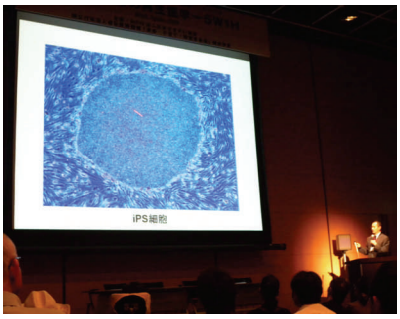
International Symposium

Regenerative Medicine for the Central Nervous System

Preliminary Reports



The international symposium, project "Walk Again in 2009" to support people with spinal cord injury, was held at the Akihabara Convention Hall in Tokyo on September 19, 2009. A total of 400 people participated in the symposium, and the proceedings were shown in real time in the second hall on the 5th floor.The details of this event will be included in the next issue of the Foundation News and in the reports to be published in December. (2009 subsidized project of the Welfare and Medical Service Agency/The Foundation Office is responsible for the wording. Titles omitted.)

Report I.

iPS Cells - Perspective and Challenge

by **Shinya Yamanaka** Professor at Kyoto University and Head of iPS Cell Research Center

Ten years ago I had a lab of my own for the first time just after taking up a new position at the Nara Institute of Science and Technology. Our research objective was to improve embryonic stem cells, and we succeeded in generating mouse iPS cells in 2006.

As our research was targeted at realizing effective human treatment, we strived to generate human iPS cells. The results of our study on human embryonic stem cells were more successful than expected, and in only a year or so, we successfully generated human iPS cells in 2007. Professor Thomson, of Wisconsin University, announced the induction of iPS cells on the same day we did. Subsequently human iPS cells have been generated in various parts of the world.

○ *Establishing a method for creating safe and effective iPS cells in a year or two*

We know now that human iPS cells can be created from various cells such as those of blood, human hair, stomach, and liver. As announced jointly with Professor Okano of Keio University a few days earlier, mouse experiments had revealed that the safety of iPS cells varies significantly depending on from which type of cells they are created. We aim to arrive at a determination within a year or two as to which cells and methods should be used to generate safe human iPS cells.

○ *Aiming to set up iPS cell banks within five years*

HLA (human leukocyte antigen) has scores of thousands of types. However, if iPS cell banks were created in Japan containing samples from 50 persons, each with a specific homologous HLA type, these cell banks would cover 90 % of the Japanese population, thereby lowering the number of rejection episodes in transplantation.

Our current goal is not to realize regenerative medicine in five years but to generate iPS cells within five years that can be used for regenerative medicine.

○ *Method close at hand for evaluating toxicity and side effects*

A method close at hand is to use iPS cells "in vitro" for researching and developing new drugs. Considering motor neuron disease (which weakens muscles), researchers and drug developers could make great progress in developing new therapies and drugs by using iPS cells grown from the patient's own cells.

Report 2.

Embryonic Stem Cells (ESC) in Transplantation-based Spinal Cord Injury Therapy

by **Hans Keirstead, Ph. D** Associate Professor, Reeve-Irvine Research Institute

I would say now is a happy time for us, as our efforts over the past few years have really come to fruition. Therefore now I would like to continue research in the field of spinal cord injury therapy, and in particular go forward the treatment of chronic spinal cord injuries (SCI).

Clinical trials on spinal cord injuries are being conducted individually for acute SCI, sub-acute SCI and chronic SCI.

○ *Phase III clinical trials for acute SCI have started!*

In 2009, Bristol-Myers Squibb, a multinational medical corporation, started the Phase III (final phase) clinical trials for several hundreds patients who have acute SCI. This trial aims to block the inflammatory response that appears within a few hours of sustaining a SCI. In the trial, we were in charge of pre-clinical trials and trials on animal subjects. When the Phase III trial is completed, it will be used to treat pa-

tients with acute SCI.

○ ***Phase I clinical trials for acute SCI by Geron.***

Clinical trials involving the transplant of oligodendrocyte progenitor cells originating from ESC were approved by the FDA this year. In this trial, we injected the cells into injured parts of sub-acute thoracic SCI patients who got injured within 10 days of the injury. The aim was to protect nerve pathways. The trial was later postponed due to a minute amount of pollutant in cell rots. However it was not found to be harmful and was removed. We expect to resume the trial imminently.

○ ***Research on the treatment of acute cervical vertebrae injury.***

I was disappointed to hear that FDA had not approved Geron to include cervical vertebrae injury in its clinical trials. However we have been researching cervical vertebrae injuries, here are the results.

We injected high-grade oligodendrocyte into the cervical vertebrae of animal test subjects and confirmed the survival of the animals.

