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Human Organic Anion Transporting Polypeptide 1B3 Applied as an MRI-Based Reporter Gene

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Objective: Recent innovations in biology are boosting gene and cell therapy, but monitoring the response to these treatments is difficult. The purpose of this study was to find an MRI-reporter gene that can be used to monitor gene or cell therapy and that can be delivered without a viral vector, as viral vector delivery methods can result in long-term complications.

Materials and Methods: *CMV promoter-human organic anion transporting polypeptide 1B3* (*CMV-hOATP1B3*) cDNA or *CMV-blank* DNA (control) was transfected into HEK293 cells using Lipofectamine. *OATP1B3* expression was confirmed by western blotting and confocal microscopy. *In vitro* cell phantoms were made using transfected HEK293 cells cultured in various concentrations of gadoxetic acid for 24 hours, and images of the phantoms were made with a 9.4T micro-MRI. *In vivo* xenograft tumors were made by implanting HEK293 cells transfected with *CMV-hOATP1B3* (n = 4) or *CMV-blank* (n = 4) in 8-week-old male nude mice, and MRI was performed before and after intravenous injection of gadoxetic acid (1.2 μL/g).

Results: Western blot and confocal microscopy after immunofluorescence staining revealed that only *CMV-hOATP1B3*-transfected HEK293 cells produced abundant OATP1B3, which localized at the cell membrane. OATP1B3 expression levels remained high through the 25th subculture cycle, but decreased substantially by the 50th subculture cycle. MRI of cell phantoms showed that only the *CMV-hOATP1B3*-transfected cells produced a significant contrast enhancement effect. *In vivo* MRI of xenograft tumors revealed that only *CMV-hOATP1B3*-transfected HEK293 tumors demonstrated a T1 contrast effect, which lasted for at least 5 hours.

Conclusion: The human endogenous *OATP1B3* gene can be non-virally delivered into cells to induce transient OATP1B3 expression, leading to gadoxetic acid-mediated enhancement on MRI. These results indicate that *hOATP1B3* can serve as an MRI-reporter gene while minimizing the risk of long-term complications.

Keywords: *Reporter gene; MRI; Gadoxetic acid; Gene therapy; OATP1B3*

INTRODUCTION

Reporter genes play essential roles in biological research, such as allowing visualization of gene expression and tracking the migration of living cells (1, 2). A variety of reporter genes are currently available, and fluorescent protein-based optical imaging is probably the method most frequently used to assess gene expression. However, in spite of its many advantages, optical imaging suffers from limited imaging depth (3, 4), which may be a trivial limitation in *ex vivo* experiments, but is an obvious shortcoming for intravital imaging. Achieving a sufficient imaging depth

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is particularly difficult in humans, as the human body is larger than that of most animals used in research, and is an important technical consideration for imaging of human patients.

Recent innovations in biology, including next-generation sequencing (5, 6), *CRISPR-Cas9* gene editing (7, 8), and induced pluripotent stem cell technology (9, 10), are enabling treatment of diseases that were previously considered incurable (11-13). However, radiological studies based on anatomical imaging may not be effective in assessing the response to treatment. A reporter gene in human patients could be used in gene and cell therapy to assess the expression of a target gene or to visualize the bio-distribution of therapeutic cells (14).

Gadoxetic acid, which is sold under the tradename Primovist® (Bayer AG, Berlin, Germany), is a clinically-approved contrast agent widely used for magnetic resonance imaging (MRI) of the liver. Approximately 50% of an injected dose of gadoxetic acid is taken up by hepatocytes, and is subsequently excreted into the bile (15, 16). Gadoxetic acid is transported from the bloodstream into hepatocytes by the organic anion transporting polypeptides (OATP) 1B1 and 1B3, which are only expressed in hepatocytes (17). Consequently, the normal liver parenchyma is selectively enhanced at the hepatobiliary phase (18). A few groups have used either mouse *OATP1a1* or human *OATP1B3* as MRI gene reporters by transfecting target cells with viral vectors (19-22).

Although viral vectors ensure higher transfection efficiency and more stable expression of genes than non-viral transfection methods, they increase the risk of complications such as insertional mutagenesis (23). Overexpression of OATP1B3 has been reported to have undesirable effects in tumors (24), therefore raise a theoretical risk of unexpected biological influence that might be caused by persistent OATP1B3 expression induced by viral vectors.

Because of these concerns, we believe that if *human organic anion transporting polypeptide 1B3 (hOATP1B3)* is to be used as a gadoxetic acid-mediated MRI-reporter gene in humans, it should be delivered using a non-viral vector. Here, we used a non-viral vector method to transfect the *hOATP1B3* gene into HEK293 cells and evaluated *hOATP1B3* as a gadoxetic acid-enhanced MRI-reporter gene. The purpose of this study was to find an MRI-reporter gene that can be used to monitor gene or cell therapy and that can be delivered without a viral vector.

MATERIALS AND METHODS

This study was approved by the institutional animal care and use committee of Yonsei University Severance Hospital (2014-0178) and Samsung Medical Center (20181013001).

Cell Lines and Cell Cultures

A HEK293 cell line was purchased from the Korean cell line bank. The cells were maintained in high-glucose Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Hyclone, Logan, UT, USA), 1% penicillin, and 1% streptomycin (Hyclone). *HOATP1B3* cDNA in the pCMV3 vector (HG13013-UT, Sino biological Inc., Beijing, China) and the control vector CV011 (Sino biological Inc.) were transfected into HEK293 cells using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). To select transfected clones, 100 μg/mL hygromycin B (Invitrogen) was added to the cell cultures, and isolated colonies were picked using microscopy and grown separately.

