Original Article

Effect of Matrix Metallopeptidase 13 on the Function of Mouse Bone Marrow-derived Dendritic Cells

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Abstract

Background: Dendritic cells are professional antigen-presenting cells found in an immature state in epithelia and interstitial space, where they capture antigens such as pathogens or damaged tissue. Matrix metallopeptidase 13 (MMP-13), a member of the collagenase subfamily, is involved in many different cellular processes and is expressed in murine bone marrow-derived dendritic cells (DCs). The function of MMP-13 in DCs is not well understood. Here, we investigated the effect of MMP-13 on DC maturation, apoptosis, and phagocytosis.
Methods: Bone marrow-derived dendritic cells were obtained from C57BL/6 mice. One short-interfering RNA specific for MMP-13 was used to transfect DCs. MMP-13-silenced DCs and control DCs were prepared, and apoptosis was measured using real-time polymerase chain reaction and Western blotting. MMP-13-silenced DCs and control DCs were analyzed for surface expression of CD80 and CD86 and phagocytosis capability using flow cytometry.
Results: Compared to the control DCs, MMP-13-silenced DCs increased expression of anti-apoptosis-related genes, *BAG1* (control group vs. MMP-13-silenced group: 4.08 ± 0.60 vs. 6.11 ± 0.87, $P = 0.008$), *BCL-2* (control group vs. MMP-13-silenced group: 7.54 ± 0.76 vs. 9.54 ± 1.29, $P = 0.036$), and *TP73* (control group vs. MMP-13-silenced group: 4.33 ± 0.29 vs. 5.60 ± 0.32, $P = 0.001$) and decreased apoptosis-related genes, *CASP1* (control group vs. MMP-13-silenced group: 3.79 ± 0.67 vs. 2.54 ± 0.39, $P = 0.019$), *LTBR* (control group vs. MMP-13-silenced group: 9.23 ± 1.25 vs. 6.24 ± 1.15, $P = 0.012$), and *CASP4* (control group vs. MMP-13-silenced group: 2.07 ± 0.56 vs. 0.35 ± 0.35, $P = 0.002$). Protein levels confirmed the same expression pattern. MMP-13-silenced groups decreased expression of CD86 on DCs; however, there was no statistical difference in CD80 surface expression. Furthermore, MMP-13-silenced groups exhibited weaker phagocytosis capability.
Conclusion: These results indicate that MMP-13 inhibition dampens DC maturation, apoptosis, and phagocytosis.

Key words: Apoptosis; Dendritic Cell; Matrix Metallopeptidase 13; Maturation; Phagocytosis

Introduction

Metastatic tumor cells and adult immune cells share many common properties such as the ability to invade surrounding tissue, enter the lymphatic system, and migrate to regional lymph nodes.[1] It has been reported that CD44, which plays a role in tumor metastasis, is also required for the function and migration of dendritic cells (DCs) through the dense extracellular matrix (ECM).[1,2] Furthermore, matrix metalloproteases (MMPs) have the capacity to support the invasion of tumor cells by degrading almost all the components of the ECM and ECM proteins.[3] Usually, the expression of MMPs is low in normal cells, which allows for healthy physiology function. Excessive expression of MMPs is associated with the pathology of many diseases.[4,5] In addition to their ability to facilitate migration, there is abundant evidence that MMPs are involved in immunological processes, such as endocytosis, major histocompatibility complex (MHC)-I presentation, and cytokine/chemokine release.[4-8] MMP-13 is an ECM-degrading endopeptidase and is primarily expressed in chondrocytes and osteoblasts, and functions in bone metabolism and homeostasis.[9,10] Studies of

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MMP-13 revealed that it is also expressed in murine bone marrow-derived DCs, but to our knowledge, very few reports have ever been published regarding the function of MMP-13 in DCs, particularly regarding its role in DC maturation, apoptosis, and phagocytosis.

Lipopolysaccharide (LPS) induces MMP-13 expression in treated DCs, and active MMP-13 exhibits increased activity in response to LPS treatment,[10,11] suggesting that DCs require MMP-13 for antigen presentation under inflammatory conditions. Yet, the function of MMP-13 in DC under normal conditions is also unknown. Here, we provide the first comprehensive analysis of MMP-13 involvement in the biology of DC and the functional changes resulting from the inhibition of MMP-13, including DC maturation, apoptosis, and phagocytosis.

