In Vivo Stem Cell Tracking with Magnetic Resonance Imaging using Superparamagnetic Iron Oxide in Rat Genitalia

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Directed by Professor Myeong-Jin Kim

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ABSTRACT

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Purpose: The purpose of this study was to determine the feasibility of in vivo stem cell tracking using magnetic resonance (MR) imaging in the rat genitalia after radical prostatectomy and in the analysis of fibrosis of external genitalia.

Methods and Materials: We studied 36 male 5 weeks-old Sprague-Dawley rats (SLC, Tokyo, Japan). They are divided into 4 weeks, 8 weeks, and 16 weeks group and each group included 12 rats. Also, 12 rats of each group were divided into control and stem cell group and each group included 6 rats. Human mesenchymal stem cells (MSC) were labeled with superparamagnetic iron oxide particle (Feridex, Berlex Laboratories, Wayne, NJ, USA). In the stem cell group (n=6), $1x10^6$ cells were injected into the corpus cavernosum

after transaction of bilateral cavernous nerves. In control group (n=6), 0.02cc of cell-free media was injected in the same manner. *In vivo* MRI was serially performed up to 16 weeks using 47mm microcoil and 1.5 T clinical scanner. After MR imaging, the penile specimens were collected and prepared for detecting the expression of transforming growth factor (TGF)-β_1 by polymerase chain reaction (PCR).

Results: Magnetic resonance imaging showed a drop in signal intensity at the site of injection in the stem cell injected group. On serial MR imaging up to 16 weeks, the size of low signal intensity area showed decreased. There was no change in signal intensity at the injection site in the control group. PCR analyses revealed a significantly higher expression of TGF-β_1 in the penile tissues of the control group than that in stem cell group in 4 weeks group.

Conclusion: Our results showed that in vivo stem cell tracking using MR imaging in a rat model of radical prostatectomy was feasible. This may be a viable method for evaluating the fate of stem cells after injection into the rat penis.

Key words: animals, magnetic resonance imaging, iron, radical prostatectomy, stem cell transplantation, rat

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Ⅰ. INTRODUCTION

In vivo monitoring of transplanted cells (stem cells) is a growing concern in cell therapy[1,2]. A reliable in vivo imaging method in localizing transplanted cells and monitoring their restorative effects will make systematic investigation of cell therapy possible. Previous researches with immunohistological staining in stem cell transplantation do not provide an opportunity to trace the migration of transplanted cells *in vivo* in the same host. The purpose of molecular imaging is to visualize targeted cells in living organisms. Through molecular imaging with nanoparticles, the biological process of transplanted stem cells can be monitored non-invasively, as materials can be evaluated without sacrifice and repeated

evaluation is possible[3,4]. There are various imaging technologies in cellular and molecular imaging including nuclear imaging, optical imaging, and magnetic resonance imaging (MRI)[5-9]. Labeling cells with superparamagnetic iron oxide (SPIO) nanoparticles or a paramagnetic contrast agent (gadolinium [Gd] or manganese [Mn]) provides the possibility of detecting even single cells or clusters of labeled cells within target tissues after either direct implantation or intravenous injection[10-14]. Through the highest spatial resolution, cellular magnetic resonance imaging (CMRI) allows the discrimination and detection of labeled cells *in vivo* in target tissues. This rapidly growing area of experimental research shows the potential of translating from bench to bedside in cellular therapy clinical trials.

Prostate cancer is the second most frequent cancer in men in USA and has the most rapidly increasing incidence in Korea. After the introduction of prostate specific antigen (PSA) for screening of prostate cancer and transrectal ultrasound (TRUS)-guided biopsy, prostate cancer is now diagnosed at an early stage[15]. Consequently the number of sexually active patients with prostate cancer is increasing.

Radical prostatectomy (RP) is regarded as the primary treatment for prostate cancer[16]. Although prostatectomy is a well standardized surgery, erectile dysfunction (ED) is a major complication after RP[17]. It is believed that either direct damage or neuropraxia of the cavernous nerve, alone or with hemodynamic

alterations leads to loss of corporal smooth muscle cells and subsequent fibrosis of the corpus cavernosum. Nerve sparing surgery has been proposed to spare the erectile function[18]. However, no satisfying results have been shown[19]. Another method of sural nerve grafting aimed in restoring innervation of carpora cavernosa also shows low success rates[20].

There have been reports that sidenafil improved the erectile dysfunction in patients with prostatectomy, but Zippe et al reported that patients who have received non-nerve sparing prostatectomy showed no response to sildenafil[21].

Previous reports have shown that stem cell injection improves erectile function in rat model erection dysfunctions[22]. However, they used immunohistochemical stain in the evaluation of stem cells. Recently, there has been a report with *in vivo* MR stem cell tracking in normal rat genitalia[23]. Until now, however, there are no studies in *in vivo* MR stem cell tracking in rat genitalia of radical prostatectomy models. The purpose of this study is to determine the feasibility of serial *in vivo* stem cell tracking using magnetic resonance (MR) imaging in the rat genitalia of a radical prostatectomy model.

