의학석사 학위논문

Study of cell therapy for ALS using neuronal stem cells from iPSCs with ectopic SOX2 expression

SOX2 과발현된 역분화줄기세포로부터 유도된 신경줄기세포를 사용한 근육위축가쪽경화증에서의 세포 치료 연구

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서울대학교 대학원
의과학과 의과학전공
김 혜 민

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by
Hye Min Kim

A thesis submitted to the Department of Biomedical Sciences in partial fulfillment of the requirements for the Degree of Master of Science in Medicine at Seoul National University College of Medicine

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2015 년 6 월

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위 원 _____ (인)

ABSTRACT

Study of cell therapy for ALS using neuronal stem cells from iPSCs with ectopic SOX2 expression

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Amyotrophic Lateral Sclerosis (ALS), a regressive neuronal disease, results in the death of motor neurons. It is known as a deadly disease and most ALS patients die within five years of diagnosis due to its rapid progression, however, there are few treatments available for ALS. In order to attempt to develop a cell therapy system for the treatment of ALS, here, we use neuronal stem cells (NSCs) overexpressing the sex determining region Y-box 2 (SOX2). NSCs are differentiated from induced pluripotent stem cells (iPSCs), in particular, SOX2 is related to the differentiation into a neuronal lineage. Therefore, we focus on the overexpression of SOX2 in relation to the potential of NSCs to differentiate into motor neurons, and

their effect on ALS in a mouse model.

NSCs contain an inducible system to overexpress SOX2, which can be controlled by the use of doxycycline, therefore we gauged the differentiation potential by the level of SOX2 expression. According to quantitative RT-PCR and immunocytochemistry, motor neurons were more differentiated with increasing levels of doxycycline.

To evaluate the functional activity of differentiated NSCs, they were transplanted into the ALS Cu/Zn superoxide dismutase 1 (SOD1) G93A transgenic mouse model (SOD1 G93A TG). The NSCs were injected at the junction between the brain and spinal cord, engrafting and surviving successfully, and further treated with doxycycline. A greater differentiation into motor neurons was expected in the group of treated mice, however the NSCs were differentiated into various other subtypes of neurons and glial cells, including astrocytes, after migration from the injection site to other areas of the brain and spinal cord. For the functional analysis of transplanted NSCs, we tested the movement and life span of SOD1 G93A TG mice engrafted with NSCs.

Compared with the untreated control mice, the motion of the

mice transplanted with NSCs was not improved, and their life span was not significantly extended. However, the NSCs prolonged the disease onset point. As the number of remaining cells reduced until death, the effectiveness of the NSCs was not carried over to the end of disease progression. With further experiments, considering the number of injection times and cells, the lifespan and motor function will likely be improved.

Therefore, the NSCs that overexpressed SOX2 were well differentiated into motor neurons, although the ALS mouse did show extensive neural differentiation of differing types. In addition, NSCs were effective in slowing the ALS disease onset.

Keywords: Amyotrophic lateral sclerosis, differentiation, induced pluripotent stem cells, SOX2, transplantation.

Student number: 2012-23666.

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LIST OF ABBREVIATIONS

ALS : Amyotrophic laterel sclerosis

BSA : Bovine serum albumin

DAPI : 4', 6-diamidino-2-phenylindole

Dox : Doxycycline

GFAP : Glial fbrillary acidic protein

iPSCs : Induced pluripotent stem cells

ICC : Immunocytochemistry

IHC : Immunohistochemistry

NPCs : Neural precursor cells

NSCs : Neural stem cells

PBS : Phosphate buffered saline

PFA : Paraformaldehyde

SOD1 : Cu/Zn superoxide dismutase 1

SOX2 : SRY (sex determining region Y)-box 2

TBS : Tris buffered saline

TLX : Tailless

INTRODUCTION

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a fatal disease in which motor neurons die exclusively in the spinal cord and brain. It usually occurs between the ages of 45 and 60 years old, and results in muscle weakness and atrophy. Due to progressive neuronal death, the vast majority of patients die from respiratory muscle paralysis within five years (1). There exist two types of ALS disease: familial ALS and sporadic ALS. Familial ALS occupies only roughly 10 % of the onset rate, including mutation of Cu/Zn superoxide dismutase 1 (SOD1), tar DNA binding protein 43 (TDP43), RNA binding protein fused in Sarcoma/Translocated in Sarcoma (FUS/TLS). The cause of sporadic ALS is not known, and to date, there is no cure for this disease.

Following extensive study of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), it has emerged that stem cell transplantation could be a potential treatment for ALS. ESCs are derived from the inner cell mass of blastocysts and have pluripotency and the ability of self-renewal and

differentiation into other types of cells (2, 3). Human iPSCs are reprogrammed by transcription factors (Oct4, SOX2, Klf4, c-Myc) from fibroblasts (4, 5), and have pluripotency and self-renewal ability equal to that of ESCs (6). Human iPSCs are reprogrammed from their own fibroblasts, thus, there is no immune rejection. Despite the fact that there is no immune rejection, delivery of reprogramming factors by lentiviral vectors is a limitation of iPSC as a source for cell therapy (7).

Before stem cell based therapy was applied in humans, it was tested in animal disease models. Various stem cells have been used in an ALS disease mouse model, showing that stem cell injection has therapeutic effects (8, 9, 10). Moreover, stem cells that were differentiated into other lineages have also been used in disease animal models. In particular, due to the fact that ALS is a neurodegenerative disease, we use neural stem cells (NSCs) in the present study. Wright *et al.,* studied NSCs isolated from human fetus and adult tissue, and Hermann *et al.,* suggested the potential of neural differentiation (11, 12).

In recent studies, researchers transplanted neural stem cells into the spinal cord of an ALS patient by cervical microinjection (13), and suggested that it has a protective effect and improved

safety. However, there exist no reports regarding the effects of iPSCs, overexpressing the sex determining region Y-box2 (SOX2), after injection.

