

Bone marrow mesenchymal stem cells attenuate 2,5-hexanedione-induced neuronal apoptosis through a NGF/AKT-dependent pathway

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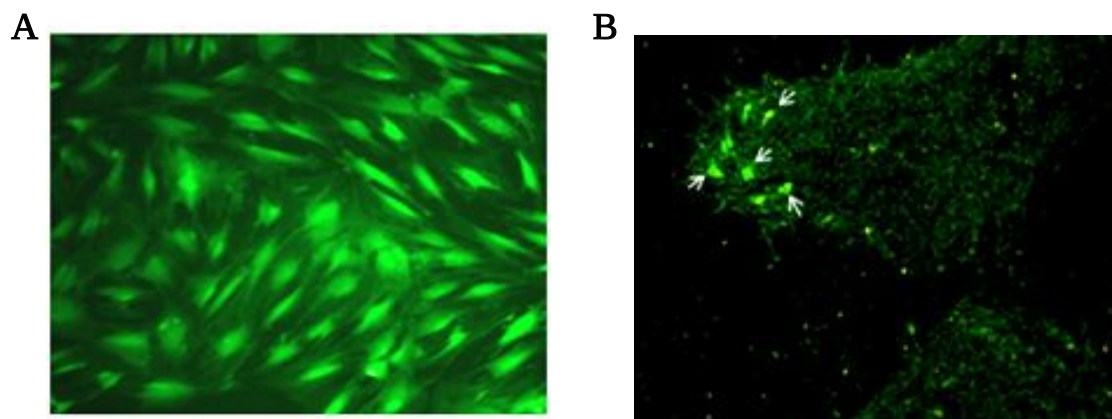
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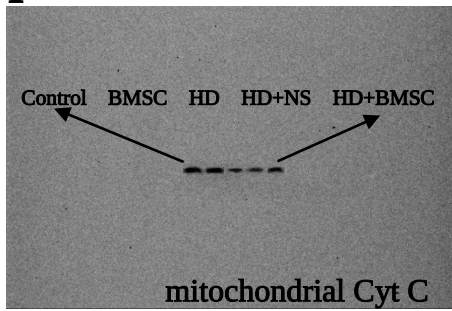
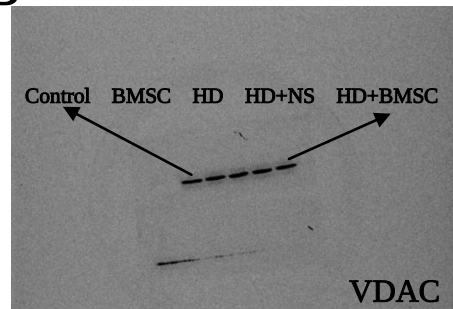
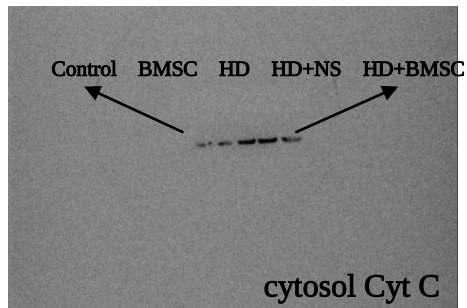
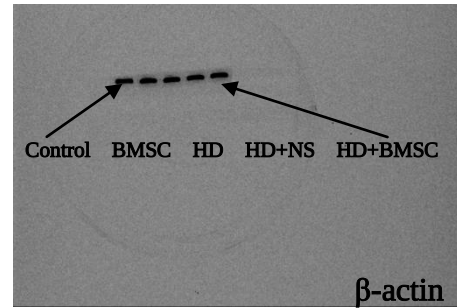
Abstract

Growing evidence suggests that the increased neuronal apoptosis is involved in *n*-hexane-induced neuropathy. We have recently reported that bone marrow-mesenchymal stem cells-derived conditioned medium (BMSC-CM) attenuated 2,5-hexanedione (HD, the active metabolite of *n*-hexane)-induced apoptosis in PC12 cells. Here, we explored the anti-apoptotic efficacy of BMSC *in vivo*. HD-treated rats received BMSC by tail vein injection 5 weeks after HD intoxication. We found that in grafted rats, BMSC significantly attenuated HD-induced neuronal apoptosis in the spinal cord, which was associated with elevation of nerve growth factor (NGF). Neutralization of NGF in BMSC-CM blocked the protection against HD-induced apoptosis in VSC4.1 cells, suggesting that NGF is essential for BMSC-afforded anti-apoptosis. Mechanistically, we found that the decreased activation of Akt induced by HD was significantly recovered in the spinal cord by BMSC and in VSC4.1 cells by BMSC-CM in a TrkA-dependent manner, leading to dissociation of Bad/Bcl-xL complex in mitochondria and release of anti-apoptotic Bcl-xL. The importance of Akt was further corroborated by showing the reduced anti-apoptotic potency of BMSC in HD-intoxicated VSC4.1 cells in the presence of Akt inhibitor, MK-2206. Thus, our findings show that BMSC attenuated HD-induced neuronal apoptosis *in vivo* through a NGF/Akt-dependent manner, providing a novel solution against *n*-hexane-induced neurotoxicity.

