Bone marrow mesenchymal stem cells differentiate into intestinal epithelioid cells through the ERK1/2 pathway

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Cite this article as: *Jiang T, Shi ML, Xia G, et al. Bone marrow mesenchymal stem cells differentiate into intestinal epithelioid cells through the ERK1/2 pathway. Turk J Gastroenterol 2020; 31(6): 459-65.*

ABSTRACT

Background/Aims: *Previous studies have found that the injection of rat bone marrow mesenchymal stem cells (rBMSCs) in a mouse model of acute hepatic failure significantly relieves intestinal damage and endotoxemia. However, the mechanism of this process remains unknown. This study demonstrated the differentiation of rBMSCs into enterocyte-like cells and possible molecular mechanisms for this with the aim of finding a new treatment for intestinal epithelial injury and endotoxemia during liver failure.*

Materials and Methods: *rBMSCs were isolated from rat femurs and tibias. Differentiation was induced by co-culturing rBMSCs with rat intestinal epithelial cells (mIEC-6) using Transwell plates; after three, seven, and ten days of induction, expression of specific differentiation molecules were quantified. To inhibit the activity of the Mitogen-activated protein kinase 1/2 (ERK1/2) signaling pathway, an inhibitor of Mitogen-activated protein kinase kinase 1/2 (MEK1/2) was added to the co-culture medium, and western blot analysis was performed after 36 or 72 h to evaluate the expression of ERK1/2 signaling pathway markers (p-MEK1/2 and p-ERK1/2).*

Results: *The rBMSCs differentiated into enterocyte-like cells when co-cultured with mIEC-6 cells. Inhibition of ERK1/2 signaling abrogated the activity of MEK1/2, but MEK increased after 72 h, and the epithelioid differentiation of rBMSCs was consistent with the change in MEK expression.*

Conclusion: *rBMSCs differentiate into intestinal epithelium after co-culture with mIEC-6 by regulation of the ERK1/2 signaling pathway. Further research is needed to elucidate the network of mechanisms.*

Keywords: *Mesenchymal stem cells, intestinal epithelial cells-6, MAP Kinase, ERK*

INTRODUCTION

Patients with terminal stage liver disease often suffer from intestinal barrier dysfunction. This leads to intestinal bacterial translocation and endotoxemia, forming a main cause of progression and poor survival (1, 2). Effective treatment of intestinal epithelial damage in liver failure is therefore crucial. A previous study demonstrated that after the injection of rat bone marrow mesenchymal stem cells (rBMSCs) into a mouse model of acute hepatic failure, the level of inflammatory factors and endotoxins significantly reduced, the intestinal epithelial barrier was restored, and the mortality rate in rats declined (3, 4). Thus, transplantation of bone marrow mesenchymal stem cells (BMSCs) may be effective in patients undergoing hepatic failure with intestinal epithelial injury. However, the mechanism by which this occurs is unclear.

BMSCs were first found in the bone marrow in the 1960s. Subsequent studies have shown that these cells can differentiate into many different cell types such as bone cells, chondrocytes, and adipocytes. BMSCs are widely used in tissue transplantation, gene therapy, and other fields because of the following advantages: easy extraction, self-renewal ability, and multi-differentiation potential (5-7). Thus, the possibility that BMSCs can differentiate into intestinal epithelial cells to repair intestinal mucosal damage during liver failure merits further investigation.

The signaling pathways involved in regulating the resting, self-renewal, proliferation, and differentiation of BMSCs include Notch (8), Wnt (9), phosphatidylinositol-4-phosphate 3-kinase/AKT serine-threonine kinase (PI3K/AKT) (10), *mitogen-activated protein kinase* (ERK) (11), and bone morphogenetic protein (BMP)(12) signaling pathways. ERK1/2 is a classical subpathway of mitogen-activated protein kinases in mammals, which was first reported by Sturgill in 1986 (13). The ERK1/2 signaling pathway is the key pathway to determine cell fate by promoting the proliferation and regulating the final differentiation

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Received: ***August 14, 2018*** *Accepted:* ***April 19, 2019***
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DOI: ***10.5152/tjg.2020.18644***

of cells when the cell is stimulated by external factors. Many reports suggest that BMSCs rely on this pathway for differentiation and proliferation (6). We therefore investigated whether BMSCs differentiate into intestinal epitheliod cells by the ERK1/2 signaling pathway.

To test our inferences, we examined the differentiation potential of BMSCs into intestinal epitheliod cells by co-culturing them with intestinal crypt epithelial cells (mIEC-6) via Transwell plates. Moreover, we investigated the role of the ERK1/2 signaling pathway in the differentiation in an effort to find a new way to treat hepatic failure patients with intestinal epithelial injury.

