Hirosaki Med. J. 71 : 120—130, 2021

ORIGINAL ARTICLE

Carbonyl reductase 1-overexpressing exosomes inhibit proliferation of ovarian cancer cells

Anna Takabayashi-Ebina[1)], Minako Yokoyama[1)], Kayo Horie[2)], and Yoshihito Yokoyama[1)]

Abstract We previously reported that a human carbonyl reductase 1 (CBR1) DNA-dendrimer complex could potentially be used in gene therapy for peritoneal metastasis of ovarian cancer. The aims of the current study were to make exosomes which overexpressed CBR1 and to examine the antiproliferative effect of using the CBR1-overexpressing exosomes on ovarian cancer cells. Endometrial stromal cells (fibroblasts) were transfected with CBR1 DNA by using Lipofectamine, the highest expression level of CBR1 was produced from the cells transfected under the condition of Lipofectamin 24μl/DNA 36μg for 48 h. Exosomes were purified from culture supernatants by exoEasy Maxi Kit. Western blot showed that CBR1 notably expressed in exosomes extracted from the stromal cells transfected with CBR1 DNA. Proliferation of ovarian cancer cell line was significantly inhibited by adding CBR1-overexpressing exosomes compared to proliferation of those cells in which exosomes without CBR1 DNA were added. We obtained the evidence that CBR1-overexpressing exosomes could function in carrying CBR1 DNA into ovarian cancer cells. Results suggested that exosomes are a useful tool of gene delivery and that a gene therapy of combining CBR1 DNA and exosomes may be promoted in the treatment of advanced and recurrent ovarian cancers with peritoneal dissemination.

Hirosaki Med. J. 71 : 120—130, 2021

Key words: Carbonyl reductase 1; exosome; gene delivery; gene therapy; ovarian cancer.

Introduction

Ovarian cancer is a representative tumor with poor prognosis among various gynecological malignancies[1)]. Approximate half of patients with the cancer are currently detected in clinical condition of stage III or IV because the ovaries are located within the pelvis and lack of early warning signs[2)]. Cytoreductive surgery followed by adjuvant chemotherapy is generally recommended as the primary treatment for advanced epithelial ovarian cancer[3)]. In recent years, molecularly targeting drugs such as bevacizumab is thought to be relatively effective against the tumor, but 5-year survival rate in advanced stages (FIGO stage Ⅲ or Ⅳ) remains at 18-46%[2)]. Epithelial ovarian cancer is highly responsive to initial chemotherapy, but approximately 50% of the patients with advanced ovarian cancer recur within 2 years and this disease results in poor prognosis due to a decreased response to chemotherapy[4)]. Therefore, new treatment strategy for epithelial ovarian cancer is required.

Carbonyl reductase 1 (CBR1) is an NADPH-dependent oxidoreductase with a broad specificity for carbonyl compounds, which reduces aldehydes and ketones to be involved in metabolism of endogenous biologically active substances, foreign compounds and drugs. CBR1 is distributed in liver, skin, kidney, breast, ovary and vascular endothelium[5)].

In our previous study, patients with decreased CBR1 expression in epithelial ovarian cancer had a worse prognosis than those with a high expression level of CBR1[6)]. Growth of ovarian

[1)] Department of Obstetrics and Gynecology, Hirosaki University, Graduate School of Medicine
[2)] Department of Bioscience and Laboratory Medicine, Hirosaki University, Graduate School of Health Sciences

Correspondence: Y. Yokoyama
Received for publication, November 18, 2020
Accepted for publication, December 2, 2020

cancer was inhibited by administering clofibric acid in *in vivo* experiment, and CBR1 was highly expressed in ovarian cancer tissues[7]. When the ovarian cancer cells in which CBR1 was highly expressed were inoculated into the back of mice, tumor did not grow enough[8]. Conversely, decreased expression of CBR1 in ovarian cancer cells promoted tumor development and increased the frequency of metastasis to other organs[9]. Although DNA can be transfected via such techniques as use of transfection reagents, electroporation or viral vectors into the cells, these techniques are difficult to use in clinical settings. Thus, attention has focused on dendrimers as a drug delivery system (DDS), and we showed that mice with peritoneal dissemination of ovarian cancer had a significantly longer survival by administering CBR1 DNA-dendrimer complex into abdominal cavity[10].