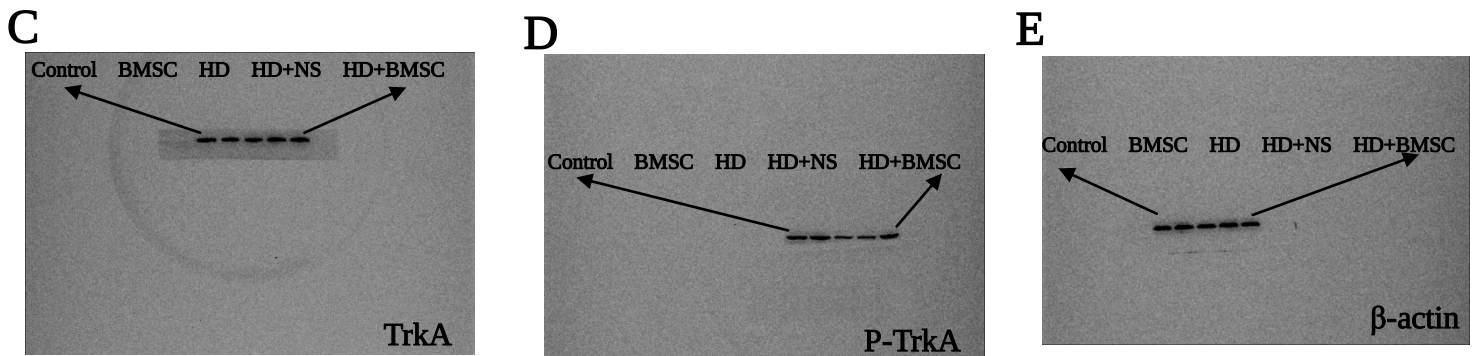
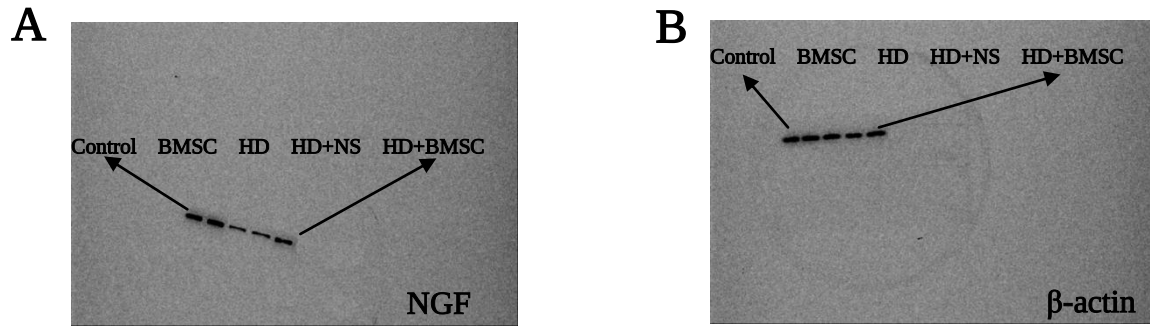
Key words 2,5-hexanedione, neurotoxicity, Apoptosis, bone marrow-mesenchymal stem cells



