

## Encapsulation Cell Therapy for Mucopolysaccharidosis Type VII Using Genetically Engineered Immortalized Human Amniotic Epithelial Cells

HIDEYUKI NAKAMA, KEIKO OHSUGI,<sup>1</sup> TAISUKE OTSUKI, ISAO DATE,<sup>2</sup>  
MOTOMICHI KOSUGA,<sup>3</sup> TORAYUKI OKUYAMA<sup>3</sup> and NORIO SAKURAGAWA<sup>4</sup>

*Department of Neurosurgery, <sup>1</sup>Department of Inherited Metabolic Disease, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan,*

*<sup>2</sup>Department of Neurosurgery, Okayama University Medical School, Okayama, Japan,*

*<sup>3</sup>Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan, and*

*<sup>4</sup>Department of Regenerative Medicine, Toho University School of Medicine, Tokyo, Japan*

NAKAMA, H., OHSUGI, K., OTSUKI, T., DATE, I., KOSUGA, M., OKUYAMA, T. and SAKURAGAWA, N. *Encapsulation Cell Therapy for Mucopolysaccharidosis Type VII Using Genetically Engineered Immortalized Human Amniotic Epithelial Cells.* Tohoku J. Exp. Med., 2006, **209** (1), 23-32 — Mucopolysaccharidosis type VII (MPSVII) is a lysosomal storage disease resulted from a deficiency of the enzyme  $\beta$ -glucuronidase (GUSB), which is necessary for degradation of glycosaminoglycans (GAGs). The deficiency of GUSB causes progressive accumulation of GAGs and subsequent lysosomal distension in multiple tissues, including the central nervous system (CNS). In murine experiments, bone marrow transplant, enzyme replacement, viral vectors, and genetically modified cells were successfully used for correction of the visceral accumulation of GAGs, but little improvement was seen in the brain, because these therapeutic agents cannot cross the blood-brain barrier (BBB). Although direct intracerebral injection of GUSB-encoding viral vectors has been developed to bypass the BBB, the possibility of tumor formation and the toxicity of over-expressed GUSB have been reported. In this study, we generated immortalized human amniotic epithelial (IHAE) cells to maintain the effect of implantation, and encapsulated these cells to prevent harmful immunological response and tumor formation and to regulate the level of GUSB expression within the host. Moreover, we generated IHAE cells that over-express and secrete human GUSB following transduction with an adenoviral vector encoding human GUSB. Therapeutic efficacy for MPSVII was evaluated in and ex vivo experiments using these encapsulated genetically engineered GUSB-encoding IHAE cells. We confirmed that encapsulated genetically engineered IHAE cells could secrete significant amounts of GUSB outside the capsule in vitro and into the cerebral

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Correspondence: Hideyuki Nakama, M.D., Department of Neurosurgery, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-higashicho, Kodaira, Tokyo 187-8551, Japan.

e-mail: nakama@ncnp.go.jp

parenchyma of C3H mice seven days after the capsule implantation. Thus, encapsulation cell therapy using genetically engineered IHAE cells is an effective armamentarium for the treatment of MPSVII. ——— mucopolysaccharidosis type VII; polymer-encapsulation; amniotic epithelial cell; immortalization;  $\beta$ -glucuronidase  
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Mucopolysaccharidosis type VII (MPSVII), a lysosomal storage disease (LSD), results from a deficiency of the lysosomal enzyme,  $\beta$ -glucuronidase (GUSB). MPSVII is characterized by short stature, mental retardation, hepatosplenomegaly, skeletal deformities, and excessive excretion of urinary mucopolysaccharides. The cause of these characteristic symptoms is the progressive accumulation of glycosaminoglycans and subsequent lysosomal distension in multiple tissues, including the central nervous system (CNS) (Glaser and Sly 1973).

