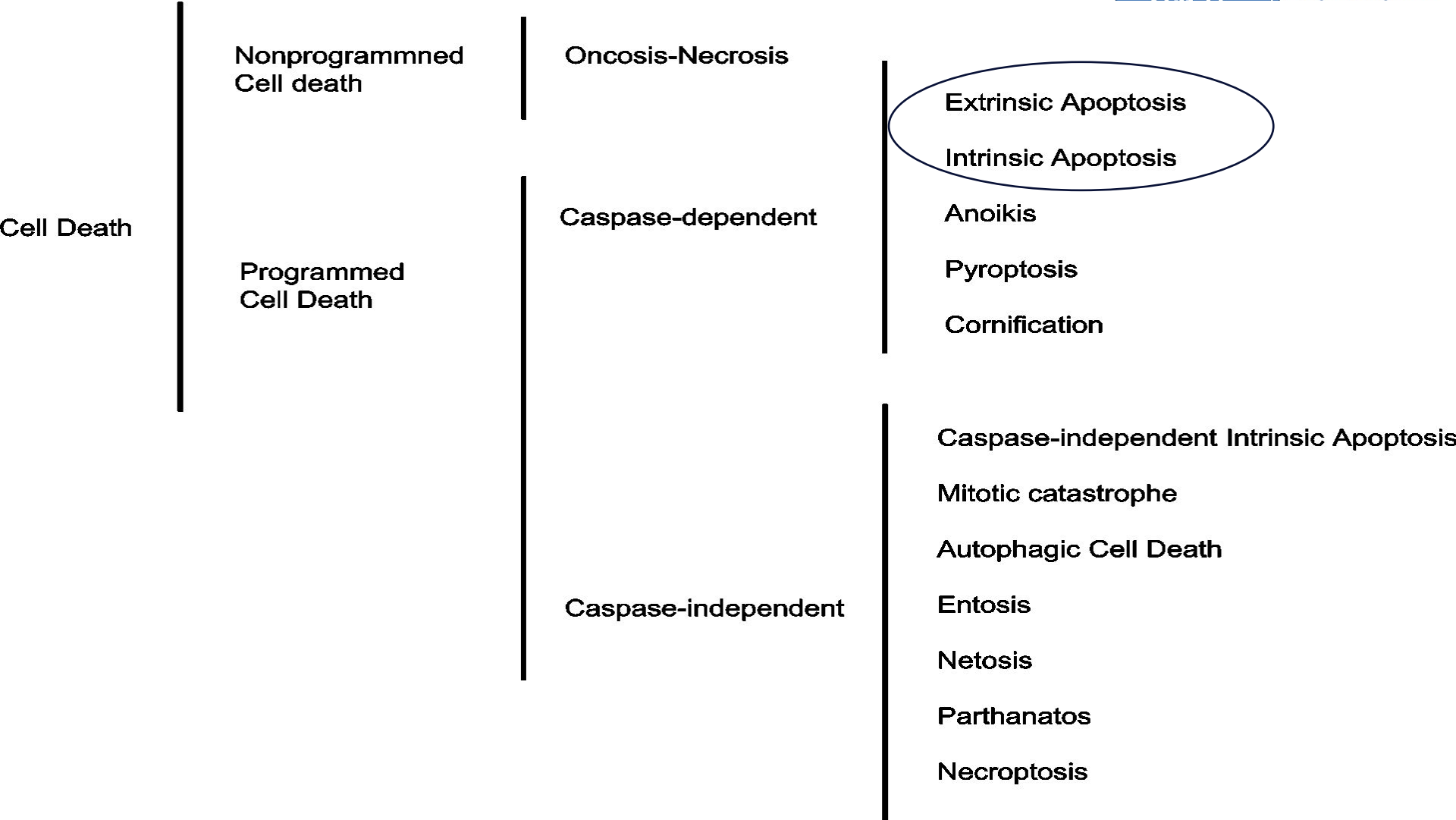


Therapeutic Effects of Human Mesenchymal Stem Cells on Traumatic Brain Injury in Rats:

Secretion of Neurotrophic Factors and Inhibition of Apoptosis

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Cell death



Cell death

Table1 | **Characteristics of different types of cell death**

Type of cell death	Morphological changes			Biochemical features	Common detection methods
	Nucleus	Cell membrane	Cytoplasm		
Apoptosis	Chromatin condensation; nuclear fragmentation; DNA laddering	Blebbing	Fragmentation (formation of apoptotic bodies)	Caspase-dependent	Electron microscopy; TUNEL staining; annexin staining; caspase-activity assays; DNA-fragmentation assays; detection of increased number of cells in subG1/G0; detection of changes in mitochondrial membrane potential
Autophagy	Partial chromatin condensation; no DNA laddering	Blebbing	Increased number of autophagic vesicles	Caspase-independent; increased lysosomal activity	Electron microscopy; protein-degradation assays; assays for marker-protein translocation to autophagic membranes; MDC staining
Mitotic catastrophe	Multiple micronuclei; nuclear fragmentation	–	–	Caspase-independent (at early stage) abnormal CDK1/cyclin B activation	Electron microscopy; assays for mitotic markers (MPM2); TUNEL staining
Necrosis	Clumping and random degradation of nuclear DNA	Swelling; rupture	Increased vacuolation; organelle degeneration; mitochondrial swelling	–	Electron microscopy; nuclear staining (usually negative); detection of inflammation and damage in surrounding tissues
Senescence	Distinct heterochromatic structure (senescence-associated heterochromatic foci)	–	Flattening and increased granularity	SA-β-gal activity	Electron microscopy; SA-β-gal staining; growth-arrest assays; assays for increased p53, INK4A and ARF levels (usually increased); assays for RB phosphorylation (usually hypophosphorylated); assays for metalloproteinase activity (usually upregulated)

CDK1, cyclin-dependent kinase 1; MDC, monodansylcadaverine; MPM2, mitotic phosphoprotein 2; SA-β-gal, senescence-associated β-galactosidase; RB, retinoblastoma protein.

Traumatic brain injury

Austria

- Incidence: 303/100,000/year
- Mortality rate: 11/100,000/year

- Occurrence highest in:
 - in male teens
 - female octogenarians

- leading cause of death in people aged 15–45 year globally

Symptoms of TBI

Mild TBI

- Fatigue
- Headaches
- Visual disturbances , Sensitivity to light and sounds
- Memory loss
- Poor attention/concentration
- Sleep disturbances
- Dizziness/loss of balance Irritability-emotional disturbances
- Feelings of depression
- Seizure
- Nausea
- Loss of smell

Symptoms of TBI

Severe TBI

- not understanding the spoken word (receptive aphasia)
- difficulty speaking and being understood (expressive aphasia)
- partial or total loss of vision
- weakness of eye muscles and double vision (diplopia)
- loss or diminished sense of smell (anosmia)
- Convulsions
- Physical paralysis/spasticity
- Chronic pain Control of bowel and bladder
- Loss of stamina Appetite changes
- Regulation of body temperature

Materials and Methods

6-8 weeks old Sprague Dawley rats

Group 1:

- Sham control group (craniotomy, without TBI)

Group 2:

- Placebo group (TBI with saline injection)

Group 3:

- Treatment group (TBI with hMSC injection)

Materials and Methods

First set of animals (8 per subgroup)

- Neurological assessment
- ELISA
- Immunohistochemistry
- Sacrifice on day 2,8,15 and 29 (total n=96)

Second set of animals (4 per subgroup)

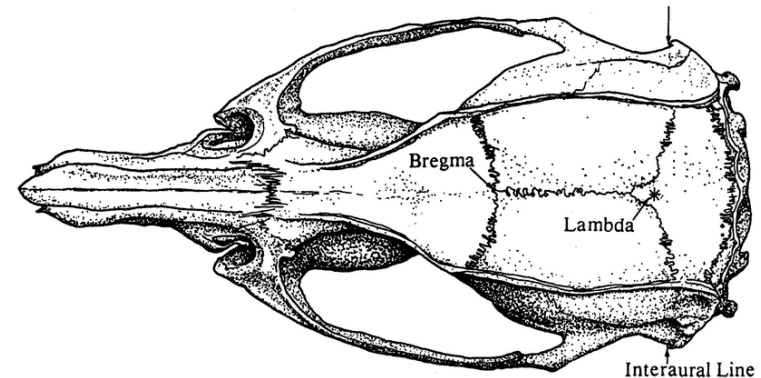
- Westernblot
- Sacrifice on day 2 and 8 (total n=24)