II. MATERIALS AND METHODS

1. Cell preparation and labeling

After mesenchymal stem cells (MSCs) were isolated from human bone marrow, they were expanded in a 37°C 5% CO_2 incubator. As a standard culture medium, we used Dulbeco's Modified Eagle Medium (DMEM, Gibco, Carlsbad, CA, USA) including 10 percent of fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA) and penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA).

We used ferumoxides (Feridex, Berlex Laboratories, Wayne, NJ, USA) in labeling MSCs as a superparamagnetic iron oxide (SPIO) nanoparticles for intracellular magnetic labeling of stem cells to monitor cell trafficking by MR imaging and protamine sulfate (American Pharmaceuticals Partner, Schaumburg, IL, USA) as a transfection agent[12 24]. We added Feridex (50μg/ml) and protamine sulfate (9μg/ml) to the standard culture medium and then mixed for 10 minutes with intermittent hand shakings. After incubating the medium containing Feridex-protamine sulfate complex for 2 hours, the equal volume of the standard culture medium was added to the adherent cell culture. Therefore, the final concentration of Feridex was 25μg/ml and protamine sulfate was 4.5μg/ml. They were kept in the incubator for overnight. After overnight incubation, the medium was discarded and the cells were washed twice with heparin containing

phosphate buffered saline. After being treated with trypsin, the cells were collected, rewashed, and counted to adjust the cell concentration for injection.

The efficiency of intracellular labeling with SPIO was checked with Prussian blue staining. The Labeled 10^5 celles were fixed with 95% alcohol, washed, incubated for 20 minutes with 10% potassium ferrocyanide (Perl's reagent for Prussian blue staining) and 3.7% hydrocholoric acid, washed again, and counterstained with nuclear fast red.

Cells were considered Prussian blue positive if intracytoplasmic blue inclusions were detected. Labeling efficiency was determined by manual counting of Prussian blue-stained and unstained cells using hemocytometer at 100x magnification.

2. Rat radical prostatectomy model and MSC injection

We studied 36 male 5 weeks-old Sprague-Dawley male rats (SLC, Tokyo, Japan). They were divided into 4 weeks, 8 weeks, and 16 weeks group and each group included 12 rats. Also, 12 rats of each group were divided into control and stem cell groups, each group including 6 rats. After anesthesia with an intraperitoneal injection of ketamine and xylazine, skin incision was made. After transection of bilateral cavernous nerves, 0.02cc of cell-free media was injected into corpus cavernosum in control group (n=18) (Figure 1). The penile base was

strangulated for 30 seconds after injection and then released. The stem cell group (n=18) received $1x10^6$ MSCs in the same manner.

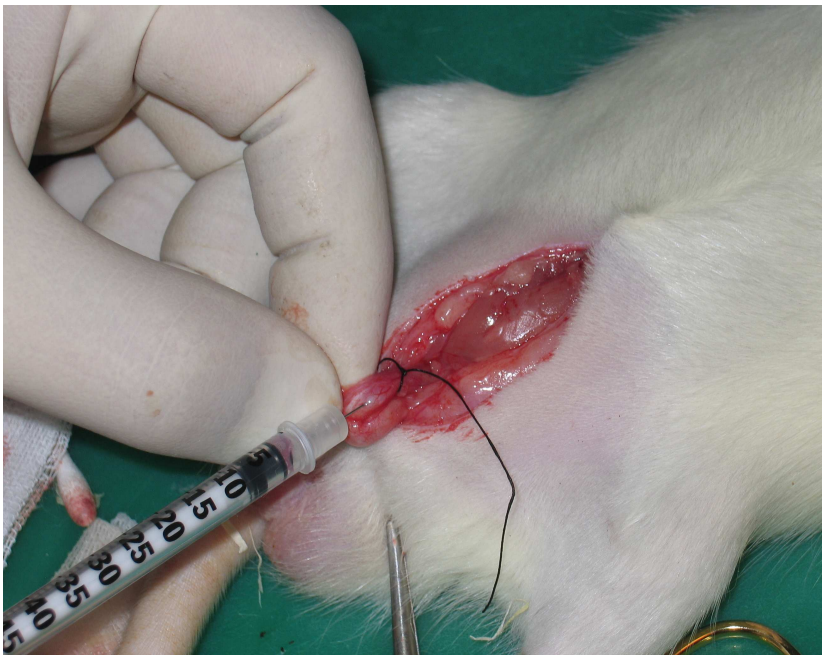


Figure.1. Stem cell injection into the corpus cavernosum.

3. In vivo MR imaging

MRI was obtained with a 1.5 T clinical MRI instrument with a micro-47 surface coil (Intera Achieva, Philips Medical Systems, Best, Netherlands).

