Stem Cells in Reproduction and Neurological Diseases

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LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

- State the current advancements in reproductive medicine together with stem cells.
- Summarize the types of stem cells and its clinical application for neurological diseases.

DISCLOSURE

- NOTHING TO DISCLOSE
Stem Cells in Reproductive Medicine

- Clinical Trials using Reproductive Organ-derived Stem Cells
  - Embryonic stem cells (20)
  - SCNT (somatic cell nuclear transfer) stem cells (1)
  - Fetal stem cells (11)
  - Cord blood stem cells (180)
  - Wharton’s jelly-derived stem cells (50)
  - Placenta-derived stem cells (90)
  - Endometrium-derived stem cells (5)
  - Spermatogonial stem cells (2)
  - Ovarian stem cells (controversial) (1)

* Numbers in parenthesis indicate numbers of clinical trials registered in clinicaltrials.gov

R&D Pipeline Overview by Indications

- Parkinson’s Disease
  - Fetal NPC
  - IIT: Currently Ongoing

- Central Palsy
  - Cord Blood
  - IIT: Completed

- Alzheimer’s Disease
  - eMNCs (Placenta)
  - Phase I/Ia: Currently ongoing

- Recurrent GBM
  - Autologous NK Cell
  - IITs currently ongoing

- Stroke
  - eCells (Cord)
  - Phase I/II: Completed

- Disc Degeneration
  - Adipose Stem Cell
  - IIT: Completed

- Cartilage Defect
  - eCells (Cord)
  - SIT: IND approved

- Intermittent Claudication
  - eCASs (Cord)
  - SIT: IND approved

- PLX-PAD (Placenta)
  - Global Phase II completed

- SMD / AMD
  - hESC RPE
  - Phase I completed for SMD
  - Phase I/IIa ongoing for AMD
  - IIT ongoing for SCNT AMD

- SMD / AMD
  - SCNT RPE

- Cerebral Palsy
  - Cord Blood
  - IIT: Completed

Clinical Applications of Embryonic Stem Cells & Somatic Cell Nuclear Transfer
Embryonic Stem Cells & SCNT-ESCs

<table>
<thead>
<tr>
<th>Embryonic Stem Cells</th>
<th>SCNT-Embryonic Stem Cells</th>
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</table>

hESC-Retinal Pigment Epithelial Cells:
Target diseases: Stargardt’s Disease, Dry Age-Related Macular Degeneration

Ongoing Clinical Trial
- March 2010: Granted Orphan Drug Status from FDA for Treatment of Stargardt’s Macular Dystrophy
- 2010 and 2011: Approval of Clinical Trial in USA and in Korea (Phase I / IIa)

Conventional ESCs

→ Establishment of optimal conditions for differentiating human ES cells into RPE

hESC-Derived Retinal Pigment Epithelial Cells:
Target diseases: Dry Age-Related Macular Degeneration, Stargardt’s Disease

Conventional ESCs

5x10^6 vs/100µl in BSS solution, 20 Gauge
Visual Acuity Changes of the Study Eye of Macular Degeneration Patients after hESC-RPE Injection

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</table>

*Visual acuity: stabilized or slightly improved and maintained, with no serious adverse events

→ Suggests that hESC-RPE is feasible therapy in treating MD patients

Song et al., STEM CELL REPORTS 2015

Preliminary Results From Transplantation of hESC-RPE into Dry AMD Patients

Dry AMD case

Formation of Pigmented Patches at Injection Site and Vision Persist until 30 month Post-Op

Pre-Op: 25 (20/320) → 30 month Post-Op: 39 (20/160)

BEFORE Injection

AFTER Injection

OCT: optical coherence tomography

Post-Op Photography and OCT (Optical coherence tomography) show pigmentations suggesting engrafted RPEs

→ The preliminary results of this study are promising in terms of safety and efficacy

Song et al., STEM CELL REPORTS 2015

First Demonstration of the Survival of Transplanted hES–Retinal Cells in Patients

Conventional ESCS

Best Corrected Visual Acuity

Baseline 25/200 (20/320) improved and maintained 10/10 (20/160) at 30mo.

