56ASM-0138 | Effect of p53 activator nutlin-3a on cytokine release in peripheral blood mononuclear cells of healthy donor and patient with multiple sclerosis

I. Ganeeva; A. Valiullina; E. Zmievskaya; A. Rizvanov; E. Bulatov
Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russia C.I.S.

Background: The role of p53 in autoimmune diseases was demonstrated in many models. Multiple sclerosis (MS) is a chronic autoimmune disease targeting myelin. At the moment, there are 44 cases of MS per 100,000 population in the world and the number of patients is constantly increasing. However, the role of p53 in MS regulation is still unclear. In this study, we explore the influence of p53 activator Nutlin-3a on the secretion of cytokines by immune cells of healthy donor and MS patient.

Materials and Methods: In this study we used peripheral blood mononuclear cells (PBMCs) separated using Ficoll gradient from whole blood of healthy donor and MS patient. PBMCs from each donor were divided into 3 groups: untreated, treated with 10 μM and 40 μM Nutlin-3a. PBMCs were incubated with Nutlin-3a for 24 h. Cell supernatants were collected and tested by multiplex assay using Bio-Plex 200 system (Bio-Rad, USA). To evaluate the effect of Nutlin-3a on p53 activation and expression of p53-dependent genes we isolated mRNA from PBMCs of each group and estimated the expression levels of p21; Bax, PUMA by RT-PCR.

Results: RT-PCR analysis demonstrated increased expression levels of p53-dependent genes (p21; Bax, PUMA). According to the multiplex cytokine analysis, Nutlin-3a in healthy donor increased IP-10 level, decreased levels of IL-10; G-CFS, MCP-1; TNF-α, VEGF and did not affect IL-1b, IL-1ra, IL-15; INF-γ, RANTES levels. In MS patient, Nutlin-3a increased levels of IL-1b and TNF-α, decreased IL-1ra, IL-10; G-CSF, VEGF and did not affect IL-15; IFN-γ, IP-10; MCP-1; RANTES.

Conclusions: Our results demonstrate that p53 activator Nutlin-3a affects cytokine secretion in PBMCs of healthy donor and MS patient. This allows the assumption that p53 protein is involved in the regulation of immune processes.

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56ASM-0150 | Cross-correction of β-hexosaminidase a deficiency using genetically modified human mesenchymal stem cells in a cell culture of tay-sachs disease patient

A. Shaimardanova; D. Chulpanova; S. Issa; A. Mullagulova; V. Solovyeva; A. Sharafieva; A. Rizvanov
Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russia C.I.S.

Background: GM2-gangliosidoses are autosomal recessive diseases caused by impaired enzymatic activity of β-hexosaminidase A (HexA); an essential enzyme for metabolism of GM2-gangliosides and other molecules containing terminal N-acetyl hexosamines. HexA deficiency causes accumulation of GM2-gangliosides mainly in the nervous system cells, leading to severe progressive neurodegeneration. Gene-cell therapy is considered a promising treatment for GM2-gangliosidoses. This study aimed to evaluate the possibility of restoring HexA deficiency in cells of Tay-Sachs disease (TSD) patient using genetically-modified mesenchymal stem cells (MSCs).

Materials and Methods: MSCs were isolated from human adipose tissue, and genetically modified with recombinant adeno-associated viruses encoding codon-optimized cDNA of α- (*HEXA*) and β-subunits (*HEXB*) genes of HexA (MSCs-HEXA-HEXB). MSCs-HEXA-HEXB were cultured with TSD patient's MSCs (mutMSCs) in a transwell system. After 7 days, the effectiveness of HexA deficiency cross-correction was studied. HEXA concentration in cell lysates was determined by ELISA. Copy number of *HEXA* and *HEXB* genes was determined using qPCR. Detection of HEXA and HEXB proteins was carried out by ICC.

Results: After HexA delivery by cross-correction in the transwell culture system, mutMSCs contained 72903.12 ± 14026.65 and 80899.7 ± 20847.92 copies of codon-optimized HEXA and HEXB genes, respectively, per 1 μg of total RNA. HEXA concentration in mutMSCs after cross-correction of HexA deficiency (0.71191 ± 0.069171 ng/μl) increased by 2.25-fold compared to native mutMSCs (0.302794 ± 0.0273096 ng/μl). Both α- and β-subunits of HexA were detected in mutMSCs using ICC after enzyme deficiency cross-correction.

Conclusions: Therefore, the effectiveness of HexA deficiency cross-correction was shown in TSD patient's mutMSCs upon interaction with MSCs-HEXA-HEXB. Such correction could be mediated by vesicular transport and HexA delivery via extracellular vesicles MSCs-HEXA-HEXB.

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