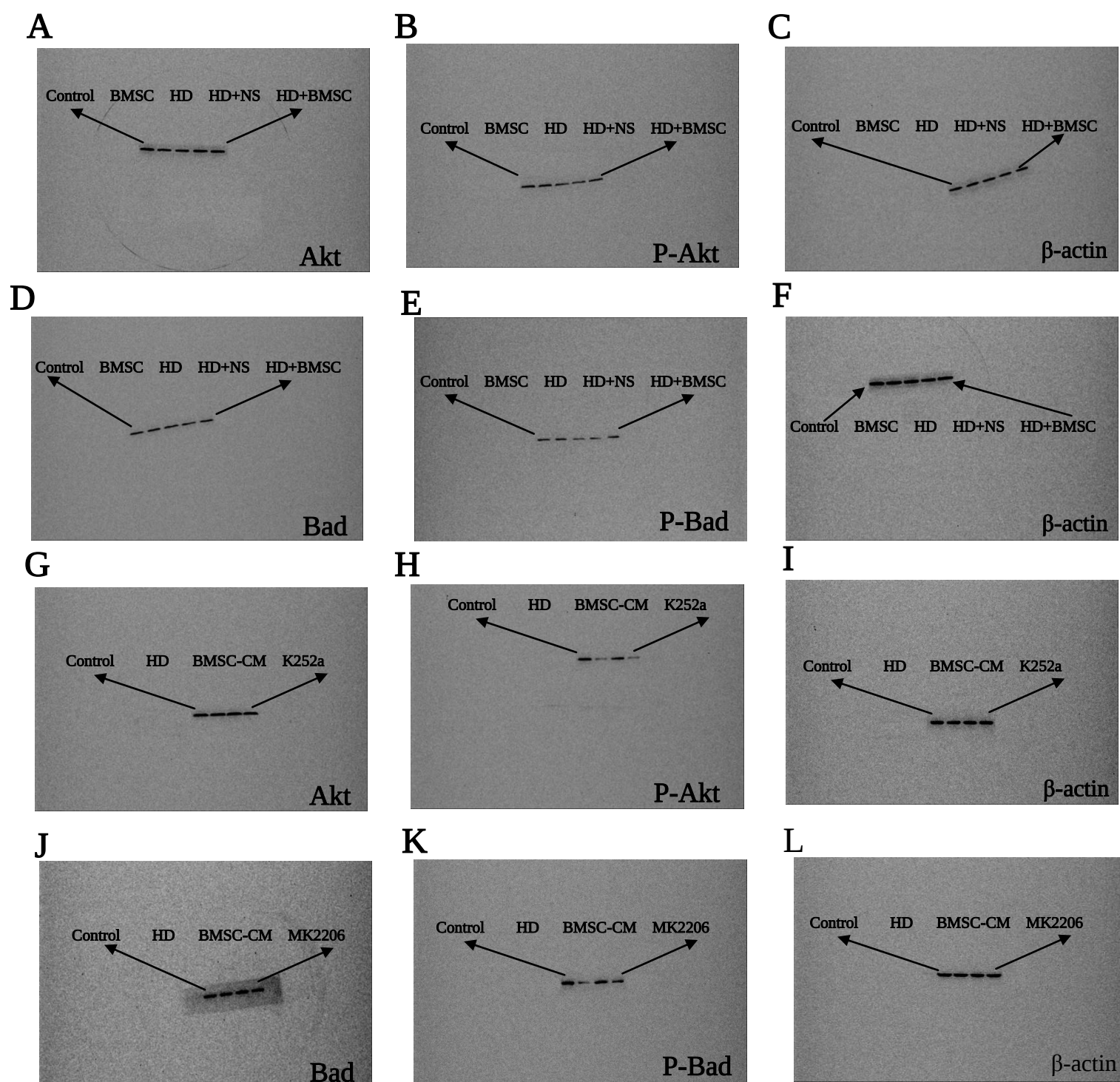
Supplementary Figure 1. (A) BMSC were labeled by CFSE before graft. Incubation with 10 $\mu\text{mol/L}$ CFSE for 15 mins, over 98% BMSC was labeled. (B) CFSE-labeled BMSCs were detected in the spinal cord of HD-intoxicated rat after BMSC transplantation 5 weeks.

A**B****C****D**

Supplementary Figure 2. The full-length gels for Figure 1C. The full-length Western blot gels of mitochondrial Cyt C and its VDAC (A, B) and cytosol Cyt C and its β -actin (C, D) in the spinal cord of HD-intoxicated rats with or without BMSC transplantation.



Supplementary Figure 3. The full-length gels for Figure 3. (A, B) The full-length Western blot gels of NGF and β -actin in the spinal cord of HD-intoxicated rats with or without BMSC transplantation; (C-E) The full-length Western blot gels of TrkA, p-TrkA and β -actin in the spinal cord of HD-intoxicated rats with or without BMSC transplantation.



Supplementary Figure 4. The full-length gels for Figure 5. (A-C) The full-length Western blot gels of Akt, p-Akt and β-actin in the spinal cord of HD-intoxicated rats with or without BMSC transplantation; (D-F) The full-length Western blot gels of Bad, p-Bad and β-actin in the spinal cord of HD-intoxicated rats with or without BMSC

transplantation; (G-I) The full-length Western blot gels of Akt, p-Akt and β -actin in HD-intoxicated VSC4.1 cells with or without K252a treatment; (J-L) The full-length Western blot gels of Bad, p-Bad and β -actin in HD-intoxicated VSC4.1 cells with or without MK2206 treatment.