学 位 論 文 の 要 旨

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学 位 論 文 名 Highly-purified Rapidly Expanding Clones, RECs, are Superior for Functional-mitochondrial Transfer

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論 文 内 容 の 要 旨

INTRODUCTION

Mitochondrial disorders are a group of multi-system diseases caused by the defects of mtDNA or nuclear DNA, which lead to mitochondria morphology and functional abnormalities.

Researchers have been interested in exogenous replacement of damaged mitochondria to prevent cell death. In 2006, it was revealed that mitochondria from human mesenchymal stem cells (MSCs) might be transported into defective cells through TNTs. Furthermore, MSCs isolated from bone marrow (BM) and fat is likely to repair the mitochondrial activity of the recipient cells by transferring their own mitochondria. However, the conventionally isolated bone marrow-derived MSCs (BMSCs) used in clinical research has shown different ability for cellular proliferation and differentiation and even contradictory results because these BMSCs always contain undifferentiated cells leading to a heterogeneous cell population with inconsistent functions.

Our previous work reported that rapidly expanding clones (RECs) were isolated as a single clone from $CD90^{high}/CD271^{high}$ population in bone marrow mononuclear cells. This clonally expanded and ultra-purified BM-MSCs, RECs display all the properties of MSCs, such as plastic adherence, differentiation capacity, and cell surface antigens, and does not exhibit lot-related variations in clinical applications. RECs have a possibility to offer many potential benefits as transplantable cells for treating several disorders related to bone, heart, peripheral nerves, brain,

and other organs. However, whether RECs can restore the bioenergetics of the cell remains unknown. Further, the health of the mitochondria transferred into mtDNA-deficient cells needs to be assessed. This study aimed to identify REC-dependent mitochondrial transport pathways and investigate whether this transfer restores cellular functions in mtDNA-deficient cells.

MATERIALS AND METHODS

We established mitochondria-deficient cell lines (ρ^0 A549 and ρ^0 HeLa cell lines) using ethidium bromide. Mitochondrial transfer from RECs/MSCs to ρ^0 cells was confirmed by PCR and flow cytometry analysis. We examined several mitochondrial functions including ATP, reactive oxygen species, mitochondrial membrane potential, and oxygen consumption rate (OCR). The route of mitochondrial transfer was identified using inhibition assays for microtubules/tunneling nanotubes, gap junctions, or microvesicles using transwell assay and molecular inhibitors.

Data analysis was performed using the GraphPad Prism software version 9.0 (San Diego, CA). Data are expressed as the mean ± standard deviation. Student's t-test or one-way ANOVA with Tukey's post hoc analysis was performed to compare the differences between two or more groups, and $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

1. Mitochondrial transfer pathways of MSCs and RECs in the direct contact systems

In this study, we focused on three pathways of mitochondrial transfer from RECs to mtDNA-deficient cells compared to that by regular MSCs (tunneling nanotubes (TNTs), connexin 43 (Cx43) gap junctions (GJs), microvesicles (MVs)). Both MSCs and RECs were capable of donating mitochondria to ρ^0 cells. This transfer is rapid, unidirectional, and is mediated by multiple mechanisms, including MVs, connexin-43 GJs, and TNTs. However, compared to MSCs, we found that the content of TNTs generated by RECs was significantly lower, consistent with previous reports. One probable explanation for this is high migration rate of RECs. The equilibrium response of G-actin incorporation and dissociation balances the synthesis of F-actin. F-actin is a critical component of microfilaments generated by the polymerization of monomeric actin (G-actin). Microfilaments anchor to the cell membrane and are involved in cytoskeletal organization, resulting in successful cell motility. Non-motile cells, on the other hand, frequently collect large bundles of microfilaments known as stress fibers. RECs have high migration, so they produce less TNTs. Through cell inhibition experiments, we also found that compared with MSCs, the mitochondrial transfer rate of RECs was much higher under TNTs inhibitors (cytochalasin D). Therefore, TNTs may not be the primary method by which RECs donate mitochondria.

2. Mitochondrial transfer pathways of RECs in the non-contact systems

In our study, we observed RECs-transferred mitochondrial via MVs in the non-contact co-culture system. TEM results showed that mitochondria in microvesicles were also found in ρ^0 cells co-cultured with RECs. These suggest that MVs might mediate the mitochondrial transfer mechanism of RECs. Using gap junction (carbenoxelone) and endocytosis/MVs (dynasore) inhibitors, the mitochondrial transfer rate of RECs was significantly reduced compared to that of MSCs. These results indicate that RECs depend more on gap junction and MVs transfer mitochondria than MSCs. And the quantitative analysis of the relative fluorescence intensity of Cx43 protein also showed that RECs produced more Cx43. According to related investigations, MSCs give their functioning mitochondria to alveolar epithelial cells via MVs in a Cx43-dependent way. Additionally, MVs participate in the transmitophagy of stressed MSCs and injured retinal ganglion cells, resulting in the self-preservation and reuse of depolarized mitochondria. These findings imply that REC's primary mitochondrial transport mechanism might be Cx43-mediated microvesicle release in the non-contact co-culture system.

3. The recovery of mitochondrial function

The use of RECs represented a significant advantage of this study because the cell source produced high-quality MSCs with minimal inter-batch variation and showed superior properties in terms of cell proliferation, cell size uniformity, and surface antigen expression compared to that of MSCs. In addition, in this study, we found that RECs could deliver more mitochondria to ρ^0 cells than MSCs in both contact and non-contact co-culture systems, and RECs was superior to MSCs in terms of mitochondrial content, mitochondrial membrane potential, and OCR. It is worth mentioning that RECs itself was superior to MSCs in mitochondrial content, mtDNA content, mitochondrial membrane potential, and OCR. This is also a strong guarantee that RECs can better restore mitochondrial function in mtDNA-deficient cells.

CONCLUSION

We have provided an alternative, efficient, homogenized, REC-based therapeutic strategy to supplement healthy mitochondria to rescue bioenergetic demands and OXPHOS-dependent processes. However, REC's efficiency and therapeutic effect in mitochondrial transfer to other cells or animal models of mitochondrial disease require further investigation.