Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

Systemic Administration of Exosomes Released from Mesenchymal Stromal Cells Attenuates Apoptosis, Inflammation, and Promotes Angiogenesis after Spinal Cord Injury in Rats

Jiang-Hu Huang *et al.*,

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Tanja Wagner

Introduction

Spinal cord injury (SCI)

- lead to severe and often permanent disability
- result in significant decrease in quality of life and heavy burden to the individuals and their families
- many therapies but all of these approaches demonstrated limited efficacy

➔compelling need to develop novel therapeutic strategies designed to improve functional recovery post-SCI

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Mesenchymal stem cells (MSCs)

- subset of non-haematopoietic cells consisting of mesenchymal stem and progenitor cells
- possess pluripotent features
- obtained from different sources such as bone marrow, peripheral blood, umbilical cord blood, adipose tissue and skin of the human body

➔ Studies showed that MSCs can serve as a promising cell source for the prelinical cell therapy in treatment of SCI in rat model

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Mesenchymal stem cells (MSCs)

However, there are several disadvantages

- After long-term culture, MSCs might become immortalized and spontaneously transform (enhanced chromosome instability, dysregulation of telomere activity and cell-cycle-related genes) →can result in tumorigenesis when injected in multiple organs

- Transplantaion of short-term MSCs cultured into mice can form malignant tumors

- Intra-aterial MSCs administration may lead to occlusion in the distal vasculature (relatively large cell size)

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Mesenchymal stem cells (MSCs)

– Small proportion of transplanted MSCs actually survived and few MSCs an differentiate into neural cells in injured spinal cord tissues

➔most of the biological and therapeutic effect of MSCs are attributed to paracrine mechanisms mainly through secreting molecules

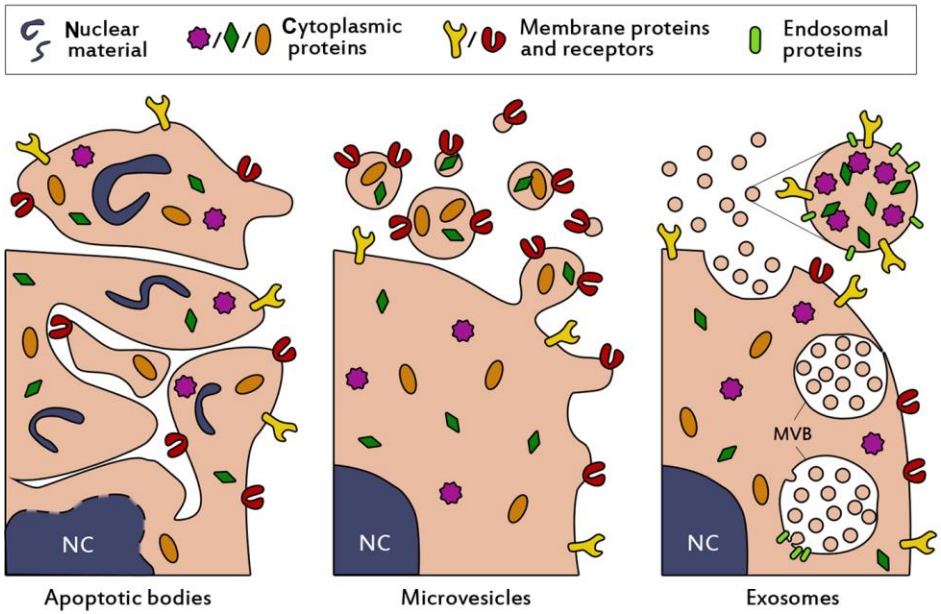
- growth factors
- chemokines
- cytokines
- extracellular vesicles

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Exosomes

- Endosomal origin small-membrane vesicles: diameter **30-120 nm**
- Originate from budding into the limiting membrane of large endosomal structures (multivesicular bodies =MVB) in the cytosol

→ MVB are able to fuse with the plasma membrane, causing the release of exosomes into the extracellular space



Kooijmans *et al.*, 2012

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Exosomes

- generated by many cell types
- contain functional messenger RNAs, micro-RNAs and proteins
- pivotal role in cell-to-cell communication

- Exosomes derived from MSCs do not proliferate and are easier to store and deliver than MSCs
- Exosomes and microvesicles derived from multipotent MSCs have therapeutic promise in cardiovascular, liver and kidney diseases
- Systemic administration of MSCs-exosomes promoted neurovascular remodeling and functional recovery post-stroke

Stoorvogel W. *et al.*, Traffic (2002)
Thery, C. *et al.*, Nat. Rev. Immunol. (2002)

Hypothesis

Whether systemic administration of exosomes generated from MSCs can promote the function recovery on the rat model of SCI *in vivo*