Experimental design

- Mononuclear cells from human bone marrow (posterior iliac crest)
- Differentiation in culture to human mesenchymal stem cells (hMSC)
- Intravenous application 24h after TBI

Traumatic brain injury

- Fixation in stereotaxic frame
- Drilling an 8mm hole
- Adjacent to central suture, between lambda and bregma
- Impacting right cortex with pneumatic piston
- 2mm of compression,
- Velocity of 3,2 m/sec



Methods

- Westernblot
- Immunoprecipitation
- Immunofluorescent staining
- Cleaved caspase 3 (apoptosis)
- Neurological severity score/ Behaviour

Modified neurological Severity Score

Raising the rat by the tail	3	Beam balance tests (normal = 0; maximum = 6)	6
1 = Flexion of forelimb		0 = Balances with steady posture	
1 = Flexion of hindlimb		1 = Grasps side of beam	
1 = Head moved >10° to the vertical axis within 30 s		2 = Hugs the beam and one limb falls down from the beam	
Walking on the floor (normal = 0; maximum = 3)	3	3 = Two limbs fall down from the beam or spins on the beam (>60 s)	
0 = Normal walk		4 = Attempts to balance on the beam but falls off (>40 s)	
1 = Inability to walk straight		5 = Attempts to balance on the beam but falls off (>20 s)	
2 = Circling toward the paretic side		6 = Falls off: no attempt to balance or hang on to the beam (<20 s)	
3 = Fall down to the paretic side		Reflexes absence and abnormal movements	4
Sensory tests	2	1 = Pinna reflex (a head shake when touching the auditory meatus)	
1 = Placing test (visual and tactile test)		1 = Corneal reflex (an eye blink when lightly touching the cornea with cotton)	
1 = Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)		1 = Startle reflex (a motor response to a brief noise from snapping a clipboard paper)	
		1 = Seizures, myoclonus, myodystony	
		Maximum points	18