As for the results of functional experiments, the injuries of animal subjects (mice) were recovered just as in cases of thoracic SCI. Especially note was that cervical vertebrae tissues were maintained with the advantage was that oligodendrocyte was not lost. The number of axons in transplanted in part of the central nervous system was more than other parts. The amount of gray matter was also maintained. Tissue was also maintained notably in the parts composed of motor neurons.

○ ***We succeeded in creating motor neuron progenitor cells***

Recently, we succeeded in differentiation of motor neuron progenitor cells with a purity of 98% or higher. These cells can be used in human. We can produce about 1 billion of those cells per week. After a SCI, motor neuron disappears but these progenitor cells can substitute for the motor neurons.

The problem was whether these motor neurons can contract or not. While we found some functionality in the connections between muscles and nerves, motor neuron progenitor cells cannot be passaged. Therefore we will continue the research.

○ ***Research on treatment for chronic SCI.***

The human fetus contains a growth factor called GDNF (glial cell derived neurotrophic factor). Motor neurons are pulled toward muscles by this factor. But GDNF disappears from the human body after birth. We attempted to generate GDNF in the muscles of some patients.

We sampled a small amount of tissue from a rat and multiplied it. Then we introduced GDNF into the muscle using the third-generation lentivirus.

We conducted an experiment on a chronic SCI subject injured one-and-a-half years ago. We prepared a mouse by paralyzing its forefeet, and then injected GDNF into the upper part of the injury. The upper part recovered but the lower part remained to be paralyzed. After six weeks, we found that secretions of GDNF increased significantly. We hope to develop this further in clinical trials.

We submitted an application of over ten thousand pages to the FDA. We are aiming to have clinical trials at first with Type I Infantile SMA, then apply it to ALS and finally to chronic SCI clinical trials.

Table 1. Developing Treatments for Spinal Cord Injury and Disease (H. Keirstead)

Acute SCI (hours to days)	Sub-acute SCI (days to weeks)	Chronic SCI (weeks to decades)
↑	↑	↑
Antibody treatment	ESC-OPC* treatment	ESC-**MNP treatment
Phase III	Phase I	pre clinical trials
Clinical trials	Clinical trials	
begin in 2009	begin in 2009	California Stem Cell
Bristol-Myers Squibb	Geron	1. Type I Infantile SMA
		2. Terminal ALS
		3. Chronic SCI

*OPC, oligodendrocyte progenitor cells ** MNP, motor neuron progenitor cells

Report 3.

Induced pluripotent stem cells from a spinal muscular atrophy patient

by **Allison Ebert, Ph. D** University of Wisconsin – Madison Assistant Scientist, Stem Cell and Regenerative Medicine Center

Pillars of stem cell research conducted at our lab

1. Modeling of neurodegenerative diseases: spinal muscular atrophy (SMA), Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Huntington's disease
2. Development of therapeutic treatments : drug screening and cell transplantation

○ ***Neurodegenerative disease modeling***

Recent advances enable the generation of iSP cells by using cells from patients with a variety of diseases: ALS, SMA, dysautonomia, Parkinson's disease, and diabetes.

In the case of ALS, iPS cells have been generated from cells derived from two patients with ALS, sisters in their eighties. This is a promising technique for generating iPS cells. Implemented to initialize somatic cells, it enbales even the cells taken from the skin of octogenarians to generate cells that are exactly like embryonic stem cells.

SMA

A person has two types of genes SMN-1 and SMN-2. In a person with SMA, the SMN-1 genes in particular are lost, thereby making It impossible to form SMN-1 protein. This especially affects spinal cord neurons, leading to muscle atrophy, paralysis, and death. SMA Type 1 is a serious disease that occurs in infants about six months after birth, causing death at ages 1 to 3.

○ ***Inducing SMA cells from iPS cells of the patient***

We obtained iPS cell colonies from fibroblast cells derived from normal skin cells from a mother and from her 3-year-old son, who had SMA Type 1. Both the cells from the mother and the mother-derived iPS cells had full-length SMA-1 protein. But the fibroblast cells from the skin of the son and the iPS cells generated from them were missing SMN-1 genes.

We cultured the iPS cells to form iPS spheres, from which neural progenitor cells were induced. By further inducing neural progenitor cells to produce mature motor neurons, we generated SMA-affected cells.

At an early stage, there was no difference in total motor neuron production between iPS cells from the mother and her son. The iPS cells remained unchanged in number even seven weeks later. However, when the culture time was lengthened, the number of motor neurons in SMA iPS cells decreased substantially, and neurons became smaller. SMA-affected cells apparently have factors that reduce the number and size of motor neurons, but this was not confirmed.

This reduction might be due to adverse effects of active oxygen, culture time, orsecretory activity. We actually observed mitochondrial dysfunction and generation of incomplete motor neurons .