Methods

Mice and dendritic cells

Six female C57BL/6 mice were obtained from Liaoning Changsheng Biotechnology Company Ltd., and kept under pathogen-free conditions. All mice were used at age of 4–6 weeks. Each experiment used three mice and was repeated three times. DCs were isolated as described by Liu and Quan.[12] DCs were cultured in RPIM-1640 medium (HyClone, Shanghai, China) with 10% fetal bovine serum. All cell lines were incubated at 37°C in a humidified incubator with 5% CO_2.

Knockdown

One short-interfering RNA (siRNA) specific for MMP-13 (MMP-13 siRNA) and a scrambled sequence (scrambled siRNA) were purchased from Invitrogen (Carlsbad, CA, USA). Cells were seeded the day before transfection in antibiotic-free medium and transfected with Silencer® Select MMP-13 siRNA (10 nmol of siRNA per well for 6-well plates) and mixed with Lipofectamine™ RNAiMAX (Invitrogen, USA) as transfection reagent according to the manufacturer's instructions. After transfection, cells were assessed for changes in messenger RNA (mRNA) and protein levels.

RNA isolation and real-time polymerase chain reaction

Total RNA was extracted using a miRNeasy mini kit (Qiagen, Stockach, Germany) and reverse-transcribed with PrimeScript™ RT reagent kit (TaKaRa, Otsu, Japan). Real-time polymerase chain reaction (PCR) was employed to evaluate the relative mRNA expression level using the following primers: *BAG1* forward: 5'-CAGGGCAGCAGTGAACCA-3', and reverse: 5'-ACCCGGCAACCATCTTGT-3'; *BCL2* forward: 5'- CTGGGAGAACAGGGTACGATAA-3', and reverse: 5'-GGCTGGGAGGAGAAGATGC-3'; *TP73* forward: 5'-CTCGGGAGGGACTTCAACG-3', and reverse: 5'-CCTGTGGTGGCTCATAGG-3'; *CASP1* forward: 5'-ATTACAGACAAGGGTGCT-3', and reverse, 5'-GAATAACGGAGTCAATCAAA-3'; *LTBR* forward: 5'- CAAAGATGAAGTTGGGAAGG-3', and reverse: 5'- GTTTCCTGCAGAGAGAAGGGTGGCT-3'; *CASP4* forward: 5'- GCACAATGGGCTCTATCT-3', and reverse: 5'-CAGTCGTTCTATGGTGGG-3'; *GAPDH* forward: 5'-AAGAGCACAAGAGGAAGAGAGAGAC-3', and reverse: 5'-GTCTACATGGCAACTGTGAGGAG-3'.

Western blotting

Cells were harvested from 100 mm dishes by rinsing twice with PBS and then lysed on the plate with 70 μl RIPA buffer (Thermo, USA), and 1× complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) on ice for 30 min. Cell lysates were centrifuged at 4°C at 12000 r/min for 20 min. The supernatant (protein) was retained; protein content was quantified. An aliquot of the total protein (30 μg) was loaded into each lane of a 12% sodium dodecyl sulfate polyacrylamide gel and then electrophoretically transferred to polyvinylidene difluoride membranes. The membrane was blocked with 5% nonfat milk in 0.01 mol/L TBS and 0.05% Tween-20 (TBST) at room temperature for 2 h before incubation with anti-BAG1, anti-BCL-2, anti-TP73 (dilution 1:1000; Santa Cruz, California, USA), anti-CASP1, anti-LTBR, or anti-CASP4 (dilution 1:1000; Abcam, Cambridge, MA, USA) antibodies at 4ºC overnight, as appropriate. After three quick washes with TBST, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Proteintech, Wuhan, China) for 2 h at room temperature. Bands were visualized using ECL Western Blotting Substrate (Pierce, Rockford, USA).

Surface expression of dendritic cells

Cells were surface stained at 4°C for 30 min with anti-CD80 (16-10A1) and anti-CD86 (GL1) monoclonal antibodies followed by donkey anti-rabbit IgG secondary antibodies conjugated to phycoerythrin (PE) and anti-mouse IgG antibodies conjugated to fluorescein isothiocyanate (FITC) for 30 min. Flow cytometry was performed on a BD LSRFortessa instrument (BD Bioscience), and the resulting data were analyzed with FlowJo 7.6.1 software (Ashland, OR, USA).