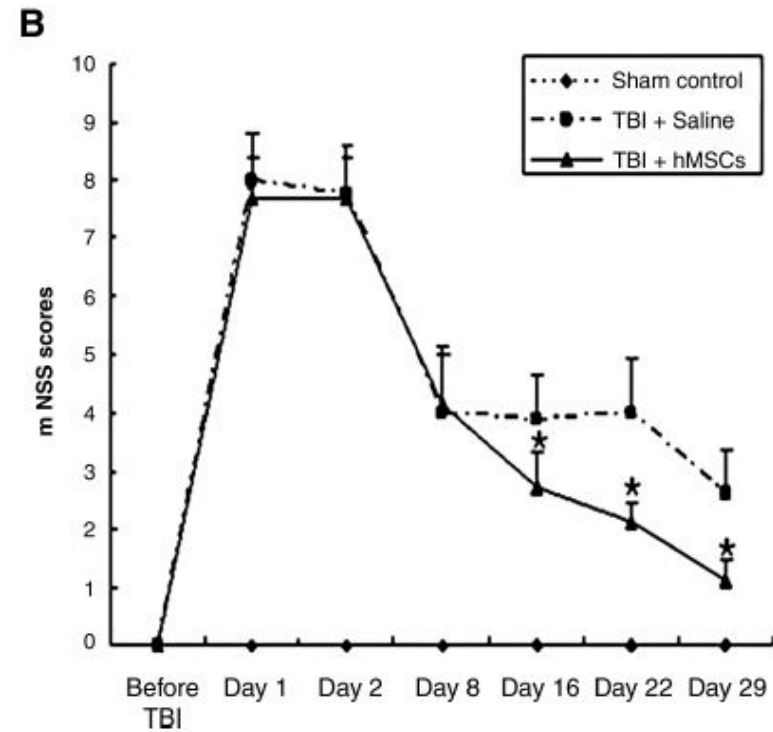
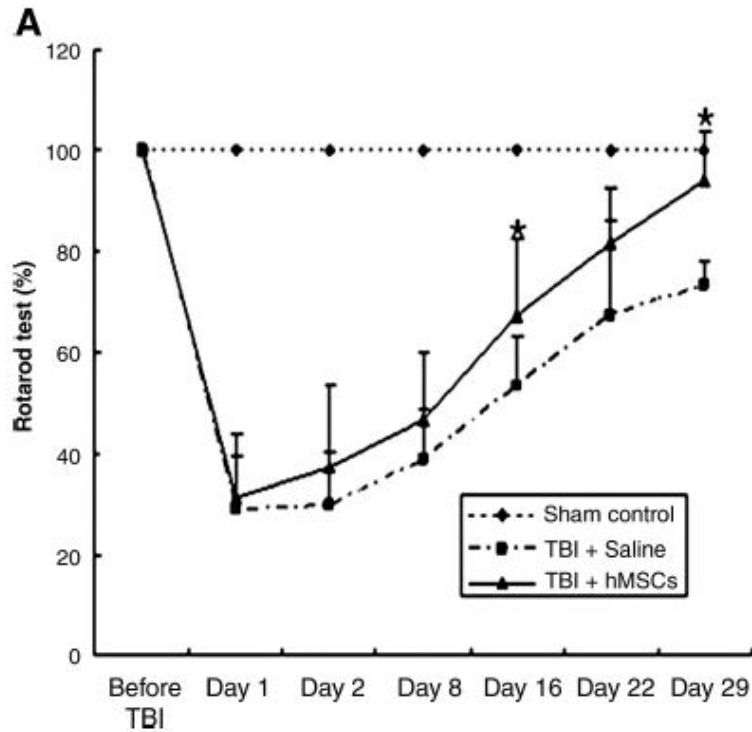
One point is awarded for the inability to perform the tasks or for the lack of a tested reflex.

13–18 = severe injury; 7–12 = moderate injury; 1–6 = mild injury.