Although research had been conducted to design a dendrimer that precisely controls adhesion to cells and tissues, we focused on exosome to seek more beneficial drug delivery tool. Exosomes are 40–100 nm membrane vesicles of endocytic origin secreted by most cell types *in vitro*, and they are also found *in vivo* in body fluids such as blood, urine, amniotic fluid, malignant ascites, bronchoalveolar lavage fluid, synovial fluid, and breast milk[11]. Interestingly, the recent studies have shown that exosomes contain both mRNA and microRNA, which can be transferred to another cell, and be functional in the new environment, and that the function of exosomes changes depending on the conditions of secretory cells[11]. Numerous studies have reported that exosomes are useful as a DDS[12, 13]. The application of exosomes as a DDS to the nervous system has been reported[14], and one study succeeded in suppressing breast cancer by using exosomes as a DDS[15].

In this study, we aimed to make exosomes which overexpress CBR1 and to determine the optimal conditions for delivering CBR1 DNA to ovarian cancer cells by using exosomes. Finally, we examined the antiproliferative effect of adding CBR1- overexpressing exosomes on ovarian cancer cells.

Materials and Methods

Cell Lines and cell culture

Fibroblast cell line, hTERT-immortalized human endometrial stromal cells (THESCs) was obtained from the American Type Culture Collection (Rockville, MD, USA). THESCs were delivered from healthy female uterus endometrium and do not secret any hormone. THESCs are a fibroblast immortalized with hTERT, and the invaluable tool for consistent *in vitro* work[16]. They were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich, St Louis, MO, USA), supplemented with 10% fetal bovine serum (NICHIREI Biosciences, Tokyo, Japan) at 37 ℃ in a water-saturated atmosphere with 5% CO_2/95% air. TOV-21G and SK-OV-3 cells were obtained from the American Type Culture Collection. TOV-21G cells are derived from human ovarian clear cell carcinoma tissues. SK-OV-3 cells are derived from human ovarian serous carcinoma ascites. Both TOV-21G and SK-OV-3 cells are commonly used to produce xenografted solid tumor[17, 18]. They were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco/Life Technologies, Carlsbad, CA,USA) supplemented with 10% fetal bovine serum at 37 ℃ in a water-saturated atmosphere with 5% CO_2/95% air.

Plasmid DNA preparation

A pCMV6-AC-GFP vector (OriGene Technologies, Inc., Rockville, MD, USA) that encodes the human CBR1, green fluorescent protein (GFP) and ampicillin resistance genes were used to optimize and obtain highly efficient transfection. For amplification, pCMV6-AC-GFP was transformed into E. coli-DH5α competent cells by

heat-shock transformation according to standard laboratory protocols. The transformed bacteria were amplified in LB-ampicillin medium. The plasmids were purified from cultured transformed bacteria using a PureLink HiPure Plasmid Filter Maxiprep Kit (Invitrogen Life Technologies, Carlsbad, CA,USA) according to the manufacturer's protocol.

Small interfering RNA preparation

The sequences of small interfering RNA (siRNA) duplexes specific to CBR1 were synthesized commercially by Invitrogen Life Technologies. CBR1 siRNA sense, 5'-AUACGUUCACCACUCU CCCTT-3' and antisense, 5'-GGGAGAGUGGUG AACGUAUTT-3' were designed to target different coding regions of the human CBR1 mRNA sequence (GeneBank Accession no. NM_001757).

Transfection

One day before transfection, THESCs cells of 1.0×10^6 cells/plate were cultured in 10ml of RPMI-1640 medium, supplemented with 10% fetal bovine serum without antibiotics per 100mm culture vessel, so that they would reach 80-90% confluence at transfection. Afterwards, CBR1 DNA was added to the cells and transfected using a Lipofectamin 3000 Reagent (Invitrogen Life Technologies) according to the manufacturer's protocol. The ratio of Lipofectamin/DNA was examined with Lipofectamin 12μl and DNA 12μg, 24μg or 36μg, and Lipofectamin 24μl and DNA 24μg, 36μg or 48μg. Transfected cells were incubated at 37℃ in a CO_2 incubator for 6h and culture fluid was exchanged to Advanced RPMI-1640 Reduced Serum Medium (Gibco/Life Technologies), and then those continued to be incubated for 72h and culture supernatant was harvested. In the same way, 600pmol of CBR1 siRNA was added to the cells and transfected using a Lipofectamin 3000 Reagent (Invitrogen Life Technologies). Transfected cells were incubated at 37℃ in a CO_2 incubator for 6h and culture fluid was exchanged to Advanced RPMI-1640 Reduced Serum Medium, and then those continue to be incubated for 48h and culture supernatant was harvested. The control group was only given the Lipofectamin 3000 Reagent. THESCs cells transfected with CBR1 DNA were evaluated for GFP expression using a laser scanning confocal microscope (BZ-X700; Keyence, Osaka, Japan) 24,48 and 72 h after transfection.