So far, several kinds of therapy (bone marrow transplant, enzyme replacement, gene and cell-mediated therapy) have been reported for murine MPSVII. Bone marrow transplant was sufficient for correction of the visceral manifestations, although little improvement was seen in brain (Birkenmeier et al. 1991). Enzyme replacement therapy corrected visceral pathology but showed little change in the lysosomal distensions in brain of adult MPSVII mice, because infused GUSB could not cross the blood-brain barrier (BBB) in mice after 14th day of age (Vogler et al. 1999). In virus- or cell-mediated gene therapy, GUSB-encoding viral vectors or genetically modified cells were transplanted into MPSVII mice, but neither improved the CNS abnormalities of adult MPSVII mice (Wolfe et al. 1992; Marechal et al. 1993; Moullier et al. 1993a, b; Ross et al. 2000; Kamata et al. 2003). Because the therapeutic agents including bone marrow, enzyme, viral vectors, genetically modified cells cannot cross the BBB, therapeutic effects for CNS lesions was limited in the neonatal period. The collective incidence of LSDs is less than 1 in 7,000 live births, with 65% affecting the CNS (Meikle et al. 1999). Since most patients of LSDs with CNS lesions are diagnosed after the neonatal period, the brain-directed gene therapy, in which viral

vectors were introduced directly into the brain, has been developed to bypass the BBB and correct CNS pathology. Although direct intracerebral injection of GUSB-encoding viral vectors using adenoviruses, adeno-associated viruses, and lentiviruses showed evidence of clearance of CNS storage (Ohashi et al. 1997; Ghodsi et al. 1999; Brooks et al. 2002; Sly and Vogler 2002), some disadvantages of gene therapy, such as the possibility of tumor formation and the toxicity of over-expressed GUSB, were reported recently (Donsante et al. 2001; Vogler et al. 2003). These reports indicate that a new strategy to control the level of GUSB expression should be developed before the clinical application.

Human amniotic epithelial (HAE) cells constituting the inner lining layer of the amniotic cavity are formed from amnioblasts on about 8th day after fertilization. It has been reported that HAE cells can secrete lysosomal enzymes and do not express human leukocyte antigen (HLA)-A, -B, -C, -DR antigens and  $\beta_2$ -microglobulin on their surfaces (Adinolfi et al. 1982). Since Akle et al. performed amniotic tissue transplantation in seven volunteers who did not show clinical signs of acute rejection, several therapeutic trials of amniotic tissue transplantation have been carried out for patients with LSDs (Akle et al. 1981, 1985). Sakuragawa et al. (1992) reported a clinical and biochemical evaluation for a patient with juvenile Gaucher disease who underwent amniotic tissue grafting twice. Remarkable clinical improvement associated with an increase in deficient enzyme  $\beta$ -glucosidase was observed 1 week after implantation. However, the effect did not last for long because the implanted amniotic tissue was absorbed eventually. Kosuga et al. (2000) reported that implantation of genetically engineered HAE cells overexpressing GUSB into the spleen of MPSVII mouse elicited pathological

correction in both liver and spleen. These results suggest that HAE cells are useful donor cells for LSDs and have the possibility as a transgene carrier.

In this study, we generated immortalized HAE (IHAE) cells to maintain the effect of implantation, and encapsulated IHAE cells to prevent harmful immunological response and tumor formation in regulating the level of GUSB expression within the body of host. Moreover, we generated IHAE cells that over-express and secrete human GUSB following transduction with an adenoviral vector encoding human GUSB. Therapeutic efficacy for MPSVII was evaluated in and ex vivo experiments using these genetically engineered GUSB-encoding IHAE cells and the encapsulation technique.

#### MATERIALS AND METHODS

##### *Animals*

C3H mice were purchased from Japan Crea, Inc. (Tokyo). All mice were maintained at the National Institute of Neuroscience, National Center of Neurology and Psychiatry (Japan). Experiments using the mice were approved by the Animal Investigation Committee of the Institute.

##### *MPSVII cells*

Human and murine fibroblasts lack of GUSB activity were originated from a patient affected with MPSVII and a model mouse of MPSVII. These cells were originally generated by Dr. T. Torii in Gifu University and Dr. W.S. Sly in St. Louis University respectively, and maintained and prepared in National Center for Child Health and Development, Japan.

