Hypoxic Preconditioning Strategy for Stem Cell Transplantation Therapy after Ischemic Stroke

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• Stroke is a leading cause of human death;

• 700,000 new cases each year, 4.5 million stroke survivors in the US;

• Failure of clinical trials using neuroprotective drugs

• Regenerative medicine provides a promising hope for the treatment of ischemic stroke and other CNS disorders such as traumatic brain injury.
Regenerative Cell-based Therapy for Brain Repair

- Cell replacement
- Trophic supports

Promoting regenerative mechanisms (Neurogenesis and Angiogenesis)

Tissue Repair and Functional Recovery
Specific major issues in stem cell transplantation therapy

- Cell types and neuronal differentiation: functional neurons and multiple cell types, ethical concerns;
- Survival of transplanted cells in the injurious environment (ischemia, ROS, inflammatory factors, excitotoxicity, apoptotic insults, autophagy and so on);
- Cell delivery and homing to the lesion site;
- Integration/engraftment with host cells/tissues: guided neural network repair;
- Functional recovery;
- .....
Neuronal Differentiation of Human ES Cells and iPS Cells

Drury-Stewart et al., 2011; Song & Yu, 2012
Human neural precursor cells show poor survival following transplantation

  (21,200 – 52,800 / 400,000  5.3 – 13.2%)

  (few to innumerable / 500,000  >10 %)

  (25,919 ± 4,756 / 200,000  10.6 - 15.3%)

  (136,726 ± 23,515 / 500,000  22.6 – 32%)

- Capowski et al. 2007. *J Neurosci Methods*; 163:338-349. (1,500 / 300,000  0.5%)
New Approach for stem cell therapy: Hypoxic Preconditioning

- Exposure to sublethal hypoxia promotes the activation of an endogenous protective phenotype.
- Protective effects of hypoxic preconditioning have been shown in virtually all cells and multiple systems, including heart, brain, and many other organs.
Adaptation to hypoxia: HIF-1 activation

Brain ischemia
↓ Blood flow
Hypoxia
Hypoglycemia

HIF-1 binding sites

“O₂ Sensor”

Target genes

VEGF
EPO
ADM
NOSi
Glycolytic enzymes
GLUT-1

Angiogenesis
Vasodilation

Neurogenesis
↑ ATP

Protection, regeneration and functional recovery

↑ Blood flow

Cell survival
BNIP3, Others...

Apoptosis
Hypoxic preconditioning of transplanted cells

Neuronal differentiation of ES cells or iPS cells

Bone marrow mesenchymal stem cells

Hypoxic preconditioning with Sublethal low O₂ (0.5-1% O₂ X 8-12 hrs)

Stem cell transplantation (Systemic and local delivery)

1% O₂

4/4+ Differentiation

Serum Deprivation

Cell death, Western blot and other assays in vitro & in vivo

1 to 30 days after SD or transplantation

ES-NPC harvest

Transplantation

8 days
Dose-response Relationship of Hypoxia Exposure and Cytoprotection in vitro

Theus et al., Exp Neurol 2007
Hypoxic Preconditioning Enhances HIF-1alpha and Surviving Factors in Conditioned Cells

Hu et al.
Am J Physiol Cell Physiol. 2011
Hypoxic Preconditioning Enhances HIF-1alpha and Trophic/Angiogenic Factors in Conditioned Cells

Hu et al.
Am J Physiol Cell Physiol. 2011
Hypoxic Preconditioning Enhances Erythropoietin and its Receptor in Conditioned Cells
Hypoxic Preconditioning Enhanced Survival of Transplanted Cells in vivo

Control Cell vs HP Cell

Caspase-3 positive ES cells

TUNEL Positive ES Cells

ES cells-derived neurons

3 Day Post-Transplantation

Ratio Caspase-3/M2-M6 Positive Cells

% TUNEL Positive Cells

Ratio NeuN/M2-M6 Positive cells
Transplantation of Hypoxic Preconditioned Cells Promotes Angiogenesis in the Ischemic Brain

Hu et al.
Am J Physiol Cell Physiol. 2011
Transplantation of Hypoxic Preconditioned Cells Suppresses Inflammatory Activities in the Ischemic Brain

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Transplantation of Hypoxic Preconditioned Cells Suppresses Inflammatory Activities in the Ischemic Brain

Wei et al., Neurobiol Dis, 2012
Low Homing Rate of Transplanted Cell to the Brain after Systemic Cell Delivery

<1% of cells can reach to the ischemic brain
Directional Migration Guided by Chemoattractants

SDF-1

CXCR4: SDF-1 receptor
Hypoxic Preconditioning Enhanced Migration Factors

Wei et al., Cell Transp., 2013

SDF-1

A

Ischemic core

SVZ

CC

SDF-1

N-BMCS

H-BMCS

CXCR4

SDF-1

β-actin

F

Fold of expression (H-BMSC/N-BMSC)

* 2.00

1.50

1.00

0.50

0.00

SDF-1

CXCR4
Hypoxic Preconditioning Increased Homing of Transplanted BMSCs

Hu et al.
Am J Physiol Cell Physiol. 2011
Hypoxic Pre-conditioning Promotes Cell Migration to Ischemic Region

Wei et al., Cell Transplantation 2012
Repair of Thalamo-cortical Connections
Functional Recovery after Stroke and Transplantation of iPS-derived Neural Progenitors

Sticky Dot Test
Hypoxic preconditioning is an effective pre-treatment for increased tolerance of stem cells and neural progenitor cells both in vitro and after transplantation into the ischemic brain.

The increased expression of angiogenic factors in preconditioned cells can promote angiogenesis in the ischemic brain.

Hypoxic preconditioning increases the expression of migration factors such as SDF-1 and its receptor CXCR4, thus enhancing cell homing to the ischemic region.

Improved cell survival, differentiation and homing to the ischemic region will benefit clinical applications of cell transplantation therapy.
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