Biochemical and Biophysical Research Communications 451 (2014) 68–73

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Direct contacts with colon cancer cells regulate the differentiation of bone marrow mesenchymal stem cells into tumor associated fibroblasts

Yanan Peng [a,1], Zongwei Li [a,1], Peng Yang [a], Ian P. Newton [c], Hua Ren [d], Lichao Zhang [a], Haili Wu [a], Zhuoyu Li [a,b,*]

[a] Institute of Biotechnology, Key Laboratory of Chemical Biology and Molecular Engineering of National Ministry of Education, Shanxi University, Taiyuan 030006, China
[b] College of Life Science, Zhejiang Chinese Medical University, Hangzhou 310053, China
[c] Division of Cell & Developmental Biology, College of Life Sciences, University of Dundee, Dundee DD15EH, Scotland, UK
[d] Shanxi College of Traditional Chinese Medicine and Western Medicine Hospital, Taiyuan 030013, China

ARTICLE INFO

Article history:
Received 9 July 2014
Available online 22 July 2014

Keywords:
Mesenchymal stem cell
Colon cancer cell
Tumor-associated fibroblast
Differentiation
Notch signaling pathway
TGF-β/Smad signaling pathway

ABSTRACT

Tumor–stroma interactions are referred to as essential events in tumor progression. There has been growing attention that bone marrow-derived mesenchymal stem cells (BMSCs) can travel to tumor stroma, where they differentiate into tumor-associated fibroblast (TAF)-like cells, a predominant tumor-promoting stromal cell. However, little is definitively known about the contributors for this transition. Here, using an *in vitro* direct co-culture model of colon cancer cells and BMSCs, we identify that colon cancer cells can induce adjoining BMSCs to exhibit the typical characteristic of TAFs, with increased expression of α-smooth muscle actin (α-SMA). Importantly, the present data also reveals that activated Notch signaling mediates transformation of BMSCs to TAFs through the downstream TGF-β/Smad signaling pathway.

1. Introduction

Most solid tumors are composed of parenchymal tumor cells and a complex array of tumor stromal cells [1]. It is increasingly appreciated that stromal cells in the tumor microenvironment exert profound effects on neoplastic progression, tumor growth, angiogenesis and metastasis [2,3]. Tumor-associated fibroblasts (TAFs), also termed cancer-associated fibroblasts, are the most frequent component of tumor stroma [4]. They distinctively express α-smooth muscle actin (α-SMA) and have been reported to participate in important aspects of solid tumor progression [5,6].

Mesenchymal stem cells (MSCs) are a population of pluripotent progenitor cells that can be isolated from a variety of adult and fetal tissues, including bone marrow, adipose tissue, umbilical cord blood, placenta and amniotic fluid [7]. These cells can self-renew *in vitro* and have the potential to give rise to multiple mesenchymal cells, such as osteoblasts, chondrocytes, adipocytes, fibroblasts and myocytes [8,9]. Importantly, MSCs exhibit an innate tropism for inflamed or damaged tissues as well as tumor sites, which are likened to wounds that never heal, due to the close proximity of factors secreted by tumors and wounds [10,11]. There is an accumulating amount of evidence demonstrating that primary and metastatic tumors attract MSCs into their microenvironment, where they become TAFs, contributing to tumor stroma formation and affecting tumor cell survival and angiogenesis [12–14]. However, the underlying regulatory mechanisms that link MSCs to TAFs remain incompletely understood. To the best of our knowledge, the majority of current research in this field is devoted to defining which cytokines and extracellular matrix proteins are involved in this process [15,16]. Little information concerning the role of cell contact-dependent signaling is available.

This study shows that direct contact with colon cancer cells can stimulate differentiation of bone marrow mesenchymal stem cells (BMSCs) to TAFs, with an *in vitro* direct co-culture model of colon cancer cells and BMSCs. Specifically, Notch and TGF-β/Smad signaling pathways are demonstrated to synergistically regulate the differentiation of BMSCs into TAFs. These findings are novel and

Abbreviations: BMSCs, bone marrow-derived mesenchymal stem cells; TAF, tumor-associated fibroblast; α-SMA, α-smooth muscle actin; MSCs, mesenchymal stem cells; FBS, fetal bovine serum; PFA, paraformaldehyde; SE, standard error; GFP, green fluorescent protein; BMMSCs, murine bone marrow-derived mesenchymal stem cells; p-Akt, the phosphorylated form of AKT; p-Smad2/3, the phosphorylated form of Smad2/3.

* Corresponding author at: Institute of Biotechnology, Key Laboratory of Chemical Biology and Molecular Engineering of National Ministry of Education, Shanxi University, Taiyuan 030006, China. Fax: +86 351 7018268.
E-mail address: lzy@sxu.edu.cn (Z. Li).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.07.074
0006-291X/

important to the understanding of stem cell biology and the developmental events that govern the initiation of TAFs from MSCs.

2. Materials and methods

2.1. Cell culture

DLD1 and SW480 (human colorectal cancer cell lines) were obtained from the Chinese Type Culture Collection and grown in RPMI-1640 (Gibco, Grand Island, NY, USA) containing 1× penicillin/streptomycin and 10% fetal bovine serum (Gibco). Human bone marrow mesenchymal stem cells were purchased from Chinese Biowit Technologies and cultured in HBMSC-GM (Biowit, China). Cells were kept at 37 °C in 5% humidified CO_2.