Methods

Experiment design

- SCI rats were randomly divided into 2 groups:
    - PBS group (control group)
    - MSCs-exosomes treatment group (exosomes group)

- Rats were subjected to SCI, then half an hour later followed by tail vein injection of MSCs-exosomes (precipitated in 0.5ml PBS, approximately $1x10^{10}$ particles) or an equal volume of PBS (0.5ml)

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Establishment of contusion spinal cord injury model in rats

- rats were anesthetized with intraperitoneal injection
- a laminectomy was performed at thoracic vertebra level 10 (T10)
- a moderate contusion injury was induced using a modified Allen`s weight drop apparatus (8g weight at a vertical height of 40mm) on the exposed dura of the spinal cord

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MSCs-exosomes generation and collection

- Bone marrow from adult male rats was mechanically dissociated
  → cells were washed and suspended in culture medium
  (modified Eagle`s medium +20% fetal bovine serum +penicillin/streptomycin)

- For exosome isolation: culture medium was replaced with an exosome depleted FBS-contained medium when cells reached 60%-80% confluence →MSCs were cultured for additional 24h

- Supernatants collected from cultured MSCs
  → filtered 0.2μm filter to remove large debris and dead cells
  → small-cell debris removed by centrifugaton at 10,000g for 30min
  → supernatants recentifugated at 100,000g for 3h

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Basso, Beattie, Bresnahan scores (BBB score)

- Locomotor activity was evaluated at 1, 3, 7, 14, 21 and 28 days post-injury using the BBB score, which measured locomotor ability for 4min

0 No observable hindlimb (HL) movement
1 Slight movement of one or two joints, usually the hip and/or knee
2 Extensive movement of one joint
or
extensive movement of one joint *and* slight movement of one other joint
3 Extensive movement of two joints
4 Slight movement of all three joints of the HL
5 Slight movement of two joints *and* extensive movement of the third
6 Extensive movement of two joints *and* slight movement of the third
7 Extensive movement of all three joints of the HL
8 Sweeping with no weight support
or
plantar placement of the paw with no weight support
9 Plantar placement of the paw with weight support in stance only (i.e., when stationary)
or
occasional, frequent, or consistent weight supported dorsal stepping and no plantar stepping
10 Occasional weight supported plantar steps, no forelimb (FL)–HL coordination
11 Frequent to consistent weight supported plantar steps *and* no FL–HL coordination
12 Frequent to consistent weight supported plantar steps *and* occasional FL–HL coordination
13 Frequent to consistent weight supported plantar steps *and* frequent FL–HL coordination
14 Consistent weight supported plantar steps, consistent FL–HL coordination; *and*
predominant paw position during locomotion is rotated (internally or externally) when it makes *initial contact* with the surface as well as just before it is *lifted off* at the end of stance
or
frequent plantar stepping, consistent FL–HL coordination, and occasional dorsal stepping
15 Consistent plantar stepping and consistent FL–HL coordination; *and*
no toe clearance or occasional toe clearance during forward limb advancement;
predominant paw position is parallel to the body at initial contact
16 Consistent plantar stepping and consistent FL–HL coordination during gait; *and*
toe clearance occurs frequently during forward limb advancement;
predominant paw position is parallel at initial contact and rotated at lift off
17 Consistent plantar stepping and consistent FL–HL coordination during gait; *and*
toe clearance occurs frequently during forward limb advancement;
predominant paw position is parallel at initial contact *and* lift off
18 Consistent plantar stepping and consistent FL–HL coordination during gait; *and*
toe clearance occurs consistently during forward limb advancement;
predominant paw position is parallel at initial contact and rotated at lift off
19 Consistent plantar stepping and consistent FL–HL coordination during gait; *and*
toe clearance occurs consistently during forward limb advancement;
predominant paw position is parallel at initial contact *and* lift off; and
tail is down part or all of the time
20 Consistent plantar stepping and consistent coordinated gait; consistent toe clearance;
predominant paw position is parallel at initial contact and lift off; tail consistently up;
and trunk instability
21 Consistent plantar stepping and coordinated gait, consistent toe clearance, predominant paw position is parallel throughout stance, consistent trunk stability, tail consistently up

Basso D.M. *et al.*, J. Neurotrauma (1995)

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling assay (TUNEL assay)

- The hallmark of apoptosis is DNA degradation
- To detect DNA fragmentation caused by cell death in the injured spinal cord

