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602.MYELOID ONCOGENESIS: BASIC

CX43-Mediated Mitochondria Transfer Enhances the Stemness of Leukemia Stem Cells Though Metabolic Remodeling

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Background : Despite improvements in treatment options for acute myeloid leukemia (AML), chemotherapy resistance remains an important clinical concern with a high recurrence rate in patients.Leukemia stem cells (LSCs) play a decisive role in the relapse resistance of leukemia. Bone marrow mesenchymal stem cells(BMSCs) protect LSCs from being completely removed from the bone marrow, but the underlying mechanisms are poorly understood.In this study, we aimed to uncover the mechanism of BMSCs-regulated the stemness of leukemia stem cells through mitochondrial transfer mediated by Connexin43(CX43), thus providing a promising clinical therapy target.

Methods: Primary BMSCs were isolated from the primary bone marrow specimens of patients with AML and cultured in MSCM culture base within 10% serum.CX43 overexpressing BMSCs (CX43-BMSCS) were constructed by lentivirus transfection.The efficiency of CX43 overexpression was verified by immunofluorescence, qPCR and Western Blot.In vitro, CX43-BMSCs and control BMSCs (EV-BMSCs) were cocultured with KG-1a cells.In some experiments,vincristine (1nM) was added to inhibit the mitochondrial transferring.Bidirectional mitochondrial transfer was detected by flow cytometry and confocal microscopy.The mechanism of mitochondria transfer was analyzed by inhibitor assays.The proliferation of KG-1a from the coculture system were assessed by Flow cytometry and EdU assay.After co-culture, the clone formation of KG-1a was detected by using semisolid cloning assay.The mitochondrial respiration of KG-1a was analyzed by using Seahorse.The transcriptomes of KG-1a after co-culture using RNA-seq.Finally,we verified our findings using LC-MS non-targeted metabolomics to detect the metabolites changes of KG-1a after co-culture.

Results: Compared with Control-BMSCs and EV-BMSCs,the mRNA and protein expression levels of CX43 in CX43-MSCs were significantly increased ($P<0.05$).In the co-culture system without serum for 48h,BMSCs transfer mitochondria to KG-1a cells but receive no mitochondria from KG-1a.Moreover,CX43-BMSCs transferred more mitochondria to KG-1a than EV-BMSCs group ($P<0.05$),while there was no obvious mitochondrial transfer was observed in the transwell and 10% serum co-culture system.In addition,compared with EV-BMSCs,CX43-BMSCs significantly increased the proliferation and adhesion of KG-1a ($P<0.05$) and clone formation ability ($P<0.05$).Vincristine significantly reduced the mitochondrial transfer from CX43-BMSCs to KG-1a,and decreased the adhesion of CX43-BMSCs to KG-1a,as well as the proliferation and clone formation of KG-1a after co-culture.The metabolic capacity of KG-1a cells in CX43-BMSCs was significantly increased compared with EV-BMSCs ($P<0.05$),and this metabolic enhancement could be reversed by vincristine.Hierarchical cluster analysis (HC) of metabolites showed that there were significant differences in metabolites among treatment groups ($p<0.05$),showed unique metabolic characteristics related to CX43-induced changes.The GO analysis revealed that the enrichments occurring in KG-1a after co-cultured with CX43-BMSCs mainly included cell adhesion and migration,cell proliferation and regulation,signal transduction,PI3K/Akt pathway,transmembrane transport and intracellular protein metabolism.Metabolism-related pathways,like nucleotide metabolism,lipid metabolism and amino acid metabolism,were enriched in KG-1a after co-cultured with CX43-BMSCs.Furthermore,KG-1a after co-cultured with CX43-BMSCs showed up-regulated of promoting the proliferation and the stemness of AML-LSCs,including N-cadherin (CDH2),CD244,SLC43A2,CYBB and IGLL1.

Conclusion: We show evidence that CX43 mediated mitochondria transfer from BMSCs to LSCs which promoting the adhesion of BMSCs to LSCs under nutrient deficiency condition,and then enhanced the proliferation and cloning ability of LSCs,altered gene expression profiles related to cell proliferation,adhesion,and energy metabolism,and increased the expression of genes related to maintaining stemness of LSCs, allows metabolic remodeling to regulate LSCs stemness, may be a potential therapeutic target for AML treatment.

Keywords: Leukemia stem cells;Bone marrow microenvironment;Connexin;Mitochondrial transfer;Metabolic remodeling

Disclosures No relevant conflicts of interest to declare.

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