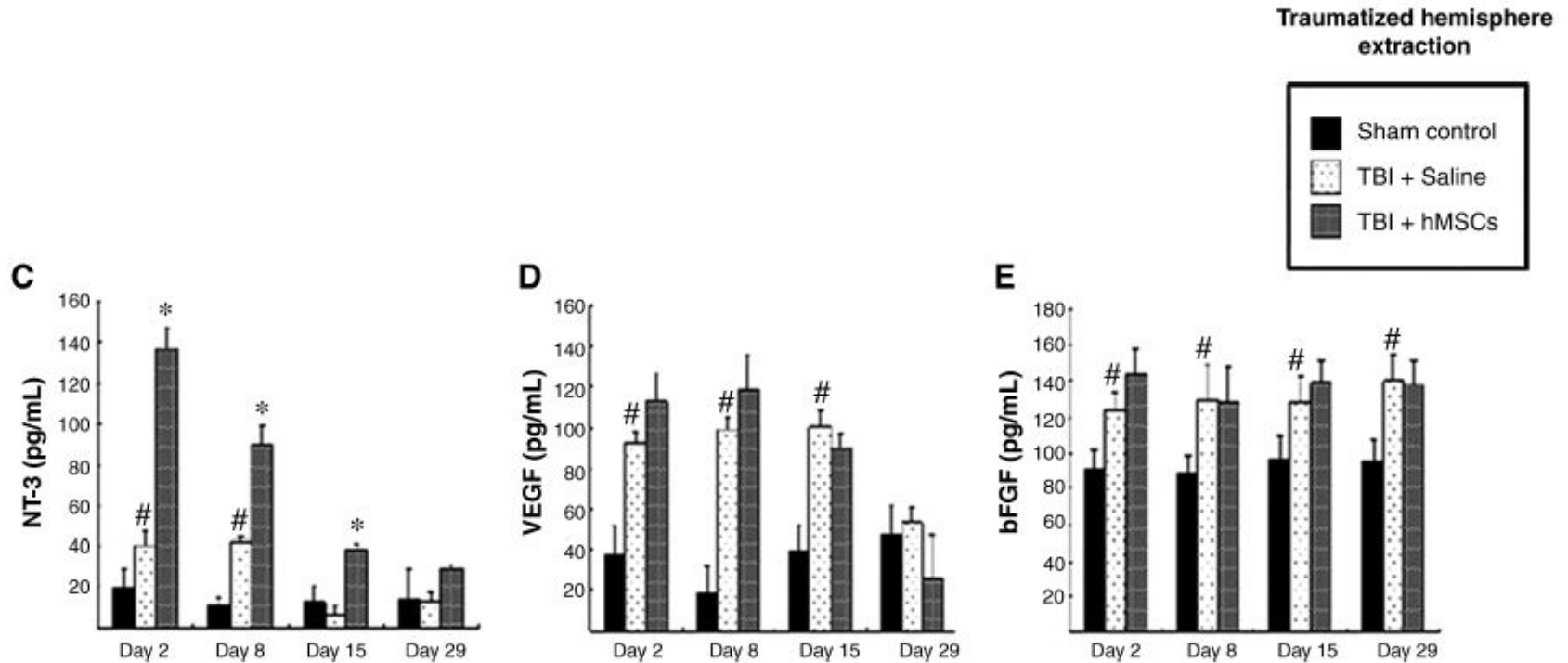
Rotarod motor test



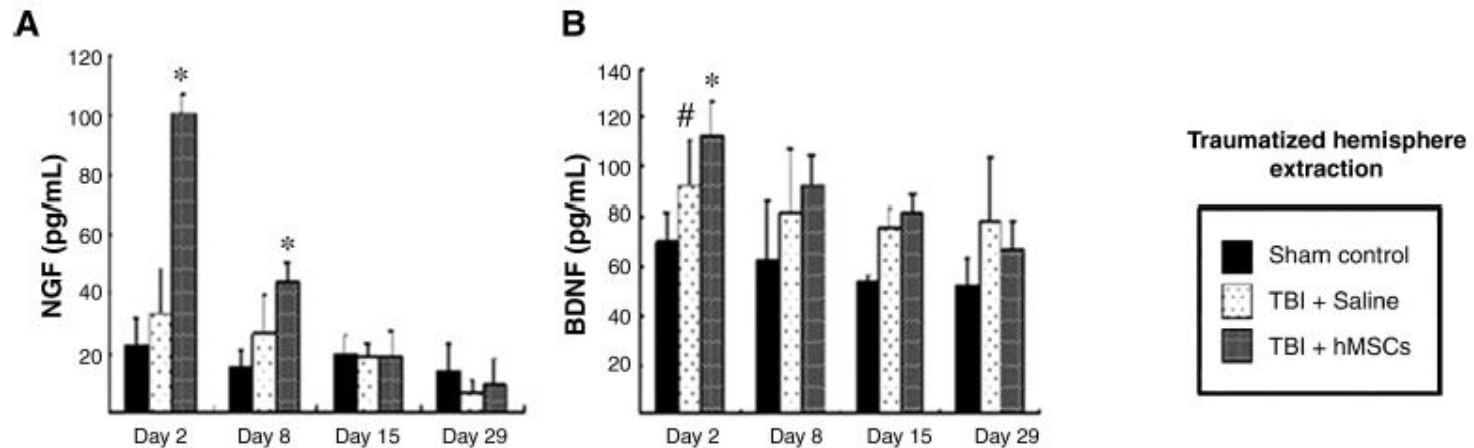
Results



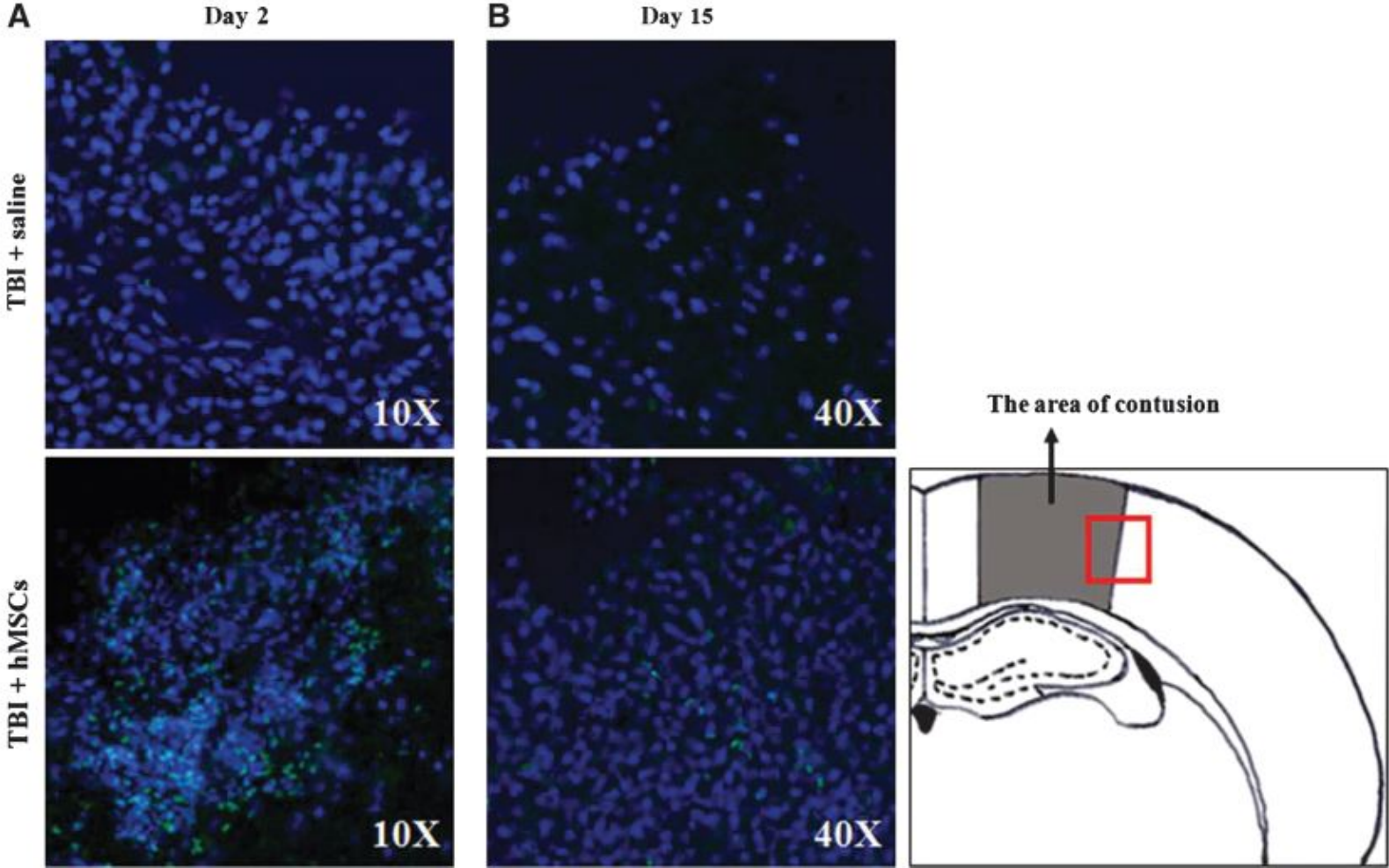
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