MATERIALS AND METHODS

Animals and cells

Three-to-five-week-old male SD rats obtained from the Laboratory Animal Unit Of Kunming Medical University were used in the experiments. mIEC-6 were from Kunming Cell Bank of Chinese Academy of Sciences (No: KCB200720YJ). All experimental procedures were conducted under the supervision of Animal Experimental Ethical Committee of Kunming Medical University (Approval number KMMU2018019) and complied with institutional guidelines. This experiment strictly adhered to the regulations of feeding and euthanasia of experimental animals.

Isolation and expansion of rBMSCs

The rBMSCs were isolated and harvested in this experiment. Briefly, male SD rats (weight, 80-100 g; age, 3-5 weeks) were euthanized, and cells were obtained from the marrow of femurs and tibias and cultured in Dulbecco's modified Eagle's medium 12 (DMEM/12; HyClone Corp, Logan, Utah, USA) supplemented with 10% fetal bovine serum (FBS; HyClone Corp, Logan, Utah, USA) at 37°C and 5% CO_2. Medium was replaced every 24 h, and the cells were observed by inverted phase-contrast microscopy every day. Then, adherent rBMSCs were harvested using 0.25% trypsin and sub-cultured when they reached 80%-90% confluence. The third generation of rBMSCs was digested by 0.25% EDTA–trypsin and transferred to tubes supplemented with configured cell cryopreservation solution (FBS: DMSO, 9:1). Then, they were stored in liquid nitrogen for the following experiments. Rewarming, centrifuging, and adjusting cell density to 2×10^6 cells/mL were performed before the next experiment.

Expansion of mIEC-6

The culture bottle was placed in a room at 37°C for 2-3 hours. mIEC-6s were harvested using 0.25% trypsin and sub-cultured when they reached 80%-90% confluence. The third generation of mIEC-6 was digested by 0.25% EDTA–trypsin and co-cultured with rBMSCs.

In vitro enterocyte-like differentiation and co-culture

Cells were divided into two groups. Briefly, in group A, rBMSCs of passage 3 were inoculated at 1×10^6 cells/cm^2 in six-well culture plates in the lower chamber of the Transwell (Costar, Corning, NY, USA). Then, the Transwell insert was placed in the hole, and mIEC-6s of passage 3 were inoculated in the upper chamber. The co-culture system of rBMSCs and mIEC-6s was established by mixing together the upper and lower chambers containing DMEM (HyClone Corp, Logan, Utah, USA). In group B, the control group, rBMSCs of passage 3 were inoculated both in upper and lower chambers in the same way. All groups were cultured at 37°C and 5% CO_2 for 3, 7, or 10 days. They were placed under a fluorescence microscope camera for immunofluorescence detection.

ERK1/2 inhibition

The experiment was divided into three groups: group I (rBMSCs and rBMSCs), group II (rBMSCs and mIEC-6s), and group III (rBMSCs, mIEC-6s, and inhibitor U0126). All groups were co-cultured using a Transwell assay. After 36 and 72 h of co-culture, western blot analysis was used to evaluate the protein expression of markers, including specific markers for the ERK1/2 signaling pathway (p-MEK1/2 and p-ERK1/2). Morphological changes of rBMSCs also were recorded using the inverted phase-contrast microscopy.

Protein isolation and western blot

After three washes with PBS (HyClone Corp, Logan, Utah, USA), considerable lysis solution (150–250 µL/well) was added for complete lysis of proteins, and then the superior solution was obtained after centrifugation. Protein concentrations were measured using a Bicinchoninic Acid (BCA) protein assay kit (Vazyme corp. Shanghai, China). The absorbance was measured by A562 nm at the wavelength of the spectrophotometer (Bio-tek, Montpellier,

MAIN POINTS

- *There is the potential for BMSCs to differentiate into enterocyte-like cells by transwell in vitro.*
- *ERK1/2 cell- signalling pathway is involved in the differentiation of BMSCs into enterocytes.*
- *Stem cell and tissue engineering show great hope for gut disease based on liver failure, further researches are needed.*

Vermont, USA), and the protein concentration of the sample was calculated according to the standard curve.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 4% concentration gels (0.5 M pH 6.8) loading equal amounts of protein per lane. After electrophoresis, separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes (GE, Fairfield, Conn, USA) and blocked with 5% non-fat milk in Tris- buffered saline tween20 (TBST) buffer for 2 h. Antibodies at 1:1,000 dilutions were then added, and the membranes were incubated overnight at 4°C. Bands were visualized by ECL. Image J, a chemiluminescent imaging system (Bio-Rad, Sacramento, CA, USA), was used to quantify protein levels in each lane.