Preparation of a Phantom Contrast Agent and *in Vitro* Phantoms

Either 12 μL of gadoxetic acid (Primovist®) or gadobenate dimeglumine (MultiHance®, Bracco Diagnostics Inc., Princeton, NJ, USA) was added to phosphate-buffered saline (PBS) to make a total volume 1 mL, and serial 1:10 dilutions were made with PBS. PBS without contrast agent was used as a negative control. Each well of a 16-well polymerase chain reaction (PCR) strip was filled with the aforementioned diluents to make MRI contrast phantoms. Then strips were sealed and dipped in a 50-mL Falcon tube containing PBS.

Gadoxetic acid or gadobenate dimeglumine in a volume of 1.2 μL was added to 1 mL of culture media, and serial 1:10 dilutions with culture media were made to prepare culture media with different concentrations of the contrast agent. Cell phantoms were then prepared by incubating *CMV promoter-human organic anion transporting polypeptide 1B3 (CMV-hOATP1B3)*- and *CMV-blank*-transfected HEK293 cells in media containing one of the two contrast agents. After 24 hours, the cells were harvested and the cell pellets were placed in 16-well PCR strips. Empty spaces in the strip were filled with PBS, and the strips were sealed and dipped in 50-mL Falcon tubes containing PBS (Supplementary Materials).

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In Vivo MRI Using of Mouse Xenograph Tumors

Five million HEK293 cells transfected with *CMV-hOATP1B3* (n = 4) or *CMV-blank* (n = 4) were implanted in 8-week old male nude mice for *in vivo* growth as xenograft tumors. All animals were anesthetized in an induction chamber using a mixture of 3% isoflurane and 97% oxygen and examined with a horizontal-bore 9.4T micro-MRI scanner (Biospec 94/20 USR, Bruker Biospin Inc., Billerica, MA, USA). During the procedure, anesthesia was maintained a mouth and nose mask. All animals were placed in a prone position in a dedicated animal cradle heated by a thermostat-driven water bath. For the contrast-enhanced study, gadoxetic acid, in a volume of 1.2 μL per g body weight, was injected through the tail vein.

MRI was performed using a linear, polarized coil with an inner diameter of 40 mm that was developed for imaging of the mouse abdomen (Bruker Biospin Inc.). Pre-contrast T1 weighted images (T1WIs) were obtained prior to gadoxetic acid injection and 5-minute, 30-minute, and 5-hour after injection.

Pre-/-post T1WIs were obtained using the T1 spin echo rapid acquisition with relaxation enhancement sequence technique with the following parameters: echo time = 8.06 ms, repetition time = 494 ms, field of view = 35 x 23 mm, matrix size = 256 x 256, slice thickness = 1 mm, flip angle = 90°, number of excitation average 5, and bandwidth 2000. The total measurement time was approximately 5 minutes (Table 1). All images were acquired with fat suppression.

Table 1. Imaging Parameters Used for MRI

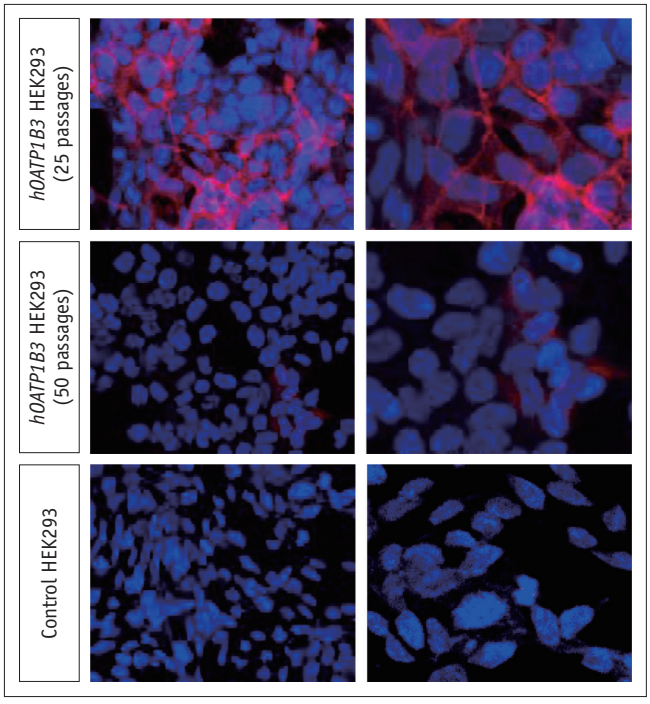
Sequence	T1 Spin Echo RARE	T1 Mapping
Pulse sequence	2D spin echo	RARE with variable TR
Matrix	256 x 256	128 x 128
TE	8.06	10
TR	494	200, 400, 800, 1500, 3000
NEX	Average 5	1
Flip angle	90	90
Bandwidth Hz/pixel	2000	100
FOV (mm)	35 x 23	80 x 40
Section thickness (mm)	1	1
Slice spacing (mm)	1	1
Fat suppression		

FOV = field of view, NEX = number of excitation, RARE = rapid acquisition with relaxation enhancement, TE = echo time, TR = repetition time, 2D = two-dimensionl

RESULTS

OATP1B3 Expression in *CMV-hOATP1B3*-Transfected HEK 293 Cells

Confocal microscopy and western blotting revealed that *CMV-hOATP1B3*-transfected HEK293 cells, but not *CMV-blank*-transfected cells, expressed hOATP1B3 protein in the cell membrane. Expression of hOATP1B3 remained high through the first 25 cell culture cycles that occurred during cell phantom preparation (Fig. 1), but gradually dropped after another 25 cycles, until only a relatively small fraction of cells expressed hOATP1B3 (Fig. 1).