In particular, we focus on the SOX2, which is a well-known transcription factor related to pluripotency and stemness in ESCs and iPSCs in early neuronal development (14). Moreover, it is associated with differentiation into neural ectoderm (15). Many researchers have studied SOX2, nevertheless much remains unknown in this area. In mouse ESCs, overexpression of SOX2 has been shown to induce cell death and differentiation (16, 17), however, surprisingly reduced SOX2 expression has also been shown to induce differentiation (18). Thus, we hypothesize that overexpression of SOX2 leads to greater differentiation into neurons.

In the present study, we use neural precursor cells from human iPSCs reprogrammed by only one factor, SOX2. The iPSCs originated from human fetus neural precursor cells and overexpressed SOX2, having a tetracycline-on system, thus doxycycline induced expression.

Here, we demonstrate the beneficial effect of neural precursor cells derived from SOX2-overexpressing human

iPSCs for ALS. We transplanted the cells in an ALS model mouse spinal cord by intrathecal injection.

MATERIALS AND METHODS

1. Cell culture and differentiation *in vitro*

Neural progenitor cells (NPCs) were kindly provided by Harvard University.

Plate surfaces were coated with poly-L-ornithine (Sigma, MO, USA) and laminin (Invitrogen, CA, USA) prior to NPC plating. The neural progenitor cell medium was Dulbecco's Modified Eagle Medium containing Nutrient Mixture F-12 (DMEM/F12; Invitrogen, CA, USA), B27 supplement (Invitrogen, CA, USA), N2 (Invitrogen, CA, USA), 1 % penicillin/streptomycin (Invitrogen, CA, USA), 20 ng/ml basic fibroblast growth factor (bFGF; Invitrogen, CA, USA), and 20 ng/ml human epidermal growth factor (Hu EGF; Invitrogen, CA, USA). Culture medium was changed every day.

For differentiation into motor neurons, the differentiation medium was neurobasal medium (Invitrogen, CA, USA) supplemented with N2 supplement, 10 ng/ml brain derived neurotrophic factor (BDNF; Sigma, MO, USA), recombinant human glial derived neurotrophic factor (GDNF; Peprotech, NJ,

USA), insulin like growth factors (IGF-1; Prospecbio, Ness-Ziona, Israel), 1 μM cAMP (Sigma, MO, USA), 0.1 μM retinoic acid (RA; Sigma, MO, USA), and 50 ng/ml recombinant human sonic hedgehog (Shh; R&D System, MN, USA). The medium was changed every day for seven days.

For the expression of SOX2, cells were treated with 1 μg/ml doxycycline (Sigma, MO, USA) for 48 hours.

2. Animal model and treatment of doxycycline

All animal experiments were performed at the Center for Animal Resource Development in the College of Medicine, Seoul National University (SNU-120908-4-2).

In the present study, the widely-used model for ALS, B6SJL SOD1 G93A transgenic mouse, was used, which was provided by the Jackson Laboratory (ME, USA). This strain generally leads to death around 120 days after birth, the survival rate at that time point is 50 %.

The control group consisted of 10 untreated mice, five of these mice were treated with doxycycline. The cell-injected

group consisted of 14 doxycycline-treated mice and 10 untreated mice.

Mice were started with the immunosuppressant FK506 (1 mg/ml; Enzo Life Sciences, NY, USA) by intraperitoneal injection, this was performed a week before transplantation.

Approximately 10 days post-transplantation, animals were treated with doxycycline (2 mg/ml; Sigma, MO, USA) supplemented with water, until death. When the animals could no longer walk, they took the gelatin with 5 % sucrose on the ground.

3. Transplantation

The animals were anesthetized, shaved between the head and cervical spinal cord, and fixed on stereotactic apparatus. The skin on the back of the head was incised and the needle positioned on the cistern magna. The needle moved down until the head bounced a little, at which point the cells were injected by the stereotactic machine. To prevent cell spillage, the needle was kept in place for 5 minutes. Finally, the wound was sutured using surgical thread. All mice underwent the same surgical

progress, with control mice being injected with the same volume of cell culture medium.

4. Tissue processing

Mice were harvested before death. They were transcardially perfused with PBS and 4 % paraformaldehyde (PFA). The spinal cord and brain were removed and filled with 4 % PFA in a conical tube at 4 ℃. The following day, the solution was replaced with 10 % sucrose, 20 % sucrose, and 30 % sucrose in PBS. The tissues were placed in a disposable mold with O.C.T compound (Tissue-Tek, Alphen aan den Rijn, Netherlands) on dry-ice and kept at -80 ℃.

For immunohistochemisty, tissues were coronal-sectioned using a cryostat microtome.

5. Immunohistochemistry and Immunocytochemistry

To confirm engraftment onto the mouse tissue, the tissue from the mouse' s spinal cord or brain was stained with human nuclei and other specific markers. Tissue sections of 12 ㎛ thickness were rinsed with tris-buffered saline (TBS),

blocked using TBS with 5 % bovine serum albumin (BSA) and 0.3 % triton X-100 (Sigma, MO, USA), and then incubated for 1 hour at room temperature. The tissues were then stained with primary antibody, human nuclei (1:200; Millipore, MA, USA) and human mitochondria (1:200; Millipore, MA, USA), rabbit polyclonal FOX3/NeuN antibody (1:700; Abcam, Cambridge, UK), and glial fibrillary acidic protein (GFAP, 1:200; Santa Cruz Biotechnology, CA, USA) and then stored overnight at room temperature. The following day, the tissue samples were washed with TBS three times, and were then incubated with the secondary antibodies Alexa Fluor 488 goat anti-rabbit IgG (H+L) (1:500; Invitrogen, CA, USA,), Alexa Fluor 555 goat anti-rabbit IgG (H+L) (1:500; Invitrogen, CA, USA), Alexa Fluor 488 goat anti-mouse IgG (H+L) (1:500; Invitrogen, CA, USA,), Alexa Fluor 555 goat anti-mouse IgG (H+L) (1:500; Invitrogen, CA, USA), and 4', 6-diamidino-2-phenylindole (DAPI, 1:200; sigma, MO, USA,) for 1 hour at room temperature. The slide was dried in the dark at room temperature. A fluorescence mounting medium (Dako, Glostrup, Denmark) was subsequently placed on the slide, and the slide

was then covered using cover-slip. All of the sections were stained with the control-mouse tissues.