Song et al., JAMA Ophthalmology. March 2017
Patient-Specific Somatic Cell Nuclear Transfer-Stem Cells (SCNT-SCs) vs. iPSCs
for overcoming immune rejection during cell replacement therapy

Human Somatic Cell Nuclear Transfer Using Adult Dermal Fibroblasts
Chung et al., Cell Stem Cells 2014

Human Somatic Cell Nuclear Transfer Using Adult Cells

Somatic Cell Nuclear Transfer–ES Cells (SCNT-ESCs)

CHA NT2
Derivation of SCNT-SC Lines and Characterization

• Expression of Stem Cell Markers, Karyotyping, and Nuclear DNA Genotyping using 16 SRT markers

Cytogenetic G-banding Analysis

Obstacles for application of the SCNT-SCs

• Low derivation efficiency
• Limited reprogramming in only specific oocytes

Chung et al., Cell Stem Cells 2014

Chung et al., Cell Stem Cells 2015
Collaboration with Yi Zhang Lab. (Harvard Univ.)

ZGA: Zygotic Genome Activation

Identification of Reprogramming Barriers by Comparing Human IVF- and SCNT-derived 8-cell Embryos

**A**

IVF

SCNT

Embryonic ICM

Skin fibroblast

Embryonic ICM

**ZGA**

Establishment of SCNT-Stem Cells using Patient Fibroblasts

RNA-Seq Analysis of IVF- and SCNT-derived 8-cell Human Embryos

**A**

**B**

**C**

Persisting H3 Lysine 9 Tri-Methylation (H3K9me3) Activity may block the Reprogramming in SCNT-derived Human Embryos (Reprogramming Resistant Regions (RRR) in Gene Expression)

mRNA Injection of KDM4A (a Histone Demethylase)

- Immunocytochemical Analysis of SCNT-derived human eggs
- Reduced Histone H3 Lysine 9 Tri-Methylation (H3K9me3)
mRNA Injection of KDM4A (a Histone Demethylase)

Chung et al., Cell Stem Cells, 2015

25 Blastocysts / 56 Oocytes = 26.8% / oocyte
8 Expanded blastocysts / 56 Oocytes = 14.3% / oocyte
4 SCNT-SC lines / 15 Blastocysts = 26.7% / blastocyst
4 SCNT-SC lines / 56 Oocytes = 7.1% / oocyte

Improvement of SCNT technology for better embryonic development

Frozen vs. Fresh oocytes

Closed mouse embryos using cryopreserved oocytes have shown increased apoptosis.

Lee AR, Hong KH et al., Stem Cell Reports, 2019

Supplementation of melatonin into culture medium increased embryonic development and implantation.

Melatonin confers beneficial effects on cell survival to SCNT-CROC, and reduced apoptosis and ROS production and enhanced development of the SCNT embryos using cryopreserved oocytes.

Lee AR, Hong KH et al., Stem Cell Reports, 2019
Our novel CPP (cell penetrating peptide)-delivery system efficiently delivered CARM1 protein into somatic cells and embryos, and regulated embryonic gene expression and development of cloned embryos.

Bang JI, Lee EH, et al., Scientific Reports, 2018

Recombinant CPP-conjugated Coactivator-associated arginine methyltransferase 1 (CARM1) protein

Improvement of SCNT technology for better embryonic development

Histone arginine methylation

Analysis of embryo development and implantation efficiency of cloned embryos treated with recombinant CPP-CARM1 protein showed increased blastocyst development and implantation rates compared to control group.

Bang JI, Lee EH, et al., Scientific Reports, 2018

Clinical Application of Somatic Cell Nuclear Transfer-Stem Cells
Clinical Application:
Production of Patient-Specific SCNT-RPE

Schematic View of Clinical Application of Patient-Specific SCNT

We established Patient-Specific SCNT-hESC lines from AMD patient’s skin fibroblasts. SCNT-hESC lines are currently registered at KCDC.