##### *An adenovirus expressing human GUSB*

An adenovirus, AxCAhGUS, encoding human GUSB gene was generated following COS-TPC method (A method for constructing recombinant adenovirus utilizing cosmid cassettes and adenovirus genome tagged with terminal protein complex). The construction method of this adenoviral vector was described previously. Briefly, a cosmid, pAxCAhGUS, which contained an expression cassette of human GUSB under the control of the CAG promoter, was constructed by subcloning the cDNA for human GUSB into a unique SmaI site of pAxCAwt. 293 cells were cotransfected with cosmid

pAxCAhGUS and the adenovirus DNA-terminal protein complex, which had already been digested at several sites with EcoT22I. A recombinant adenovirus was generated through homologous recombination in the 293 cells (Kosuga et al. 2001).

##### *Preparation of immortalized human amniotic epithelial cells*

Primary human amniotic epithelial cells were prepared as described previously (Sakuragawa et al. 1992, 1995). Briefly, the human amniotic epithelial tissue was mechanically peeled free from the chorion of a placenta obtained from an uncomplicated elective caesarian section with the informed consent of each donors. The HAE cell layer was extensively scraped out to remove the underlying tissue such as the spongy and fibroblast layers to eventually obtain a pure epithelial layer with the basement membranes. The layer was then treated with 0.25% trypsin three times for 20 min each. HAE cells, obtained usually after the third treatment, were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO, Carlsbad, CA, USA) under a humidified atmosphere of 5% CO<sub>2</sub> in air at 37°C. The primary cultures were transfected with the plasmid bearing origin-defective SV40 T-antigen gene by an electroporation method (Sakuragawa et al. 1997). The transformed cultures were incubated in the same condition as the primary cultures and clones of transformed cells, identifiable after 3 weeks, were selected and recloned. The cells surviving more than 12 months and showing the positive telomerase activity detected by reverse transcriptase-polymerase chain reaction (RT-PCR) were used as IHAE cells in the following experiments.

##### *Encapsulation of immortalized human amniotic epithelial cells*

IHAE cells were washed twice with phosphate buffered saline (PBS) and incubated in 0.125% trypsin (Sigma, St. Louis, MO, USA) /PBS at 37°C for 20 min. The cells were gently harvested and centrifuged at 1,000 rpm. Prior to encapsulation, the cells were treated with trypan blue to determine cell viability and counted with a hemocytometer. The results indicated that more than 95% cells were viable after this procedure. Then, the cell suspension in growth medium was prepared at a concentration of  $1 \times 10^8$  cells/ml and mixed with the same volume of 0.3% collagen solution (Cellmatrix 1-A, Nitta Gelatin Inc., Osaka) for final concentration of  $5 \times 10^7$  cells/ml. Devices for cell loading were prepared from dried polymer (polysulfon) hollow fibers (inner diameter

1.1 mm,  $7 \pm 0.5$  mm in length). These devices had a septal fixture at the proximal end for cellular loading access and were sealed at the distal end with light-cured resin. After infusing 10  $\mu$ l of the IHAE cell suspension, the septum was removed and the access port was sealed with light-cured resin. These IHAE cell-loaded capsules were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS).

#### *Lysosomal enzyme assay*

After centrifugation at 5,000 rpm for 5 min, the clear medium was stored at  $-20^{\circ}\text{C}$  until assay. The cells were harvested by scraping with a rubber policeman, washed PBS, centrifuged, and stored as pellets at  $-20^{\circ}\text{C}$  until assay. The frozen pellets or the brain tissue of mice were thawed and agitated with distilled water, using a Vortex mixer (Scientific Industries, Bohemia, NY, USA). The mixture was sonicated three times for 20 sec each time on ice and treated with frozen and thawing three times. These suspensions were assayed as cell extracts. Activities of lysosomal enzymes,  $\alpha$ -glucosidase,  $\beta$ -glucosidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, hexosaminidase, *N*-acetyl-glucosaminidase, and arylsulfatase A, were determined respectively. Protein concentration was determined by Lowry's method (Lowry et al. 1951). For the assessment of the heat-stability of the GUSB, the enzyme activity was assayed after 2-hrs incubation at  $65^{\circ}\text{C}$ .

#### *MPSVII cells uptake of $\beta$ -glucuronidase secreted from the encapsulated genetically engineered IHAE cells*

We studied the uptake of the human GUSB secreted

from the encapsulated genetically engineered IHAE cells into MPSVII cells (both human-origin and mouse-origin MPSVII cell line). We cocultured MPSVII cells with encapsulated IHAE cells infected with AxCAhGUS at a multiplicity of infection of 20 for these experiments. MPSVII cells were cultured with or without 10 mM mannose 6-phosphate (Man-6-P) and harvested 72 hrs later.