Dextran-fluorescein isothiocyanate uptake assay

DCs were transfected with scrambled siRNA or MMP-13 siRNA for 24 h. The cells were washed with PBS, resuspended in culture media, and treated with 1 mg/ml of dextran-FITC (Molecular Probes, Eugene, OR, USA) for an additional 1 h at 4°C to measure nonspecific uptake as a negative control. Fluorescence intensity of the cells was assessed by flow cytometry (FlowJo software).

Statistical analysis

All experiments were repeated at least three times, and statistical analyses were performed using GraphPad Prism (GraphPad software, San Diego, CA, USA). For experiments with only two groups, an unpaired *t*-test or Mann-Whitney test was performed for comparison of group means. A $P < 0.05$ was considered statistically significant.

Results

Downregulation of matrix metallopeptidase 13 expression decreased dendritic cell apoptosis

To investigate the effect of MMP-13 on murine bone marrow-derived DC apoptosis, we knocked down MMP-13 expression using siRNA. Western blotting and real-time PCR analysis revealed significant decreases at both the protein and mRNA level after transfection as compared with transfection using scrambled siRNA [Figure 1a and 1b]. In subsequent experiments, DCs were pretreated with scrambled siRNA or MMP-13 siRNA for 24 h, total RNA and protein were extracted, and then real-time PCR and Western blotting were performed. Compared with scrambled siRNA, the expression of anti-apoptosis-related genes/proteins (BAG1, BCL-2, and TP73) increased while apoptosis-related genes/proteins (CASP1, LTBR, and CASP4) decreased in MMP-13-silenced cells [Figure 1c and 1d].

Downregulation of matrix metallopeptidase 13 expression inhibited dendritic cell maturation

Given the specific function of MMP-13 in immunological processes, we analyzed the levels of CD80 and CD86 expression in both groups of DCs by flow cytometry. We found that transfection with MMP-13 siRNA did not alter the level of CD80 ($P = 0.8053$) while it significantly decreased the level of CD86 ($P = 0.006$) in DCs when compared to scrambled siRNA [Figure 2].

Downregulation of matrix metallopeptidase 13 expression inhibited phagocytic capacity in dendritic cell

The ability of cells to uptake of soluble dextran-FITC as a measure of phagocytosis was assessed *in vitro* after transfection with either MMP-13 siRNA or scrambled siRNA. Analysis was performed by flow cytometry; results showed that the phagocytic capacity of MMP-13-silenced cells was substantially decreased by nearly 10% as compared to scrambled siRNA group ($P = 0.016$) [Figure 3].

Discussion

MMP proteolytic activity is critical for various physiologic and pathologic inflammatory processes; it functions by regulating physical barriers and inflammatory mediators.[13] MMP-13 is expressed by several cell types, including DCs. DCs are professional antigen-presenting cells, building a bridge between innate and adaptive immunity. DC dysfunction has been reported in a wide range of diseases.[12,14-16] A recent study revealed a novel role for MMP-13 in endocytosis, MHC-I presentation, and T-cell induction of cytokine release by DCs; inhibition of MMP-13 was able to diminish the DCs' capacity to activate T-cell through the MHC-I pathway.[10] In this study, we further investigated the effect of MMP-13 on DCs' function.

For the functional assays, we used siRNA to inhibit MMP-13 expression. Then, we tested the apoptotic, maturation,