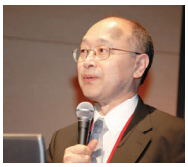
○ ***Development of novel drug compound: 007***

We developed drug compound 007, and, after adding it to iPS SMA cells, observed gems (protein spheres) increasing drastically in number within a few days. The interesting thing about 007 is that owing to this addition, motor neurons in mouse models of SMA have increased in number and lived longer.

Thus iPS cells can be generated directly from patients and, with their genetic specificity retained, can contribute to identifying disease mechanisms.

Report 4.

What we can do for ALS patients



by **Yasuto Itoyama** Professor, Department of Neurology, Tohoku University School of Medicine

ALS (amyotrophic lateral sclerosis) is the most severe of all the major diseases in the field of neurology. The characteristics of ALS are 1) selective motor neuron death (while other neurons remain intact), 2) progressive general muscular atrophy, and 3) a hopeless therapeutic situation.

While there are no reliable treatments for ALS, the “Miyagi prefectural association of medical network systems for intractable disease” was established as a community support system. It integrates the medical profession, patients, and administration and is the model used for the nationwide network being launched by the Ministry of Health, Labor and Welfare.

Our ALS Therapy Development History

1993: Detected mutant SOD1 gene in familial ALS.
2001: Developed ALS rat model.
2007: Validated HGF protein treatment using ALS rat model.
2008: Confirmed safety of intrathecal administration of HGF to primates.
2010–11: Aiming at clinical trial of administering HGF to ALS patients.

○ ***ALS Therapy Development History***

ALS Pathogenesis:

Although there are many etiologies of ALS, we focused on the Cu/Zn superoxide dismutase (SOD1). Our latest research suggests that in ALS, SOD1 accumulated in the neurons impairs cell key function, resulting in apoptosis (programmed cell death)., orsubstances produced by neighboring glia cells injure the motor neurons .

Model Animal: We have developed an ALS rat model 20 times larger than the mouse one. When human SOD1 was injected intraspinally in the ALS rat model, ALS was developed approximately 70 days after injection and the model died approximately 70 days later.

HGF: The HGF growth factor was discovered by Toshikazu Nakamura, Ph.D., at Osaka University. HGF is widely expressed in the nerve system and plays a role, for example, in the regeneration of hepatocytes and the proliferation of endothelial cells. It also affects the antifibrosis function and other important biological functions, particularly the anti-apoptotic and neurotrophic functions. Basic experiments proved that the neurotrophic function is effective in the protection of motor neurons.

Intrathecal Administration of HGF in ALS Rats: ALS rat models showed motor neuron reduction 99 days after birth, developed ALS 123 days, and died 140 days. Administration of HGF to rats from the onset of ALS extended their lifespan by 63%. This corresponds to extending human life by about two years.

Challenges in Planning for Clinical Trial: Humanized recombinant HGF formulation satisfying the Good Manufacturing Practice (GMP) standards is produced by Kringle Pharma, Inc. Safety tests of HGF intrathecal treatment using small primates, marmosets, conducted jointly with Keio University, confirmed its safety and effectiveness. A marmoset costs about 4 million yen, so the testing using ten marmosets was expensive. Safety tests satisfying Good Laboratory Practices (GLP) standards and using crab-eating monkeys are in the planning stage.

The most difficult stage, the so-called "valley of death", is the fund-raising stage, and it still must be cleared. However, we would like to overcome the challenges somehow.

Report 5.

Regenerative Therapy for Spinal Cord Injuries– Reviews of Recent Studies and Future Prospects –

by **Hideyuki Okano** Professor, Department of Physiology, School of Medicine, Keio University

In spinal cord injuries (SCI), it is essential to develop the new treatments in consideration of the posttraumatic damage progress with the time after injury.

Goals to Achieve Functional Recovery after Central Nerve System Damage

Therapeutic Targets or Clinical Goals	Timeline for Human Application after SCI	Selected Underlying Biological Mechanisms
Protection	First few weeks	Edema Secondary cell death Inflammation Immune system responses
Repair	Weeks to months	Angiogenesis Myelination Astrocyte responses
Regeneration*	Weeks to years	Cell transplants Biocompatible substrates Axonal outgrowth
Recovery	Weeks to years	Axonal sprouting Synaptic plasticity

*Sometimes grouped with Repair

○ *Regenerative Therapy for SCI – Our Study*

Acute stage: Injecting anti-IL6 antibody into SCI mouse models to suppress the inflammatory reaction was reported to be a remarkably effective treatment (approved as an antirheumatic drug in 2008 by Chugai Pharmaceutical Co., Ltd.).

HGF is being aimed for mutual safety trials in SCI by Keio University and ALS by Tohoku University. We are also aiming for clinical trials in HGF for SCI within 2 years.