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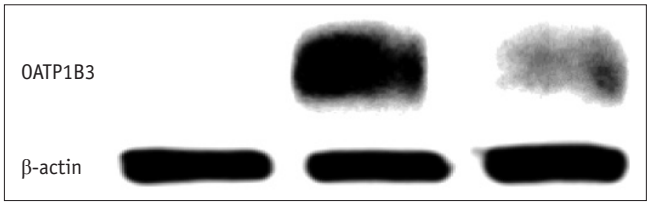

B

Fig. 1. Generating *CMV-hOATP1B3* overexpressing HEK293 cells.
A. Immunofluorescence staining and confocal microscopy of HEK293 cells passed for 25 (upper row) or 50 (middle row) cycles after transfection with *CMV-hOATP1B3*; (lower row) *CMV-blank* transfected HEK293 cells for negative control (left column: x 20, right column: x 40, hOATP1B3 [red], DAPI [blue]). **B.** Western blot analysis of hOATP1B3 (upper row) and β-actin (lower row) in HEK293 cells transfected with (left) *CMV-blank* (negative control) or with *CMV-hOATP1B3* and passed for 25 (middle) or 50 (right) cycles. *CMV-hOATP1B3 = CMV promoter-human organic anion transporting polypeptide 1B3*

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MRI of *in Vitro* Cell Phantoms

We tested the function of two commercially available hepatocyte-selective gadolinium contrast agents (gadoxetic acid and gadobenate dimeglumine) known to be transported by OATP1 (25). Using a 9.4T micro-MRI, we first scanned a phantom containing each of the contrast agents diluted to various concentrations and confirmed that the agents both generated sufficient T1 contrast (Supplementary Fig. 1). Next, to determine if hOATP1B3-expressing HEK293 cells can functionally induce the OATP1B3-mediated contrast enhancement effect, we made cell phantoms with the pellets of either *CMV-hOATP1B3*- or *CMV-blank*-transfected HEK293 cells that had been cultured for 24 hours either with gadoxetic acid or gadobenate dimeglumine. In MRI scans, only *CMV-hOATP1B3*-transfected HEK293 cell pellets cultured in the presence of gadoxetic acid (1.2 μL/mL media) demonstrated perceivable contrast enhancement (Fig. 2A, Supplementary Fig. 2). The pellets of cells cultured at lower gadoxetic acid concentrations and the pellets of cells cultured in gadobenate dimeglumine did not show significant contrast enhancement (Fig. 2, Supplementary Figs. 2, 3).

MRI of *in Vivo* Xenograft Tumor-Bearing Mice

Based on the cell phantom results, we proceeded with *in vivo* studies using gadoxetic acid. To mimic the conditions of the cell phantom, the HEK293 cells were passed through approximately 25 culture cycles after *CMV-hOATP1B3*-transfection and then implanted into the flanks of nude mice. *CMV-blank*-transfected HEK293 xenograft tumors were also implanted as a control. *In vivo* MRI was performed using a 9.4T scanner before and after an intravenous bolus injection of gadoxetic acid (1.2 μL/g body weight). The dosage of gadoxetic acid was determined from a previous reference, which translated the equivalent human dose from other species according to body surface area (26).

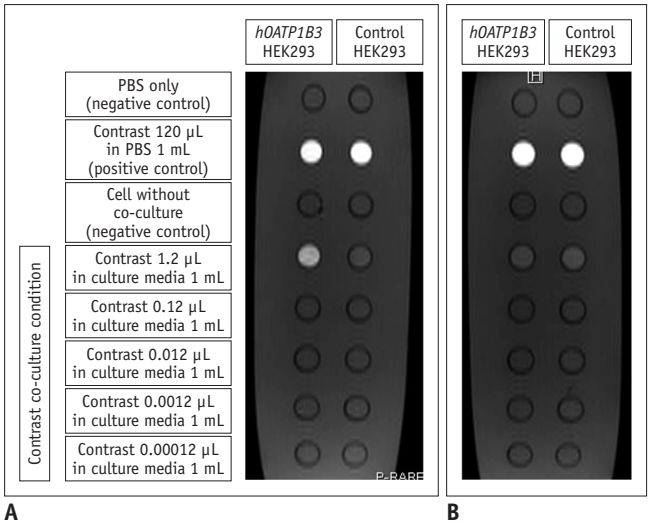

Fig. 2. 9.4T MRI T1WIs of (A) gadoxetic acid and (B) gadobenate dimeglumine based cell phantoms. Either *CMV-hOATP1B3* transfected HEK293 cells (left column) or *CMV-blank* transfected control HEK293 cells (right column) were pelleted. PBS = phosphate-buffered saline, T1WI = T1 weighted image

Liver parenchymal enhancement was observed in scans taken approximately 30 minutes after the gadoxetic acid injection in both *CMV-hOATP1B3*-transfected HEK293 xenograft and *CMV-blank*-transfected HEK293 xenograft tumor-bearing mice (Fig. 3). We used this liver enhancement (27) as an internal reference for physiologic mouse OATP expression in hepatocytes. Both the *CMV-hOATP1B3*-transfected HEK293 xenograft tumors and the control xenograft tumors showed heterogeneous enhancement, although enhancement of the *CMV-hOATP1B3*-transfected HEK293 xenograft appeared to be more prominent (Fig. 3). However, when we re-scanned the mice approximately 5 hours later, only the *CMV-hOATP1B3*-transfected HEK293 xenograft and not the control xenograft maintained perceivable enhancement (Fig. 3) and enhancement in the mouse liver was no longer observed (Fig. 3).

Immunohistochemistry was used to detect OATP1B3 protein in the xenograft tumors. In *CMV-hOATP1B3*-transfected xenografts, both OATP1B3 positive- and negative-cells were present. In OATP1B3 positive cells, the protein signal was localized at the cell membrane (Fig. 4).