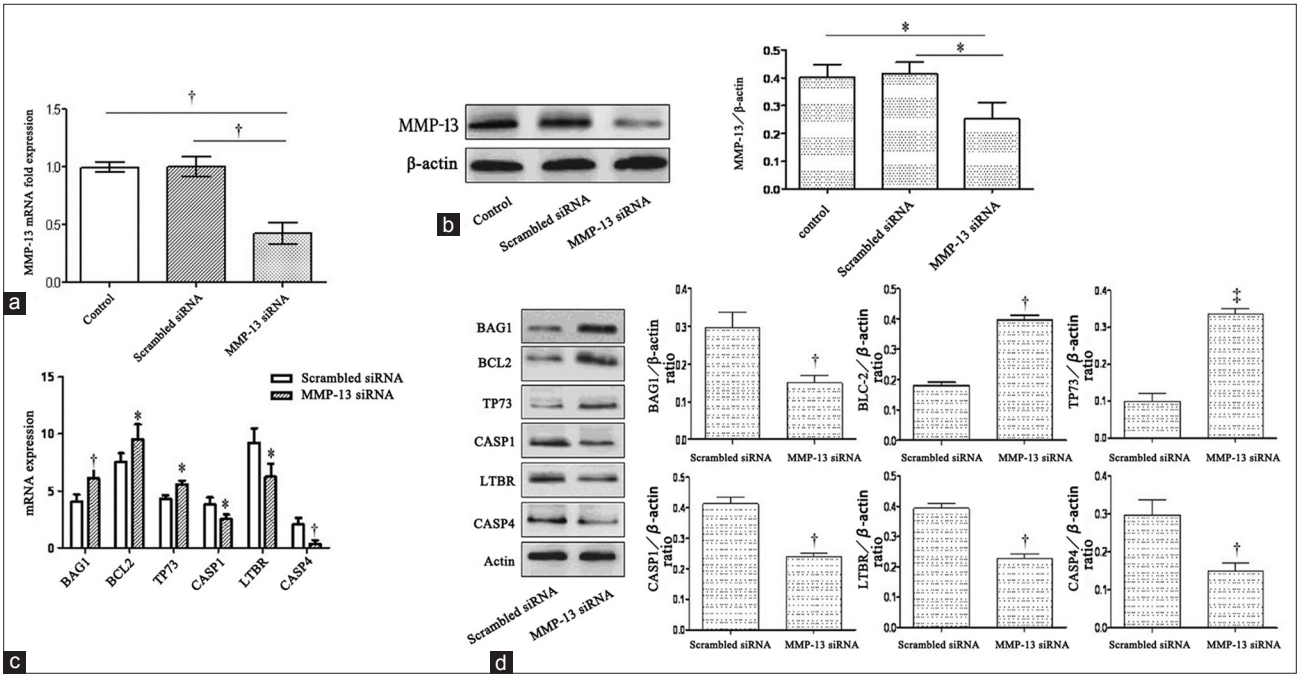

Figure 1: Downregulation of MMP-13 decreased DCs apoptosis. (a) Establishment of DCs transiently expressing MMP-13 short-interfering RNA and scrambled short-interfering RNA was carried out as described in Materials and Methods. The mRNA expression of MMP-13 in DCs was examined with quantitative real-time PCR. (b) Western blotting confirmed MMP-13 protein expression. (c and d) The expression of *BAG1*, *BCL-2*, *TP73*, *CASP1*, *LTBR*, and *CASP4* was examined by real-time PCR and Western blotting. The expression of actin served as a loading control. The experiment was replicated three times. * † ‡ Statistically significant between two groups (*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$). MMP-13: Matrix metallopeptidase 13; DC: Dendritic cell; PCR: Polymerase chain reaction; mRNA: Messenger RNA.