Exosome extraction

The exosomes were purified from culture supernatants using an exoEasy Maxi Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The kit is extracted with buffer XE. When 5% of XE buffer is added to cells, no cytotoxicity is found, so we administered XE buffer to the TOV-21G and SK-OV-3 cells at a concentration of 5% and it was confirmed that no cytotoxicity was found as with the non-treated group. Finally, eluted solution concentration used in this study was 4%.

Western blot analysis

Cell lysates (30 μg protein) were prepared from THESCs cells transfected with CBR1 DNA or CBR1 siRNA cultured for 48h and 72h, electrophoresed using a 12% sodium dodecyl sulfate polyacrylamide gel onto Poly Vinylidene Di-Fluoride (PVDF) membranes (Bio-Rad Laboratories, Tokyo, Japan). The protein concentration was determined using the UV absorption method. The blots were probed for 4℃ overnight with the diluted antibodies against the following proteins: CBR1 (Catalog number SC-390554, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:100, CD63 (Catalog number SC-5275, Santa Cruz Biotechnology) at 1:100, and β-actin (Catalog number A5441, Sigma-Aldrich) at 1:2,000. The membranes were then incubated for 2 h with anti-mouse immunoglobulin G HRP-

linked Antibody (Catalog number 7076, Cell Signaling Technology, Beverly, MA, USA), and protein bands were visualized using enhanced chemiluminescence (Image Quant LAS4000 system, GE Healthcare Life Sciences, MA, USA) according to the manufacturer's procedure.

Addition of exosomes to ovarian cancer cells and evaluation of cell proliferation

TOV-21G and SK-OV-3 cells were cultured 24h in 60mm dish 1.0×10^5 cells/plate with 5 ml of DMEM medium. Afterwards, 200 μl of exosomes solution including CBR1-DNA or CBR1-siRNA were added to TOV-21G and SK-OV-3 cells. Only pure exosomes derived from THESCs cell culture supernatants were added to those cells as a control. The cells were incubated at 37℃ in a CO_2 incubator for24, 48 and 72h. After 24, 48, and 72 h, the cells were washed with 2 ml of PBS, trypsinized and sufficiently detached. To distinguish between living and dead cells, the cells were stained with 0.1% trypan blue solution (NACALAI TESQUE, Inc., Kyoto, Japan), and the cells were counted by using a hemocytometer (Bio medical science, Tokyo, Japan). The cell proliferation experiment was performed in triplicate and the cell numbers were shown as average ± standard deviation.

Statistical analysis

Differences in the number of living cells between exosomes including CBR1 DNA, those including CBR1 siRNA and those without both (control) were evaluated using Student's t-test. A result was deemed significant at a P value < 0.05.

Results

CBR1 expression levels in THESCs cells

Expression levels of CBR1 was compared in THESCs cells transfected with CBR1 DNA at various Lipofectamin/DNA ratios. The uptake of DNA increased over time for up to 48 h, and the efficiency was highest at a ratio of Lipofectamin 24μl/DNA 36μg (Figure 1). Western blot analysis confirmed that the strongest expression of CBR1 was produced in THESCs cells transfected with CBR1 DNA at a ratio of Lipofectamin 24μl/DNA 36μg for 48 h (Figure 2A). Inherent CBR1 of 32 kDa is expressed in control cells, while CBR1 DNA-transfected cells showed strong expression of CBR1 with GFP of 64 kDa as well as inherent CBR1 of 32 kDa. On the other hand, CBR1 siRNA-transfected cells showed decreased inherent CBR1 expression (Figure 2B).

Exosome extraction

The exosomes were purified from culture supernatant of THESCs cells using an exoEasy Maxi Kit (QIAGEN). The vesicle size of the collected sample was measured with the nano tracking analysis system NanoSight (Quantum Design Japan, Inc. Tokyo, Japan). Western blot analysis showed that expression of CD63, an exosome marker, in samples extracted from the cells transfected with CBR1 DNA or CBR1 siRNA and the control cells (Figure 3A). The expression level of CBR1 was higher in exosomes extracted from CBR1 DNA-transfected cells and lower in those extracted from CBR1 siRNA-transfected cells than in those extracted from the control cells (Figure 3B).