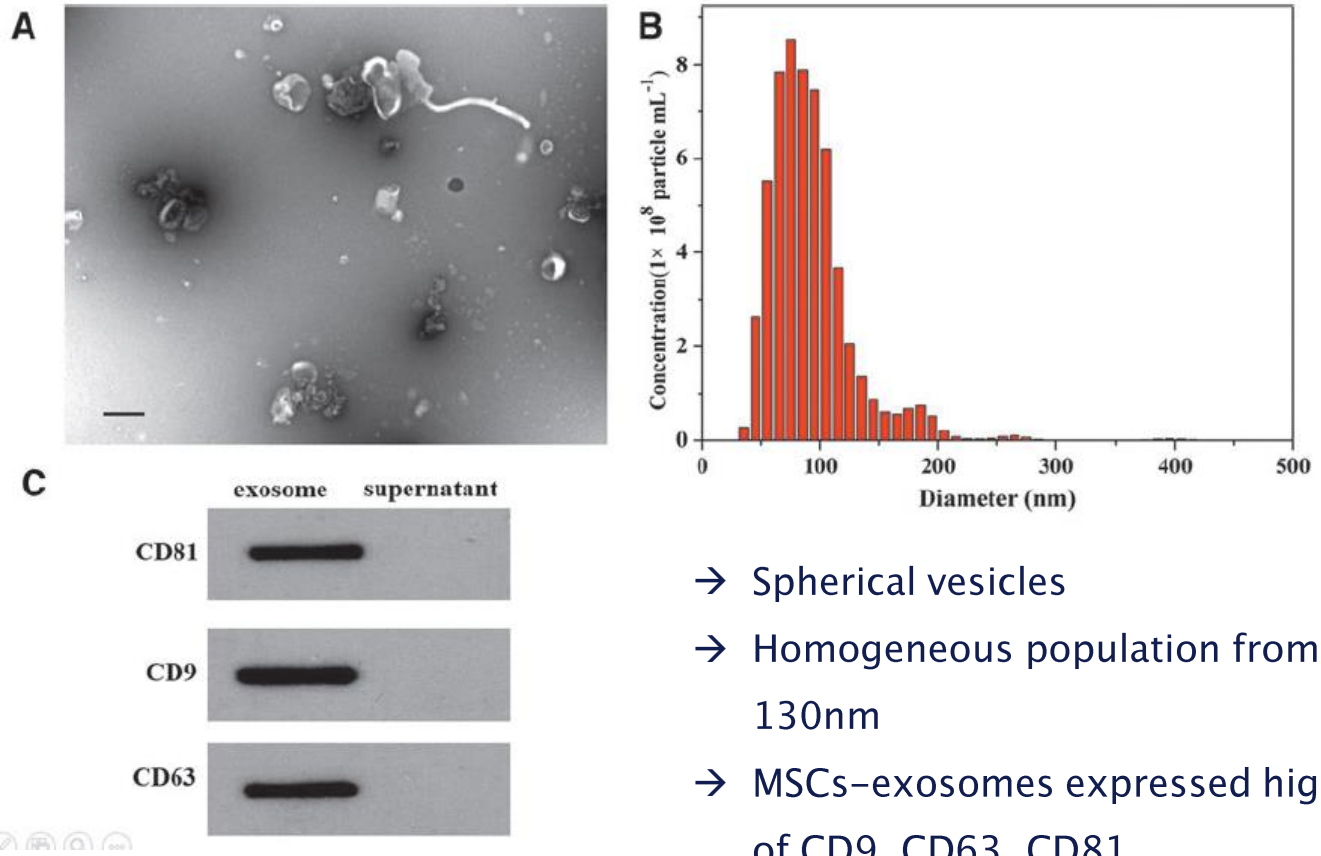
- DNA breaks (nicks) can be detected by labeling the free 3′-OH termini with modified nucleotides (e.g., biotin-dUTP, fluorescein-dUTP) in an enzymatic reaction
- The enzyme terminal deoxynucleotidyl transferase (TdT) catalyzes the polymerization of deoxyribonucleotides (dUTP) to the 3′-end of single- and double-stranded DNA

  ➔named **TUNEL** (**T**dT-mediated d**U**TP-X **n**ick **e**nd **l**abeling)
- Labeled sections were scanned with a light microscope