For *in vivo* penile MRI, the rats were anesthesized with the same procedure used in surgery. *In vivo* MRI to visualize Feridex-labeled MSCs were conducted by gradient echo sequence with the following parameters: T1-turbo field echo sequence, shortest TR and TE, flip angle=15°, matrix=256 x 256, slice

thickness=3mm, number of averages=6, field of view=80mm.

MRI was taken up to 16 weeks after normal media or MSCs injection.

4. Fibrosis analysis with reverse transcriptase polymerase chain reaction

The rats were sacrificed and the penile tissues collected, 4, 8, and 16 weeks after normal media or MSCs injection. The expression of endogenous of transforming growth factor (TGF)-β1 mRNA in the penis was detected by reverse transcriptase polymerase chain reaction (RT-PCR) 4, 8, and 16 weeks after intracavernous injection of stem cells. First-strand cDNA was synthesized by using reverse transcriptase with oligo (dT) primers, and PCR was performed with a forward primer specific for the sequence encoding the rat TGF-β1 (5'-GAT GAG ATC GAG TAC ATC TT-3') and reverse primer (5'-CAC CGC CTC GGC TTG TCA CAT-3') for 30 cycles. β-actin was used as an internal control for RT-PCR. The set of primers for β-actin included the following: forward primer (5'-TCT ACA ATG AGC TGC GTG TG -3') and reverse primer (5'-AAT GTC ACG CAC GAT TTC CC- 3'). All signals were visualized and analyzed by densitometry. To compare the difference of TGF-β1 between control and stem cell group, statistical analysis was done (p value=0.05).

5. Histological Analysis

The rats were sacrificed and the penile tissues were collected, 4, 8, and 16 weeks after normal media or MSCs injection. The penile tissues were retrieved and fixed in 4% paraformaldehyde. The specimens at the injection sites were cut into 4μm sections in the short-axis plane and stained with hematoxylin and eosin (H&E). Prussian blue stain was performed to detect Feridex-labeled MSCs.

III. RESULTS

1. Cell labeling

Photomicrographs of Prussian blue staining of Feridex-labeled MSCs are shown in Figure 2. MSCs showed abundant intracytoplasmic blue inclusions on Prussian blue staining. Labeling efficiency was approximately 100% and extracellular iron complexes were rarely seen.

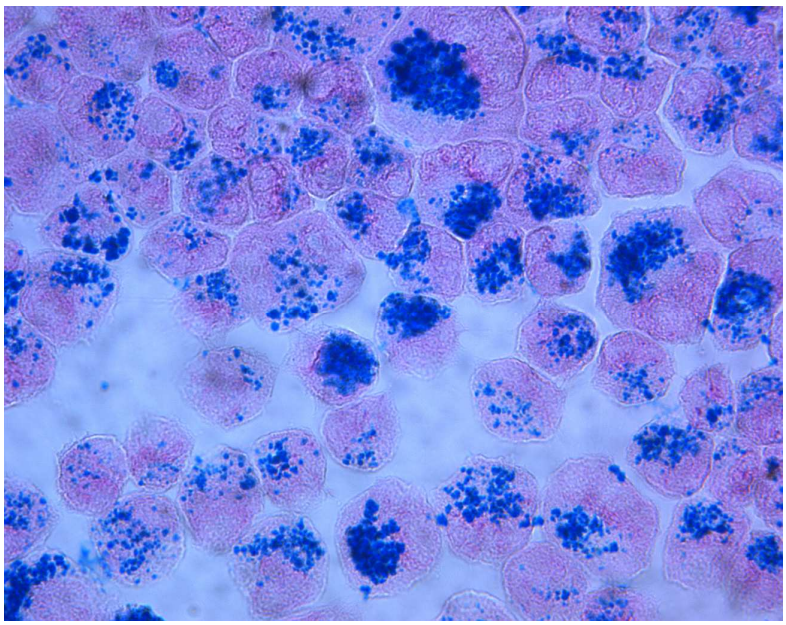


Figure 2. Prussian blue-stained Feridex-labeled MSCs (magnification x 1000).

2. In vivo MR tracking of MSCs

Two rats (one in 4 weeks and one in 8 weeks) of MSC injection group died during the study. *In vivo* MRI of the rat penis with transaction of bilateral

cavernous nerves was successful in remaining 34 rats. In the MSCs injection group, signal void area corresponding to the injection site was demonstrated in MR images of the entire MSC injection group (Figure 3). As time went by, the size of signal void area became to be decreased. After 12 weeks, most signal void areas were difficult to define. On the other hand, this signal void was not observed at the injection site in the control group injected with cell-free media (Figure 4).