Antiproliferative effect of CBR1

Necrotic cells were notable in both TOV-21G cells and SK-OV-3 cells in which exosomes extracted from CBR1 DNA-transfected cells were added compared to the control (Figure 4). Figure 5 A and B show differences of proliferations of TOV-21G cells and SK-OV-3 cells according to time by adding CBR1-overexpressing exosomes or those transfected with CBR1 siRNA. Proliferations of both of TOV-21G cells and SK-OV-3 cells was significantly inhibited by adding CBR1 DNA-exosomes complex compared

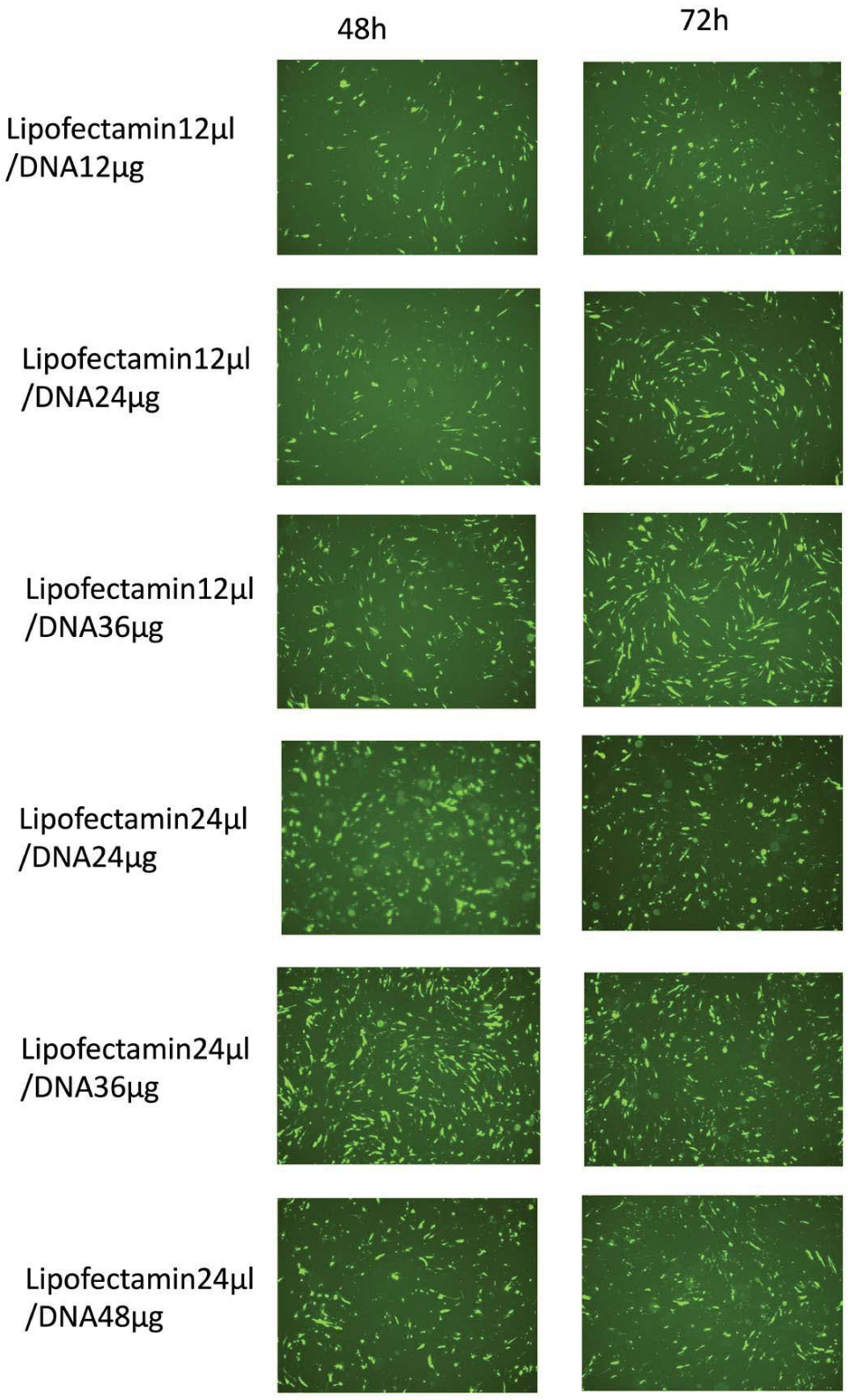

Figure 1 Transfection of carbonyl reductase 1 (CBR1) DNA into hTERT-immortalized human endometrial stromal cells (THESCs).