Flow cytometry analysis

Cell surface antigens were analyzed by flow cytometry. Briefly, cells treated with trypsin were harvested by centrifugation, washed with phosphate buffer saline (PBS; HyClone Corp, Logan, Utah, USA) and divided into three tubes. Adding different antibodies to each tube, labeled tubes were washed with PBS and then analyzed by a flow cytometer (Partec, Hamburg, Germany).

Adipogenically induced differentiation

The third generation of rBMSCs was cultured at 37°C and 5% CO_2. Cells were harvested using 0.25% trypsin and inoculated at 2×10^6 cells/cm^2 in six-well culture plates containing 2 mL culture fluid per well when they reached 90% confluence. The liquid was changed every three days until the fusion level reached 100%. Then, the liquid was sucked out, the rBMSCs were washed thrice with PBS (HyClone Corp, Logan, Utah, USA), and 2 mL adipogenically induced differentiation liquid A (SAIYE, Guangzhou, China) was added into every well. After a three-day induction, liquid A was replaced with liquid B (SAIYE, Guangzhou, China) and then liquid B was replaced with liquid A after 24 h. After 3-5 cycles between liquids A and B (approximately 12-20 days), the lipid droplets in the cells became large and round enough. They were placed under a microscope camera for detection by oil red O staining.

Statistical Analysis

Experiments were performed at least in triplicate, and results were expressed as the mean±SD. Statistical analysis was performed using the Statistical Packages for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). Differences of $p<0.05$ were considered statistically significant.

RESULTS

Morphological characterization of rBMSCs

Morphological changes of rBMSCs were observed by electron microscopy. As shown in Figure 1, one day after the primary culture, spindle-shaped and polygonal cells adhered and formed colonies in the culture flask. With the increase in culture time, adherent cells became larger and began to proliferate. The proliferation rate was significantly faster than that of the original cells. These cells could be sub-cultured within 3-5 days.

Identification of rBMSCs

To date, no specific antigenic markers for identifying BMSCs have been identified. The commonly accepted methods of identification are: (a) typical morphology; (b) the expression of surface antigens CD29, CD90, or CD105 but without expression of surface antigens CD34, CD45, or CD106; and (c) potential for multidirectional differentiation and self-renewal. Only when cells satisfy the above three conditions are they considered BMSCs (17).

Studies show that the surface marker CD29 is an important antigenic marker for BMSCs. Therefore, in this study, to identify rBMSCs, CD29 (positive) and CD34 (nega-

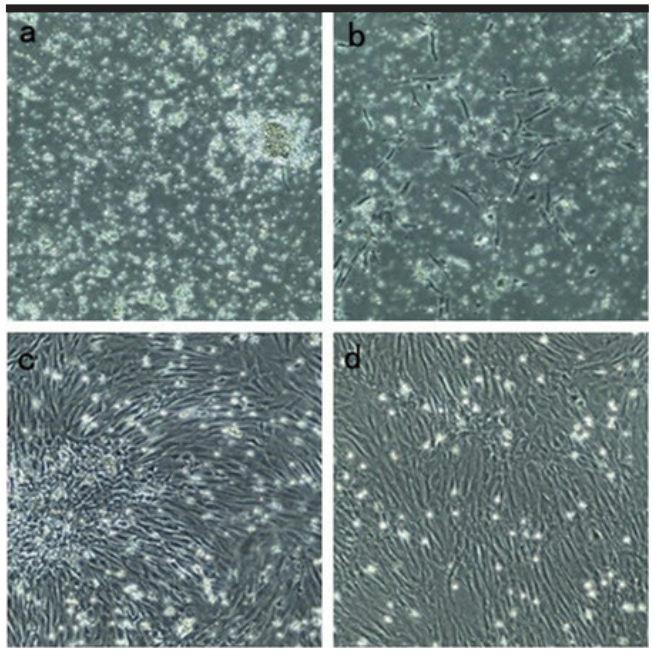

Figure 1. a-d. Morphology of rBMSCs. a) 1 day; b) 4day; c) 6day; d) 9day. 40×)