Clinical Application:
Production of Patient-Specific SCNT-RPE

Functional analysis of SCNT-PRE before clinical trial

ESC vs. SCNT-SCs

Morphological characterizations and function (polarization of Na+/K+‐ATPase, VEGF and PEDF secretion, and anti-oxidative stress) of SCNT-RPEs are very similar to those of ESC-RPEs.

Autologous-SCNT-RPE- Ongoing Clinical Trials
Target diseases: Age-Related Macular Degeneration

1. Establishment of autologous SCNT-ESC
2. RPE production
3. Characterization
4. Functional analysis
**Autologous-SCNT-RPE- Ongoing Clinical Trials**

**Target diseases: Age-Related Macular Degeneration**

**Approval of IND (IIT) in Korea (2016)**
One patient was transplanted (2017) and in follow-up stage

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**Clinical Application of Fetal Midbrain-Neural Precursor Cells (NPCs)**

New Method: Grafting Expanded Cells from ONE Embryo into Striatum
- Expanded Cells and their Safety have been successfully characterized

Traditional Method: Grafting Cells from 5-6 Embryos into Striatum
(NEJM. 344: 710-9, 2001)

- **Several Embryos**
  - Limited sources
  - Ethical issues
  - Heterogeneous NPCs
- **ONE Embryo to cure more than 1,600 patients**
  - Overcome Limited Sources
  - Reduce Ethical Issues
  - Have Homogeneous NPCs

**Human Fetal Midbrain-Derived Neural Precursor Cells for Treatment of Parkinson's Disease**

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Efficacy and safety of expanded fetal NPC cell lines have been successfully characterized for clinical trials.

Human Fetal Midbrain-Derived Neural Precursor Cells (FMD-NPCs) for Treatment of Parkinson’s Disease

First clinical trial in Parkinson’s disease using Human Fetal Mesencephalic Dopamine Neuronal Precursor Cells in a GMP-compliant, serum-free, long-term cultivation process

10 patients analyzed / 12 month follow-up (56-year-old women, Stage 3 UPDRS, Hoehn & Yahr Staging)

Significantly better performance in MTP has been found

MTP is a fine motor function using CAPSIT-PD test
Human FMD-NPCs
-Fetal Midbrain Derived-Neural Precursor Cells

Significantly better performance in PS, another fine motor function using CAPSIT-PD test

Pre-Op | Post-Op (1 year) | Post-Op (2 year)

PS: Pronation Supination

CAPSIT-PD: The Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease

Pre-Op 12-month Difference

UPDRS part.III (mid dose, drug off)

0
10
20
30
40

Low dose: 4*10^6 cells/250ul, 4 points
Middle dose: 12*10^6 cells/250ul, 4 points

MDS-UPDRS: Movement Disorder Society Unified Parkinson's Disease Rating Scale

Interim Analysis Following Transplantation of Human FMD-NPCs

10 patients analyzed, 12 months follow up
No serious or mild adverse events
Patients transplanted with the FMD-NPCs showed significant improvement in PS and MTP measuring fine motor functions using CAPSIT-PD test

Low Dose Group, n = 5
Middle Dose Group, n = 5

In submission

Interim Analysis Following Transplantation of Human FMD-NPCs

10 patients analyzed, 12 months follow up
MDS-UPDRS scores showed significant improvement of motor function 12 months after transplantation in the middle dose group
6 out of 10 patients showed significant improvement in UPDRS motor scores

In submission
- Radiotracer targeting dopamine transporter (DAT).
- Striatal [18F]FP-CIT uptake is correlated with DAT density.
- DAT is specific to dopaminergic neurons and best correlates with the density of dopaminergic neurons.
Human fetal mesencephalic dopamine neuronal precursor cells (FMD-NPCs) can be generated in large quantities through a good manufacturing practice-compliant and serum-free cultivation system.

Transplantations of human FMD-NPCs for the treatment of patients with PD showed favorable clinical outcomes with no AE during the 12-month follow-up period.