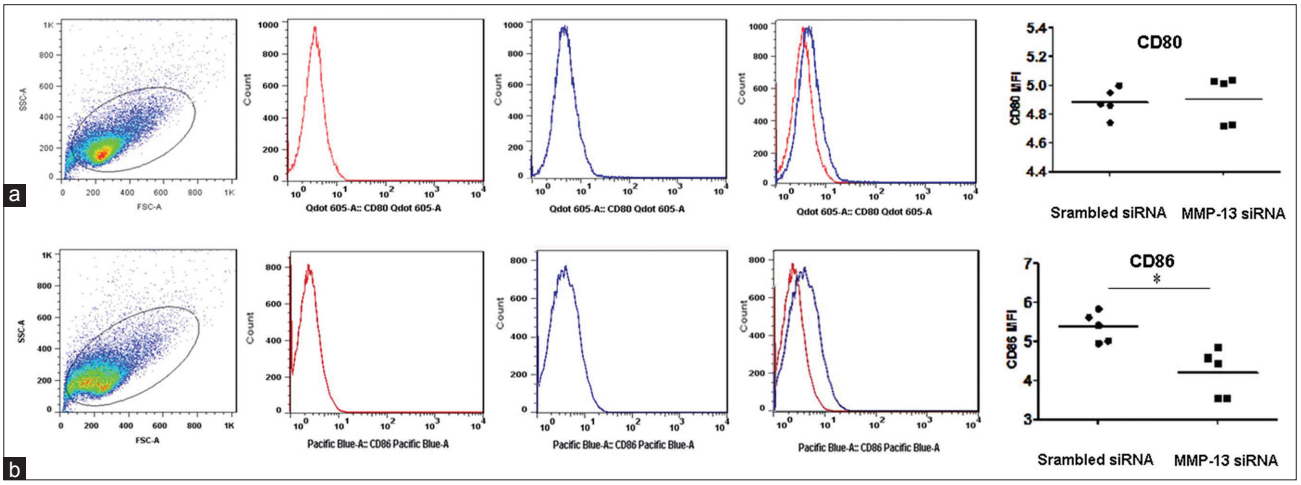

Figure 2: Downregulation of MMP-13 regulated DCs' maturation. (a and b) for detection of CD80 and CD86, the results are expressed as the difference between scrambled short-interfering RNA group and MMP-13 short-interfering RNA group. Red represented scrambled short-interfering RNA group, and blue represented MMP-13 short-interfering RNA group. *t*-test was performed. *$P < 0.05$. MMP-13: Matrix metallopeptidase 13; DC: Dendritic cell.

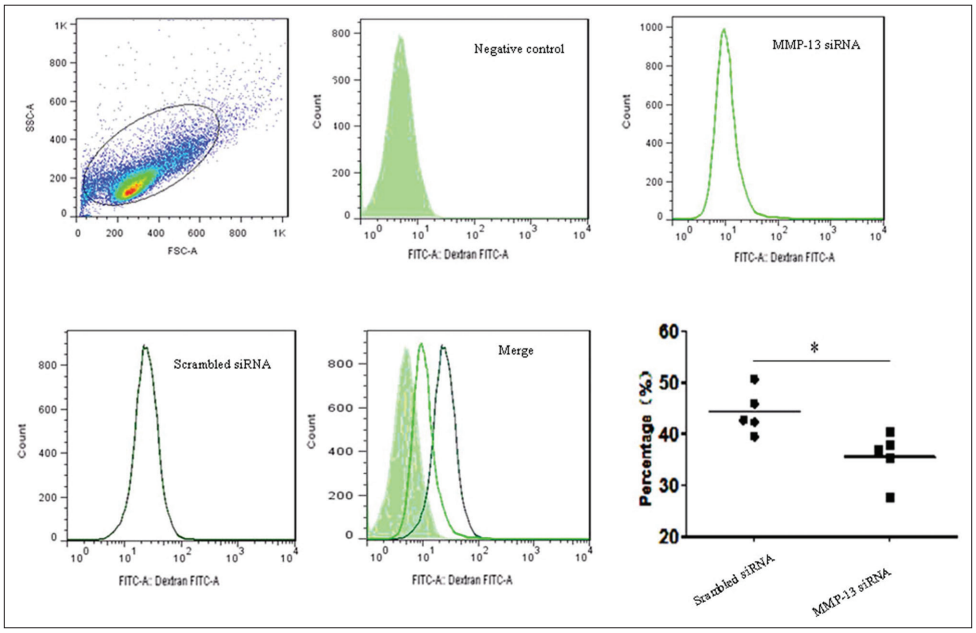

Figure 3: Inhibition of matrix metallopeptidase 13 decreased the capacity of phagocytosis in dendritic cells; the experiment was replicated three times. *Statistically significant between two groups (*$P < 0.05$).

and phagocytic capacity of DCs. The data indicate that knockdown of MMP-13 blocks the expression of apoptosis-related proteins, indicating that MMP-13 is likely a protective factor for DC survival [Figure 1].

Several previous studies reported an involvement of MMP-13 in the maturation of various cells or tissues although studies reported inconsistent results. Study regarding skeletal muscle regeneration found that MMP-13 could not affect myoblast maturation, while MMP-13 was reported to regulate postnatal testis maturation as wells as maturation of myofibroblasts after wound healing.[17-19] In the present study, we observed that MMP-13 inhibition could impair the maturation of DCs [Figure 2].

To date, there is only a single report regarding the involvement of MMP-13 in phagocytosis, in which MMP-13 inhibition decreased the capability of DCs.[10] In this experiments, we also found that downregulation of MMP-13 could diminish phagocytic capacity in DCs by nearly 10% [Figure 3].

The fascinating conclusion from this study is that MMP-13 is involved in regulating apoptosis, maturation, and phagocytic capacity in DCs. The ability to influence the function of DCs is critical for the delivery of effective oncotherapy. Thus, further exploration and understanding of MMP-13 function in DCs, with a particular focus on defining its substrates, signaling pathway, and associated cytokines, will have important implications for health and disease.

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Conflicts of interest

There are no conflicts of interest.