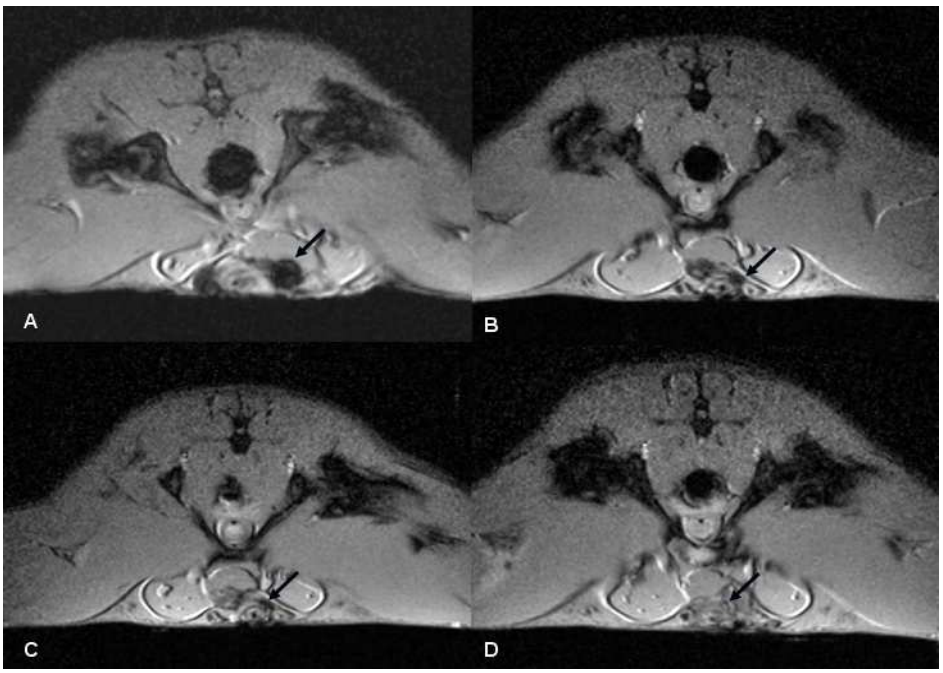


Figure 3. In vivo MRI with MSCs injection after transaction of bilateral cavernous nerves. (A-D) Short axis T1 weighted images of the rat with Feridex-labeled MSC injection shows the distinct signal dropout (arrow in A) at the penis in 4 weeks after injection . The size of signal dropout decrease along the 8 (B), 12 (C), and 16 weeks.

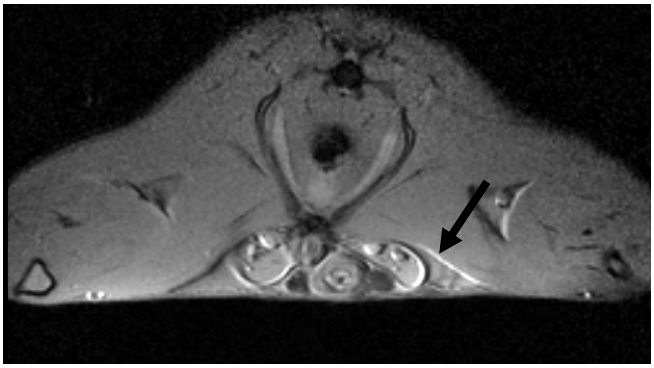
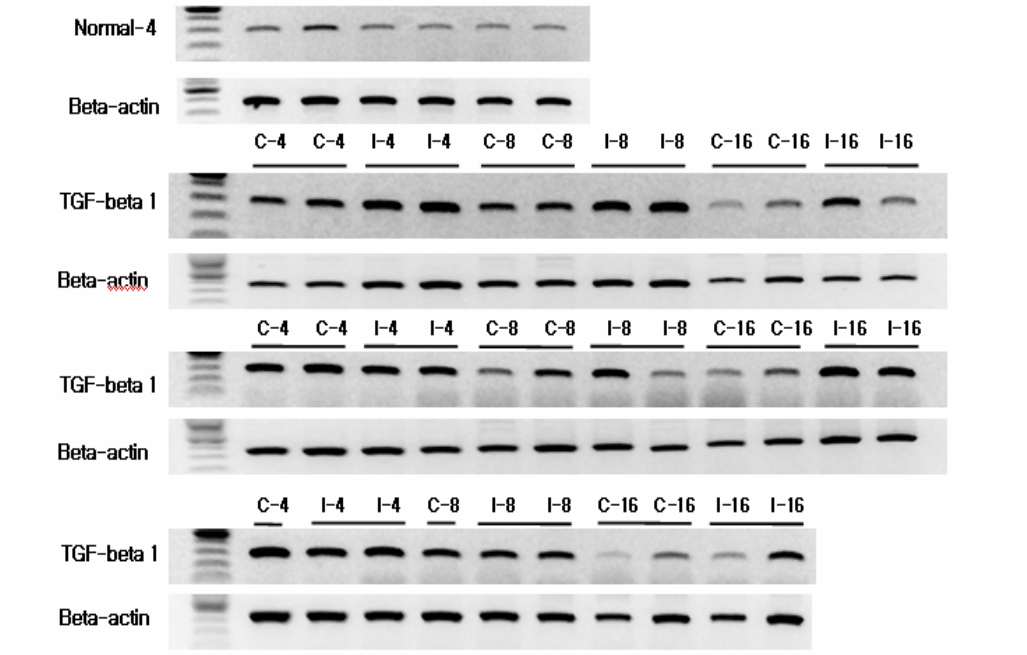