2.2. Lentiviral experiments

To select for DLD1 and SW480 stably expressing green fluorescence protein (GFP), pLVX-AcGFP1-N1 (Clontech), psPAX2 and pMD2.G were co-transfected into 293T cells at a ratio of 15:10:5 μg using the calcium phosphate transfection method. Thirty-six hours later the viral supernatant was collected and concentrated using 100 kDa ultrafiltration membranes (Millipore). Rapidly proliferating DLD1 and SW480 were infected with the concentrated virus in the presence of polybrene (8 μg/ml) for 24 h and then subjected to selection using 5 μg/ml puromycin. This method resulted in >90% infection efficiency, as determined by the percentage of cells labeled with GFP.

We utilized the lentivirus-based shRNA expression plasmid pLL3.7 to knockdown the endogenous expression of Jagged1 and DLL1 in the colon cancer cell line DLD1. shRNA oligonucleotides for Jagged1 and DLL1 were chemically synthesized, annealed and cloned into the PLL3.7 lentivector utilizing HpaI and XhoI restriction sites. Correct insertions of shRNA cassettes were confirmed by direct DNA sequencing. Recombinant lentivirus was generated by co-transfecting 293T cells with three plasmids: pLL3.7-Jagged1/DLL1 shRNA (experimental virus) or pLL3.7-control shRNA (control virus), plus psPAX2 and pMD2.G. The infection of colon cancer cells was performed according to the protocol above. Hairpin sequences in these shRNA constructs are depicted in Supplementary Table 1.

2.3. Co-culture assay

BMSCs were plated in 12-well plates (Corning Costar Co., NY, USA) with gelatin coated glass slides for immunofluorescence analysis or in 75 cm^2 tissue culture flasks (Corning Costar Co., NY, USA) for protein or RNA isolation. After 24 h, colon cancer cells were loaded directly onto the BMSCs cultures. The direct co-culture systems were maintained for 72 h in HBMSC-GM medium. Some of the co-cultures were treated with 30 μM γ-secretase inhibitor DAPT (Cayman Chemical) or 10 μM transforming growth factor-β type I receptor kinase inhibitor SB431542 (Cayman Chemical).

2.4. Immunofluorescence staining

After co-culturing, the cells were fixed in 4% paraformaldehyde (PFA) at room temperature for 30 min, then permeabilized with 0.3% Triton X-100 in PBS for 10 min. Next, the slides were blocked in 3% BSA (Sigma) for 1 h and then incubated with the primary antibody for α-SMA (1:100, Biotime, China) at 4 °C overnight. Anti-mouse-TRITC secondary antibody was used for primary antibody detection. After three PBS washes, the slides were mounted in gelvatol for the confocal immunofluorescence analysis.

2.5. Western blotting and quantitative RT-PCR

Western blotting and quantitative RT-PCR assays were performed as previously described [17]. Antibodies used for western were as follows: α-SMA (1:1000; Biotime, China), β-catenin (1:1000; Abmart, Shanghai, China), NF-κB (1:1000; Bioworld, Nanjing, China), Phospho-AKT (1:500; Bioworld, Nanjing, China), Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (1:1000; Cell Signaling), Smad2/3 (1:500; Bioworld, Nanjing, China), GAPDH (1:5000; Abmart, Shanghai, China), secondary anti-mouse and anti-rabbit antibodies (1:1000; Invitrogen, Carlsbad, CA). The primers for qPCR used in this study were depicted in Supplementary Table 2.

2.6. Statistical analysis

Data are presented as mean ± standard error (SE). Student's *t*-test was used to analyze differences between two groups. We considered probability (P) values <0.05 as significant.

3. Results

3.1. Human colon cancer cells induce α-SMA expression in BMSCs through direct cell–cell contacts

To investigate whether colon cancer cells can trigger differentiation of BMSCs into TAFs through direct cell–cell contacts, we first established direct co-cultures by placing colon cancer cells in the same well with human bone marrow mesenchymal cells (HBMSCs) on primaria slides for 3 days. Two colon cancer cell lines labeled with lentivirus expressing green fluorescent protein (GFP), GFP-DLD1 and GFP-SW480, were selected for our co-culture assay. Then, expression of α-SMA, the most reliable marker of TAFs in HBMSCs was examined by immunofluorescence staining. As shown in Fig. 1, clear expression of α-SMA was observed in HBMSCs adjacent to colon cancer cells, indicating that direct cell–cell contacts between colon cancer cells and HBMSCs can promote the differentiation of HBMSCs into TAFs.

To further support the notion above, the parallel experiments were performed with murine bone marrow-derived mesenchymal stem cells (BMMSCs). Consistent with observations from HBMSCs, BMMSCs in co-culture were also found to display strongly positive α-SMA staining at sites of cell contact with human colon cancer cells (Supplementary Fig. 1).

3.2. Notch and Smad-dependent signaling pathways are activated in the co-culture system

The Notch signaling pathway is known as an evolutionarily ancient cell–cell interaction mechanism [18]. As a primary candidate, Notch signaling molecules were examined to investigate whether signaling through Notch receptors could control the induced differentiation of HBMSCs to TAFs by direct contacts with colon cancer cells. We first co-cultured HBMSCs with GFP-DLD1 and GFP-SW480 respectively. After culturing for 3 days, the ratio of the two cell types in co-cultures was determined by flow cytometry. Next, corresponding colon cancer cells and HBMSCs cultured separately at the same time, were mixed to the final cell ratio as in the co-culture system and used for control groups. Quantitative RT-PCR analysis showed that both the direct co-cultured groups resulted in dramatically increased mRNA expression of the downstream Notch effector Hes1 (Fig. 2A).