Transfection of HEK293 Cells with an Overexpressing *hOATP1B3* Gene

Next, to increase the sensitivity of the reporter gene and to enable co-express of green fluorescent protein, we designed a modified *hOATP1B3* gene with an enhanced capacity for hOATP1B3 expression. This modified gene, which was named *CMV-Kozak-(codon-optimized) hOATP1B3-IRES-GFP* (Supplementary Materials), was transfected into HEK293 cells. Confocal microscopy revealed that, unlike normal hepatocytes or *CMV-hOATP1B3*-transfected HEK293 cells, in which OATP1B3 is localized in the membrane (Fig. 1) (28), in *CMV-Kozak-(codon-optimized) hOATP1B3-IRES-GFP*-transfected cells, hOATP1B3 was localized mostly in the cytoplasm (Supplementary Fig. 4). MRI was performed on cell phantoms containing pellets of *CMV-Kozak-(codon-optimized) hOATP1B3-IRES-GFP*-transfected cells that had been cultured with either gadoxetic acid or gadobenate

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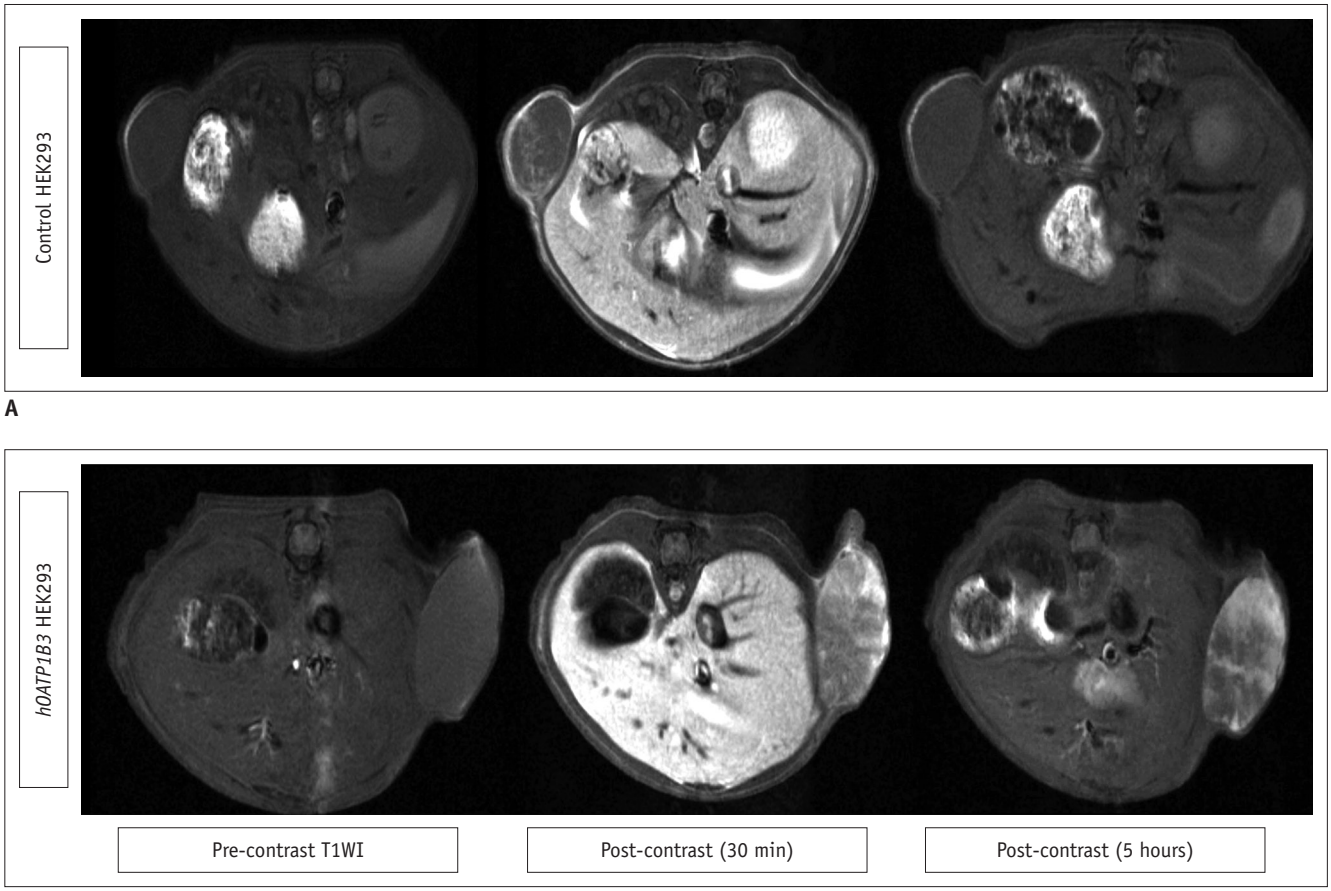

Fig. 3. 9.4T MRI T1WIs of (A) *CMV-blank* transfected xenograft tumor and (B) *CMV-hOATP1B3* transfected xenograft tumor-bearing mice. Pre-contrast images (left), post-contrast images obtained 30 minutes (middle), and 5 hours (right) after gadoxetic acid intravenous injection.

dimeglumine, but no significant T1 enhancement was observed (Supplementary Figs. 5, 6).

DISCUSSION

In this study, our goal was to identify a reporter gene that can safely be used in humans undergoing gene or cell therapy to monitor gene expression or track genetically labeled cells (2). Human imaging is more challenging than animal imaging because 1) the human body is much larger than the bodies of most animal used in research, making it more technically challenging to achieve a sufficient imaging depth; 2) imaging methods used on humans need to be both robust and convenient if they are to be integrated into the daily workflow; 3) for safety reasons, in humans, the reporter gene should preferably be an endogenous human gene and not an exogenous gene such as a luciferase or fluorescent protein (2); and 4) if possible viral vectors should not be used to deliver the reporter gene in humans, as integration of virus DNA into chromosomes can cause unexpected complications (23).