#### *Implantation of the encapsulated IHAE cells into mouse brains*

Recipient mice (7 weeks of age) were anesthetized and placed in a stereotactic apparatus. A small straight scalp incision was made to expose the coronal and sagittal sutures on the right side. For implantation into the corpus striatum, a 1.5-mm burr hole was made + 2 mm lateral to the Bregma. A capsule containing  $3.5 \times 10^5$  IHAE cells was stereotactically inserted via the burr hole into the right corpus striatum to the depth of 3.5 mm from the brain surface. In the control study, a vacant capsule without IHAE cells was also implanted in the same manner.

#### *Statistical analysis*

Regarding Figs. 1 and 2, the results were assessed with student *t*-test. The level of significance (\*) was set at  $p < 0.05$ .

## RESULTS

#### *Secretion of lysosomal enzymes from HAE and IHAE cells*

We studied the extracellular secretion of

TABLE 1. Activity of lysosomal enzymes in HAEC and IHAEC

	HAEC	IHAEC
$\beta$ -glucuronidase (GUSB)	$4.77 \pm 1.31$	$28.87 \pm 3.16$
$\alpha$ -galactosidase	$6.17 \pm 2.37$	$69.63 \pm 2.84$
$\beta$ -galactosidase	$85.17 \pm 4.51$	$292.10 \pm 42.17$
$\alpha$ -glucosidase	$31.73 \pm 1.46$	$14.80 \pm 0.52$
$\beta$ -glucosidase	$21.93 \pm 1.01$	$18.00 \pm 0.52$
Hexosaminidase A	$287.73 \pm 44.70$	$566.53 \pm 68.98$
<i>N</i> -Ac-glucosaminidase	$1,187.27 \pm 99.44$	$2,849.17 \pm 210.48$
Arylsulfatase A	$33.47 \pm 3.95$	$13.57 \pm 2.65$

Units: nmol/hour/mg cell protein

Both primary cultured HAE cells and immortalized HAE cells can produce lysosomalenzymes including GUSB.

Data are expressed as mean  $\pm$  s.d.

TABLE 2. Activity of lysosomal enzymes in the medium of HAEC and IHAEC

	HAEC	IHAEC
$\beta$ -glucuronidase (GUSB)	0.16 $\pm$ 0.03	0.15 $\pm$ 0.07
$\alpha$ -galactosidase	N.D.	N.D.
$\beta$ -galactosidase	0.15 $\pm$ 0.06	0.02 $\pm$ 0.01
$\alpha$ -glucosidase	0.13 $\pm$ 0.03	0.09 $\pm$ 0.06
$\beta$ -glucosidase	0.01 $\pm$ 0.01	N.D.
Hexosaminidase A	12.37 $\pm$ 2.42	12.26 $\pm$ 2.04
N-Ac-glucosaminidase	3.73 $\pm$ 0.58	3.51 $\pm$ 0.47
Arylsulfatase A	8.00 $\pm$ 5.57	9.60 $\pm$ 0.55

Units: nmol/hour/ml

Both primary cultured HAE cells and immortalized HAE cells can secrete lysosomal enzymes including GUSB.

Data are expressed as mean  $\pm$  s.d.

lysosomal enzymes from HAE, IHAE, and encapsulated IHAE cells (Tables 1 and 2). The GUSB activity in cells and medium was measured using samples for 3 days' culture. The GUSB activity in both HAE and IHAE cells is relatively low.

### Generation of IHAE cells overexpressing GUSB

We generated IHAE cells overexpressing human GUSB by transducing IHAE cells with AxCAhGUS, which is an adenovirus expressing human GUSB under the control of the CAG pro-

motor. When IHAE cells were infected with AxCAhGUS at MOI of 1, 5, and 20, GUSB activities in the infected cells increased with the titers of AxCAhGUS added to the medium. The activity in IHAE cells infected with AxCAhGUS at MOI of 20 was increased approximately 23-fold (Fig. 1). High levels of GUSB also detected in the medium used for the culturing the infected IHAE cells (Table 3). These results indicate that an adenoviral vector can transfer an exogenous gene into IHAE cells with extremely high efficiency.