Fluorescent photographs of THESCs transfected with pCMV6-AC-GFP expressing human CBR1 taken 48 and 72 h after transfection. The efficiency of uptake was best at a Lipofectamin 24μl/DNA 36μg than at other Lipofectamin/DNA ratios.

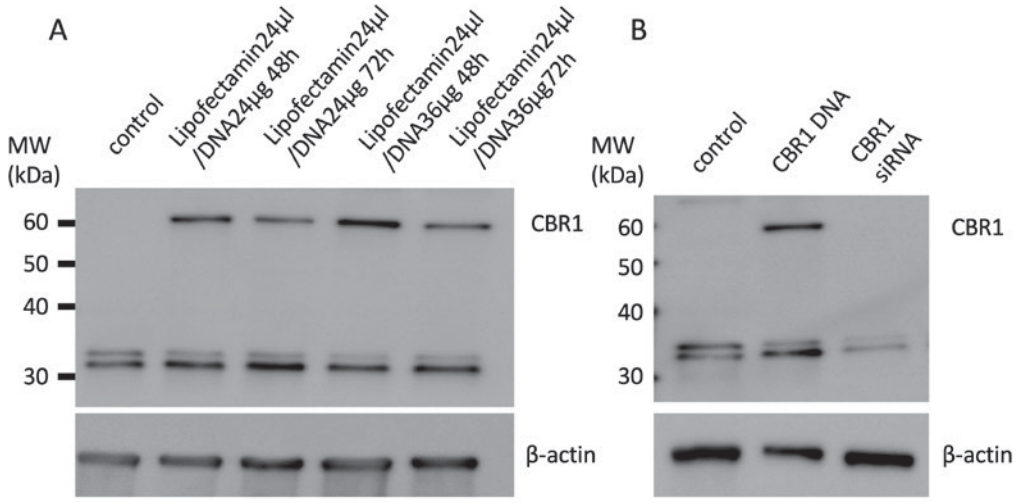

Figure 2 Confirmation of carbonyl reductase 1 (CBR1) expression in hTERT-immortalized human endometrial stromal cells (THESCs) transfected under various conditions.

A. Western blot analysis for the CBR1 expression levels to confirm the optimal Lipofectamin/DNA ratio and incubation time. The level of CBR1 expression was the strongest in THESCs cells transfected with CBR1 DNA at a Lipofectamin/DNA ratio of a Lipofectamin 24μl/DNA 36μg for 48h. β-actin was used as an internal control.

B. Western blot analysis for the CBR1 expression levels. Transfection of THESCs with CBR1 DNA or CBR1 siRNA. CBR1 expression level was higher in CBR1 DNA-transfected cells and lower in CBR1 siRNA-transfected cells than in the control cells. β-actin was used as an internal control.

with the control, whereas proliferations of both of TOV-21G cells and SK-OV-3 cells was significantly reduced by adding CBR1 siRNA-exosomes complex compared with the control.

Discussion

The results of this study showed that by transfection CBR1 DNA into stromal cells (fibroblasts), exosomes overexpressing CBR1 could be created and that by adding the exosomes, the proliferation of TOV-21G and SK-OV-3 cells was significantly inhibited compared with the addition of exosomes from fibroblasts without transfection of CBR1 DNA.

Intraperitoneal administration of a CBR1 DNA-dendrimer complex improved intraperitoneal dissemination of ovarian cancer in peritoneal carcinomatosis mice model and significantly prolonged the survival period of mice[10]. Even if ovarian cancer cells transfected with CBR1 DNA were inoculated on the back of nude mice, the tumor development was significantly inhibited compared to the tumor without transfection of CBR1 DNA[19]. Caspase-8 and TNFR1 were highly expressed immunohistochemically in the tumor overexpressing CBR1, suggesting that CBR1 could induce apoptosis in ovarian cancer cells[19]. The Research using artificial human peritoneal tissue (AHPT) demonstrated that proliferation of ovarian cancer cells transfected with CBR1 DNA was significantly inhibited on AHPT, and necrosis was evident[20]. Thus, previous studies have shown the inhibitory effect of CBR1 on cancer cell proliferation, tumor growth and dissemination. However, if CBR1 DNA is applied to treatment of ovarian cancer patients as a gene therapy, transfection reagents, electroporation and viral vector may not be realistic clinically as a delivery tool to the cancer cells.