Extensive studies indicate that α-SMA gene transcription is regulated by the interplay between a variety of signal transduction pathways [19,20]. To address this we performed Western blot

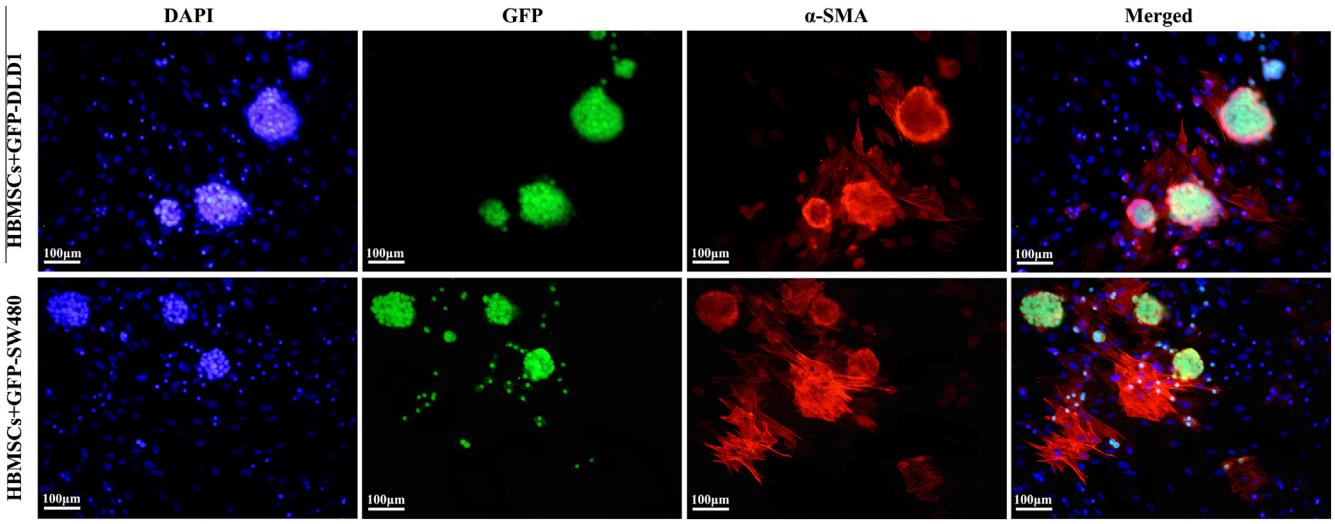

Fig. 1. Analysis of TAF-like characteristics of HBMSCs co-cultured with colon cancer cells. HBMSCs were cultured in the same well with two human colon cancer cell lines labeled with GFP (GFP-SW480 or GFP-DLD1) for 3 days, respectively. Positive immunofluorescence staining of TAF marker protein α-SMA (in red) was observed in HBMSCs that were located close to GFP-SW480 or GFP-DLD1. The nuclei of all cells were counterstained blue with 4′,6-diamidino-2-phenylindole (DAPI). Images were obtained using an Olympus upright confocal microscope (20× objective). Scale bars represent 100 μm. Representative images obtained from at least three independent experiments are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