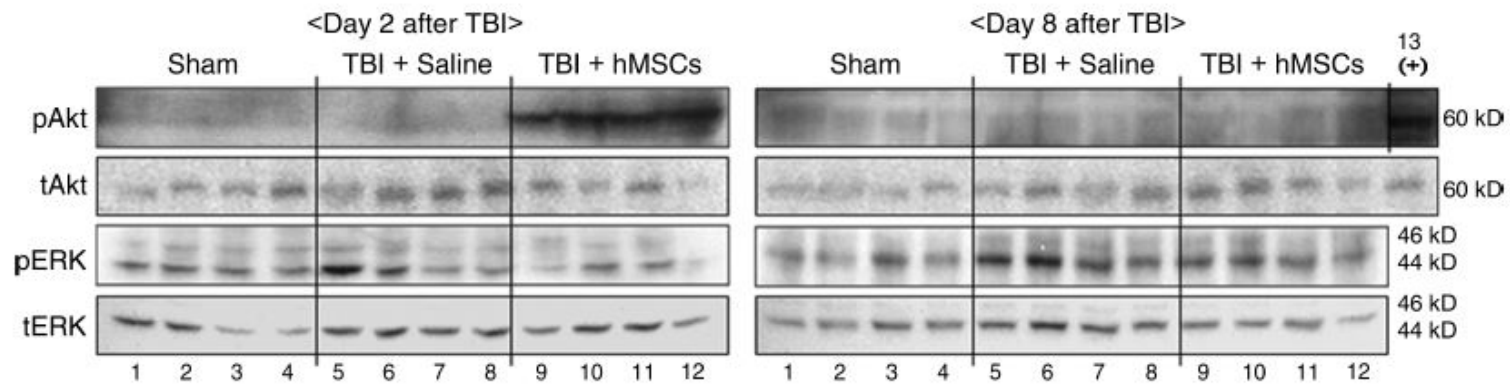
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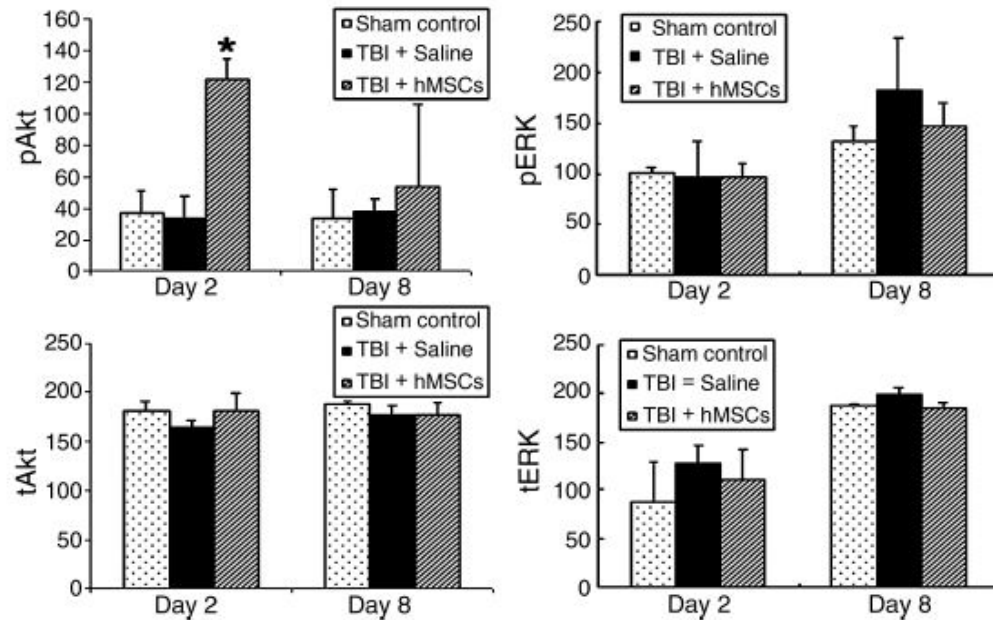
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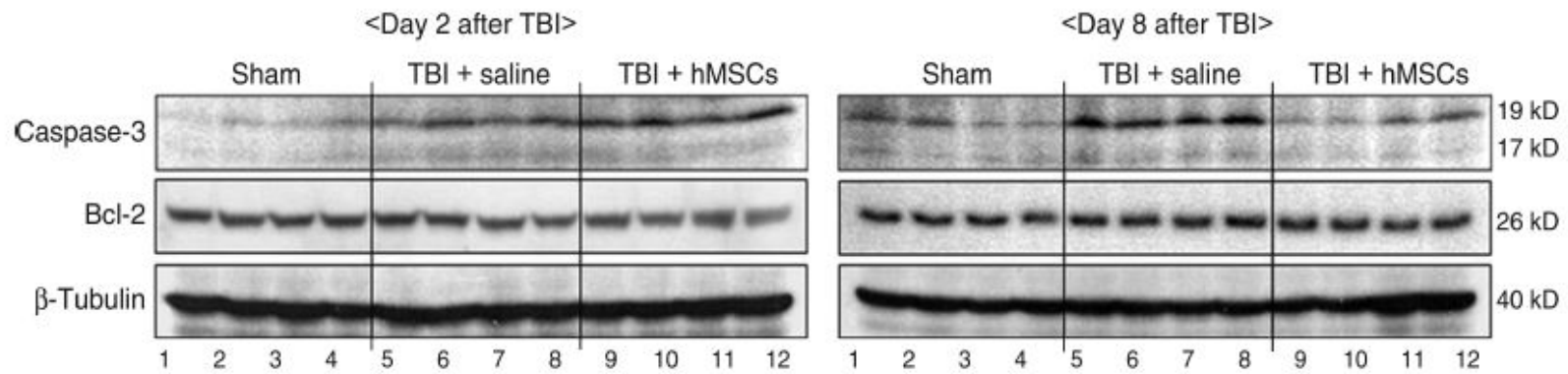