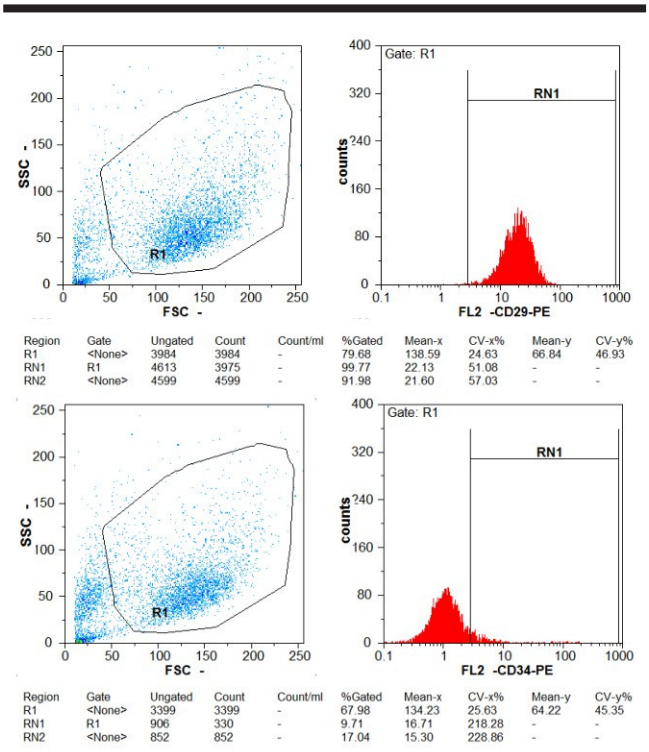

Figure 2. Flow cytometry of rBMSCs.

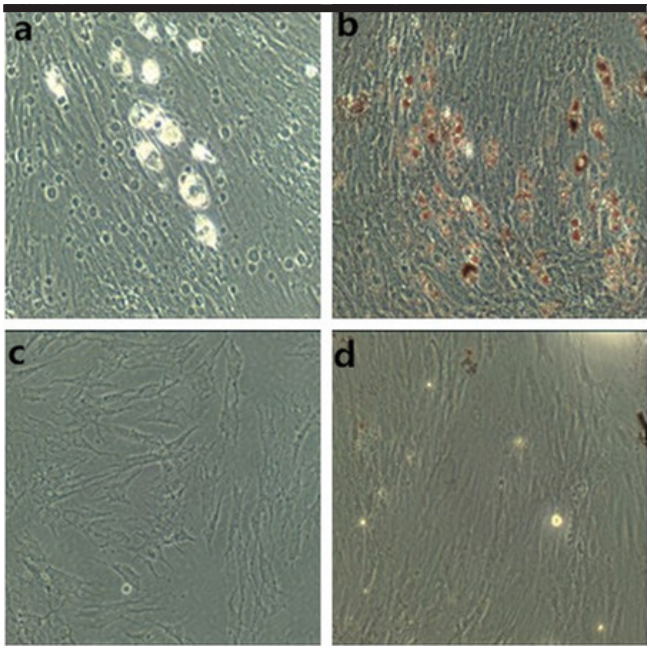

Figure 3. a-d. Adipogenic differentiation of rBMSCs. a) Pre-staining in induction group; b) After staining in induction group; c) Pre-staining in control group; d) After staining in control group. 100×.

tive) were used as BMSC surface markers to recognize third-generation BMSCs. As shown in Figure 2, the cells were positive for CD29 and negative for CD34.

To confirm the multiple differentiation potential of the extracted cells, we carried out adipogenically induced differentiation experiments.

Figure 3 demonstrates that through adipogenically induced differentiation, the third generation of rBMSCs had the potential for multidirectional differentiation and self-renewal. Microscopically, a number of red fat droplets in the induced group were observed, and oil red O staining was positive, whereas oil red O staining was negative in the control group.

In summary, these results demonstrated that the high-purity cells harvested herein are identified as rBMSCs.

Co-culture using Transwell

As shown in Figure 4, in this part, the differentiation was induced by co-culture (rBMSCs+mIEC-6) using Transwell plates. Cells were divided into two groups: group A (rBMSCs and mIEC-6) and control group B (rBMSCs and rBMSCs).

After three, seven, or ten days of induction, morphology and specific expression molecules were examined under a fluorescence microscope by immunofluorescence detection. CK and Villin are specific expression molecules of epithelioid cells and were used for the identification of epithelioid cells in this experiment.

Figures 5 and 6 show that CK and Villin were recognized three days after co-culture of the P3 generation (5a and 6a). After seven days of co-culture (5c and 6c), some rBMSCs cells had epithelioid changes. After 10 days of co-culture (5e and 6e), two kinds of proteins were expressed continuously, and most of the cells became

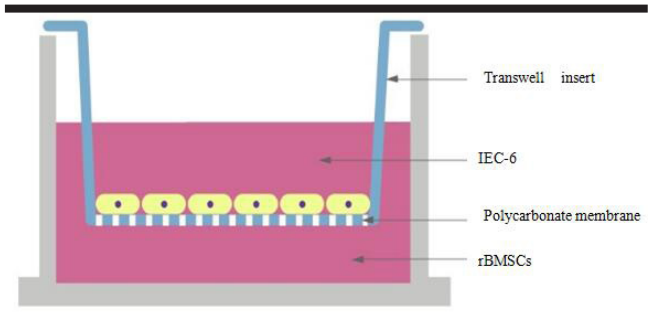

Figure 4. Co-culture sketch map using Transwell.