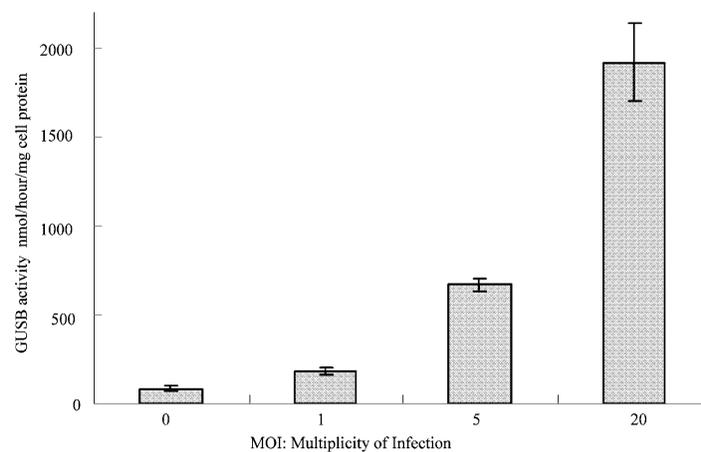


Fig. 1. GUSB activities of IHAE cells transduced with different titers of AxCAhGUS.

IHAE cells were infected with different titers of adenovirus expressing human GUSB (AxCAhGUS). GUSB activities significantly increased as the viral titers added to the medium increased. MOI: multiplicity of infection. The mean values  $\pm$  s.d. are shown ( $n = 3$ ).

TABLE 3. Activity of lysosomal enzymes in the medium of encapsulated IHAEC and AxGUSB-IHAEC

	IHAEC	AxGUSB-IHAEC
$\beta$ -glucuronidase (GUSB)	8.17 $\pm$ 1.99	107.63 $\pm$ 94.49
$\alpha$ -galactosidase	0.16 $\pm$ 0.05	0.11 $\pm$ 0.03
$\beta$ -galactosidase	1.97 $\pm$ 0.62	1.80 $\pm$ 0.62
$\alpha$ -glucosidase	1.73 $\pm$ 0.28	1.68 $\pm$ 0.30
$\beta$ -glucosidase	1.97 $\pm$ 0.65	1.96 $\pm$ 0.66
Hexosaminidase A	2.41 $\pm$ 0.66	1.81 $\pm$ 0.78
N-Ac-glucosaminidase	281.00 $\pm$ 119.05	259.98 $\pm$ 118.53
Arylsulfatase A	25.22 $\pm$ 3.78	24.15 $\pm$ 2.60

Units: nmol/hour/ml

Encapsulated immortalized HAE cells can secrete lysosomal enzymes including GUSB into the medium. After transduction of AxCAhGUSat MOI 20, immortalized HAE cells can secrete significant amount of GUSB into the medium.

Data are expressed as mean  $\pm$  s.d.

#### Uptake of GUSB into MPSVII cells

The uptake of GUSB enzyme from the genetically engineered encapsulated IHAEC cells was studied using both human and murine MPSVII fibroblasts. When the genetically engineered encapsulated IHAEC cells were added to cultured MPSVII fibroblasts, significant amounts

of the enzyme was taken up from the medium. However, GUSB activity was significantly reduced when the cells were cultured in the presence of 10 mM Man-6-P, suggesting that the secreted enzyme was transferred to MPSVII cells mainly via Man-6-P-related receptors (Fig. 2). These results demonstrate that MPSVII cells are

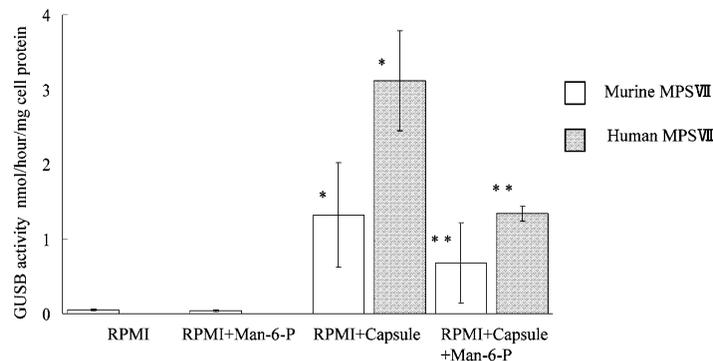


Fig. 2. Cellular uptake of GUSB secreted from cocultured encapsulated AxCAhGUS-transferred IHAEC cells via Man-6-P related receptors.