References

1. Fieber C, Baumann P, Vallon R, Termeer C, Simon JC, Hofmann M, *et al.* Hyaluronan-oligosaccharide-induced transcription of metalloproteases. J Cell Sci 2004;117:359-67. doi: 10.1242/jcs.00831.
2. Weiss JM, Renkl AC, Sleeman J, Dittmar H, Termeer CC, Taxis S, *et al*. CD44 variant isoforms are essential for the function of epidermal Langerhans cells and dendritic cells. Cell Adhes Commun 1998;6:157-60. doi: 10.3109/15419069809004472.
3. Saalbach A, Janik T, Busch M, Herbert D, Anderegg U, Simon JC. Fibroblasts support migration of monocyte-derived dendritic cells by secretion of PGE2 and MMP-1. Exp Dermatol 2015;24:598-604. doi: 10.1111/exd.12722.
4. Vincenti MP. The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. Transcriptional and posttranscriptional regulation, signal transduction and cell-type-specific expression. Methods Mol Biol 2001;151:121-48. doi: 10.1385/1-599259-046-2:121.
5. Brinckerhoff CE, Rutter JL, Benbow U. Interstitial collagenases as markers of tumor progression. Clin Cancer Res 2000;6:4823-30.
6. Elkington PT, O'Kane CM, Friedland JS. The paradox of matrix metalloproteinases in infectious disease. Clin Exp Immunol 2005;142:12-20. doi: 10.1111/j.1365-2249.2005.02840.x.
7. Parks WC, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 2004;4:617-29. doi: 10.1038/nri1418.
8. Nissinen L, Kähäri VM. Matrix metalloproteinases in inflammation. Biochim Biophys Acta 2014;1840:2571-80. doi: 10.1016/j.bbagen.2014.03.007.
9. Leeman MF, Curran S, Murray GI. The structure, regulation, and function of human matrix metalloproteinase-13. Crit Rev Biochem Mol Biol 2002;37:149-66. doi: 10.1080/10409230290771483.
10. Bartmann J, Frankenberger M, Neurohr C, Eickelberg O, Noessner E, von Wulffen W. A novel role of MMP-13 for murine DC function: Its inhibition dampens T-cell activation. Int Immunol 2016;28:473-87. doi: 10.1093/intimm/dxw008.
11. Chen Z, Gordon JR, Zhang X, Xiang J. Analysis of the gene expression profiles of immature versus mature bone marrow-derived dendritic cells using DNA arrays. Biochem Biophys Res Commun 2002;290:66-72. doi: 10.1006/bbrc.2001.6147.
12. Liu X, Quan N. Immune cell isolation from mouse femur bone marrow. Bio Protoc 2015;5. pii: E1631. doi: http://doi.org/10.21769/BioProtoc.1631.
13. Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol 2008;19:34-41. doi: 10.1016/j.semcdb.2007.07.003.
14. Collins LE, DeCourcey J, Soledad di Luca M, Rochfort KD, Loscher CE. An emerging role for SNARE proteins in dendritic cell function. Front Immunol 2015;6:133. doi: 10.3389/fimmu.2015.00133.
15. Ward C, Eger K, Diboll J, Jones D, Haniffa MA, Brodlie M, *et al.* Bronchial epithelial cells cultured from clinically stable lung allograft patients promote the development of macrophages from monocytes rather than dendritic cells. Thorax 2009;64:430-5. doi: 10.1136/thx.2008.104067.
16. Yousem SA, Ray L, Paradis IL, Dauber JA, Griffith BP. Potential role of dendritic cells in bronchiolitis obliterans in heart-lung transplantation. Ann Thorac Surg 1990;49:424-8. doi: http://dx.doi.org/10.1016/0003-4975(90)90248-5.
17. Toriseva M, Laato M, Carpén O, Ruohonen ST, Savontaus E, Inada M, *et al.* MMP-13 regulates growth of wound granulation tissue and modulates gene expression signatures involved in inflammation, proteolysis, and cell viability. PLoS One 2012;7:e42596. doi: 10.1371/journal.pone.0042596.
18. Huh MI, Jung JC. Expression of matrix metalloproteinase-13 (MMP-13) in the testes of growing and adult chicken. Acta Histochem 2013;115:475-80. doi: 10.1016/j.acthis.2012.11.003.
19. Lei H, Leong D, Smith LR, Barton ER. Matrix metalloproteinase 13 is a new contributor to skeletal muscle regeneration and critical for myoblast migration. Am J Physiol Cell Physiol 2013;305:C529-38. doi: 10.1152/ajpcell.00051.2013.