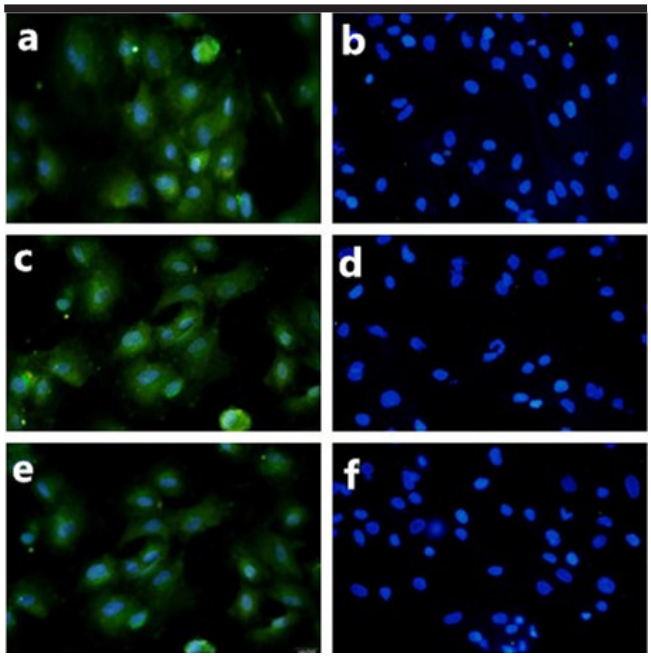

Figure 5. a-f. Expression of CK. a) 3 days in rBMSCs+mIEC6; c) 7days in rBMSCs+mIEC6; e) 10days in rBMSCs+mIEC6; b) 3 days in rBMSCs+rBMSCs; d) 7days in rBMSCs+rBMSCs; f) 10days in rBMSCs+rBMSCs. 40×.

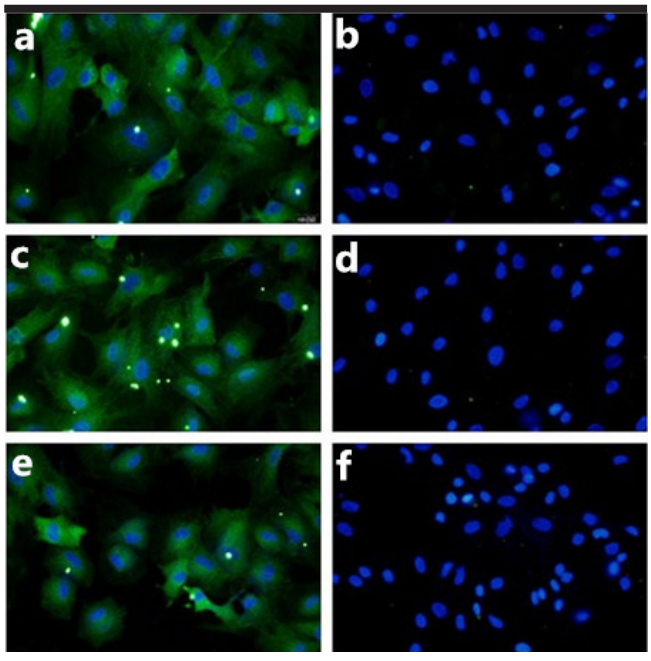

Figure 6. a-f. Expression of villin. a) 3 days in rBMSCs+mIEC6; c) 7days in rBMSCs+mIEC6; e) 10days in rBMSCs+mIEC6; b) 3 days in rBMSCs+rBMSCs; d) 7days in rBMSCs+rBMSCs; f) 10days in rBMSCs+rBMSCs. 40×.

round and oval. The isolated cells displayed typical epithelial morphology. Control group B was always negative (5b, 5d, 5f and 6b, 6d, 6f).

Effects of ERK1/2 inhibition

The experiment was divided into three groups: group I (rBMSCs and rBMSCs), group II (rBMSCs and mIEC-6) and group III (rBMSCs, mIEC-6, and U0126). The cells were co-cultured for 36 or 72 h using Transwell plates. Then, the ERK1/2 signaling pathway molecules were measured by western blotting.

As seen in Figure 7, p-MEK1/2 and p-ERK1/2 were not expressed in group I. After 36 h, compared with the group II, p-MEK1/2 and p-ERK1/2 bands became weaker, and the expression of p-MEK1/2 and p-ERK1/2 proteins decreased in group III. However, after 72 h, the expression of p-MEK1/2 and p-ERK1/2 proteins of group III somewhat recovered.