Sub-acute stage: The ideal stage for transplanting neural stem cells. Seikagaku Corporation is developing chondroitinase ABC to enzymologically destroy glial scar which is formed at this stage.

Dainippon Sumitomo Pharma Co., Ltd is developing a semaphorin 3A inhibitor to induce axonal regeneration. We reported in *Nature Medicine* about 3 years ago that administering semaphorin 3A to rats with completely a ruptured spine resulted in the growth of axons and improvement in motor function of the limbs. We are aiming at bringing this therapy to the clinical stage.

The next step is to transplant the substance into primate SCI models.

○ *SCI: Other Past Phase II Trials*

To date, no global regulatory approval has been made. No statistically significant difference was found with Sygen (GM-1 ganglioside). GK-11 (NMDA antagonist) was tested as a neuroprotective treatment at the acute stage (the primary outcome might be set too high). 4-aminopyridine (a K^+ channel-blocker) was tested to improve conduction along surviving axons at the chronic stage. No significant difference was found in it. Procord (transplant of autologous activated macrophages) was tested as a neuroprotective and/or regenerative therapy at the acute stage (no benefit compared with the control groups). Upon reviewing the evaluation methods, these studies might show significant differences compared with the control groups.

○ *Transplantation of iPS-derived Neural Stem/Progenitor Cells*

Neural stem/progenitor cells derived from human iPS cells were transplanted into SCI mouse models 9 days after the injury. The effectiveness was observed after transplanting neural stem/progenitor cells into the injured lesion of the paralysed animals. The control showed

no recover of hind limb paralysis at 49 days after the injury. However, the SCI mouse models with transplanted neural stem/progenitor cells derived from iPS cells recovered their motor function to walk around by putting weight on the limbs (April 2008).

The major concern for the application of iPS cells is neoplastic development, however, safe non-tumourigenic iPS cells can be prepared in advance.

We are continuing the study with the aim of making iPS cells derived treatments available in the clinical application in about 5 years.

○ *Early Phase Clinical Trials on SCI Abroad*

- Lithium - Phase I/IIa: Often used as an affordable drug for bipolar disorders, and now suggested to provide neuroprotection and/or promote functional repair after acute SCI. (W. Yang et al, People's Republic of China)
- Riluzol - Phase I/IIa: Has an anti-apoptotic effect, and currently expected to be effective as a neuroprotector for acute SCI. (Canada and USA)
- Minocycline - Phase IIb: Used as an anti-apoptotic therapy for acute SCI (within 12 hours). Phase I/IIa trials showed promise in a small (50 patients) study. (Trial location: Canada)
- C3 Rho inhibitor (Cethrin) - Phase IIb: Used as a neuroprotective and/or axonal sprouting/regenerative therapy. Rho is small protein mediating axonal regenerative inhibitory action. Administering Cethrin that inhibits the Rho action showed some functional recovery in 37 patients at Phase I/IIa trials (no control groups observed). Phase IIb is awaiting funding. (Canada and USA)
- Humanised anti-NOGO antibody against CNS myelin (ATT-355) – Phase II: Used as a neuroprotective and/or axonal sprouting and/or therapy for acute SCI (within 14 days of injury). Phase I study has showed safety. (Europe)
- OEG (olfactory ensheathing glia) autografting: Used as a substrate for axonal sprouting and/or regeneration after SCI. The medical activities performed in China are for profit, and are therefore not regarded as clinical trials. In Phase I/IIa trials involving 6 chronic SCI subjects, 3 of them showed safety in the autografting but with little efficacy. (Trial location: Australia)
- Human embryonic stem cell oligodendrocyte progenitor cells –Phase I: Conducted by Geron Corporation. (Refer the report by H. Keirstead.)
- Autologous bone marrow (stromal) cell infusion transplant – Phase I/II: Used as a substrate for neuroregeneration and/or neuroprotection at the acute stage. (Czech Republic, Brazil and India)

○ *The Importance of Regenerative Medicine in Connection with Rehabilitation Training*

In animal models, the groups which started rehabilitation training in early stages demonstrated a significant difference in improvement. Combining cell therapy with rehabilitation training can achieve efficacy. It is considered that rehabilitation training induces axonal sprouting from undamaged neurons; the regenerated axons can then develop signalling pathways among neurons at proximal and distal sites by-passing the injured sites. What is developed with cell transplant can also occur with rehabilitation training.

Rehabilitation training for the sub-acute and the chronic stages includes treadmill use, restraint therapy of non-hemiplegic upper limbs, LOKOMAT (automated robotic walking trainer) and BMI (brain-machine interface).

〔Translator〕**The Japanese Red Cross Language Service Volunteers**; Susumu SHINTANI, Kaoru MAMIYA, Maki UEDA, Mariko KUWABARA