evaluate the role of the Notch pathway in cell migration in mono- and co-culture systems, the expression level of Hey1 and Hes1 was examined by Western blot and qPCR. Hey1 and Hes1 mRNA exhibited greater expression in co-culture groups (Figure 6A,B). The expression of Hey1 and Hes1 in Western blot was remarkably higher in co-culture systems in the 1, 2, 3 and 5 days and this result further

FIGURE 3 The migration of adipose-derived stem cells (ADSCs) and chondrocytes by real-time cell analysis migration (RTCA). A, Cell migration index was detected by RTCA system. B, Histogram representation of the migration index of migration of ADSCs and chondrocytes at 12, 24 and 36 h in mono- and co-culture conditions. Data are presented as mean $\pm$ SD ($n = 3$). Statistical analysis: * $P < .05$, ** $P < .01$, *** $P < .001$

established the association between the Notch signalling pathway and culture systems (Figure 6C).

4 | DISCUSSION

The structural scaffolds of cells consist of three different filamentous components, ie actin filaments, microtubules and intermediate filaments. They are combined into a complex network and regulate a variety of cell behaviours.³⁵ The assembly and depolymerization of actin filaments and microtubules are highly dynamic and allow cell movement. The elongation of microfilaments produced by the polymerization of actin and the contraction of myosin-mediated microfilaments at the tail of the cell are considered the main drivers of animal cell migration.³⁶ The finger-like extensions that composed of parallel actin bundles in migrating cells is called filopodia.³⁷⁻³⁹ The filopodia could help cells in sensing their external environment and follow chemical clues.³⁹⁻⁴¹ Moreover, it could also promote cell adhesion and therefore it is important for traction forces.^{39,42} Studies have shown that cell migration was reduced because of the inhibition of filopodia formation.^{39,43} In our study, the immunofluorescence analysis of F-actin showed more filopodia on the surfaces of migrating cells in the mono-culture group than the co-culture groups. This observation showed that the migration of cells in the single culture groups is more obvious. Thus, we used wound healing experiments and RTCA

to detect horizontal and vertical migration. The results confirmed our conjecture that the migration of cells differs significantly under different culture systems.

Vinculin, an adhesion protein of 117 kDa, is present in cell-cell junctions and in focal adhesion complexes.⁴⁴ The amino-terminal and carboxy-terminal of Vinculin bind to talin⁴⁵ and filamentous F-actin⁴⁶ respectively. Therefore, Vinculin is thought to transmit forces between the ECM and the actin cytoskeleton.³¹ Vinculin restrains the cellular migration velocity.^{32,33} Studies of mouse embryonic fibroblasts isolated from vinculin-inactivated mutant embryos have shown that migration rates over these substrata are 2-fold higher and the level of focal adhesion kinase (FAK) activity is 3-fold higher.⁴⁴ In our transwell co-culture system, rat ADSCs and CHs exhibit indirect contact. The expression level of Vinculin was significantly up-regulated in co-culture systems than in mono-cultures which restrained the cellular migration velocity.

ECM remodelling is essential to cell migration. It coordinates haptic and mechanical guidance cues, two crucial factors in the process of cell migration.^{47,48} Cells secrete proteolytic enzymes to cleave matrix proteins during the process of migration.⁴⁹ Matrix metalloproteinases are an enzyme family with similar structures. They are named after the need for Ca^{2+} and Zn^{2+} as a cofactor. The members of the gelatinase subgroup of matrix metalloproteinases are MMP-2 and MMP-9. MMP-2 is widely expressed in most tissues and cells.^{50,51} However, the expression of MMP-9 is usually limited to monocytes,

FIGURE 4 The abundance of Vinculin in ADSCs and chondrocytes. A, Immunostaining images of cell nuclei (blue) and Vinculin (red) at day 3 post-seeding. Scale bars are 50 μm. B, Protein expression of Vinculin measured by Western blotting in ADSCs and chondrocytes

macrophages, polymorphonuclear cells and keratinocytes.^{50,52} MMP-2 degrades components of the extracellular matrix,⁵³ and confers an important role in ECM remodelling and cell migration processes. In our study, MMP-2 activity was detected by gelatin zymography. As the culture time increased, MMP-2 activity was enhanced. However, this activity was significantly lower than that observed in mono-culture conditions. On the basis of previous studies, we have proposed that the down-regulation of MMP-2 in co-cultured groups may cause reduced migration.