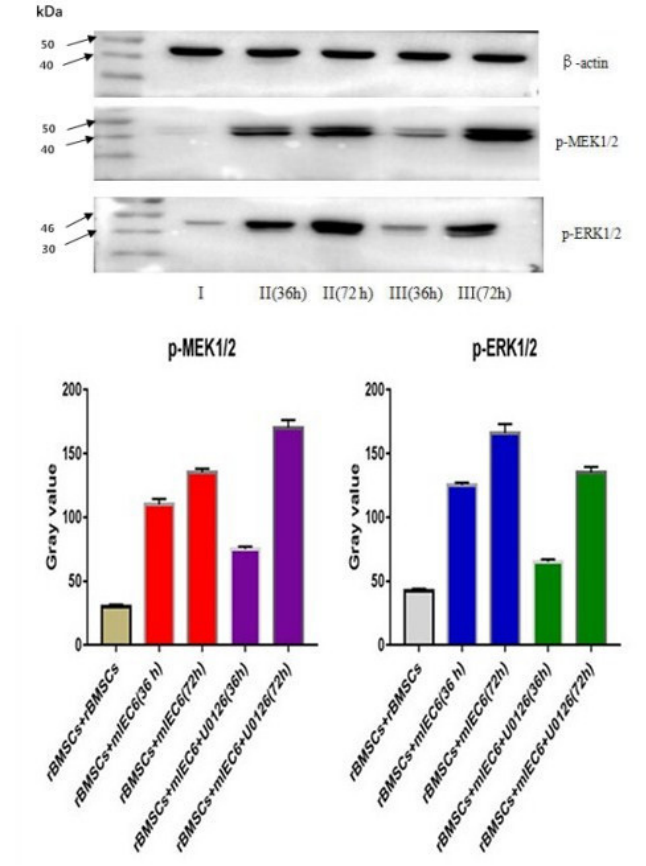

Figure 7. Expression of p-MEK1/2 and p-ERK1/2 by western blotting.

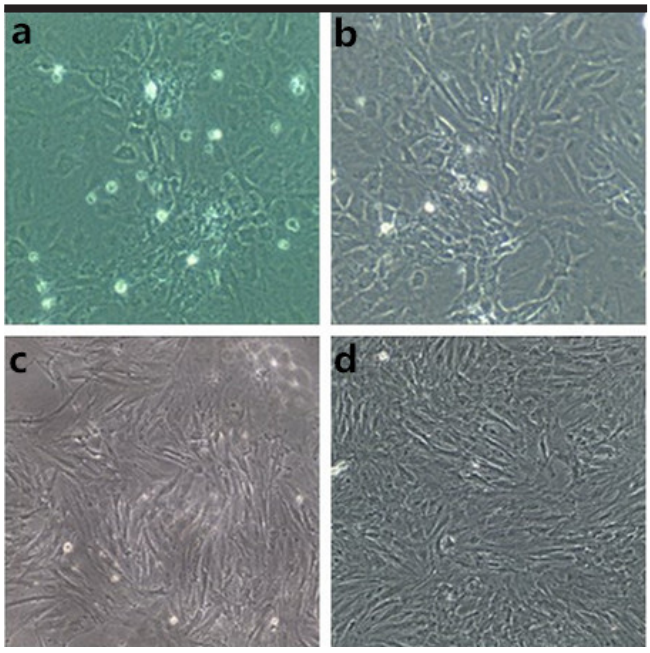

Figure 8. a-d. Morphology of rBMSCs after U0126. a) group II 36h; b) group II 72h; c) group III 36h; d) group III 72h. 40×.

Moreover, fewer epithelioid morphological changes occurred in group III after 36 h compared with group II (Figure 8). Interestingly, after another 36 h, cells showed epithelioid morphology, and some of the cells displayed an ovoid shape in group III as compared with the previous morphology.

DISCUSSION

BMSCs have attracted much attention in tissue repair, organ transplantation, and immunotherapy due to their multidirectional differentiation potential. Previous studies have indicated that BMSCs take advantage of this to repair degenerated or damaged tissues. For example, the differentiation of BMSCs into myofibroblast, cardioimyocyte-like cells, and motor neurons has been confirmed (18-20). An intestinal mucosal lesion with loss of intestinal epithelial cells is progressively invaded by pathogens and harmful factors to form endotoxemia; this results in liver dysfunction and eventual liver failure or even death. Stem cell transplantation was used as a useful treatment with endotoxemia in liver failure (1).