Targeted delivery approaches for cancer therapeutics have shown a steep rise over the past few decades[21]. Extracellular vesicles (EVs) including exosome may be applied in diagnostics, either as pathological biomarkers or to follow

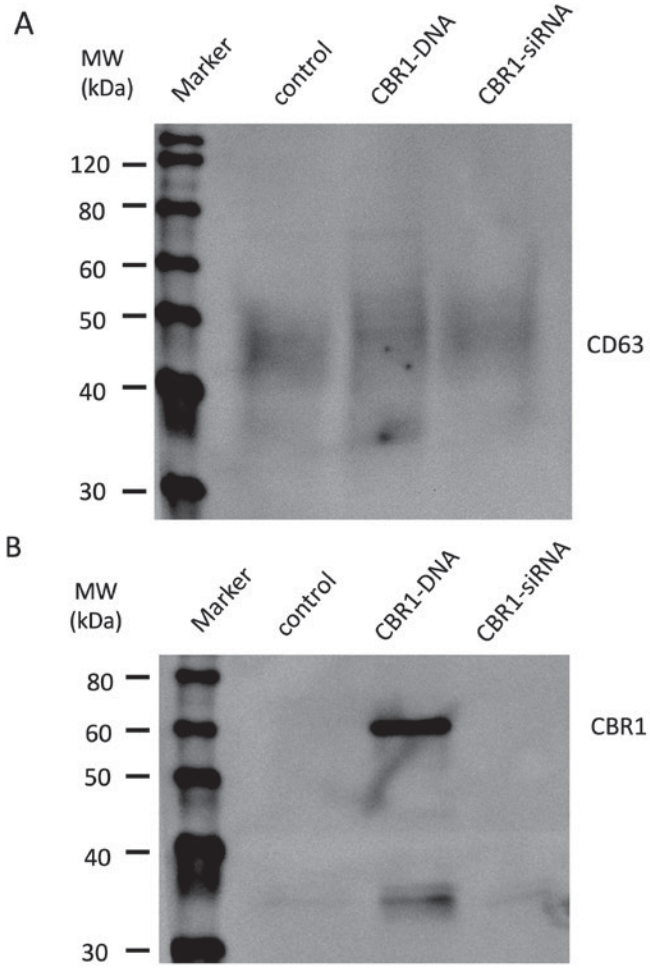

Figure 3 Exosome extraction.

A. Identification of CD63 expression by Western blot analysis. Western blot confirmed expression of CD63 indicating existence of exosomes derived from the cells transfected with carbonyl reductase 1 (CBR1) DNA or CBR1 siRNA and the control cells.

B. Carbonyl reductase 1 (CBR1) expression levels by Western blot analysis. The expression level of CBR1 was higher in exosomes extracted from the cells transfected with CBR1 DNA and lower in exosomes extracted from the cells transfected with CBR1 siRNA than in exosomes extracted from the control cells.

treatment efficacy[22]. The discovery that EVs make up a natural mechanism for information transfer between cells has stimulated interest into their potential use as a new drug delivery platform[11]. Researches have been conducted as a DDS for therapeutic agents for various diseases such as cancer[23] and brain diseases[13].

Because exosomes are stable in the circulation and do not induce immune rejection, their therapeutic applications as drug delivery systems have grown into an attractive research area[24]. Advances of nanotechnology enable the encapsulation of therapeutic agents such as chemotherapeutic drugs, small molecules, miRNAs, and siRNAs into exosomes[25]. In ovarian cancer, exosomes derived from adipose mesenchymal stem cells inhibited the cell proliferation of A2780 human ovarian cancer cells by blocking the cell cycle and activating mitochondria-mediated apoptosis signaling, indicating the importance of exosomal miRNAs in this inhibitory pathway[26]. However, a DDS using exosomes in ovarian cancer treatment. The present study showed a potential of exosomes to