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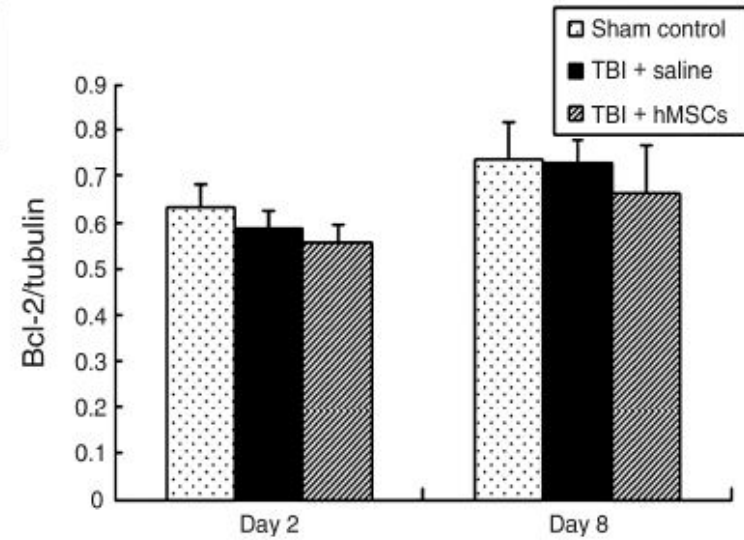
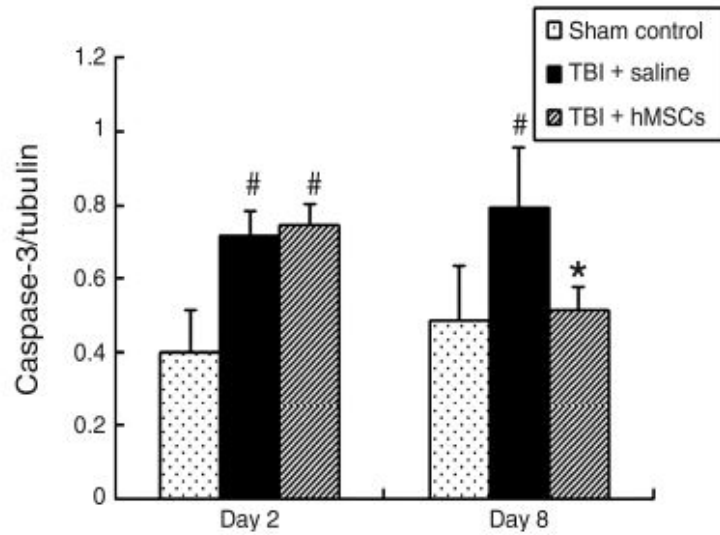
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Results



Results



Discussion

- hMSC reach the lesional area
- Surrogate effect on tissue regeneration not sufficient
- Activation of neurotrophic factors
- Amelioration of neurological function due to paracrine effect