We believe that gadoxetic acid-enhanced MRI can be exploited to meet the aforementioned conditions. MRI is a widely used diagnostic imaging technique that provides sufficient imaging depth sufficient to scan the whole body (29). Moreover, MRI can be used to produce a detailed anatomical evaluation and soft tissue characterization, and this information, along with information about the expression of a target gene, can be useful in assessing the effects of gene or cell therapy on patients.

Several groups have made significant progress in developing MRI-reporter genes and using these genes in biomedical research (14, 30-34). To be useful, an MRI-reporter gene must be capable of detecting expression of the target gene at an adequate signal-to noise-ratio. Most MRI-reporter genes developed to date have disadvantages, including low detection sensitivity, inadequate signal changes, and requirement of substrates with undesirable

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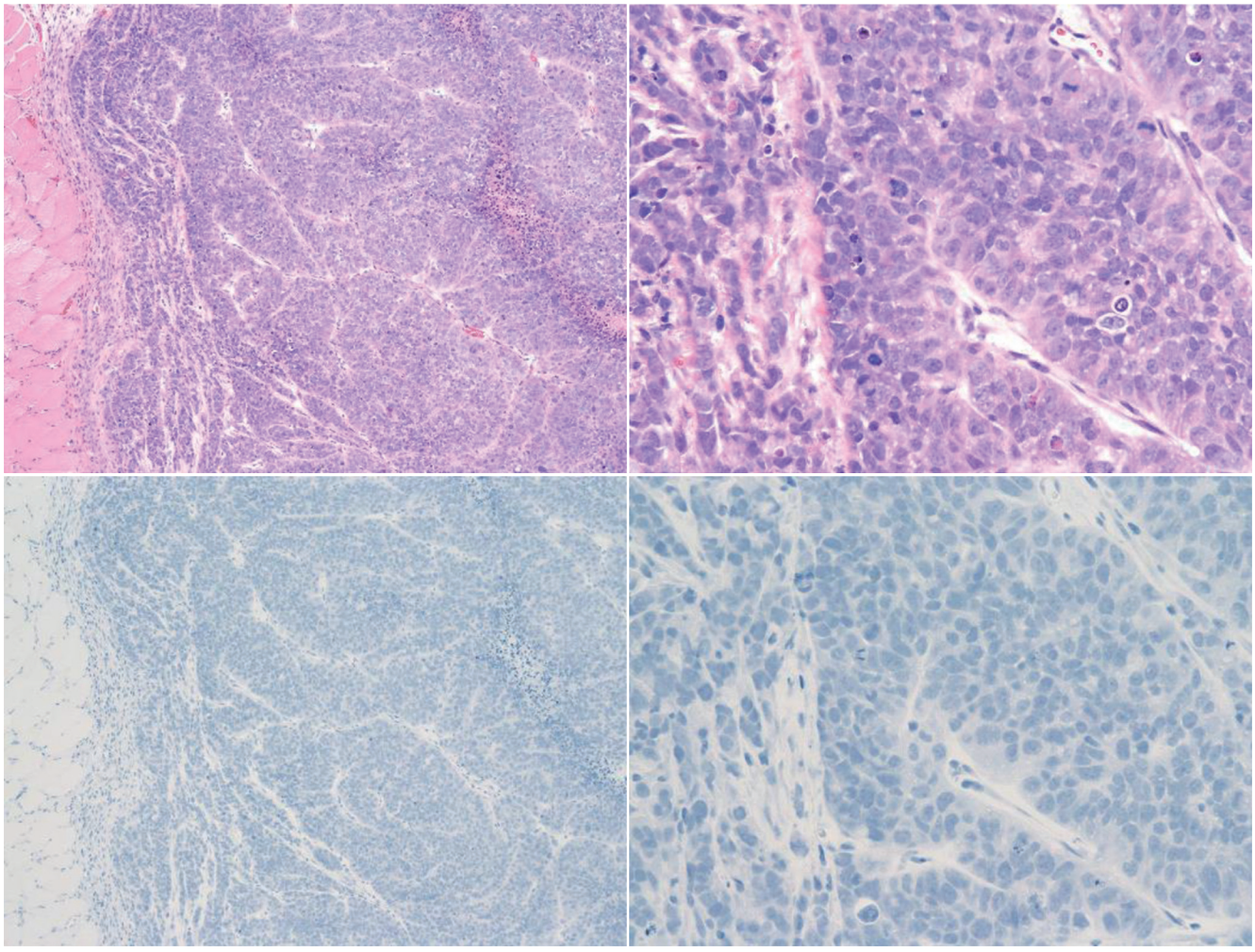

A

Fig. 4. Hematoxylin and eosin staining (upper: x 10, x 40) and hOATP1B3 immunohistochemistry (lower: x 10, x 40) of *in vivo* xenograft tumor specimens.
(A) Control *CMV-blank* transfected HEK293 xenograft tumor and **(B)** *CMV-hOATP1B3* transfected HEK293 xenograft tumor.

pharmacokinetics, that have limited their usefulness (14, 35). Many of these MRI-reporter genes, which have primarily been used for research, mostly require genes exogenous to human and/or metal-complex injection which may raise safety issues if applied to human patients.

Recent progress has been made using *OATP1a1* from mouse and *OATP1B3* from humans as gadoxetic acid-dependent MRI-reporters (19-22). But these studies have focused on the imaging in animals, and the viral vector delivery method used in these studies may be potentially unsafe for humans for the following reasons. First, viral vectors can cause insertional mutagenesis, leading to malignant transformation of cells (23, 36). Second, persistent OATP1B3 expression induced by viral vectors may have adverse effects such as reducing transcriptional activity of p53, which can lead to tumor apoptotic resistance (24). Moreover, as OATP1B3 is responsible for the cellular transportation of many drugs (37), the non-physiologic, sustained overexpression of this gene may alter the action of medications taken by human patients.