http://www.sigmaaldrich.com

Results

Characterization of mesenchymal stem cells exosomes

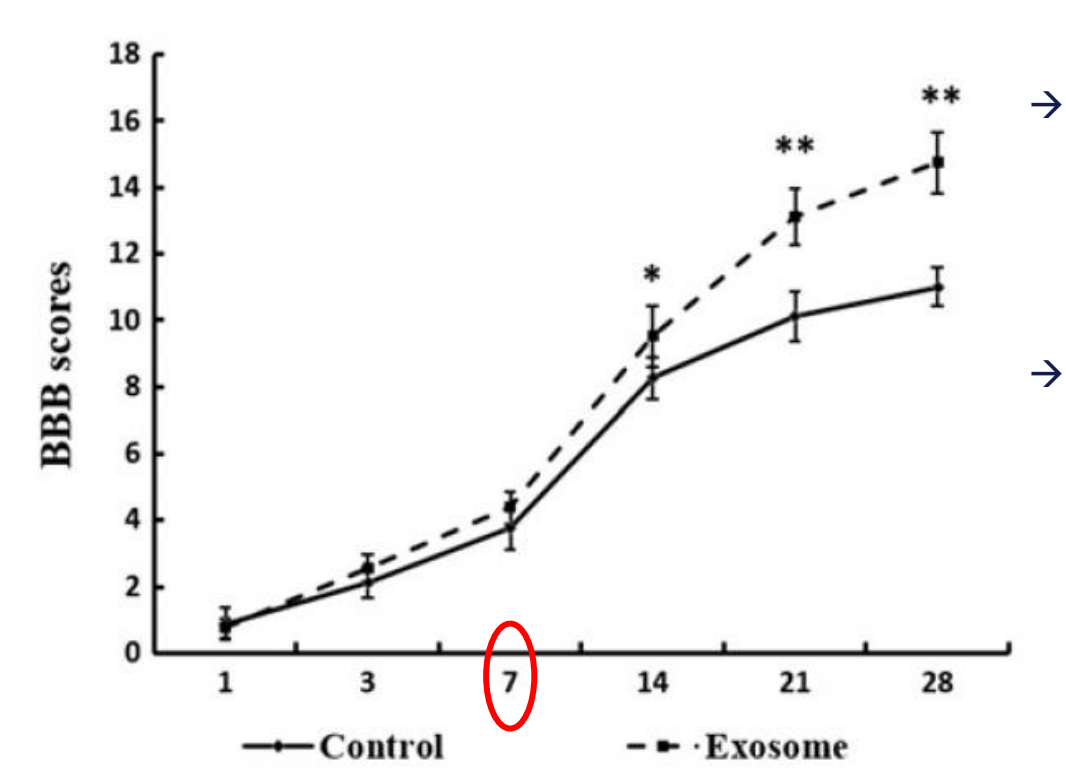


- → Spherical vesicles
- → Homogeneous population from 20 to 130nm
- → MSCs-exosomes expressed high levels of CD9, CD63, CD81

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

MSCs-exosomes improved function recovery after SCI

Post-SCI: starting all rats were paralyzed in both hindlimbs

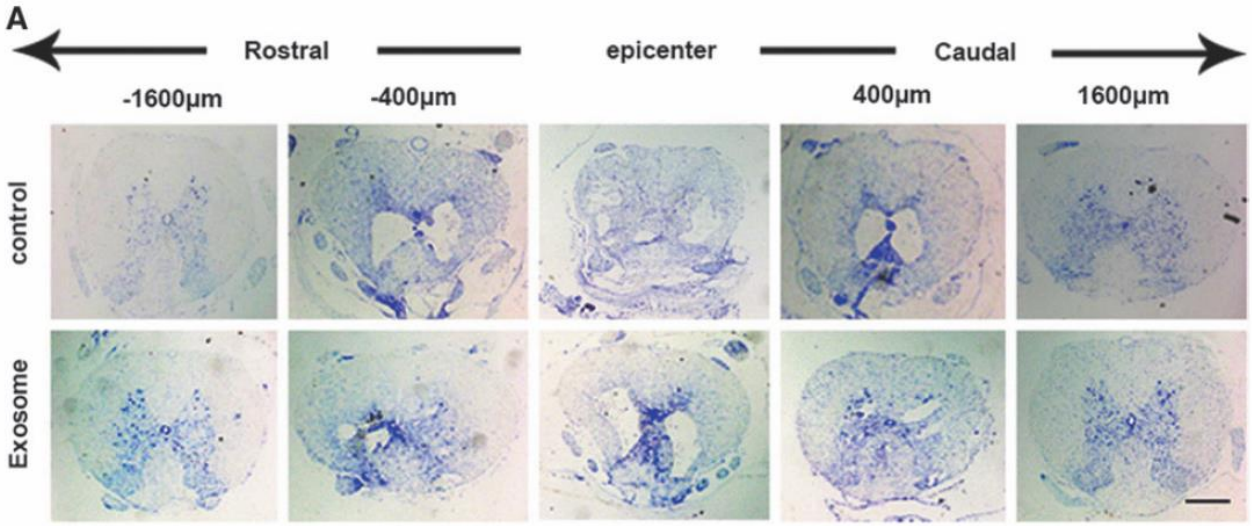


→ Hindlimb locomotor activity improved gradually over time (BBB scores gradually increased)

