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Co-culture of Umbilical Cord Mesenchymal Stem Cells and Bovine Mammary Epithelial Cells Restrains Apoptosis

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Abbreviations: UC-MSCs: Umbilical Cord Mesenchymal Stem Cells; IGF-I: Insulin Like Growth Factor-I; DMEM-F12: Dulbecco's Modified Eagle Medium-F12; FBS: Fetal Bovine Serum

ABSTRACT

The goal of the present study is to investigate the capacity of co-cultured Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) and BMECs to restrain BMECs apoptosis. In the current study, the Insulin Like Growth Factor-I (IGF-I) expression, cell proliferation, and apoptosis-related gene expression were evaluated between monoculture and co-culture systems. Meanwhile, the signal pathways related to apoptosis were examined to analyze the potential anti- apoptotic effects that co-culturing with UC-MSCs has on BMECs. Results showed that Co-culture of BMECs with UC-MSCs can significantly promote IGF-I secretion and BMEC proliferation in comparison to that of BMECs in monocultures. UC-MSCs can effectively restrain BMECs apoptosis by reactivating IGF-I through the PI-3K/AKT/mTOR signaling pathway, as well as the JAK/STAT pathway. Herein, it was found that IGF-I can up-regulate the expression of the anti-apoptotic geneBcl-2 and down-regulate the expression of the pro-apoptotic genes Caspase-3 and Bax, effectively restraining BMEC apoptosis. This study demonstrates that a co-culture BMEC/UC-MSC system is a promising strategy for simultaneously restraining BMEC apoptosis while promoting their proliferation in a more effective manner than exogenous supplementation of IGF-I alone. Furthermore, the co-culture system described herein has potential utility for other future animal husbandry applications as well.

Keywords: Umbilical Cord Mesenchymal Stem Cells; Bovine Mammary Epithelial Cells; Insulin Like Growth Factor-I; Lactogenic Capacity; Co-Culture

Introduction

The aim of the present study is to investigate the potential anti-apoptotic effects on BMECs which result from their interactions with UC-MSCs in co-culture. Specifically, the relevant anti-apoptotic mechanism was evaluated by exploring the underlying signaling pathways. For this purpose, IGF-I/ IGF-IR expression, as well as the expression profiles of both proliferation and apoptosis-related genes were examined and compared between mono- and co-culture systems. In particular, the signaling pathways associated with apoptosis were analyzed to clarify the anti- apoptosis effect of co-culture on BMECs.

Materials and Methods

Cell Culture

Holstein-Friesian UC-MSCs were isolated and identified according to our previous work [1]. Briefly, UC-MSCs were subcultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F12 (DMEM-F12) supplemented with FBS (15%, v/v) and penicillin–streptomycin (1%, v/v). Holstein-Friesian BMECs were purchased from Jennio biological technology (Guangzhou, China) and subcultured in RPMI 1640 Medium supplemented with Fetal

Bovine Serum (FBS) (15%, v/v), and penicillin–streptomycin (p/s, 1%, v/v). Cells at passage 5–8 were used in the subsequent experiments in order to ensure the cell bioactivity. The media and supplements used in the experiments were obtained from Gibco (Thermal fisher, USA). All cells were incubated at 37 °C, 5% CO2, and 95% relative humidity.

The Effect of Co-Culture on IGF-I/ IGF-IR Expression

Direct-/ indirect- contact co-culture systems were employed to investigate the effects of UC-MSC/BMEC co-culture on IGF-I/ IGF-IR expression, as well as to confirm the primary source of IGF-I. The direct contact co-cultures used the classical mixed culture method and the indirect method utilized Transwell® solution (0.4μm, Corning, USA). Specifically, the co-culture of UC-MSCs and BMECs adopted an optimal cell ratio (UC-MSCs: BMECs =1:2) in accordance to our previous experience [1]. In order to investigate the primary source of secreted IGF-I in the cultures, the cells in the insert chamber of the Transwell were collected for detecting the IGF-I/ IGF-IR gene expressions by a Real Time-PCR assay. Total RNA was extracted using RNeasy Mini Kit (Qiagen, USA) per the manufacturer's instructions.