We believe that a non-viral method is a safer than a viral method for delivering the OATP1B3 into humans. However, because non-viral vectors are often inefficient in gene delivery and induce transgenic expression transiently (23), doubts have been raised as to whether non-viral methods of gene delivery will be technically feasible. Here, we show that *OATP1B3* performs adequately as a reporter gene when delivered using non-viral vector.

Our analysis was performed using the human *OATP1B3* gene constitutively activated with a CMV promoter (*CMV-hOATP1B3*). We chose to use the human *OATP1B3* gene rather than the mouse *OATP1a1* gene because the human

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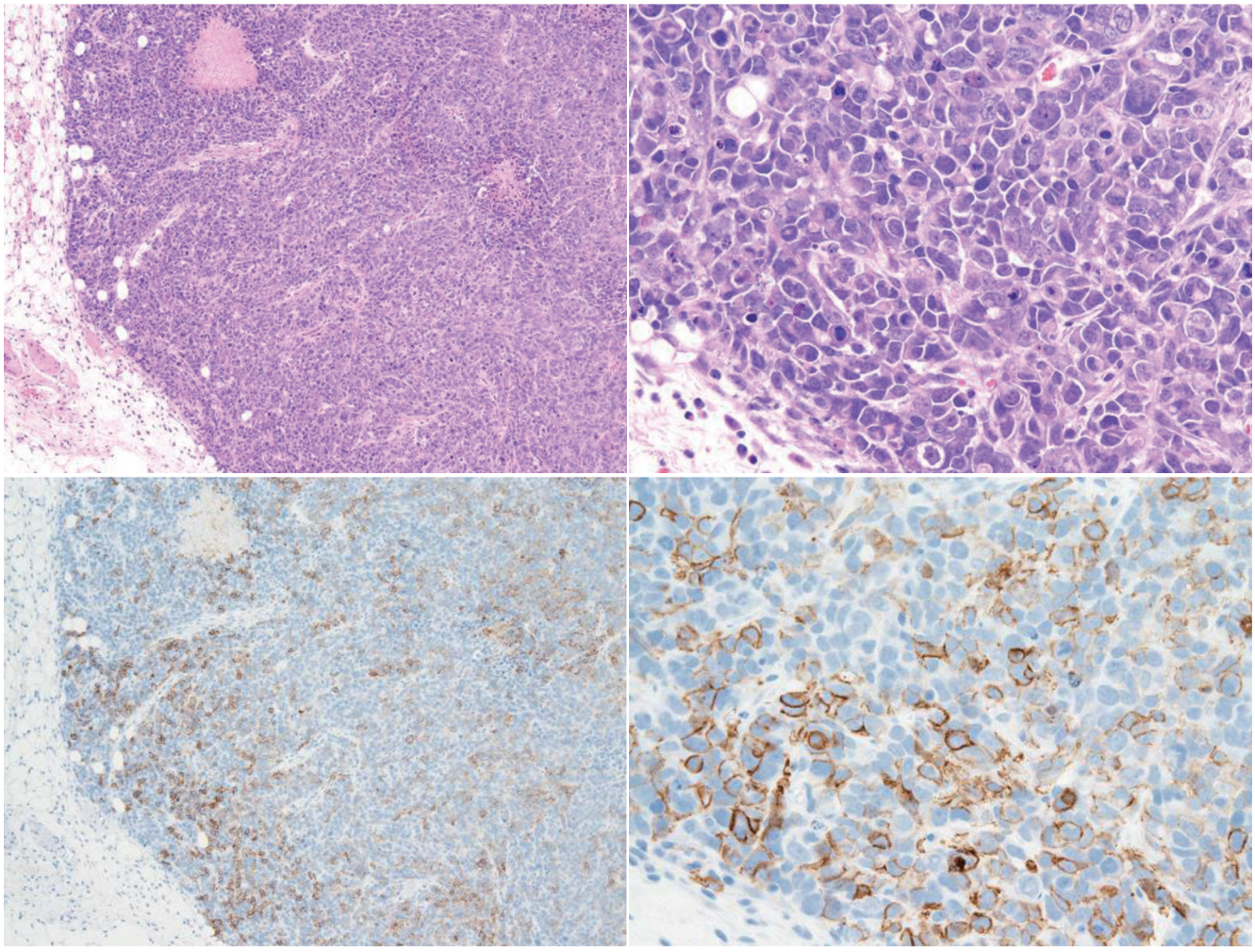

B

Fig. 4. Hematoxylin and eosin staining (upper: x 10, x 40) and hOATP1B3 immunohistochemistry (lower: x 10, x 40) of *in vivo* xenograft tumor specimens.
(A) Control *CMV-blank* transfected HEK293 xenograft tumor and **(B)** *CMV-hOATP1B3* transfected HEK293 xenograft tumor.

endogenous gene is less likely to elicit an immune response in patients (2). The *OATP1B3* reporter gene was transfected into HEK293 cells using Lipofectamine, a non-viral delivery method. Theoretically, a gene transfected using Lipofectamine is unlikely to become integrated into the chromosome; therefore, its expression is likely to be transient. We observed that in most cells hOATP1B3 expression levels remained steady for 25 *in vitro* subculture cycles, and then gradually faded away (Fig. 1). The 25 culture cycles through which hOATP1B3 was expressed allowed ample time to perform MRI imaging studies, and we believe that the duration of hOATP1B3 expression will be sufficient to establish therapeutic protocols in humans. An ideal reporter gene should be biologically inert and have little or no influence on the cells in which it is expressed (35). We recognize that it is possible that hOATP1B3 overexpression may have unintended consequences and believe that our strategy of limiting the expression of hOATP1B3 to a limited period after gene delivery will reduce the risk of undesirable side effects. We anticipate that our method may be adapted for the monitoring of therapeutic cells. For example, the *OATP1B3* gene could be non-virally delivered into induced pluripotent stem cells or immune cells *in vitro* so that the cells could be visualized by gadoxetic acid-enhanced MRI after injection into the body.