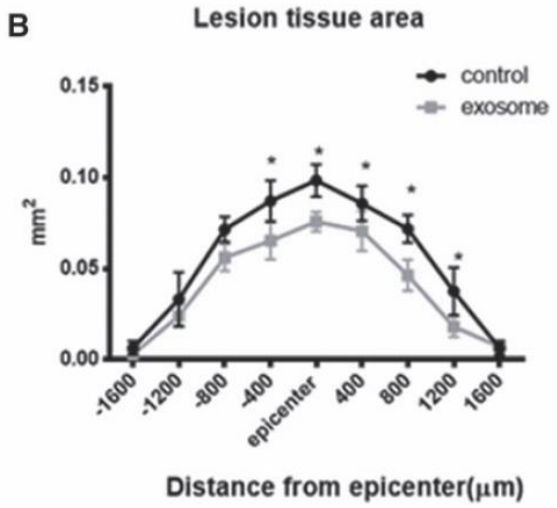
→ Exosome-group showed significantly improved hindlimb activity score from day 7 post-injury

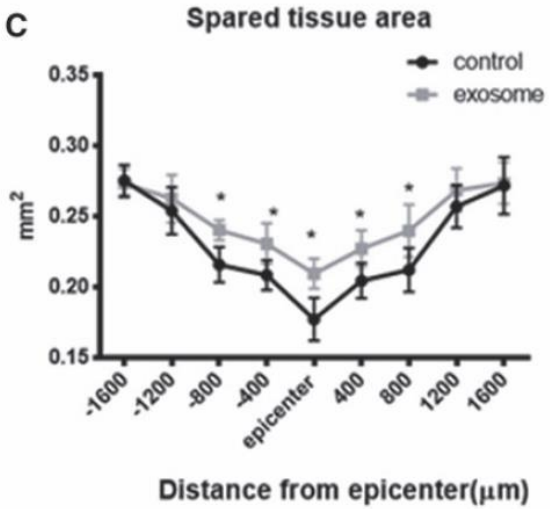
Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

MSCs-exosomes promoted tissue sparing and reduced lesion tissue after SCI



28 days post-injury:
cresyl- violet-stained section





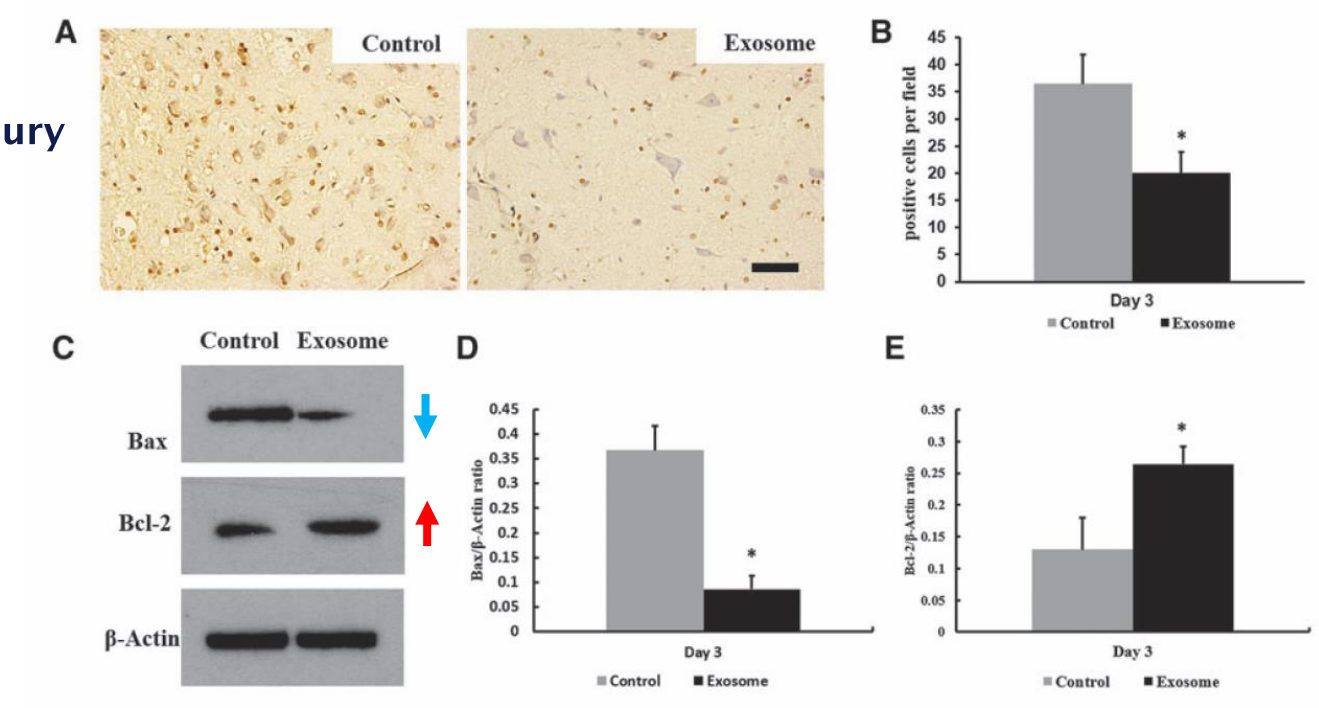
→ Lesion area was smaller in the exosomes-treated group