To perform the Immunocytochemistry, the cells with the cover-slip were rinsed with PBS and fixed by 4 % PFA for 15 minutes in a dish. The cells were blocked using 5 % BSA with 0.25 % Triton x-100 in PBS overnight at 4 ℃. The following day, the dishes were stained using the primary antibody, Olig2 (1:500; Santa Cruz Biotechnology, CA, USA), Anti-MNX1 (HB9, 1:200; Millipore, MA, USA), and beta III-tubulin (1:200; Millipore, MA, USA) for 2 hours at 37 ℃. The samples were then rinsed three times with PBS. Sections were stained with each secondary antibody (1:500) and DAPI (1:200) for 1 hour at room temperature. Finally, the sections were dried and covered on the slide with the fluorescence mounting medium.

6. Behavioral test and disease onset

To test motor neuronal function, animals performed rotarod testing every other day, running on the machine 3 times for a maximum of 5 minutes each.

The disease onset point of the animals was assessed using a neurological score developed by the Jackson Laboratory. One

point onset indicates that the mouse did not overturn within 30 seconds. Two point onset indicates dragging the legs on the ground. Three point onset indicates when the tails pick-up and the legs are folded or trembling. Rotarod failure indicates that the mouse failed to run on the machine for 15 seconds. The end point was the day of death.

7. Quantitative real-time PCR

Total RNA was isolated using Trizol reagent (Invitrogen, CA, USA).

To synthesize cDNA from total RNA, the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, CA, USA) was used with SYBR PCR Master Mix (Qiagen, Limburg, Netherlands). The PCR primers for the reaction are listed in Table 1. All the reactions were run in triplicate. The Rotor-gene 3000 (Corbett Research, Sydney, Australia) detection system was used.

8. Statistical analyses

Statistical analysis was carried out using SPSS version 18.0 (SPSS Inc., IL, USA). The survival function data were analyzed

by the Kaplan–Meier method. The other data were analyzed using a t–test in Microsoft Excel version 2007. All data are expressed as the mean ± SEM, and findings were considered statistically significant at $p < 0.05$.

RESULTS

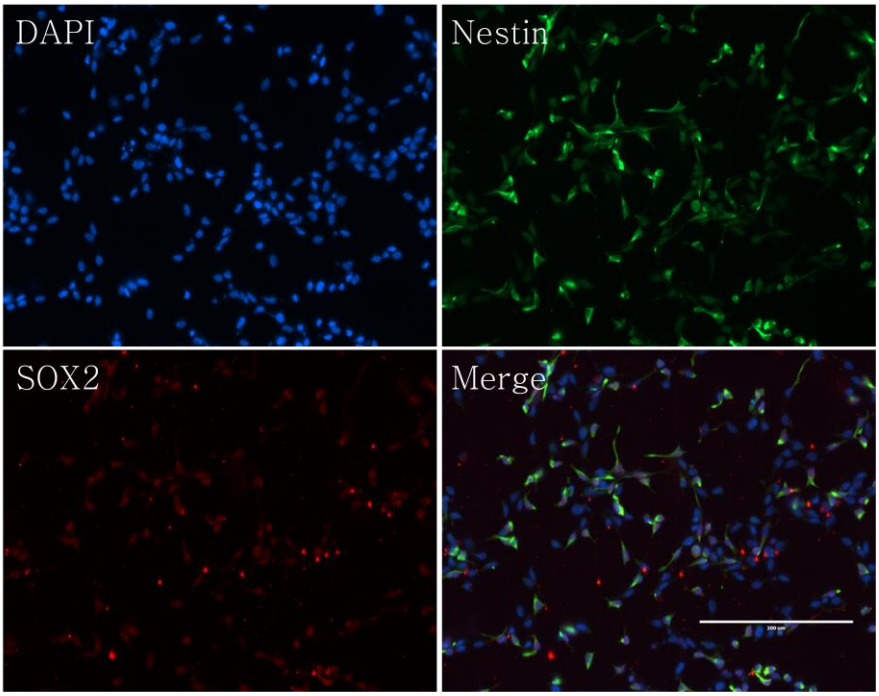
The SOX2 over-expressing NSCs differentiated into motor neurons.

Before the differentiation into motor neurons, the cells were assessed for expression of the neural stem cell markers, nestin and SOX2 (Figure 1A). Additionally, to confirmation SOX2 overexpression, we performed the quantitative real-time PCR which showed that SOX2 expression was significantly high at $p = 0.005$, as shown in Figure 1C (1 ± 0.40 for control vs 4.35 ± 0.52 for SOX2-O/E).

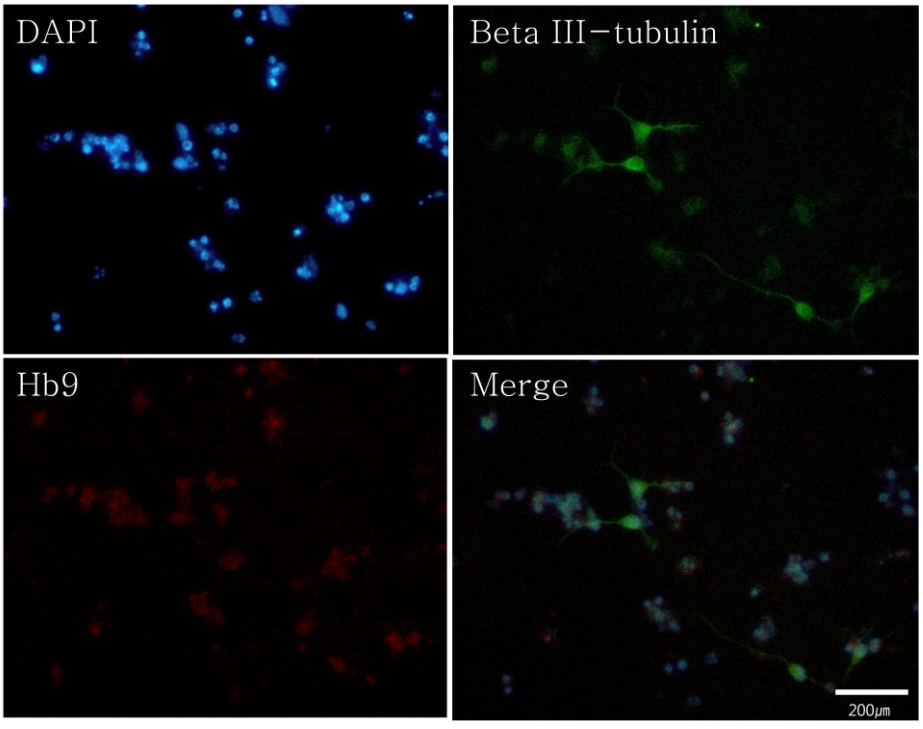
The NSCs were allowed to differentiate into motor neurons with GDNF, BDNF, ascorbic acid, and IGF-1 in neurobasal medium for seven days. Subsequently, we confirmed the differentiation ability of SOX2-overexpressing NSCs. The neural stem cell marker βIII-tubulin and the motor neuron specific marker Hb9 were positively stained in the differentiated NSCs (Figure 1B). In addition, we carried out the qrt-PCR for the digitization, and showed that differentiation