When *in vivo* MRI of xenograft-bearing mice was performed 30 minutes after gadoxetic acid injection, the liver displayed enhancement as expected, given the physiological role of mouse OATP expression in hepatocytes (27). *CMV-hOATP1B3*-transfected xenograft tumors displayed heterogeneous enhancement until 5 hours after the gadoxetic acid injection. In contrast, the

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minimal enhancement of *CMV-blank*-transfected xenograft tumors that was observed 30 minutes after gadoxetic acid injection, which was presumably a non-selective interstitial enhancement, disappeared after 5 hours. Enhancement of the liver was also lost after 5 hours, because hepatocytes express not only mouse OATP, which transports gadoxetic acid into the cells, but also multi-drug resistant proteins that excrete the contrast agent into bile (38), resulting in a loss in enhancement over time. However, since multi-drug resistant proteins were not expressed in tumor cells, gadoxetic acid would have accumulated in the tumor resulting in long-term enhancement.

Most previously described MRI-reporter genes have exhibited relatively low sensitivity (35). In an effort to increase the sensitivity of our reporter gene, we designed an artificial construct, named *CMV-Kozak-(codon-optimized) hOATP1B3-IRES-GFP*, to enhance the efficiency of OATP synthesis. Codon optimization of the *hOATP1B3*-coding sequence of this construct was performed to increase translational efficiency, a Kozak sequence was added to the 5′ end to increase transcription efficiency, and an *IRES-GFP* sequence was added to enable GFP synthesis (Supplementary Fig. 4). Although a large amount of hOATP1B3 protein was produced, confocal imaging indicated that the protein was distributed in the cytoplasm and not in the membrane, as is normally observed in hepatocytes (28). The cytoplasmic distribution of hOATP1B3 observed here is similar to the cytoplasmic distribution of aberrantly expressed hOATP1B3 observed in some tumors (24). In an *in vitro* cell phantom study of HEK293 cells transfected with artificially over-expressed *hOATP1B3*, no significant enhancement was observed in MRI scans (Supplementary Fig. 5), presumably because hOATP located in the cytoplasm could not transport gadoxetic acid into the cells.

Our results indicate that hOATP1B3 shows promise as an MRI-reporter gene. However, more work needs to be done to assess the safety and efficacy hOATP1B3 as a reporter gene in humans. First, further research needs to be done to evaluate possible adverse effects of hOATP1B3 overexpression, although, as our protocol induces transient expression of hOATP1B3, such adverse effects may be limited. Second, the sensitivity of the hOATP1B3 reporter gene needs to be further evaluated as it is unclear from our results if a small number of hOATP1B3-transfected cells can be detected. Third, the pharmacokinetics of gadoxetic acid accumulated within the target cells will need to be evaluated.

In conclusion, we show here that the human endogenous *OATP1B3* gene can be used as a gadoxetic acid-mediated MRI-reporter gene, and that this gene can be transiently expressed in target cells without using viral vectors, which minimizes the risk of long-term complications. We believe that the *hOATP1B3* gene can be used to monitor gene expression during human gene or cell therapy, and can also be used to assess the bio-distribution of therapeutic cells.

Supplementary Materials

The Data Supplement is available with this article at https://doi.org/10.3348/kjr.2019.0903.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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REFERENCES

1. Lyons SK, Patrick PS, Brindle KM. Imaging mouse cancer models in vivo using reporter transgenes. *Cold Spring Harb Protoc* 2013;2013:685-699
2. Brader P, Serganova I, Blasberg RG. Noninvasive molecular imaging using reporter genes. *J Nucl Med* 2013;54:167-172
3. Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat Biotechnol* 2004;22:1567-1572
4. Ntziachristos V. Going deeper than microscopy: the optical imaging frontier in biology. *Nat Methods* 2010;7:603-614
5. Anderson MW, Schrijver I. Next generation DNA sequencing