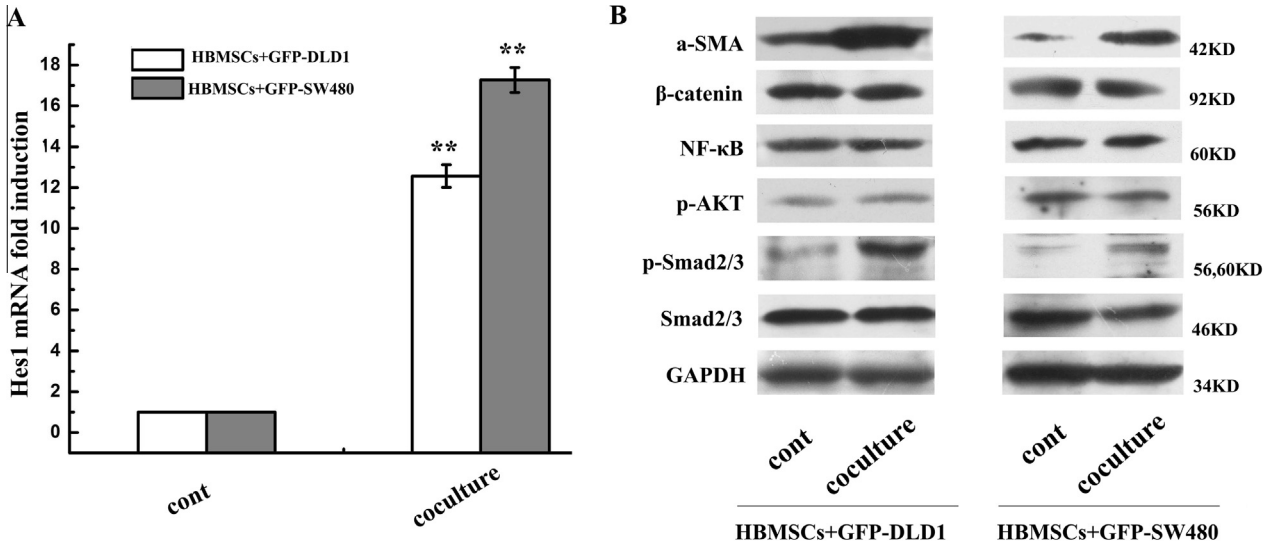

Fig. 2. Activation of Notch and Smad-dependent signaling pathways in co-cultures of HBMSCs with GFP-DLD1 or GFP-SW480. (A) Quantitative RT-PCR analysis of the Hes1 mRNA expression, a well known Notch target gene, in the co-cultures. mRNA extracted from admixtures of HBMSCs with GFP-DLD1 or GFP-SW480 that were initially cultured alone and mixed to the ratio of that in corresponding co-culture system were used as control. Hes1 transcript was highly up-regulated in both direct cultures. $^{**}p < 0.01$, compared with control group. (B) Involvement of other differentiation related signalings were assessed by Western blotting using antibodies against β-catenin for the Wnt pathway, NF-κB for the NF-κB pathway, phosphorylated AKT (p-AKT) for the PI3K/AKT pathway, or Smad2/3 and phosphorylated Smad2/3 for the Smad-dependent pathway. GAPDH was used as the protein loading control.

analysis against β-catenin, NF-κB, the phosphorylated form of AKT and Smad2/3 (p-Akt and p-Smad2/3), which are indicators of Wnt, NF-κB, PI3K/AKT and Smad pathway activation, respectively. This would test whether these cell differentiation associated pathways functioned synergistically with Notch signaling to modulate HBMSCs–TAFs transformation. The results showed that direct co-culture of HBMSCs with GFP-DLD1 or GFP-SW480 only led to notable up-regulated expression of p-Smad2/3, in contrast to no obvious activation of the other pathways (Fig. 2B). Taken together, all these results suggest that the cell contact effects on HBMSCs could potentially be regulated by Notch- and Smad-dependent pathways.

3.3. Notch ligand Jagged-1 is responsible for initiating differentiation of HBMSCs into TAFs

DAPT is a chemical inhibitor of γ-secretase, which is typically required for intramembranous cleavage of Notch receptor as well as induced sequence biological effects [21]. For the sake of verifying the essential role of Notch signals in induced HBMSCs–TAFs transformation, we examined α-SMA expression in HBMSCs co-cultured with GFP-DLD1 or GFP-SW480 in the presence of 30 μM DAPT for 3 days. As shown in Fig. 3A, DAPT treatment attenuated differentiation of HBMSCs cultured in direct contact with GFP-DLD1 or GFP-SW480, with weaker α-SMA staining. In addition,