In this study, we have shown the potential and molecular modulation of BMSCs to differentiate into epithelioid cells. Specifically, we found rBMSC differentiation into epithelioid cells via co-culturing with IEC-6 cells, and that transiently inhibiting ERK1/2 in the co-cultured system induces a decrease in differentiation into epithelioid cells. This strongly indicates that the ERK1/2 pathway is involved in this differentiation process.

rBMSCs and IEC-6 were co-cultured under the same external environment though a Transwell chamber. Western blot detection showed the expression of p-ERK1/2 and p-MEK1/2 were significantly reduced in the ERK1/2-inhibition group treated with U0126 (36 h). Also, with decreased amounts of p-ERK1/2 and p-MEK1/2, the epithelioid cells also decreased lower than that of the normal co-cultured group, indicating that the differentiation of rBMSCs into epithelioid cells was impeded and closely related to the ERK1/2 pathway. Interestingly, the expression of p-ERK1/2 and p-MEK1/2 in the inhibitor group (72 h) were not suppressed and even showed high expression. Meanwhile, the rBMSCs gradually differentiated into epithelioid cells. We next investigated whether this phenomenon is caused by the lack of U0126 inhibition or the involvement of other unknown differentiation mechanisms.

U0126 has traditionally been regarded as an inhibitor of the ERK1/2 pathway. However, a recent study demonstrated a novel function for U0126 in promoting osteogenic differentiation of rBMSCs (2). Whether inhibiting or promoting differentiation shows completely distinct groups or two ends of a line remains to be further studied, but this indicates that the ERK1/2 pathway directly or indirectly plays a key role in orchestrating the differentiation of rBMSCs.

The picture of molecular modulation of BMSC differentiation may be more complex, and further studies are needed to reveal the underlying mechanism of this phenomenon. Also, as a common factor, MEK1/2 could be regulated to participate in many signaling pathways to influence the differentiation and proliferation of stem cells. Moreover, in addition to active proteins, non-coding RNA can also regulate the differentiation, proliferation, and apoptosis of stem cells (3, 4). Together these data indicate that complex modulation of BMSC differentiation probably interweaves into a working network; specifically, the ERK1/2 pathway is one of the most important components. Notably in this study, the ERK1/2 pathway is an important factor for the differentiation of rBMSCs into epithelioid cells.

In conclusion, herein we co-cultured rBMSCs and IEC-6 through Transwell chambers and successfully induced the differentiation of BMSCs into intestinal epithelial

cells, providing a reference for future studies of BMSCs and ERK signaling pathway. However, further investigation is still needed to uncover the complex network of differentiation regulation mechanisms of BMSCs.

Ethics Committee Approval: Ethics committee approval was received for this study from the Animal Experimental Ethical Committee of Kunming Medical University (Date: January 9, 2018; Approval number: KMMU2018019).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – T.J., M.S.; Design - T.J., M.S..; Supervision - Y.T., J.Y.; Resource - Y.T., J.Y.; Materials - Y.T., J.Y.; Data Collection and/or Processing - T.J., M.S., G.X.; Analysis and/or Interpretation -Y.Y., J.X., Y.L.; Literature Search - T.J., M.S., G.X.; Writing - T.J.; Critical Reviews - Y.T., J.Y.

Conflict of Interest: The authors have conflict of interest to declare.

Financial Disclosure: The work was financially supported by the National Natural Science Foundation of China (grant no. 81760107) and Scientific research fund project of Yunnan Provincial Department of Education (grant no. 2020Y0127).

REFERENCES

1. Zhang T, Sun K, Wang Y, Huang L, Lang R, Jiang W. Disruption of the gut-liver axis in the pathogenesis of acute-on-chronic liver failure. Eur J Gastroenterol Hepatol 2018; 30: 130-5. [CrossRef]

2. Harputluoglu MMM, Temel I, Demirel U, et al. Methylprednisolone prevents bacterial translocation in thioacetamide-induced liver failure in rats. Turk J Gastroenterol 2017; 28: 394-400. [CrossRef]

3. Zheng S, Yang J, Tang Y, et al. Effect of bone marrow mesenchymal stem cells transplantation on the serum and liver HMGB1 expression in rats with acute liver failure. Int J Clin Exp Pathol 2015; 8: 15985-92.

4. Zheng S, Yang J, Yang J, et al. Transplantation of umbilical cord mesenchymal stem cells via different routes in rats with acute liver failure. Int J Clin Exp Pathol 2015; 8: 15854-62.