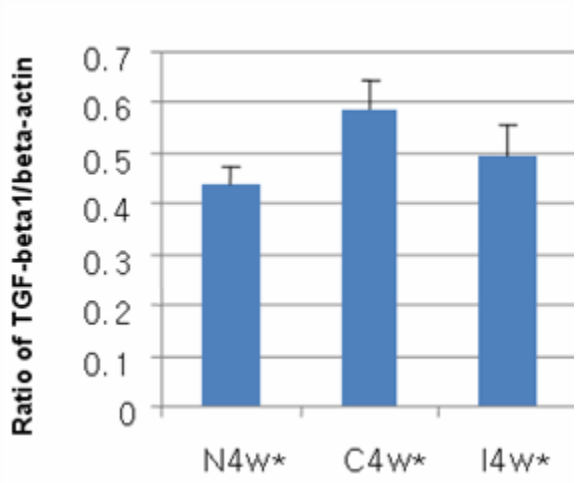
Figure 4. In vivo MRI with normal media injection after transaction of bilateral cavernous nerves. Short axis T1 weighted images of the rat with normal media injection shows no signal change at the penis (arrow).

3. PCR analysis

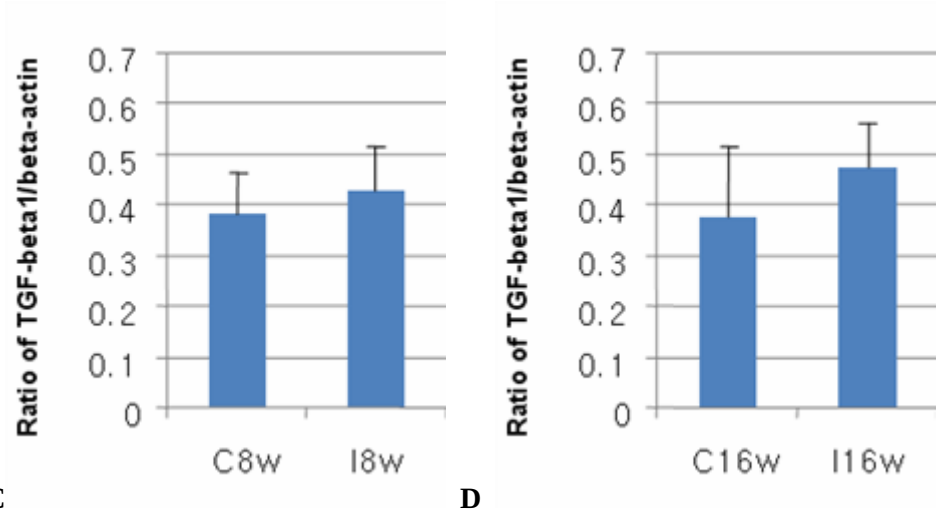
Total RNA extracted from the penile tissues of rats was analyzed by RT-PCR to investigate the mRNA expression of TGF- β_1. The ratio of band density of the TGF- β_1 to β-actin was significantly higher ($P<0.01$) in the control group than those of the stem cell group of 4 weeks group (Figure 5). However, there is no significant difference ($P>0.05$) between the control group and the stem cell group of 8 and 16 weeks group (Figure 5)


Normal-4
Beta-actin
C-4 C-4 I-4 I-4 C-8 C-8 I-8 I-8 C-16 C-16 I-16 I-16
TGF-beta 1
Beta-actin
C-4 C-4 I-4 I-4 C-8 C-8 I-8 I-8 C-16 C-16 I-16 I-16
TGF-beta 1
Beta-actin
C-4 I-4 I-4 C-8 I-8 I-8 C-16 C-16 I-16 I-16
TGF-beta 1
Beta-actin

A


Ratio of TGF-beta1/beta-actin
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
N4w*
C4w*
I4w*

B



C **D**

Figure 5. PCR analysis for TGF-β_1 mRNA expressions. (A) Products of PCR for TGF-β_1 and β-actin in 4, 8, and 16 weeks stem cell injection (I) and control (C) groups. (B-D)) Analysis for band integrated optical density. The bars represent means±SE of the ratio of TGF-β_1 mRNA to β-actin mRNA The ratio of band density of the TGF- β1 to β-actin was only significantly higher (P<0.01) in the control group than that in the stem cell group of 4 weeks group.