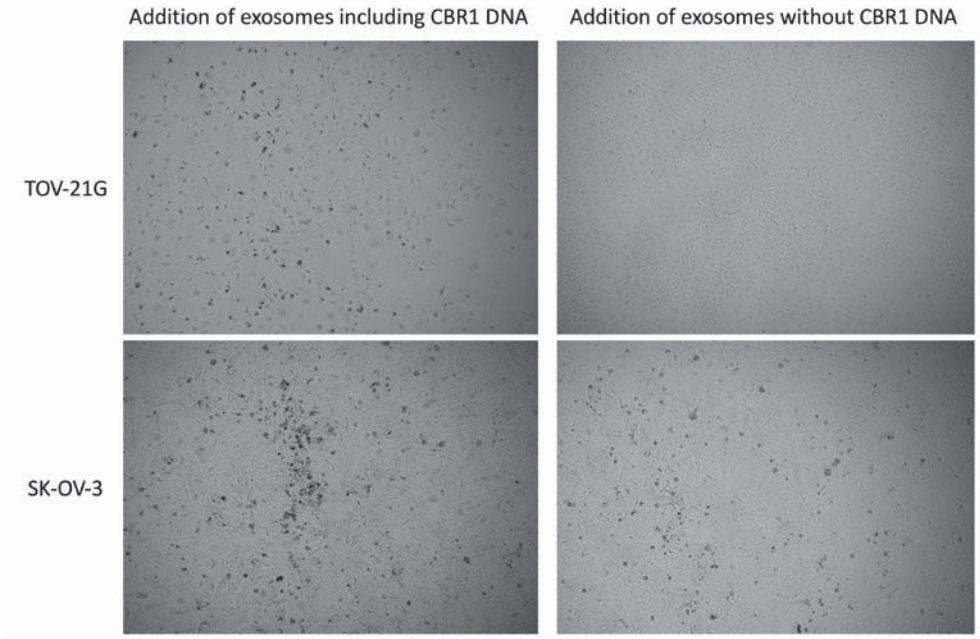

Figure 4 Cell proliferation.
Carbonyl reductase 1 (CBR1)-overexpressing exosomes or ones transfected with CBR1 siRNA were added to TOV-21G and SK-OV-3 cells. Both TOV-21G and SK-OV-3 cells in which CBR1-overxpressing exosomes were added showed cell necrosis notably compared to the control.

carry CBR1 DNA into the ovarian cancer cells, suggesting that gene therapy combining exosomes and CBR1 DNA may be promoted clinically against advanced ovarian cancers. Because the function of exosomes changes depending on the condition of secretory cells[11], we examined the optimal transfection ratio that would result in high CBR1 expression and found that Lipofectamin 24μl/DNA 36μg was the best ratio to deliver CBR1 DNA into the cells. As shown in Figure 3C, CBR1 expressed most strongly in exosomes transfected with CBR1-DNA and it expressed most weakly in those transfected with CBR1-siRNA. Differences of proliferations of TOV-21G cells and SK-OV-3 cells according to time by adding exosomes transfected with CBR1-DNA or ones transfected with CBR1-siRNA were significant (Figure 5A and B). Necrosis as well as apoptosis due to CBR1 may cause the significant difference of cell proliferation.

Nanocarriers, such as liposomes, have favorable advantages with the potential to further improve cancer immunotherapy and even stronger immune responses by improving cell type-specific delivery and enhancing drug efficacy[27]. Liposomes can offer solutions to common problems faced by several cancer immunotherapies, including the following combinational therapy[27]. Liposomes are ideal vehicles for the simultaneous delivery of drugs to be combined with other therapies, including chemotherapy[27]. For example, pegylated liposomal doxorubicin represents a new class of chemotherapy delivery system that may significantly improve the therapeutic index of doxorubicin[28]. However, even its new technology cannot still overcome peritoneal dissemination of ovarian cancer.

There are some limitations in this study. First, this study was fulfilled only by an *in vitro* experiment. Hereafter, *in vivo* experiments using peritoneal carcinomatosis mice model must be performed to apply to the clinical settings in the future. Second, we must compare a potential of