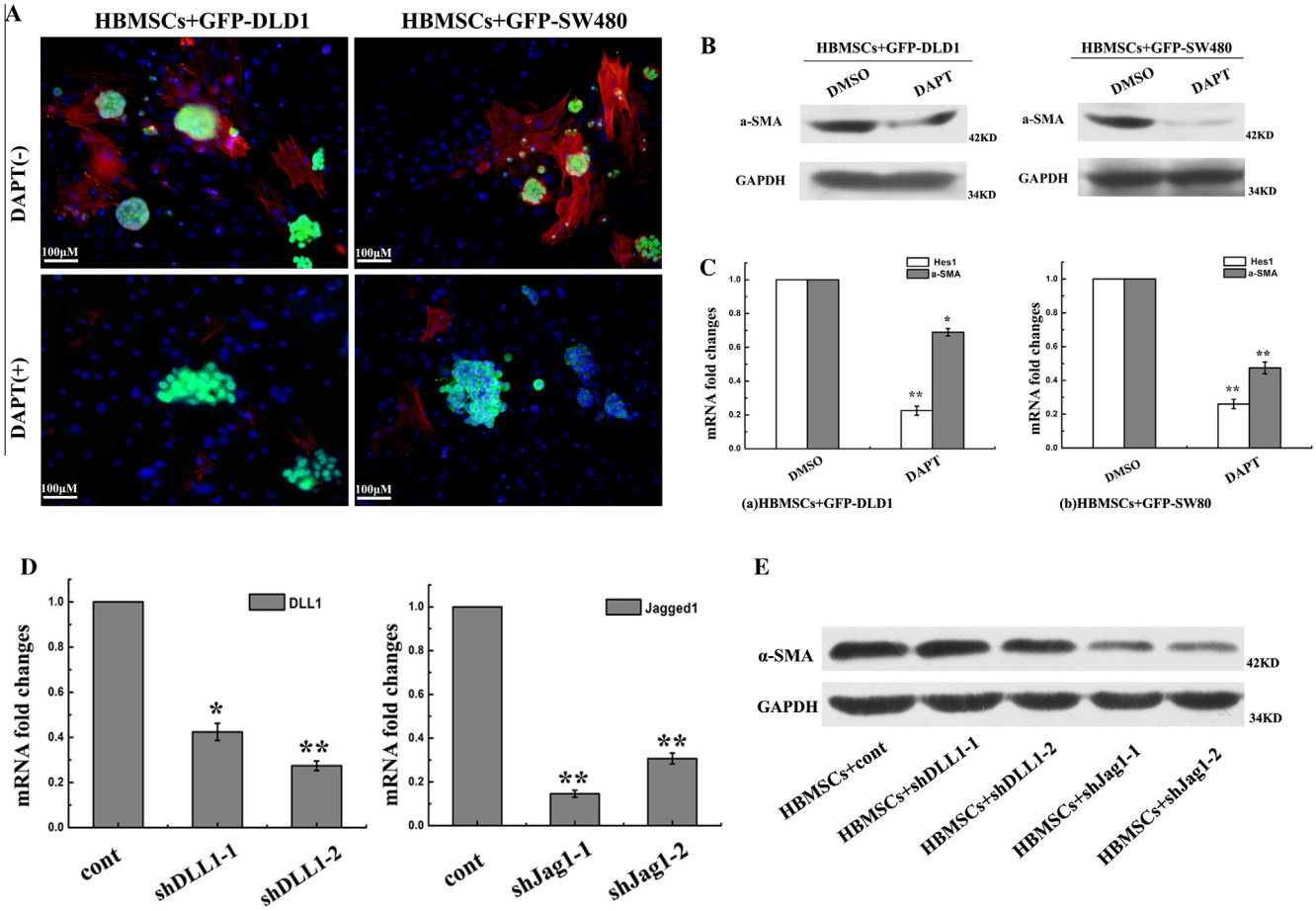

Fig. 3. Cell–cell contacts induced TAF differentiation from HBMSCs were blocked by chemical inhibition of Notch signaling and by gene silencing of Jagged1 in colon cancer cells. (A) HBMSCs were co-cultured with GFP-DLD1 or GFP-SW480 in the presence or absence of 30 μM γ-secretase inhibitor DAPT for 3 days and then stained for α-SMA. (B) Western blotting showed the effects of DAPT treatment on α-SMA protein expression in the co-cultures. (C) Quantitative RT-PCR analysis of mRNA transcription level of α-SMA and Hes1 in the co-cultures treated with DAPT. $^{*}p < 0.05$ versus control group, $^{**}p < 0.01$ versus control group. (D) DLL1 and Jagged1 gene silencing in DLD1 cells. The efficiency of each lentiviral vector-mediated Notch ligand knockdown was assessed by quantitative polymerase chain reaction. (E) HBMSCs were co-cultured with DLL$^{(-)}$ DLD1 or Jagged1$^{(-)}$ DLD1, respectively. After 3 days, the expression of α-SMA in co-cultures was compared to those of HBMSCs in direct co-culture with DLD1 infected with scrambled shRNA expressing lentivirus by Western blotting.

results of Western blotting and qPCR also displayed a marked decrease in α-SMA expression coinciding with an effective inhibition of the Notch signaling pathway, demonstrated by approximately 80% reduction of Hes1 expression (Fig. 3B and C).

After chemical inactivation of Notch signaling, we then introduced lentivirus-mediated Notch ligands (Jagged-1 and DLL-1) gene silencing into our study (derived from colon cancer cells), to further show the participation of Notch signaling in HBMSCs differentiation towards TAFs. The efficacy of each Notch ligand-shRNA knockdown, compared to scrambled shRNAs, was confirmed by qPCR analysis (Fig. 3D). Our data showed that the protein expression level of α-SMA was significantly reduced when HBMSCs and Jagged-1$^{(-)}$ DLD1 were co-cultured, compared to when HBMSCs and control-transfected DLD1 were co-cultured. In contrast, no obvious effect on α-SMA protein expression was observed with DLD1 cells transfected with DLL-1 shRNA-expressing lentivirus (Fig. 3E). Hence, Jagged1-Notch interaction is critical for induced α-SMA expression in HBMSCs.

3.4. Notch signaling mediates TAFs differentiation of HBMSCs via TGF-β/Smad signaling pathway

TGF-β is an important factor in inducing differentiation of TAFs from MSCs and expression of functional markers, notably α-SMA [22]. TGF-β signaling induces phosphorylation of Smad2/3, which then dimerises with Smad4 and translocates into the nucleus to drive the transcription of target genes [19]. To assess whether TGF-β/Smad signaling is implicated in BMSCs–TAFs transition, we firstly analyzed TGF-β1 expression in the co-culture system. Western blotting analysis revealed that the TGF-β1 protein level in direct co-culture of HBMSCs with SW480 was higher than that in the control group (Fig. 4A). This change was also reflected at the mRNA level (Fig. 4B). Moreover, 10 μM of SB431542, a specific inhibitor of TGF-β receptor type I/Alk5 kinase, could abrogate the cell contact effect on HBMSCs differentiation, as shown by α-SMA immunofluorescence staining, Western blotting and qPCR analysis (Fig. 4C–E). Collectively, these findings suggest that the TGF-β/Smad signaling pathway also contributes to inducing HBMSCs differentiation into TAFs.