4. Histological Analysis.

In the penis from the rats injected with Feridex-labeled MSCs, Prussian blue stain showed dense intracellular iron labeled cells, with iron exclusions from the nucleus. After 8 week of injection, blue colored cells were detected in sections corresponding to the signal void lesions seen on MRI (Fig. 6A and 6B). Apart from the injection site, Feridex-labeled cells were seen with elongated shape and parallel in same direction (Fig. 6B).

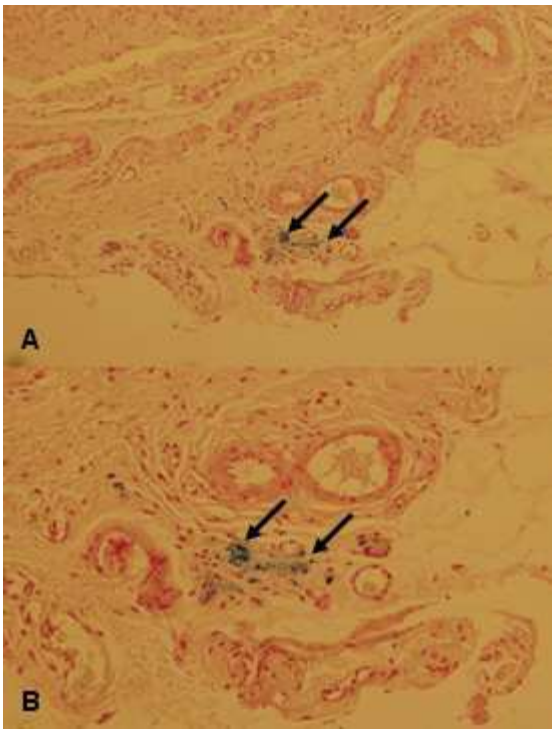


Figure 6. Histologic section with Prussian blue stain.

(A) and (B): Histologic section with Prussian blue stain at 8 week after Feridex-labeled MSC injection. Histological section with Prussian blue stain corresponding to signal void regions on MRI shows multiple cells with blue iron inclusions (arrow).

IV. DISCUSSION

Cellular therapy is an interesting era in the treatment of disease. However, noninvasive imaging methods to monitor the temporal–spatial migration into a targeted organ and nontoxic chemical or genetic agents for labeling cells are essential prerequisite for stem cell or other cell-based therapies under investigation in preclinical studies or clinical studies targeting tissues in the body. As previously mentioned, a reliable in vivo imaging method in localizing transplanted cells and monitoring their restorative effects will make a systematic investigation of cell therapy possible. Various cells, including stem cells, genetically engineered cells, immune cells, and other mammalian cells, are used in experimental models to determine if these cells will home to target sites as part of restoration of treatment strategies. There have been vigorous researches to find techniques able to determine the location of therapeutic cells infused or transplanted into the organs and to track these cells over an extended period of time. However, there are limitations in current imaging techniques. Clinical nuclear medicine approaches show relatively short monitoring period of 2-3days following the infusion of labeled cells because of the radioisotope half-life and also radioactivity may damage DNA[25]. Optical and bioluminescent imaging requiring the introduction of genes or potentially toxic agents (i.e., Qdots) into

the cell are only possible in preclinical experiments[26,27].

On the other hand, cMRI of labeling cells with SPIO nanoparticles potentially has several potential advantages over existing techniques. MRI has the high spatial resolution and SPIO nanoparticles are nontoxic to the cells because the iron oxide nanoparticles are biodegradable and metabolized by cells. Through noninvasive techniques such as MRI, long-term monitoring of transplanted cells can potentially allow to translate cell-based therapy into the clinic[28].

The majority of MR contrast agents and transfection agents for cell labeling are not approved for clinical use. In our study, we used two FDA-approved commercially available agents, ferumoxides (Feridex) and protamine sulfates, for labeling cells and it was successful. Ferumoxides, a suspension consisting of dextran-coated SPIO, is highly negative charged SPIO nanoparticles and polycationic transfection agents should be used for efficient intracellular labeling[11,13]. Transfection agents include classes of agents such as dextrans, phosphates, artificial lipids(e.g., Lipopectamine), proteins(e.g., poly-L-lysine and poly-L-arginine), and dendrimers (e.g., Superpect)[24]. Polycationic transfection agents bind to the dextran coat of ferumoxides through electrostatic interactions, thereby modifying the ferumoxides' distribution of positive and negative surface charges that can adhere to the cell membrane[10,11].

We used protamine sulfate as a transfection agent. In our study, we could verify

that cell was effectively labeled with ferumoxides-protamine sulfate without residual extracellular iron complexes. Protamine sulfate is a naturally occurring peptide containing approximately 60-70% of arginine that is clinically used worldwide as an antidote to heparin-induced anticoagulation and is well-tolerated by cells, with a high therapeutic window[29]. Arbab AS et al recently reported that magnetic labeling of cells with ferumoxides-protamine sulfate complexes is comparable or superior to other methods and addition of heparin to cell washes after labeling can completely dissolve surface bound particles of ferumoxides-protamine sulfate complex through competition, therefore very clean labeling is possible with minimum extracellular iron complexes[12,24]. These facts were concordant with our results.