and the future of genomic medicine. *Genes (Basel)* 2010;1:38-69
6. Esplin ED, Oei L, Snyder MP. Personalized sequencing and the future of medicine: discovery, diagnosis and defeat of disease. *Pharmacogenomics* 2014;15:1771-1790
7. Coombe L, Kadri A, Martinez JF, Tatachar V, Gallicano GI. Current approaches in regenerative medicine for the treatment of diabetes: introducing CRISPR/CAS9 technology and the case for non-embryonic stem cell therapy. *Am J Stem Cells* 2018;7:104-113
8. Karimian A, Azizian K, Parsian H, Rafieian S, Shafiei-Irannejad V, Kheyrollah M, et al. CRISPR/Cas9 technology as a potent molecular tool for gene therapy. *J Cell Physiol* 2019;234:12267-12277
9. Dadheech N, James Shapiro AM. Human induced pluripotent stem cells in the curative treatment of diabetes and potential impediments ahead. *Adv Exp Med Biol* 2019;1144:25-35
10. Nagoshi N, Tsuji O, Nakamura M, Okano H. Cell therapy for spinal cord injury using induced pluripotent stem cells. *Regen Ther* 2019;11:75-80
11. Del Pozo-Rodríguez A, Rodríguez-Gascón A, Rodríguez-Castejón J, Vicente-Pascual M, Gómez-Aguado I, Battaglia LS, et al. Gene therapy. *Adv Biochem Eng Biotechnol* 2020;171:321-368
12. Mollanoori H, Teimourian S. Therapeutic applications of CRISPR/Cas9 system in gene therapy. *Biotechnol Lett* 2018;40:907-914
13. Steffin DHM, Hsieh EM, Rouce RH. Gene therapy: current applications and future possibilities. *Adv Pediatr* 2019;66:37-54
14. Gilad AA, Winnard PT Jr, van Zijl PC, Bulte JW. Developing MR reporter genes: promises and pitfalls. *NMR Biomed* 2007;20:275-290
15. Van Beers BE, Pastor CM, Hussain HK. Primovist, Eovist: what to expect? *J Hepatol* 2012;57:421-429
16. Bartolozzi C, Crocetti L, Lencioni R, Cioni D, Della Pina C, Campani D. Biliary and reticuloendothelial impairment in hepatocarcinogenesis: the diagnostic role of tissue-specific MR contrast media. *Eur Radiol* 2007;17:2519-2530
17. Leonhardt M, Keiser M, Oswald S, Kühn J, Jia J, Grube M, et al. Hepatic uptake of the magnetic resonance imaging contrast agent Gd-EOB-DTPA: role of human organic anion transporters. *Drug Metab Dispos* 2010;38:1024-1028
18. Huppertz A, Balzer T, Blakeborough A, Breuer J, Giovagnoni A, Heinz-Peer G, et al. Improved detection of focal liver lesions at MR imaging: multicenter comparison of gadoxetic acid-enhanced MR images with intraoperative findings. *Radiology* 2004;230:266-275
19. Nyström NN, Hamilton AM, Xia W, Liu S, Scholl TJ, Ronald JA. Longitudinal visualization of viable cancer cell intratumoral distribution in mouse models using oatp1a1-enhanced magnetic resonance imaging. *Invest Radiol* 2019;54:302-311
20. Patrick PS, Hammersley J, Loizou L, Kettunen MI, Rodrigues TB, Hu DE, et al. Dual-modality gene reporter for in vivo imaging. *Proc Natl Acad Sci U S A* 2014;111:415-420
21. Wu MR, Hsiao JK, Liu HM, Huang YY, Tseng YJ, Chou PT, et al. In vivo imaging of insulin-secreting human pancreatic ductal cells using MRI reporter gene technique: a feasibility study. *Magn Reson Med* 2019;82:763-774
22. Wu MR, Liu HM, Lu CW, Shen WH, Lin IJ, Liao LW, et al. Organic anion-transporting polypeptide 1B3 as a dual reporter gene for fluorescence and magnetic resonance imaging. *FASEB J* 2018;32:1705-1715
23. Glover DJ, Lipps HJ, Jans DA. Towards safe, non-viral therapeutic gene expression in humans. *Nat Rev Genet* 2005;6:299-310
24. Lee W, Belkhiri A, Lockhart AC, Merchant N, Glaeser H, Harris EI, et al. Overexpression of OATP1B3 confers apoptotic resistance in colon cancer. *Cancer Res* 2008;68:10315-10323
25. Filippone A, Blakeborough A, Breuer J, Grazioli L, Gschwend S, Hammerstingl R, et al. Enhancement of liver parenchyma after injection of hepatocyte-specific MRI contrast media: a comparison of gadoxetic acid and gadobenate dimeglumine. *J Magn Reson Imaging* 2010;31:356-364
26. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* 2008;22:659-661
27. Bang DH, Park SH, Jun HY, Moon HB, Kim SU, Yu DY, et al. Gd-EOB-DTPA enhanced micro-MR imaging of hepatic tumors in H-ras 12V transgenic mice. *Acad Radiol* 2011;18:13-19
28. Park SH, Kim H, Kim EK, Kim H, Choi DK, Chung YE, et al. Aberrant expression of OATP1B3 in colorectal cancer liver metastases and its clinical implication on gadoxetic acid-enhanced MRI. *Oncotarget* 2017;8:71012-71023
29. Schmidt GP, Reiser MF, Baur-Melnyk A. Whole-body MRI for the staging and follow-up of patients with metastasis. *Eur J Radiol* 2009;70:393-400
30. Ahrens ET, Bulte JW. Tracking immune cells in vivo using magnetic resonance imaging. *Nat Rev Immunol* 2013;13:755-763
31. Genove G, DeMarco U, Xu H, Goins WF, Ahrens ET. A new transgene reporter for in vivo magnetic resonance imaging. *Nat Med* 2005;11:450-454
32. Louie AY, Hüber MM, Ahrens ET, Rothbächer U, Moats R, Jacobs RE, et al. In vivo visualization of gene expression using magnetic resonance imaging. *Nat Biotechnol* 2000;18:321-325
33. Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, et al. In vivo magnetic resonance imaging of transgene expression. *Nat Med* 2000;6:351-355
34. Kim HS, Woo J, Lee JH, Joo HJ, Choi Y, Kim H, et al. In vivo tracking of dendritic cell using MRI reporter gene, ferritin. *PLoS One* 2015;10:e0125291
35. Choi Y, Huh J, Woo DC, Kim KW. Use of gadoxetate disodium for functional MRI based on its unique molecular mechanism. *Br J Radiol* 2016;89:20150666
36. Li Z, Düllmann J, Schiedlmeier B, Schmidt M, von Kalle C, Meyer J, et al. Murine leukemia induced by retroviral gene marking. *Science* 2002;296:497

https://doi.org/10.3348/kjr.2019.0903

37. Obaidat A, Roth M, Hagenbuch B. The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. *Annu Rev Pharmacol Toxicol* 2012;52:135-151
38. Tsuboyama T, Onishi H, Kim T, Akita H, Hori M, Tatsumi M, et al. Hepatocellular carcinoma: hepatocyte-selective enhancement at gadoxetic acid-enhanced MR imaging--correlation with expression of sinusoidal and canalicular transporters and bile accumulation. *Radiology* 2010;255:824-833

 https://doi.org/10.3348/kjr.2019.0903