ability into motor neurons in the presence of doxycycline is better than in its absence. The islet1 is the marker for early differentiation into motor neurons. The differentiate rate with doxycycline is relatively high (1 ± 0.03 for control vs. 1.87 ± 0.07 for SOX2-O/E, p = 0.0003; Figure 1D); therefore, we confirmed that the SOX2 affect to differentiate into motor neuron.

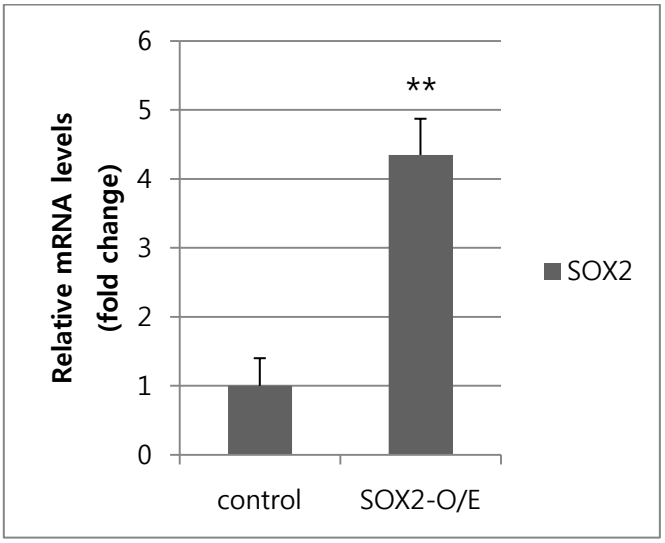
(A)


DAPI
Nestin
SOX2
Merge

(B)


DAPI
Beta III-tubulin
Hb9
Merge
200μm

(C)



(D)

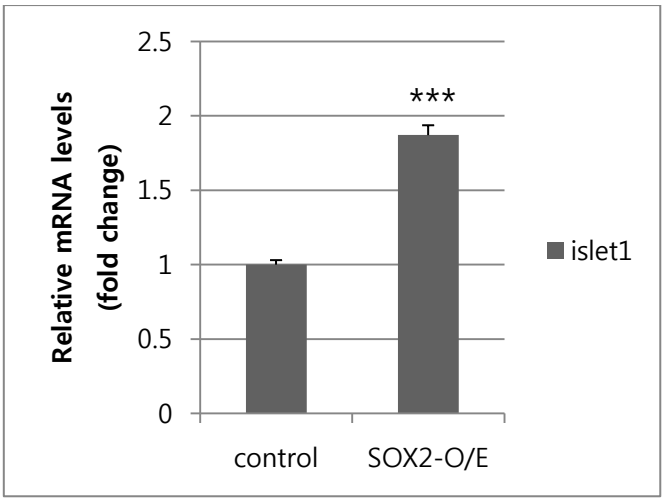


Figure 1. The SOX2-overexpressing NSCs differentiated into motor neurons in the presence of doxycycline.

(A) Confirmation that the NSCs express Nestin (green) and SOX2 (red).

(B) The nuclei stained with DAPI, βIII-tubulin marked neurons, and the motor neuron specific marker Hb9.

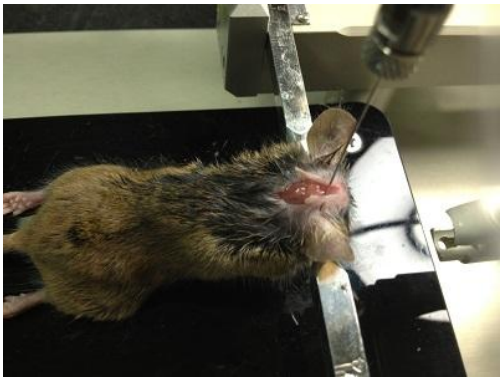
(C, D) The qRT-PCR results of SOX2 overexpression and differentiation into motor neurons in the presence of doxycycline. ** Significant at $p < 0.001$. *** Significant at $p < 0.0001$.

Transplantation of the SOX2-overexpressing neural precursor cells in ALS mouse model.

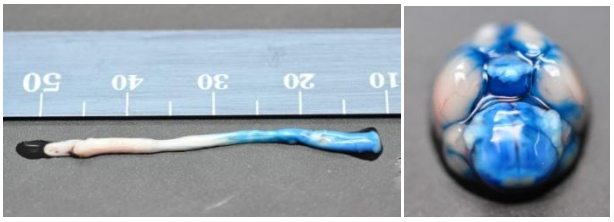
In the present study, the SOD1 G93A transgenic mouse was used as a familial ALS model. We first divided the mice into 2 groups, those injected with cells and those injected with culture medium. Each of the 2 groups was further divided into 2 groups, doxycycline treated and untreated. We injected approximately 400,000 cells per 3.5 μl cell culture medium into the cisterna magna, near the medulla (Figure 2A). The morphology of the cells was observed by phase contrast microscopy (Figure 2C). The control mice were injected with 3.5 μl cell culture medium. A number of mice in each group were treated with doxycycline (2 mg/ml) in their water, from one week post-injection until death. The doxycycline induced ectopic cellular expression of SOX2. It was expected that the neural precursor cells more easily differentiate into motor neurons than other cell subtypes. The mice were transplanted during the pre-symptomatic period, at roughly 60 days old. The animals were injected daily with the immunosuppressant FK506 (1 mg/ml), which was started a week before transplantation.