The main cause of erectile dysfunction is thought to be the damage of penile cavernous smooth muscle cells and sinus endothelial cells by various metabolic conditions or mechanical manipulations. To maintain and regulate erectile function, the integrity of cavernous endothelial cells and smooth muscle cells is critical, therefore accelerating intact cavernous smooth muscle cells and sinus endothelial cells repair can be a novel treatment for erectile dysfunction. There have been *in vitro* and *in vivo* in animal and/or human studies of several drugs, and showed relaxant activity in cavernous smooth muscle and an increase in the percent of smooth muscle[30,31].

It is widely accepted that cavernosal nerve (CN) injury is the main cause of the ED after RP, although RP is presently regarded as the dominant therapy for clinically localized prostate cancer[32]. A bilateral nerve-sparing approach can not protect most patients from ED after the procedure[33, 34]. Currently, the documented data on molecular pathophysiological process of ED after RP are insufficient, although there were several initial reports. Fraiman et al. [35] and Savoie et al. [36] reported that many patients following RP lost erectile ability coupled with a gradual reduction in the size of the penis. Klein et al. [37] reported increased apoptosis in penile tissue of rats after denervation, which provide some explanation at the molecular level for the mechanisms of ED and decrease in penile size. They suggested that ischemia in penile tissue was resulted from a change in the penile blood flow in response to denervation and it was a possible mechanism responsible for apoptosis after denervation.

In the present study we ablated both CNs in a rat model, which mimics patients after RP with no nerve sparing. The expression of TGF-β1 was increased in neurectomy rats than the normal rats in 4 weeks group. According to previous studies, this neuretomy model showed that the expression of TGF-β1 was much higher in the neurectomy than in the sham-operated group, both at the mRNA and protein level. The present results show an increase in the synthesis of a high expression of TGF-β1 in the penile tissue of rats after cavernosal neurectomy,

which may be induced by loss of nocturnal and normal erection. Erectile function would be further compromised by fibrosis of the penile tissue [38]. An increased expression of TGF-β1 in penile tissue which promotes the synthesis of collagen may be one of the important factors for the erectile dysfunction caused by bilateral CN ablation. Similar pathophysiological processes may occur in the corpus cavernosum of patients after radical prostatectomy.

The explanation that hypoxia in penile tissue could be induced by CN ablation are as follows: The penile blood partial pressure of oxygen reached 90–100 mmHg during erection, whereas it is only 25–40 mmHg during flaccidity [39]. Healthy men usually have three to five phases of nocturnal penile erection, each lasting 30–45 min[40]. The total duration of 1.5–3 hours of nocturnal penile erection every night could maintain a high level of penile blood partial oxygen pressure, which may promote the synthesis of PGE1 and inhibit the synthesis of collagen induced by TGF-β1[41]. Thus it is important to have normal nocturnal penile erections to protect the penis from fibrosis and maintain normal erectile function. A decrease in nitric oxide synthase-positive nerve fibers which was responsible for relaxing some minor arteries in the corpus cavernosum after denervation might be another potential factor [42,43].

TGF-β1 is one of cytokines with a chemotactic role in inflammatory cells and fibroblasts, and that can modulate extracellular matrix by increasing the synthesis

of its components and inhibiting the expression of hydrolytic proteinases[44, 45]. Several studies reported a relationship between hypoxia and cavernosal fibrosis. Moreland et al.[41] reported that TGF-β1, which is induced by hypoxia, can increase collagen synthesis in human corpus cavernosal smooth muscle cells in culture, and prostaglandin E1 (PGE1) can suppress the induction of collagen synthesis by TGF-β1 in smooth muscle of human corpus cavernosum. Afterwards, Moreland[46] further claimed that hypoxia may induce the expression of TGF-β1 and inhibit the synthesis of PGE1 in penile tissue. A high expression of TGF-β1 suggests that ischemia and hypoxia may have occurred in the penile tissue of rats after denervation[46]. There was a report that plasma TGF- β1 level was significantly increased in patients with ED, especially those with a vasculogenic cause[47]. These studies implicated that the increase in TGF- β1 is the cause or has an effect on cavernosal fibrosis and ED but a vast clinical study with simultaneous analysis of blood TGF- β1 and cavernosal fibrosis in a large patient population would be need to verify them.

Montorsi et al reported a 67% recovery of spontaneous erections sufficient for sexual intercourse after a nerve-sparing RP, by early use of intracavernosal alprostadil injections three times weekly for 3 months, compared with only 20% recovery in a group with no alprostadil injections after the procedure[48]. The authors hypothesized that injecting vasoactive substances could improve

cavernosal oxygenation, thereby limiting the development of hypoxia-induced tissue fibrosis during the period of postoperative neurapraxia.