Different modes of cross talk between the TGF-β and Notch signaling pathways in regulating α-SMA expression have been reported, which are either mutually regulated or in simple upstream and downstream-dependent ways [19,23]. To confirm the epistasis between Notch and TGF-β signals in the HBMSCs differentiation process, we firstly examined the expression of TGF-β1 and p-Smad2/3 in the co-culture of HBMSCs with SW480 when exposed to DAPT. The result showed that activated p-Smad2/3 and TGF-β1 expression were effectively reversed by the addition

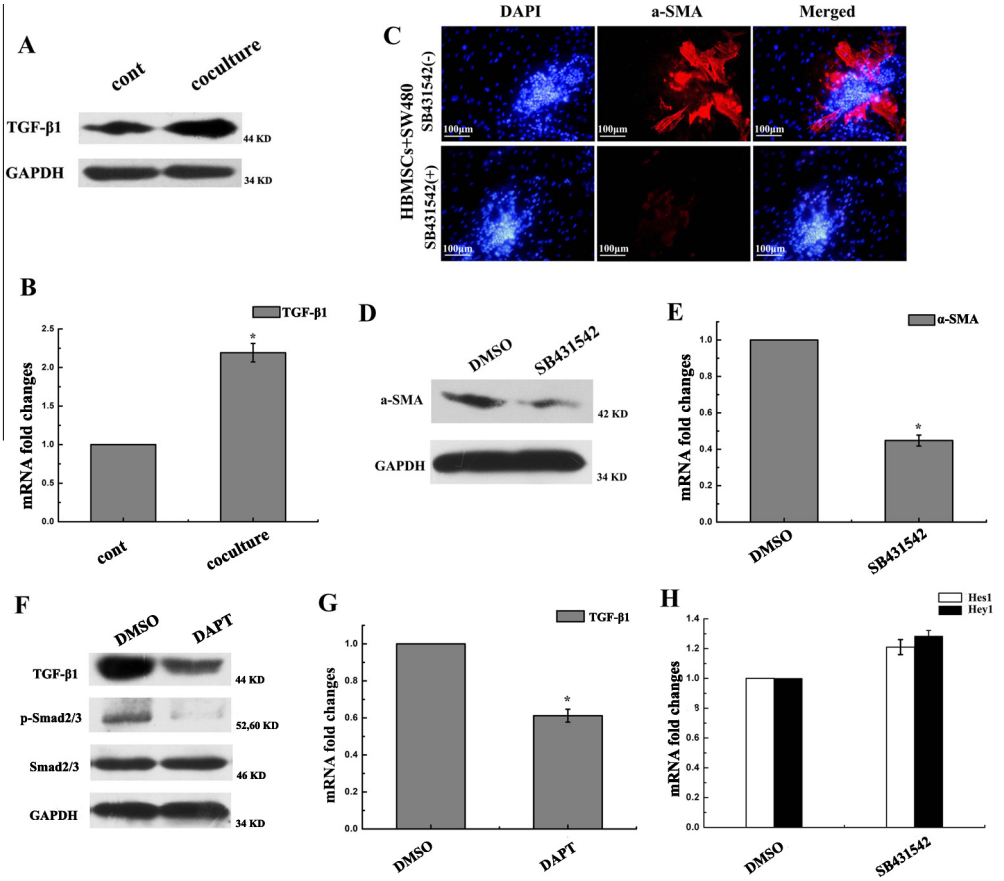

Fig. 4. TGF-β/Smad signal acts the downstream of Notch signal in induced TAF differentiation of HBMSCs. TGF-β1 protein expression level (A) and mRNA transcriptional level (B) were significantly elevated in the co-culture of HBMSCs and SW480. (C) HBMSCs were co-cultured with SW480 in the presence or absence of 10 μM SB431542, a specific inhibitor of TGF-β receptor type I/Alk5 kinase, for 3 days and stained for α-SMA. (D and E) Western blotting and quantitative RT-PCR analysis of α-SMA expression in co-culture of HBMSCs and SW480 untreated (control) or with SB431542. (F) The activated TGF-β1 and p-Smad2/3 protein expression in the co-culture of HBMSCs and SW480 were inhibited by DAPT treatment. (G) The activated TGF-β1 mRNA transcription was suppressed by DAPT in the co-culture of HBMSCs and SW480. (H) mRNA expression of Hes1 and Hey1, two Notch signaling target genes, in co-culture of HBMSCs with SW480 in the presence or absence of SB431542.

of DAPT in the system (Fig. 4F and G). Then we scored mRNA expression of two Notch signaling target genes Hes1 and Hey1 in the co-culture, by quantitative RT-PCR, after SB431542 treatment. As shown in Fig. 4H, pharmacological inhibition of TGF-β/Smad signaling by SB431542 showed no effect on Notch signaling molecules in the co-culture system. Together, these experiments argue that Notch signaling acts upstream of TGF-β/Smad signaling in HBMSCs–TAFs transition.

4. Discussion

Among various tumor stromal cells, TAFs are a predominant, active player in communications between the tumor cells and the microenvironment [24]. However, the origins of TAFs in the tumor stroma remain controversial. In present experiments, we demonstrate that HBMSCs are a source of TAFs in the colon tumor stroma. The conclusion is based upon the observation that direct exposure of HBMSCs to different colon cancer cell lines *in vitro* induces a phenotype that resembles the reported TAFs-like phenotype, as judged by increased expression of α-SMA. This is in agreement with a recent report indicating that circulating MSCs *in vivo* are able to migrate to orthotopic colon tumor as well as metastatic liver tumor and then incorporate into tumor stroma expressing α-SMA and promote the growth and metastasis of colon cancer [25].