Prior to cell transplantation, we practiced injection into the mice using trypan-blue dye instead of cells. One day after injection, we sacrificed the mice and analyzed the spinal cord and brain. We confirmed that the dye had spread to regions on both sides of the brain and to the spinal cord (Figure 2B).

(A)



(B)



(C)

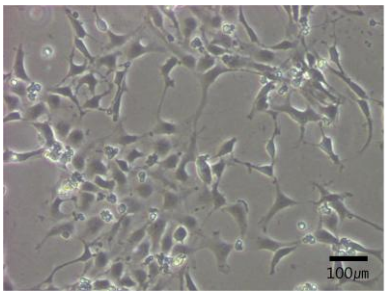


Figure 2. Transplantation into the ALS mouse model.

(A) The mouse is located in stereotactic apparatus for transplantation. (B) Spreading of the trypan-blue dye into the spinal cord and brain was confirmed one day after transplantation. (C, D) The SOX2-overexpressing neural precursor cells *in vitro*. Scale bar=right, 100㎛.

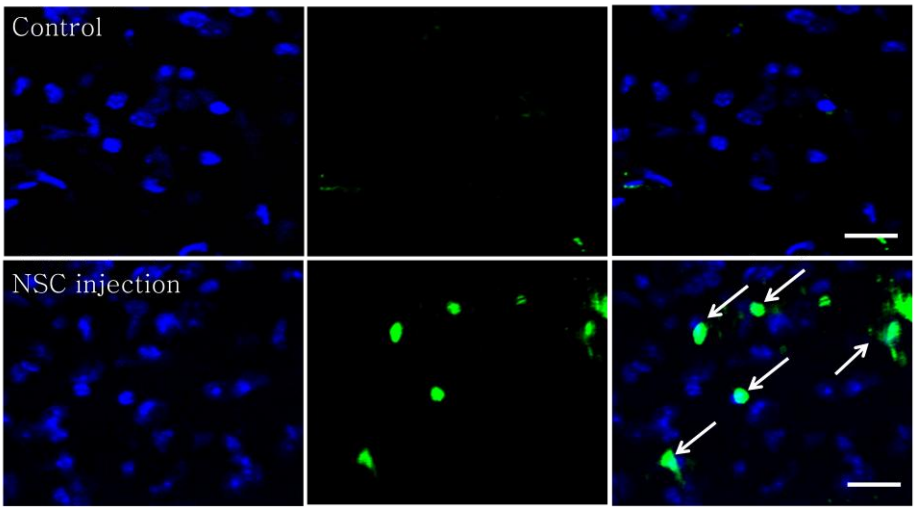
The hNPCs were well-engrafted onto the spinal cord and brain, and a low proportion is differentiated into other cell types.

The cells were found to be well-engrafted onto the mouse tissue. This was confirmed by immunohistochemistry (IHC). Due to the fact that the cells originated from a human source, we stained the cells for human nuclei (Figure 3-1A) and human mitochondria (Figure 3-1B). In particular, we confirmed the amount of cells that remained in the spinal cord, by counting the cells in the mouse spinal cord tissue sections using confocal microscopy. Only small amounts of the cells remained and no difference was observed between the doxycycline-treated (48.67 ± 8.51) and non-treated tissue sections (44.5 ± 8.66; Figure 3-1C).

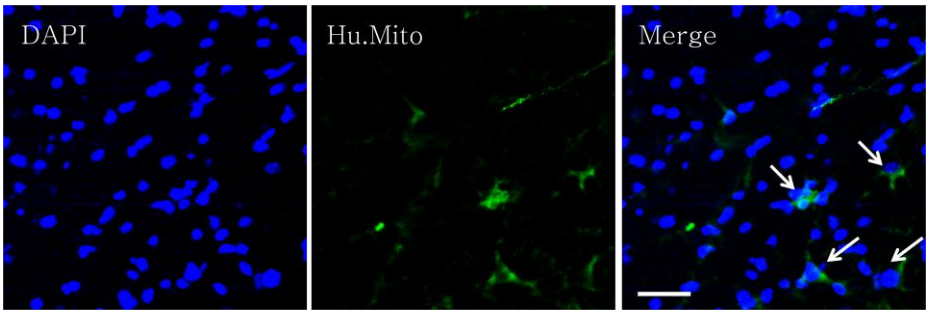
In addition, we also investigated the differentiation of these cells *in vivo.* Thus, we stained the tissues with GFAP for astrocytes and NeuN for neural nuclei (Figure 3-2A, 2B). According to the IHC results, these cells show a low proportion of differentiation into other cell types including motor neurons. In particular, we counted the remaining cells that differentiated

into neurons (Figure 3-2C). There were no differences between the doxycycline-treated (16.81 ± 1.61) and untreated groups (16.17 ± 1.09).