5. Alfaro MP, Vincent A, Saraswati S, et al. sFRP2 Suppression of Bone Morphogenic Protein (BMP) and Wnt Signaling Mediates Mesenchymal Stem Cell (MSC) Self-renewal Promoting Engraftment and Myocardial Repair. J Biol Chem 2010; 285: 35645-53. [CrossRef]

6. Boland GM, Perkins G, Hall DJ, Tuan RS. Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. J Cell Biochem 2004; 93: 1210-30. [CrossRef]

7. Javazon EH, Beggs KJ, Flake AW. Mesenchymal stem cells: paradoxes of passaging. Exp Hematol 2004; 32: 414-25. [CrossRef]

8. Li HM, Tong Y, Xia X, et al. Retracted: Bone Mesenchymal Stem Cell-Conditioned Medium Regulates the Differentiation of Neural Stem Cells Via Notch Pathway Activation. Cellular Reprogramming 2018. [CrossRef]

9. Mei Y, Liu YB, Cao S, Tian ZW, Zhou HH. RIF1 promotes tumor growth and cancer stem cell-like traits in NSCLC by protein phosphatase 1-mediated activation of Wnt/β-catenin signaling. Cell Death Dis 2018; 9: 942. [CrossRef]

10. Lin S, Zhu B, Huang G, Zeng Q, Wang C. Microvesicles derived from human bone marrow mesenchymal stem cells promote U2OS cell growth under hypoxia: the role of PI3K/AKT and HIF-1a. Human Cell 2018; 32: 64-74. [CrossRef]

11. Jeong YM, Cheng XW, Lee S, et al. Preconditioning with far-infrared irradiation enhances proliferation, cell survival, and migration of rat bone marrow-derived stem cells via CXCR4-ERK pathways. Sci Rep 2017; 7. [CrossRef]

12. Kan C, Ding N, Yang J, et al. BMP-dependent, injury-induced stem cell niche as a mechanism of heterotopic ossification. Stem Cell Res Ther 2019; 10: 14. [CrossRef]

13. Sturgill T, Ray L. Muscle proteins related to microtubule associated protein-2 are substrates for an insulin-stimulatable kinase. Biochem Biophys Res Commun 1986; 134: 565-71. [CrossRef]

14. Baumgartner C, Toifl S, Farlik M, et al. An ERK-Dependent Feedback Mechanism Prevents Hematopoietic Stem Cell Exhaustion. Cell Stem Cell 2018; 22: 879-92.e6. [CrossRef]

15. Tian R, Yao C, Yang C, et al. Fibroblast growth factor-5 promotes spermatogonial stem cell proliferation via ERK and AKT activation. Stem Cell Res Ther 2019; 10. [CrossRef]

16. Berardi DE, Raffo D, Todaro LB, Simian M. Laminin Modulates the Stem Cell Population in LM05-E Murine Breast Cancer Cells through the Activation of the MAPK/ERK Pathway. Cancer Res Treat 2017; 49: 869-79. [CrossRef]

17. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315-7. [CrossRef]

18. Cao C, Li L, Li H, He X, Wu G, Yu X. Cyclic biaxial tensile strain promotes bone marrow-derived mesenchymal stem cells to differentiate into cardiomyocyte-like cells by miRNA-27a. Int J Biochem Cell Biol 2018; 99: 125-32. [CrossRef]

19. Lecarpentier Y, Schussler O, Sakic A, et al. Human Bone Marrow Contains Mesenchymal Stromal Stem Cells That Differentiate In Vitro into Contractile Myofibroblasts Controlling T Lymphocyte Proliferation. Stem Cells Int 2018; 2018: 1-15. [CrossRef]

20. Shirian S, Ebrahimi-Barough S, Saberi H, et al. Comparison of Capability of Human Bone Marrow Mesenchymal Stem Cells and Endometrial Stem Cells to Differentiate into Motor Neurons on Electrospun Poly(ε-caprolactone) Scaffold. Mol Neurobiol 2015; 53: 5278-87. [CrossRef]

21. Zare H, Jamshidi S, Dehghan MM, Saheli M, Piryaei A. Bone marrow or adipose tissue mesenchymal stem cells: Comparison of the therapeutic potentials in mice model of acute liver failure. Journal of Cellular Biochemistry 2018; 119: 5834-42. [CrossRef]

22. Xu L, Liu Y, Hou Y, et al. U0126 promotes osteogenesis of rat bone-marrow-derived mesenchymal stem cells by activating BMP/Smad signaling pathway. Cell Tissue Res 2014; 359: 537-45. [CrossRef]

23. Li Z, Yan M, Yu Y, et al. LncRNA H19 promotes the committed differentiation of stem cells from apical papilla via miR-141/SPAG9 pathway. Cell Death Dis 2019; 10: 130. [CrossRef]

24. Chen S, Xu Z, Shao J, Fu P, Wu H. MicroRNA-218 promotes early chondrogenesis of mesenchymal stem cells and inhibits later chondrocyte maturation. BMC Biotechnology 2019; 19: 6. [CrossRef]