This study demonstrates the safety and efficacy of FMD-NPCs in patients with PD and

Provide for the first time that the therapeutic cell strategy with FMD-NPC for PD is dosage dependent.
Cerebral Palsy: Cord Blood Stem Cells

1. Preclinical Studies: Newborn Cerebral Palsy in a Rat Model with Improved Neurological Effects

Meier et al., Pediatric Research, 2006, IP infusion
Pimentel-Coelho PM et al., Stem cell Dev, 2009, IP infusion

<table>
<thead>
<tr>
<th>ID</th>
<th>Institution</th>
<th>State</th>
<th>Route</th>
<th>Start</th>
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<td></td>
<td>NCT01147653</td>
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2. Ongoing Studies in Other Sites

since Open-label Trial in 2009 (Auto & Allo) and Double-blind RCT in 2010 (Allo),CHA

Cord Blood Stem Cells

Min et al., Stem Cells, 2013

GMPM: Gross Motor Performance Measure
BSID-II, measure levels of Developmental Mental and Motor status

pUCB: Allogeneic UCB + rhEPO + Rehabilitation
EPO: Placebo UCB + rhEPO + Rehabilitation
Control: Placebo UCB + Placebo rhEPO + Rehabilitation

Motorscale
Mentalscale

Improvement in Motor Functions

An 11-month-old with mixed (spastic & dyskinetic) type Cerebral Palsy by Hypoxic Brain Injury, who had received Active Rehabilitation for More than 6 Months Before UCB Transplantation with Very Slow Functional Gain

UCB can be beneficial to improve the motor functions of children with CP.
Changes in Fiber Tractography
(All Fibers that Pass Through Mid Pons)

Robust growth of the nerve fiber through mid pons measured by Diffusion Tensor Imaging Tractography (DTI)

Changes in Fiber Tractography
(All Fibers that Pass Through Mid Pons)

2nd Clinical Trial on CP

Involvement of Immune Responses in the Efficacy of Cord Blood Cell Therapy for Cerebral Palsy

Pathomechanism in CP Brain
- Main Cause of CP is "Periventricular Leukomalacia"
- Augmented Inflammatory Response has been observed in Periventricular White Matter

Targetted Therapeutic Mechanism
- Reducing Inflammation in the Brain Tissue

2nd Clinical Trial on CP

Changes in Brain 
\(^{18}\text{F}-\text{FDG-PET} 2\) weeks after Transplantation

Red: Increased Activity
Blue: Decreased Activity
(P-value < 0.05)

Significant reduction in inflammation after UCB treatment in Periventricular White Matter
Combining Efficacy of Umbilical Cord Blood and Erythropoietin Therapy in Subacute Stroke Model of Rat
(invited for publication in Frontiers in Neurology)

The efficacy: UCB+EPO>>UCB>EPO>>Saline treatment

- Enhanced angiogenesis
- Decreased astrogliosis
- Enhanced neurogenesis

Combining Efficacy of Umbilical Cord Blood and Erythropoietin Therapy in Subacute Stroke Model of Rat

Conclusion in Cerebral Palsy Application

- Treatment with allogeneic UCB alone improved motor outcomes in children with CP.
- Assays of inflammatory markers in the blood indicated that innate immune responses potentially mediate the therapeutic efficacy of UCB.
- With the treatment with UCB combined with EPO, we found better efficacy in young children and with animal model we found that the mode of action is through more neurogenesis as well as with enhanced angiogenesis.

List of Clinical Trials with Cord Blood

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Title</th>
<th>Target Diseases</th>
<th>Interventions</th>
<th>Study Design</th>
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<td>NCT01639404</td>
<td>Umbilical Cord Blood Therapy for Children With Cerebral Palsy</td>
<td>Cerebral Palsy</td>
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<td>NCT02025972</td>
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<td>NCT03130816</td>
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<td>NCT02236065</td>
<td>Combination Therapy of Cord Blood and G-CSF for Patients With Brain Injury or Neurodegenerative Disorders</td>
<td>Brain Injury, Cerebral Palsy, Amyotrophic Lateral Sclerosis, Parkinson's Disease</td>
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<td>Open Label, Translational</td>
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