The Effect of UC-MSCs on BMEC Proliferation

The effect of UC-MSCs on BMEC proliferation was investigated by direct-/indirect- contact culture methods. Briefly, the culture medium of UC-MSCs at exponential phase was collected as conditioned medium. Subsequently, BMECs or UC-MSCs were dissociated with Trypsin-EDTA (0.25%) followed by suspension with SFM. In the direct culture group, UC-MSCs and BMECs were mixed at the ratio of 1:2 and seeded in the well plates for co-culture. In the indirect culture group, BMECs were cultured with UC-MSCs conditioned medium. The monoculture of UC-MSCs or BMECs was used as control group.

The Effect and Mechanism Of UC-MSCs On BMECs' Apoptosis

In order to further investigate the potential anti-apoptotic effect of UC-MSCs on BMECs in the co-culture system, multiple inhibitors of IGF-IR and signal pathway were used in the series of experiments.

Experiment Design: UC-MSCs and BMECs were seeded at a density of 1 × 105 cells/well (ratio: 1:2) in the insert and basolateral chambers of Transwell, respectively. AG1024 (Alexis, USA) (inhibitor of IGF-IR), AG490 (Sigma, USA) (inhibitor of JAK2/STAT5 signal pathway), and LY294002 (Sigma, USA) (inhibitor of PI3/KAKT/mTOR signal pathway) were employed for treating the BMECs at concentrations of 10 μmol/L, 50 μmol/L, and 25 μmol/L, respectively [2,3]. The untreated groups were added with DMSO as a control. The details of the groups are outlined in below. After 24 hours of culturing, the supernatants and cells of various groups were collected for biochemical analysis. Control group: BMECs; Experimental group: BMECs/UC-MSCs; Treatment Ⅰ group: BMECs+AG1024;

Treatment Ⅱ group: (BMECs+AG1024)/UC-MSCs;

Treatment Ⅲ group: BMECs+AG490;

Treatment Ⅳ group: (BMECs+AG490)/UC-MSCs;

Treatment Ⅴ group: BMECs+AG490+AG1024;

Treatment Ⅵ group: (BMECs+AG490+AG1024)/UC-MSCs;

Treatment Ⅶ group: BMECs+LY294002;

Treatment Ⅷ group: (BMECs+LY294002)/UC-MSCs;

Treatment Ⅸ group: BMECs+AG1024+LY294002;

Treatment Ⅹ group: (BMECs+AG1024+LY294002)/UC-MSCs.

Apoptosis was examined by flow cytometry [4]. Apoptosis analysis was performed by real-time quantitative PCR. Repetitive ANOVA and SPSS 18.0 software were used to determine statistical significance ($P < 0.05$) between groups.

Conclusion

In summary, the UC-MSCs and BMECs co-culture system overcome the challenges and shortcomings of BMEC degeneration and apoptosis in BMECs monoculture. Co-culture of UC-MSCs and BMECs can significantly promote the IGF-I secretion in comparison to that of BMEC monocultures, as the UC-MSCs provide an endogenous source of IGF-I. Moreover, the IGF-I and IGF-IR mRNA expression of BMECs were demonstrably enhanced through co-culture with UC-MSCs Additionally, UC-MSCs have been found to promote BMEC proliferation within the co-culture system, with directly co-cultured cells having higher proliferation rates than those that have been co-cultured indirectly. This observable phenomenon is suggested to have a positive correlation with IGF-I expression and secretion. Furthermore, UC-MSCs can effectively restrain BMEC apoptosis by reactivating IGF-I through the PI-3K/AKT/mTOR and JAK/STAT signaling pathways. The ultimate mechanism of protective action that UC-MSCs have on BMECs likely involves the up-regulation of the expression of the BMEC anti-apoptotic gene Bcl-2 and the downregulation of thee pro-apoptotic genes Caspase-3 and Bax. This study has demonstrated that BMEC/UC-MSC co-culture of system is a promising approach to restrain BMEC apoptosis while promoting their differentiation and proliferation in a more effective manner than what is possible with current techniques which rely solely on exogenous supplementation of BMEC cultures with IGF-I.

Ethics Approval and Consent to Participate

not applicable

Consent for Publication

not applicable

Availability of Data and Material

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare no competing financial interests.

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Author Contributions

WS, YK Z and HZ conceived and supervised the study. WS, YK Z and LW W designed the experiments. WS, LW W, KL W and LY X performed the experiments. WS, DH W and CL L analyzed the data. WS and YX revised the paper .WS wrote the paper.

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Appendix A. Supplementary Data

Supplementary data associated to this article can be found online.

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