→ Exosome-treated group had significantly larger spared tissue areas

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

MSCs-exosomes attenuated cell death after SCI

3 days post-injury



→ MSCs-exosomes significantly decreased the number of TUNEL-positive cells

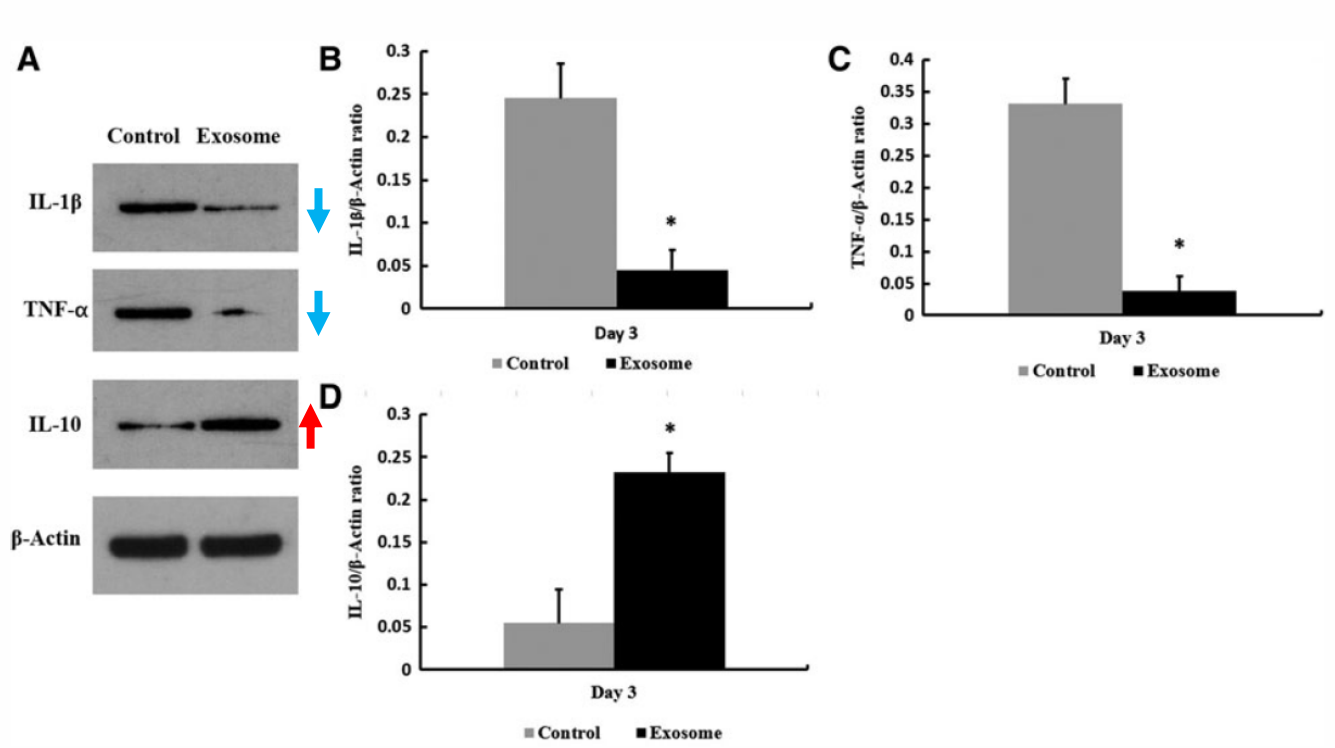
→ MSCs-exosomes **reduced Bax (pro-apoptotic)** expression

→ MSCs-exsosomes **upregulated Bcl-2 (anti-apoptotic)** expression

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

MSCs-exosomes reduced inflammation after SCI

3 days post-injury



→ **Pro**-inflammatory cytokines (**IL-1β and TNF-α**) were **downregulated** after exosomes treatment

→ **Anti**-inflammatory cytokine (**IL-10**) was **upregulated** in the exosome-treated group