In the current study, we aimed to identify transplanted cells *in vivo* using clinical MR scanner in rat model of radical prostatectomy. The serial in vivo MR stem cell tracking was successful in rat penis of radical prostatectomy mode and we were able to detect intracavernosal MSCs injection sites in rat penis by *in vivo* MRI. Injection sites at the penis appeared as signal void lesions on MRI after Feridex-labeled MSCs injection,. The SPIO nonoparticles shorten the T1, T2 and T2* relaxation times of water or tissue and have a high T2 relaxivity/T1 relaxivity ratio and significant capacity to reduce MRI signal, which can be emphasized by using spin echo sequences with longer echo time and by using gradient echo sequences [49]. Therefore, intracellular SPIO of transplanted cell could be readily detected as a signal void area (dark lesion) by MR imaging. On the other hand, the signal void lesions showed decrease in size along the serial MR images. They were considerably decreased around 12 weeks and most spots became tiny spot, and were difficult to be detected. The current study did not include elucidating the causes of decreased signal void size because the primary goal of this study was to determine if magnetically labeled MSCs could be imaged *in vivo* in a rat model of radical prostatectomy. The possible cause of it might be death, migration, and differentiation of stem cell. In a recent report,

stem cell injected into cavernosum of old aged rat showed survival up to 21 days and differentiation into the smooth muscle and endothelial cell [50]. This topic needs further investigation.

One of the limitations of the MR imaging was that magnetically labeling cells cannot provide the in formation of the transplanted labeled cells' viability, function or ability to differentiate towards a desired lineage. Multimodal imaging approaches combining MRI with other imaging modalities including nuclear medicine modalities (e.g., positron emission tomography) or optical and bioluminescent imaging in experimental models may be useful to determine the restored functional status of the target tissue. Cellular MRI could be used to identify the migration and homing of administered cells at the site of interest and then nuclear medicine or other imaging modalities could be used to determine the functional improvement of the target tissues or organs [28].

In summary, our results demonstrate that MSCs labeled with Feridex can be tracked *in vivo* in the rat genitalia of radical prostatectomy model. The ability to perform noninvasive tracking of labeled cells suggest that MRI might be a viable tool for tracking and therapeutic monitoring in the future clinical study of stem cell therapy in erectile dysfunction

V. CONCLUSION

MRI allows tracking of injected stem cells *in vivo* in the rat genitalia of radical prostatectomy model. In the experimental study and clinical trial of cell transplantation in erectile dysfunction disease, MRI could be a potential tool for *in vivo* tracking and monitoring of therapy.

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ABSTRACT (IN KOREAN)

백서 생식기에서 초상자성 산화철을 이용한 줄기세포의 생체 내 추적 자기공명영상

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오영택

연구목적: 이 연구의 목적은 자기적으로 표지된 간엽줄기세포를 백서의 근치전립샘절재술모델에 주입하였을 때 생체내에서 자기공명영상(MRI)을 이용하여 주입된 세포를 영상화하고 생식기의 섬유화 차이를 알아보기 위해서이다.

연구대상 및 방법: 36마리 수컷 백서(SD rat)를 4주, 8주, 16주 군으로 나눈 후의 양측 해면 신경 (cavernos nerve)을 절단한 후 초자기적 산화철 입자인 Feridex로 표지된 인간간엽줄기세포를 18 마리 백서의 음경 해면체에 주입하였고 18마리 대조군에는 세포가 포함되지 않은 배지를 같은 방법으로 주입하였다. 1.5 테슬라 MRI기계와 47mm 의 미세코일을 이용하여 16주까지 백서가 살아있는 상태에서 영상을

얻었다. 자기공명영상 촬영 후에, 음경 조직을 적출하여 transforming growth factor (TGF)-β1 TGF-β_1 발현을 polymerase chain reaction (PCR) 방법으로 조사하였다.

연구결과: Feridex로 표지된 간엽줄기세포는 생체 내 MRI에서 주입 후 신호감소영역으로 관찰되었다. 신호감소영역은 시간이 경과함에 따라 크기가 작아짐을 보였다. PCR 검사 결과 간엽줄기세포 4주군에서 주입군에 비해 비교군의 TGF-β_1 발현이 통계적으로 유의하게 높게 나타났다.

결론: MRI는 근치전립샘절재술 백서 모델에서 음경에 주입된 세포를 생체 내에서 용이하게 추적할 수 있음을 보여주었으며, 이 방법은 이러한 발기부전 백서 음경에 주입된 간엽줄기세포의 운명을 조사하는데 유용한 방법으로 생각된다.

핵심되는 말: 자기공명영상, 산화철, 근치전립샘절재술, 백서, 줄기세포