The Notch signalling pathway is a highly conserved network involved in many developmental processes.^{24,25} Previous studies have examined the mechanisms of cell migration,²⁰ but the role of Notch signalling has not been fully defined. It has been reported that Notch1 is specifically reduced during the healing of the corneal epithelium. Additionally, corneal epithelial cell migration is enhanced by the exogenous inhibition of Notch.⁵⁴ This is consistent with our observation that in co-culture systems, cell migration is accompanied by the up-regulation of *Hey1* and *Hes1*. However, studies have shown that the overexpression of Notch1 in transgenic mice enhances wound healing. These contradictory results may be explained by the more rapid proliferation of the transgenic

epithelium.⁵⁵ In addition, Notch inhibition induced changes in the cytoskeleton of actin, consistent with the increased migration phenotype. In particular, Notch inhibition in cells resulted in enhanced lamellipodia, accompanied by the loss of marginal actin bundles.⁵⁵ In our research, we showed the up-regulation of two effectors, *Hes-1* and *Hey-1*, of the Notch signalling pathway. To assess whether the Notch signalling pathway regulates co-culture-dependent changes in migration, it will be important to employ additional in-depth analyses of other effectors of the Notch signalling pathway in future studies.

In conclusion, the overall study showed that the migration of ADSCs and chondrocytes differs significantly depending on culture conditions. Adipose-derived stem cells and chondrocytes exhibited less migration in the co-culture group than in mono-culture groups. The mechanisms underlying these differences in migration include a decrease in the secretion of MMP-2 and an increase in the expression of Vinculin under co-culture conditions. In addition, the expression levels of *Hey1* and *Hes1* in the Notch signalling pathway were also detected. By studying the migration changes caused by co-culture, we have a new understanding of the crosstalk between ADSCs and chondrocytes.

FIGURE 5 Adipose-derived stem cells (ADSCs) and chondrocytes secrete more MMP-2 in mono-culture conditions. A, Zymography showed MMP-2 secreted in ADSCs and chondrocytes. B, Quantification of MMP-2 secreted in ADSCs and chondrocytes was performed using the OD method. C, Real-time qPCR analyses of the gene expressions of MMP-2 in day 5 post-seeding. Results were normalized in respect to expression levels of the endogen reference gene GAPDH. Data are presented as mean $\pm$ SD ($n = 3$). Statistical analysis: * $P < .05$, ** $P < .01$, *** $P < .001$

FIGURE 6 Role of the Notch pathway in cell migration in mono- and co-culture systems. A, Hey1 and Hes1 gene expression level in adipose-derived stem cells (ADSCs) and chondrocytes. GAPDH levels set as internal normalized control. The samples were collected on day 5. The results shown are representative of three different experiments ($n = 3$). Data are presented as means $\pm$ SD, * $P < .05$, ** $P < .01$, *** $P < .001$. B, Protein expression of Hey1 and Hes1 measured by Western blotting in ADSCs and chondrocytes

5 | CONCLUSION

In conclusion, our study focused on migration of ADSCs and chondrocytes in mono- and co-culture systems. Our results illustrated that ADSCs and chondrocytes exhibited less migration in the co-culture group. The mechanisms underlying the differences could be explained in following three aspects: (i) Attenuated filopodia restrained migration in co-culture groups; (ii) The down-regulated MMP-2 decreased ECM remoulding in co-culture groups; and (iii) The up-regulated Vinculin restrain cellular migration in co-culture group. Moreover, the Notch signalling pathway may be involved in this process. Accordingly, by studying changes in migration caused by co-culture, we obtained new insight into the crosstalk between ADSCs and chondrocytes.

CONFLICTS OF INTERESTS

The authors declare no conflict of interests.

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