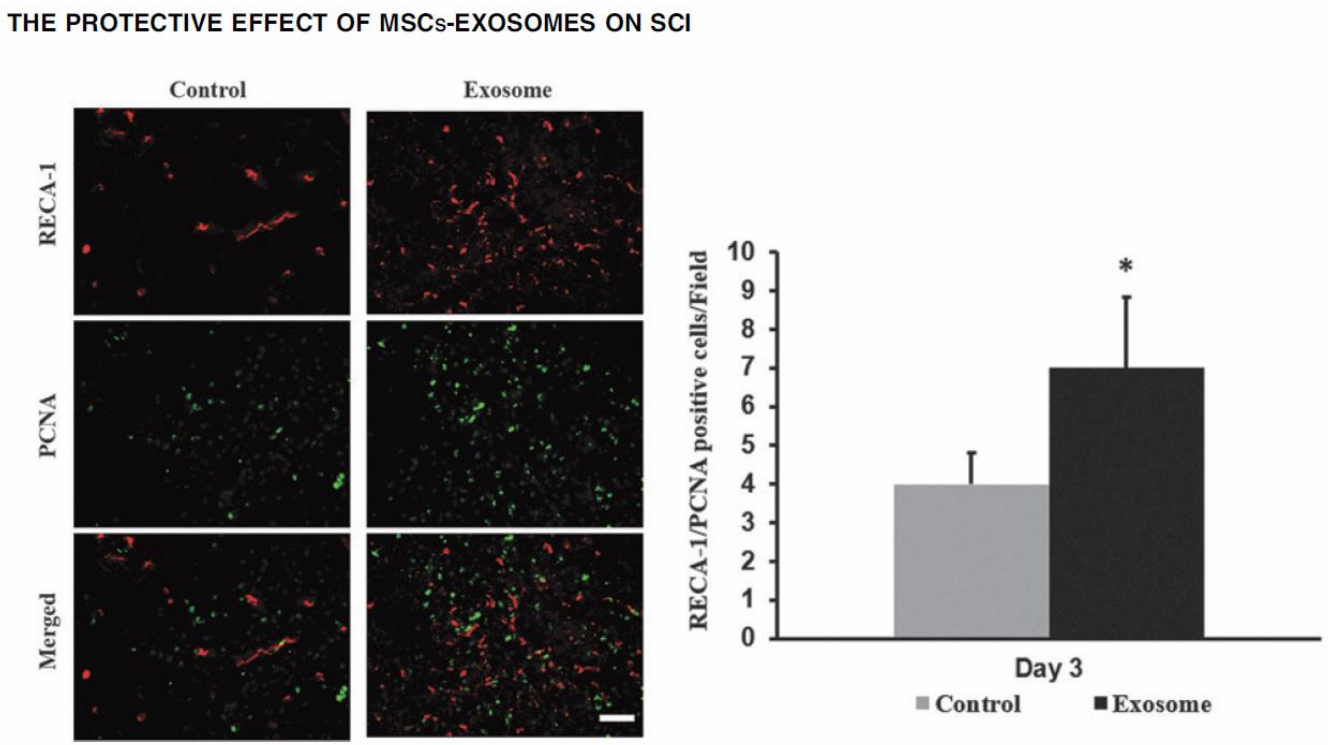
Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

MSCs-exosomes promoted angiogenesis after SCI

3 days post-injury

Rat endothelial cell antigen

Proliferating cell nuclear antigen



→ Number of proliferating vessels was significantly increased in the exosome-treated group

→ MSCs-exosomes increased endothelial cell proliferation post-SCI

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

Discussion

Mesenchymal stem cells

MSCs derived from different tissues appears to have neuroprotective effects on the spinal cord post-injury

➔on the hypothesis that it homes and engrafts into injured spinal cord tissues then differentiates into cells to replace damaged cells

➔HOWEVER, <**1%** of transplanted MSCs actually migrated to their target tissue and a large proportion of MSCs were trapped in the liver and lung after intravenous administration

Morita T. *et al.*, Neuroscience (2016)
Aras Y. *et al.*, Neurosurg. (2016)
Melo F.R. *et al.*, Neurobiol. (2017)
Phinney,D.G. *et al.*, Stem Cells (2007)

➔MSCs exerted their effects by secretion a broad spectrum of molecules (paracrine effect)

➔MSCs secreted growth factors, cytokines, chemokines, and extracellular microvesicles into their surrounding microenvironments, which subsequently benefited cell regeneration or angiogenesis

Ratajczak M.Z. *et al.*, Pol. Arch. Med. Wewn. (2014)
Kwon H.M. *et al.*, Vasc. Pharmacol. (2014)

MSCs-exosomes treatment after SCI

- attenuated cell apoptosis and inflammation
- promoted angiogenesis
- downregulated pro-apoptotic (Bax) mediator and pro-inflammatory cytokines (IL-1β and TNF-α)
- upregulated anti-apoptotic (Bcl-2) and anti-inflammatory cytokine (IL-10)

➔ MSCs-exosomes could improve the pathological hallmarks induced by trauma and promote functional recovery after SCI