In both murine and human MPSVII fibroblasts, GUSB activity is not detectable or very low. IHAEC cells were infected with AxCAhGUSat MOI 20. Encapsulated AxCAhGUS-transferred IHAEC cells were transferred to the dishes containing human or murine cultured fibroblasts, which had been established from a human MPSVII patient or murine model of the disease, respectively. \*A significant increase ( $p < 0.05$ ) in GUSB activity was observed in the fibroblasts after the treatment. However, \*\* significant decrease ( $p < 0.05$ ) in GUSB activity was observed when the fibroblasts were precultured in the presence of 10 mM Man-6-P.

Data are expressed as mean  $\pm$  s.d. Capsule: encapsulated AxGUSB-transferred IHAEC cells.

able to take up significant levels of human GUSB, which is secreted from the genetically engineered IHAE cells.

#### *Heat-stable GUSB activities in C3H mouse brain after the capsule implantation*

One week after the capsule implantation, the C3H mice were sacrificed. The mouse brains were divided into two hemispheres and the GUSB activities were assayed in each hemisphere after 2-h incubation at 65°C. Human  $\beta$ -glucuronidase (GUSB) is heat-stable, and was reduced by only 30% after 2-hrs incubation at 65°C. In contrast, the GUSB of C3H mice is heat-labile, and its activity is almost completely eliminated by the same treatment (Kamata et al. 2003). There was significant difference between the control group and AxCAhGUS-transduced IHAE cells group in the GUSB concentration of the implanted side brain tissue (Fig. 3).

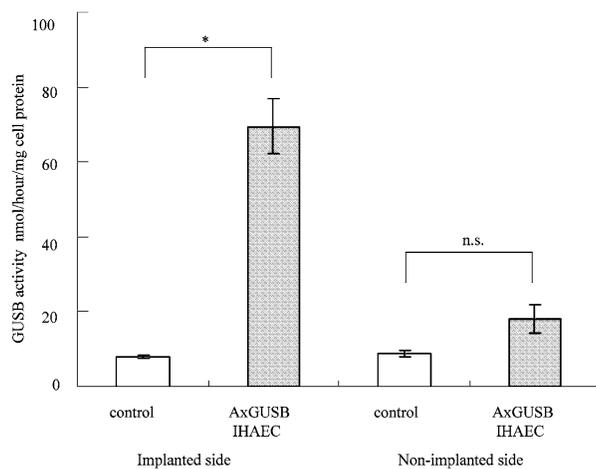


Fig. 3. GUSB activity in the brain of C3H mouse one week after the implantation of encapsulated AxGUSB-IHAE.

In the implanted side hemisphere, GUSB activities in the AxGUSB-IHAE group are significantly higher than those in control group.

\* Significant difference ( $p < 0.05$ ).

## DISCUSSION

Human amniotic epithelial cells can produce and secrete lysosomal enzymes including  $\beta$ -glucuronidase. Since HAE cells don't express major

histocompatibility complex (MHC) class II with mild expression of class I antigens on their surfaces resulting in no acute rejection after allotransplantation, they have been used as donor cells in transplantation therapy for patients with several LSDs (Adinolfi et al. 1982; Akle et al. 1985; Sakuragawa et al. 1992). Although there are some effective case reports of the amniotic membrane transplantation for LSDs, these effects are transient because the transplanted amniotic membrane is absorbed by the host immune system eventually. HAE cells have many other unique characters, production and secretion of several neurotransmitters such as catecholamines and acetylcholine, and several neurotrophic factors, e.g. nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3) (Sakuragawa et al. 1995, 1997; Elwan and Sakuragawa 1997; Uchida et al. 2000). If a stable and long-term supply of HAE cells were realized, we could apply them to the transplantation therapy for LSDs and a wide variety of diseases other than LSDs (Kakishita et al. 2000; Ohsugi et al. 2000; Kosuga et al. 2001; Naganawa et al. 2002). From this point of view, we used immortalized HAE cells for polymer-encapsulated gene-engineered cell therapy in the treatment of Mucopolysaccharidosis type VII.