The transition from mesenchymal stem cell to TAFs is a complex biological process, which may involve cell-to-cell interaction, cytokines and extracellular matrix proteins in the tumor microenvironment. Whereas the MSCs–TAFs transformation has encouraged ample investigations into possible mechanisms, the focus of literature to date has been on the participation of paracrine signaling molecules derived from tumor cells and the initiation of autocrine signaling cascades in MSCs. One study suggests that ovarian cancer-derived lysophosphatidic acid elicits differentiation of human adipose tissue-derived MSCs (hADMSC) into TAFs through the autocrine TGF-β1–Smad2 signaling loop [15]. Another report reveals that *HOXA9*, a Müllerian-patterning gene expressed in epithelial ovarian cancer cells, can induce differentiation of adipose- and bone marrow-derived MSCs to TAFs via its induction of tumor-derived TGF-β2 and an autostimulatory production of TGF-β ligands in the stroma [26]. Notably, we found when HBMSCs are exposed to colon cancer cells in a manner which allows direct cell–cell contacts, those HBMSCs closely adherent to colon cancer cells display significantly higher α-SMA immunofluorescence staining, compared with other HBMSCs existing in the co-culture system. As paracrine regulatory influences are not eliminated in our experiment, the obtained consequences imply the potential that direct dialogues between tumor cells and HBMSCs play more important roles in driving the switch of TAFs from HBMSCs.

Among several cell differentiation associated pathways examined, concurrent activation of Notch and TGF-β/Smad signalings was observed in the co-culture system. The involvement of these two signaling pathways in the MSCs–TAFs transformation process is verified by subsequent examination of influences on induced α-

SMA expression after treatment with pharmacologic inhibitors or colon cancer cell-derived Notch ligand gene knockdown. More interestingly, we also note that blocking Notch signaling affects TGF-β1 and p-Smad2/3 expression in the co-culture, whereas there are no detectable changes in expression of Notch signaling molecules when treated with TGF-β/Smad inhibitor, which clearly implies that TGF-β/Smad signaling pathway works as an intermediate step in Notch signaling-induced upregulation of TAF marker in HBMSCs. A possible explanation for this phenomenon may be that proposed by Kurpinski and co-workers in their latest study, which shows that Notch activates TGF-β signaling by inducing the expression of the TGF-β family, which in turn phosphorylates Smad3 and induces the expression of α-SMA, possibly through the formation of a Smad3/RBP-Jk/serum response factor (SRF)/myocardin complex [19]. However, it is not fully investigated in our study whether the elevated TGF-β1 was from MSCs or colon cancer cells. More work will be needed to fully elucidate the exact mechanisms of Notch-mediated TGF-β/Smad signaling activation in our model.

In conclusion, we demonstrate that colon cancer cell-derived Notch signal can induce TAF differentiation of BMSCs through activating the TGF-β/Smad signaling. However, it is uncertain whether such regulation mechanism exists in other types of tumors. A large body of research has reported the key role of dysregulated Notch and TGF-β/Smad signalings in tumor–stromal cell interactions. Jie Zhang et al. showed that the positive expression of Notch1, TGF-β1 in human papillary thyroid carcinoma (PTC) was significantly higher than that in nodular goiter and normal thyroid tissues. Moreover, the high expression of Notch and TGF-β1 was closely related with increased α-SMA levels in the stromal cells surrounding the cancer cells, suggesting the possibility of cell contact-mediated smooth muscle gene expression during TAFs differentiation of BMSCs in the PTC stroma [27]. Kamdje et al. also indicated that Notch signaling was significantly activated in the co-culture of human chronic lymphocytic leukemia (CLL) cells and HBMSCs, promoting the survival and chemoresistance of CLL cells [28]. Therefore, Notch induced TAFs differentiation appears not to be colon cancer specific, despite that molecular networks involved in this process may be not exactly the same in different cases. This novel mechanism will contribute to developing strategies for improved tumor therapy that takes into account the influence of tumor microenvironment on tumor survival and growth.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Nos. 31271516, 31201072), Shanxi Province Science Foundation for Youths (2012021028-4) and oversea scientists (20111009/20111010), Research Fund for the Doctoral Program of Higher Education of China (20111401110011).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.074.