(A) DAPI Human Nuclei Merge



(B)



(C)

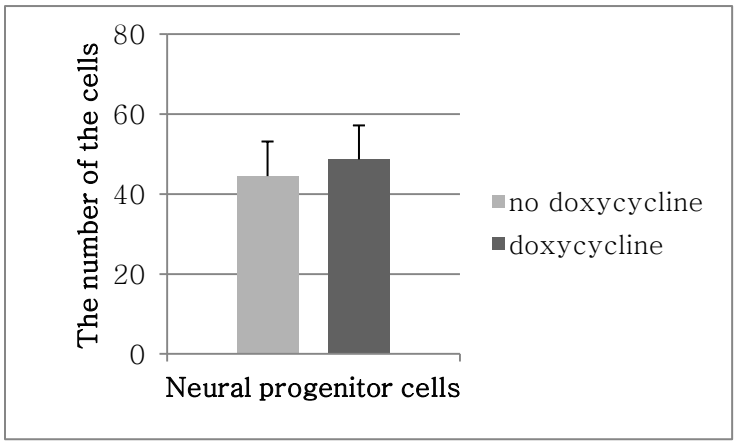
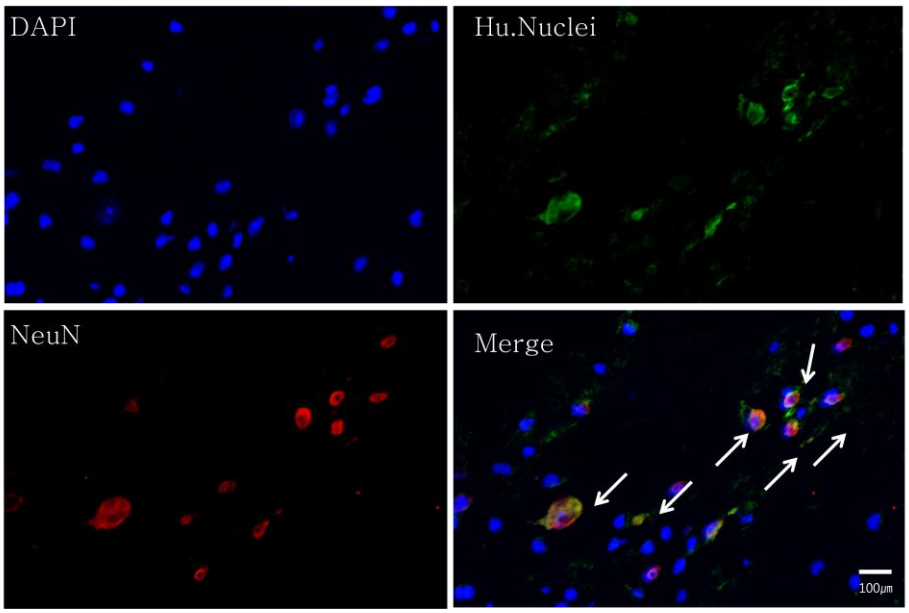


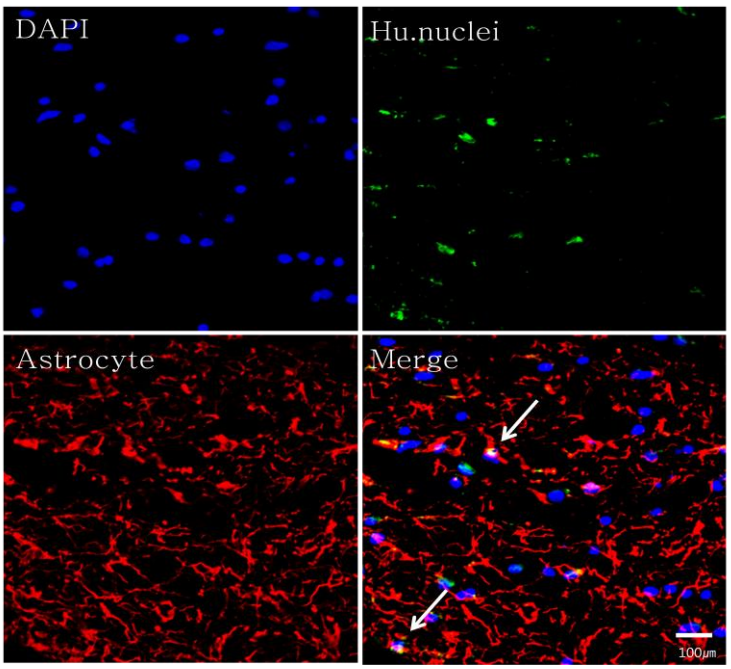
Figure 3-1. Confirmation of engraftment by IHC.

The NSCs engrafted onto the ALS mouse spinal cord tissue confirmed by human nuclei and human mitochondria antibodies. (A) Human nuclei stained green and all nuclei stained blue by DAPI. Scale bar = right, 50μm. (B) Blue indicates nuclei, green indicates human mitochondria, and merged cells are the NPCs. Scale bar = left, 100μm. (C) The average total cell number in the tissues between doxycycline-treated and untreated groups. A small amount of cells remained in spinal cord tissue and showed no differences between the doxycycline-treated and untreated groups.

(A)


DAPI
Hu.Nuclei
NeuN
Merge
100μm

(B)


DAPI
Hu.nuclei
Astrocyte
Merge
100μm

(C)

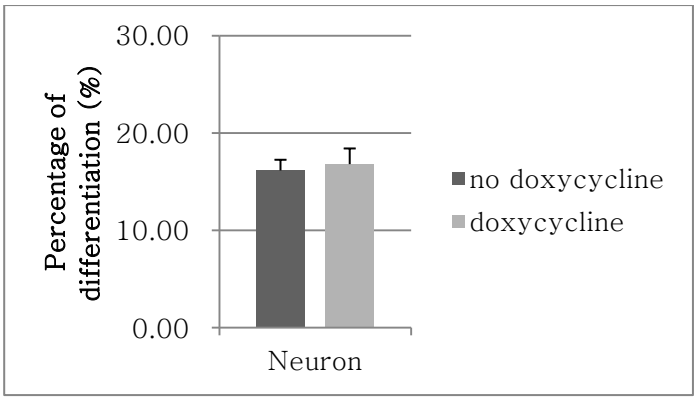


Figure 3-2. Differentiation into astrocytes and neurons in mouse spinal cord tissue.

(A) The NSCs differentiated into neurons. Nuclei are stained blue with DAPI, human mitochondria green, and neural nuclei red. (B) The NPCs differentiated into astrocytes. Nuclei are stained blue with DAPI, human mitochondria green, and astrocytes red. (C) The percentage of differentiation into neurons for the doxycycline-treated and untreated groups.