Addition of GUSB can prevent and correct the accumulation of GAGs in fibroblasts from MPSVII patients. Uptake of GUSB largely depends on cell surface receptors that recognize Man-6-P residue on the enzyme (Kaplan et al. 1977). We confirmed that GUSB secreted from the encapsulated vector-transduced IHAE cells is taken up by human or murine fibroblasts from MPSVII through Man-6-P receptors. For successful cell-mediated therapy for MPSVII, GUSB secreted from the donor cells have to be taken up by recipient cells via Man-6-P receptors expressed on the cell surface membrane. This in vitro finding shows the therapeutic potential for MPSVII or other LSDs using encapsulated vector-transduced IHAE cells.

A wide variety of cell lines, both allogeneic and xenogeneic, can be encapsulated in a semi-

permeable membrane with a controlled pore size, allowing the inward diffusion of nutrients and the outward diffusion of the secreted bioactive agents. Also, this encapsulation technique provides immunoprotection by selectively restricting the passage of cellular and humoral elements of the host immune system, and prevents tumor formation by physically isolating active dividing cells from host parenchyma. Although this encapsulation cell therapy supposed to be a promising technique, one problem has been pointed out about the possibility that endogenous retrovirus present in xenogeneic cells could infect human cells and lead to new, transmissible human diseases (Lysaght and Aebischer 1999). To solve this problem, we encapsulated allogeneic, non-infected IHAE cells for transplantation cell therapy. It is confirmed that encapsulated IHAE cells can secrete lysosomal enzymes outside capsule. Although Tohyama et al. (1997) reported the tumorigenesis of IHAE cells after the implantation into the rat subcutaneous tissue, no tumor formation was detected in the presenting experiments.

In the present study, we confirmed that the encapsulated AxCAhGUS-transduced IHAE cells can secrete significant amount of GUSB into the cerebral parenchyma of C3H mice. In the treatment of MPSVII, deficient enzyme replacement therapy cannot correct the lysosomal distension in adult brain because infused GUSB did not cross the blood-brain barrier in mice after 2 weeks of age (Vogler et al. 2003). Subsequently, the brain-directed gene therapy, in which viral vectors were introduced directly into the brain, has been developed to bypass the BBB and correct CNS pathology. Although this direct intracerebral injection of GUSB-encoding viral vectors using adenoviruses, adeno-associated viruses (AAV), and lentiviruses showed evidence of clearance of CNS storage, some disadvantages of gene therapy were reported. The effect of adenovirus as a vector is generally transient. Donsante et al. reported that a significant incidence of hepatocellular carcinomas and angiosarcomas was discovered after GUSB encoding recombinant AAV in the murine experiment of MPSVII (Donsante et al. 2001).

Although a stable and long-term effect can be expected by the use of lentivirus as a vector, Vogler et al. (2003) reported the side effect of GUSB overexpression. They detected tumor formation in the transgenic mice overexpressing human GUSB. These reports indicate that a new strategy should be developed to control the level of GUSB expression in vivo. Implantation of the encapsulated GUSB-encoding IHAE cells can control the secretion level of GUSB. If an overexpression of GUSB was elicited after the implantation, we can regulate the amount of enzyme secretion through changing the size of capsule or retrieval of implanted capsule. Moreover, since encapsulated cells cannot invade the cerebral parenchyma beyond the capsule wall, tumor formation outside the capsule is impossible. Although the further investigation for the long-term effect in vivo experiments is essential, we believe that the encapsulation cell therapy using genetically engineered IHAE cells is a promising approach to treat LSDs including MPSVII.

### CONCLUSION

We testified that IHAE cells have the abilities of production and secretion of GUSB. The secretion level of GUSB can be preserved after encapsulation, and IHAE cells can produce and secrete higher amount of GUSB by transducing GUSB cDNA. Encapsulation cell therapy enables us to solve the immunological problem associated with transplantation cell therapy and to expect a stable and long-term supply of deficient enzymes for inborn errors of metabolism.

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