References

[1] M. Quante, S.P. Tu, H. Tomita, T. Gonda, S.S. Wang, S. Takashi, G.H. Baik, W. Shibata, B. DiPrete, K.S. Betz, Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth, Cancer Cell 19 (2011) 257–272.
[2] N.A. Bhowmick, E.G. Neilson, H.L. Moses, Stromal fibroblasts in cancer initiation and progression, Nature 432 (2004) 332–337.
[3] J.A. Joyce, J.W. Pollard, Microenvironmental regulation of metastasis, Nat. Rev. Cancer 9 (2008) 239–252.
[4] K. Pietras, A. Östman, Hallmarks of cancer: interactions with the tumor stroma, Exp. Cell Res. 316 (2010) 1324–1331.
[5] A. Orimo, P.B. Gupta, D.C. Sgroi, F. Arenzana-Seisdedos, T. Delaunay, R. Naeem, V.J. Carey, A.L. Richardson, R.A. Weinberg, Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion, Cell 121 (2005) 335–348.
[6] A. Östman, M. Augsten, Cancer-associated fibroblasts and tumor growth–bystanders turning into key players, Curr. Opin. Genet. Dev. 19 (2009) 67–73.
[7] Y. Shi, J. Su, A.I. Roberts, P. Shou, A.B. Rabson, G. Ren, How mesenchymal stem cells interact with tissue immune responses, Trends Immunol. 33 (2012) 136–143.
[8] A. Friedenstein, R. Chailakhjan, K. Lalykina, The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells, Cell Prolif. 3 (1970) 393–403.
[9] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop, E. Horwitz, Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement, Cytotherapy 8 (2006) 315–317.
[10] H.F. Dvorak, Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing, N. Engl. J. Med. 315 (1986) 1650–1659.
[11] H.Y. Chang, J.B. Sneddon, A.A. Alizadeh, R. Sood, R.B. West, K. Montgomery, J.-T. Chi, M. van de Rijn, D. Botstein, P.O. Brown, Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds, PLoS Biol. 2 (2004) e7.
[12] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature 420 (2002) 860–867.
[13] S. Kidd, E. Spaeth, J.L. Dembinski, M. Dietrich, K. Watson, A. Klopp, V.L. Battula, M. Weil, M. Andreeff, F.C. Marini, Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging, Stem Cells 27 (2009) 2614–2623.
[14] W. Zhu, W. Xu, R. Jiang, H. Qian, M. Chen, J. Hu, W. Cao, C. Han, Y. Chen, Mesenchymal stem cells derived from bone marrow favor tumor cell growth in vivo, Exp. Mol. Pathol. 80 (2006) 267–274.
[15] E.S. Jeon, H.J. Moon, M.J. Lee, H.Y. Song, Y.M. Kim, M. Cho, D.S. Suh, M.S. Yoon, C.L. Chang, J.S. Jung, Cancer-derived lysophosphatidic acid stimulates differentiation of human mesenchymal stem cells to myofibroblast-like cells, Stem Cells 26 (2008) 789–797.
[16] S. Liu, C. Ginestier, S.J. Ou, S.G. Clouthier, S.H. Patel, F. Monville, H. Korkaya, A. Heath, J. Dutcher, C.G. Kleer, Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks, Cancer Res. 71 (2011) 614–624.
[17] Y. Peng, Z. Li, Z. Li, GRP78 secreted by tumor cells stimulates differentiation of bone marrow mesenchymal stem cells to cancer-associated fibroblasts, Biochem. Biophys. Res. Commun. 440 (2013) 558–563.
[18] M. Nemir, T. Pedrazzini, Functional role of notch signaling in the developing and postnatal heart, J. Mol. Cell. Cardiol. 45 (2008) 495–504.
[19] K. Aoyagi-Ikeda, T. Maeno, H. Matsui, M. Ueno, K. Hara, Y. Aoki, F. Aoki, T. Shimizu, H. Doi, K. Kawai-Kowase, Notch induces myofibroblast differentiation of alveolar epithelial cells via transforming growth factor-β–Smad3 pathway, Am. J. Respir. Cell Mol. Biol. 45 (2011) 136–144.
[20] S.G. Ball, C.A. Shuttleworth, C.M. Kielty, Platelet-derived growth factor receptor-α is a key determinant of smooth muscle α-actin filaments in bone marrow-derived mesenchymal stem cells, Int. J. Biochem. Cell Biol. 39 (2007) 379–391.
[21] M.E. Boulton, J. Cai, M.B. Grant, Γ-Secretase: a multifaceted regulator of angiogenesis, J. Cell Mol. Med. 12 (2008) 781–795.
[22] A. De Boeck, A. Hendrix, D. Maynard, M. Van Bockstal, A. Daniëls, P. Pauwels, C. Gespach, M. Bracke, O. De Wever, Differential secretome analysis of cancer-associated fibroblasts and bone marrow-derived precursors to identify microenvironmental regulators of colon cancer progression, Proteomics 13 (2013) 379–388.
[23] K.C. Nyhan, N. Faherty, G. Murray, L.B. Cooey, C. Godson, J.K. Crean, D.P. Brazil, Jagged/notch signalling is required for a subset of TGFβ1 responses in human kidney epithelial cells, Biochim. Biophys. Acta, Mol. Cell. Res. 1803 (2010) 1386–1395.
[24] M. Allinen, R. Beroukhim, L. Cai, C. Brennan, J. Lahti-Domenici, H. Huang, D. Porter, M. Hu, L. Chin, A. Richardson, Molecular characterization of the tumor microenvironment in breast cancer, Cancer Cell 6 (2004) 17–32.
[25] K. Shinagawa, Y. Kitadai, M. Tanaka, T. Sumida, M. Kodama, Y. Higashi, S. Tanaka, W. Yasui, K. Chayama, Mesenchymal stem cells enhance growth and metastasis of colon cancer, Int. J. Cancer 127 (2010) 2323–2333.
[26] S.Y. Ko, N. Barengo, A. Ladanyi, J.-S. Lee, F. Marini, E. Lengyel, H. Naora, HOXA9 promotes ovarian cancer growth by stimulating cancer-associated fibroblasts, J. Clin. Investig. 122 (2012) 3603.
[27] J. Zhang, Y. Wang, D. Li, S. Jing, Notch and TGF-β/Smad3 pathways are involved in the interaction between cancer cells and cancer-associated fibroblasts in papillary thyroid carcinoma, Tumor Biol. 35 (2014) 379–385.
[28] A.N. Kamdje, G. Bassi, L. Pacelli, G. Malpeli, E. Amati, I. Nichele, G. Pizzolo, M. Krampera, Role of stromal cell-mediated notch signaling in CLL resistance to chemotherapy, Blood Cancer J. 2 (2012) e73.