The SOX2-overexpressing NSCs affected the first onset point, but there were no relative differences in the end stage of disease.

To test the motor function of the mice, we used a rotarod machine. In addition, we assessed the disease onset point in order to study the progression. In the present study, the NPC-injected group showed a relative difference between the doxycycline-treated and untreated sub-groups. The 3-point onset of the cell-injected group without doxycycline (100 ± 1.03) began later than the control group without doxycycline (97 ± 0.93, p = 0.03; Figure 4). Moreover, the cell-injected group with doxycycline (102 ± 1.12) began slower than the control group with doxycycline (98 ± 1.02, p = 0.02; Figure 4). Thus, the SOX2-overexpressing NSCs affected the progress of the disease-onset age of ALS.

However, the disease progression was the same as that of the control group at the second, third, and end-point.

In particular, there was a significant difference in the end-point. In the stem cell-injected groups, the average end point in

the doxycycline-treated group was relatively high (129 ± 1.45 for non-treated vs. 136 ± 2.20, p = 0.02; Figure 4).

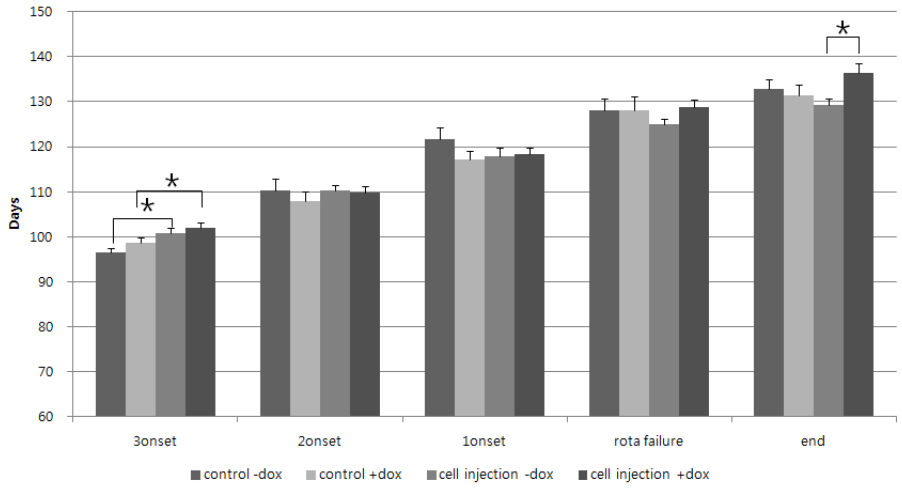


Figure 4. The animal life spans and disease-onset graph.

Comparison of the average disease onset point, rotarod failure, and end point between doxycycline-treated and untreated groups. * Significant at $p < 0.05$.

However, the life span of the mice was longer in the group treated with doxycycline than those in the untreated group, regardless of the presence of injected cells (Figure 5). In this study, we expected that the differentiated cells would replace the dying motor neurons, resulting in the animals having a longer lifespan. However, the doxycycline did not improve the differentiation into motor neurons or other cell subtypes, thus, no differences in motor function or life span between the doxycycline-treated and untreated groups were observed.

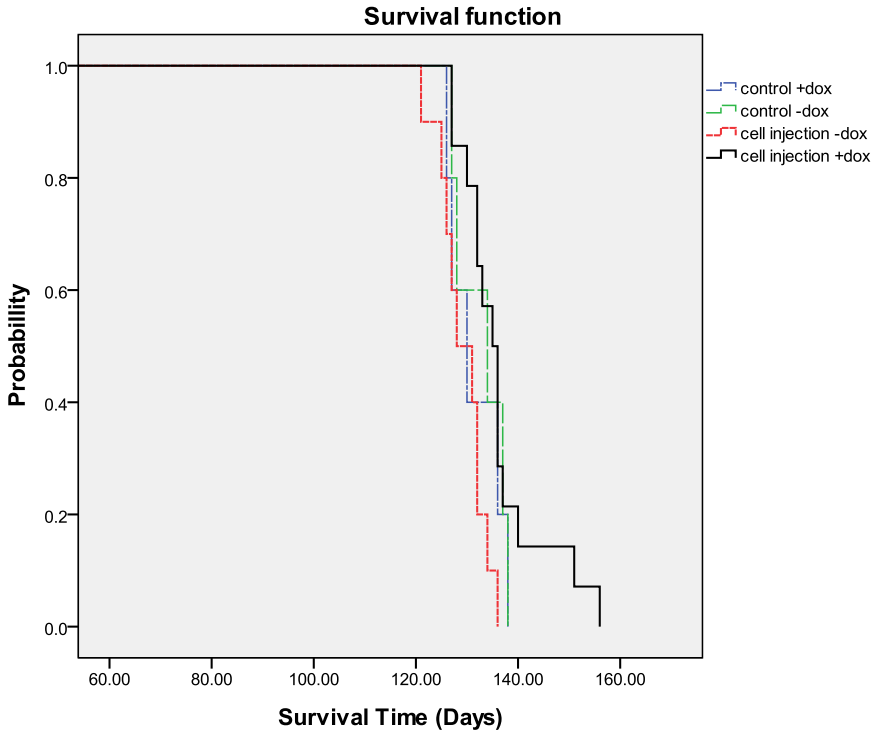


Figure 5. Survival curves of the NSC-injected group and control group.

The probability of survival between the control group and cell-injection group are not significantly different.

Table 1. Quantatitive real-time pcr primer set

Gene	Forward Sequence	Reverse Sequence
SOX2	CCCACCTACAGCATGTCCTACTC	TGGAGTGGGAGGAAGAGGTAAC
ISLET1	GCAGCATCGGCTTCAGCAAG	GTAGCAGGTCCGCAAGGTG
GAPDH	TGAAGGTCGGAGTCAACGGA	GATGGCATGGACTGTGGTCAT

DISCUSSION

In previous studies, SOX2 has been shown to be a regulator of early development and differentiation (14). Thus, in the present study, we focused on differentiation into neurons. Since ALS is a neurodegenerative disease, we expected that the transplanted cells would directly or indirectly affect the dying neurons.