Jiang-Hu Huang *et al.*, JOURNAL OF NEUROTRAUMA (2017)

→ MSCs-exosomes shows great potential as a cell-free strategy with promising therapeutic effects on SCI

→ to trace and measure that the substantive numbers of MSC-exosomes actually reach the spinal cord that aid to elucidate their superiority

➔These findings suggest that MSCs-derived exosomes is a potential new therapeutic intervention for SCI

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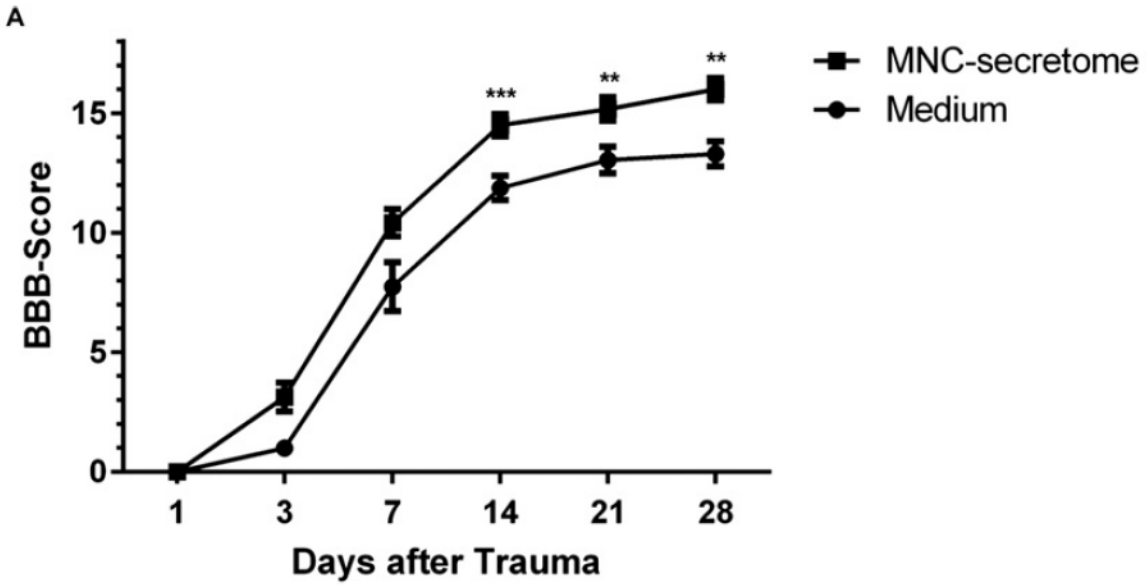
Thank you for your attention!

Secretome from apoptotic PBMCs

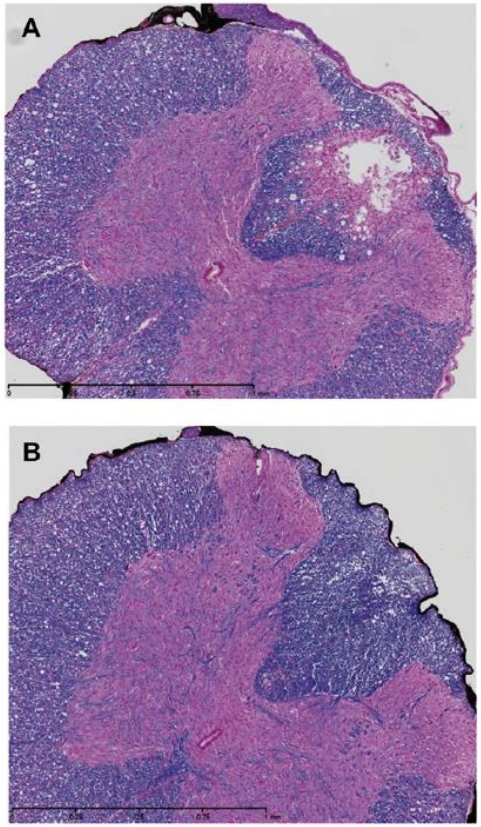


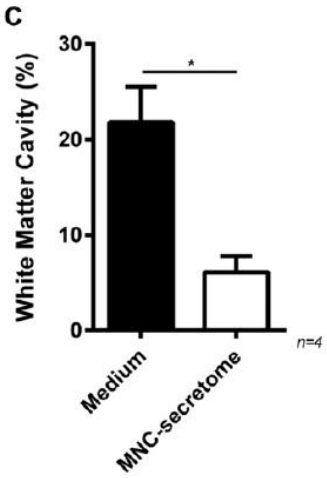
Haider T *et al.*, Experimental Neurology (2015)

Secretome from apoptotic PBMCs

medium treated

secretome treated





Haider T *et al.*, Experimental Neurology (2015)