According to our results, the neural precursor cells were well-engrafted on the mouse's spinal cord and had differentiated. In addition, we confirmed by immunohistochemistry that the SOX2-overexpressing cells survived until the death of the mice. Furthermore, the difference between the control group without doxycycline and the cell-injected group with doxycycline was a delay in the first onset of ALS in the SOD1 G93A transgenic mice. Therefore, we suggest that the time point of stem cell injection should be carried out before disease progression.

However, here, the SOX2-overexpressing cells did not prolong the lifespan of the mice in the ALS disease mouse model. The effects of the cells did not continue until death.

After the cells were engrafted, the number of living cells was small, and the migration was limited, thus, the effects were not ongoing. Zhang *et al.,* found that multiple injections into the mouse spinal cord caused the migration of a small number of cells to the lumbar spinal cord (8). It has not been reported how long the migration to spinal cord continued following only a single intrathecal injection. In our current experiments, the cells migrated to cervical level 4 or 5 as a result of a single injection. Thus, we thought that the number of injections would be considered for qualitative improvement.

Based on these results, the ectopic-expressed SOX2 in NSCs does not influence neural differentiation *in vivo*. Some reports have stated that the regulatory region of SOX2 is activated in the state of NSCs and ESCs (25, 27). In addition, SOX2 is associated with nuclear receptor tailless (TLX), a gene that controls self-renewal in NSCs. We have previously suggested that overexpression of SOX2 activates TLX, leading to maintenance of the self-renewal ability of NSCs (26). Thus, SOX2-overexpressing neural stem cells would not be effective on dying neurons in an ALS mouse.

In the present report, SOX2-overexpressing NSCs were found to delay disease onset. Thus, we suggest a new possibility to treat ALS using a cell therapy system.

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국문 초록

서론: 신경 퇴행성 질환의 하나인 근위축가쪽경화증(ALS)은 운동 신경의 죽음을 초래한다. 이는 급속한 질병의 진행으로 대부분 5 년 이내에 호흡근의 마비로 사망에 이른다. 하지만, 지금까지 ALS 를 치료할 방법은 거의 없다. 지금까지 다양한 방법으로 치료를 시도하였지만, 그 중에 줄기세포를 이용한 세포 치료 방법은 잠재력이 가장 큰 방식이다. 유도만능줄기세포는 네 가지 전사인자를 이용하여 체세포를 배아줄기세포와 유사한 전분화능 세포로 역분화 시킨다. 그 전사인자 중의 하나인 SOX2 는 배아 줄기세포 상태에서 분화능에 관련이 있고, 초기 배아 발생에서 신경으로의 분화에 연관되어있다. 특히 주목한 것은 운동 신경으로의 분화능이다. SOX2 를 과발현시켰을 때, 운동 신경으로의 분화가 얼마나 잘 일어나는지 관심을 가지게 되었다. 특히 분화된 운동 신경 세포가 특이적으로 죽는 ALS 환자의 치료에 도움이 될 것으로 기대한다.

방법: 먼저 이식할 신경 줄기 세포의 SOX2 의 발현 정도와 분화 조건으로 배양했을 때 어느 정도 분화가 진행 되는지 확인 하고자 real time PCR 을 진행하였다. 반면 체외에서 분화된 신경줄기세포의 기능성을 확인하기 위해서 ALS 모델 마우스인 SOD1 G93A 유전자 변형 마우스에 세포를 이식하였다. 신경줄기세포는 마우스의

뇌와 척추의 연결 지점에 주입하였다. SOX2 는 테트라사이클린-온 시스템으로 발현이 되도록 하였고 과발현을 위해서 독시사이클린을 마우스에 처리하였다. 운동 신경 능력을 측정하기 위해 로타로드 기계를 사용하였고, 질병의 진행 정도를 확인하기 위하여 마우스의 다리 마비 여부로 확인하였다. 또한 세포의 생착 여부와 분화 여부를 확인하기 위하여 면역염색방법을 진행하였다.

결과: 체외에서 분화된 신경줄기 세포는 SOX2 과발현 하였을 때 운동 신경 세포로 분화가 더 잘 되었다. 그리고 마우스에 이식 하였을 때 신경줄기세포는 세포를 주입한 곳으로부터 뇌와 척추 부근으로 이주하여 신경 세포와 성상세포를 포함한 교질세포로 분화하였음을 확인하였다. 그리고 마우스의 질병 진행 상태를 확인하였을 때, 질병의 시작이 유의하게 느려졌음을 확인할 수 있었다. 독시사이클린을 처리한 세포 주입 군과 독시사이클린을 처리하지 않은 대조군을 비교했을 때, 세포 주입 군의 질병 발생 시점이 더 늦어졌다. 모두 독시사이클린을 처리 하지 않은 대조군과 실험군을 비교하면, 역시 세포 주입 군의 질병 발생이 느리게 시작하였다. 하지만, 대조군과 비교해 보았을 때, 신경세포가 이식된 마우스의 행동은 크게 향상되지 않았으며, 수명 또한 유의하게 늘어나지 않았다.

결론: ALS 질병 모델 마우스에서 과발현된 SOX2 가 신경줄기세포를 운동 신경으로의 분화를 촉진하여 죽어가는 운동 신경 세포에 영향을 주어 질병의 진행을 늦추거나 운동 능력의 향상을 예상하였다. 그러나 SOX2 가 과발현 된 신경줄기세포는 ALS 질병의 시작은 늦출 수 있었지만, 그것이 장기적으로 효과를 주지는 못했다. 이식된 세포가 질병이 완전히 진행되기까지 살아있지 못하기 때문이다. 장기적으로 효과를 보기 위해서는 한 번의 주입이 아닌 여러 번의 주입이 필요하다고 생각한다. 또한 SOX2 가 세포의 분화능 뿐만 아니라 줄기세포능에도 관여하고 있기 때문에 분화된 세포를 이식 하는 것이 더 효과적일 것으로 생각된다.

주요어 : 근육위축가쪽경화증, 분화, 유도만능줄기세포, SOX2, 이식
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