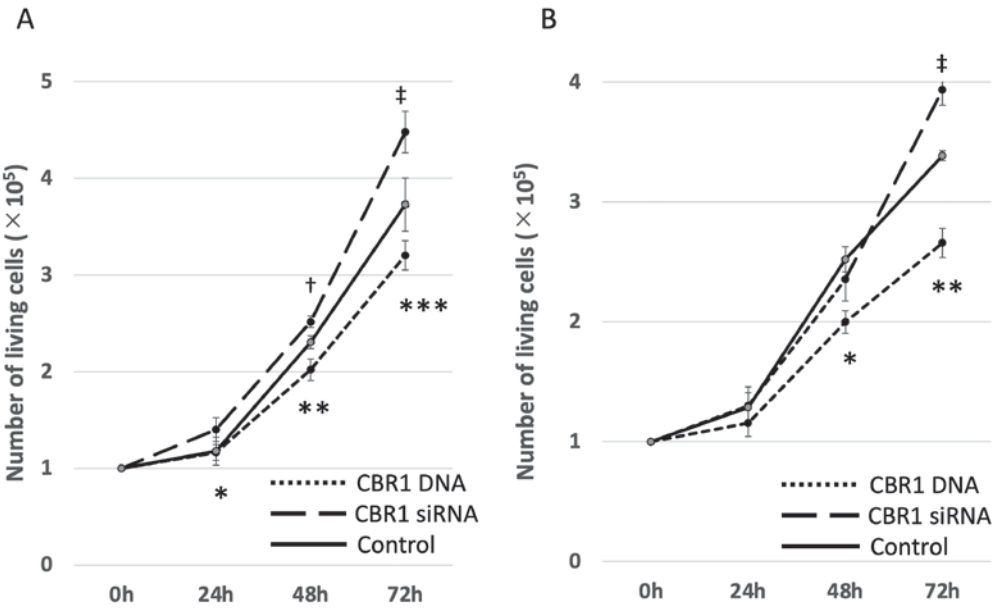

Figure 5 Antiproliferative effect of carbonyl reductase 1 (CBR1).

A. TOV-21G cells: CBR1-overxpressing exosomes significantly inhibited cell proliferation compared to those transfected with CBR1 siRNA from 24h and the control from 48h. On the other hand, exosomes transfected with CBR1 siRNA significantly promoted cell proliferation compared to the control from 48h. * CBR1 DNA vs. CBR1 siRNA, $P < 0.05$; ** CBR1 DNA vs. the control and CBR1 siRNA, $P < 0.01$; *** CBR1 DNA vs. the control and CBR1 siRNA, $P < 0.01$. † CBR1 siRNA vs. the control, $P < 0.01$, ‡ CBR1 siRNA vs. the control, $P < 0.05$.

B. SK-OV-3 cells: CBR1-overxpressing exosomes significantly inhibited cell proliferation compared to those transfected with CBR1 siRNA as well as the control from 48h. On the other hand, exosomes transfected with CBR1 siRNA significantly promoted cell proliferation compared to the control at 72h. * CBR1 DNA vs. CBR1 siRNA and the control, $P < 0.01$; ** CBR1 DNA vs. CBR1 siRNA and the control, $P < 0.001$; ‡ CBR1 siRNA vs. the control, $P < 0.001$.

exosomes to liposome and dendrimer as a DDS with regard to therapeutic effect, production efficiency and reproducibility of drug delivery. The field of exosome-based drug delivery still lacks substantial experimental validation in *in vivo* to elucidate its potential superiority to liposome or nanocarriers such as dendrimer.

In the present *in vitro* experiments, CBR1-overexpressing exosomes were created and its administration successfully inhibited proliferation of ovarian cancer cells. These findings suggest that exosomes are a useful tool of gene delivery, and that a gene therapy with combination of CBR1 DNA and exosomes may be promising treatment strategy for advanced and recurrent ovarian cancers.

Disclosure

There are no conflicts of interest to be disclosed.

Acknowledgements

This study was supported by a Grant-in Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan (20K09589 to Dr. Y. Yokoyama).

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7-30.

2) Heintz APM, Odicino F, Maisonneuve P, Quinn MA, Benedet JL, Creasman WT, Ngan HYS, et al. Carcinoma of the ovary. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet. 2006;95:S161-92.

3) Bookman MA, Brady MF, McGuire WP, Harper PG, Alberts DS, Friedlander M, Colombo N, et al.

Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a phase III trial of the Gynecologic Cancer Intergroup. J Clin Oncol. 2009;27:1419-25.

4) Yokoyama Y, Futagami M, Watanabe J, Sato N, Terada Y, Miura F, Sugiyama T, et al. Redistribution of resistance and sensitivity to platinum during the observation period following treatment of epithelial ovarian cancer. Mol Clin Oncol. 2014;2:212-8.

5) Wermuth B, Bohren KM, Heinemann G, von Wartburg JP, Gabbay KH. Human carbonyl reductase. Nucleotide sequence analysis of a cDNA and amino acid sequence of the encoded protein. J Biol Chem. 1988;263:16185-8.

6) Umemoto M, Yokoyama Y, Sato S, Tsuchida S, Al-Mulla F, Saito Y. Carbonyl reductase as a significant predictor of survival and lymph node metastasis in epithelial ovarian cancer. Br J Cancer. 2001;85:1032-6.

7) Yokoyama Y, Xin B, Shigeto T, Umemoto M, Kasai-Sakamoto A, Futagami M, Tsuchida S, et al. Clofibric acid, a peroxisome proliferator-activated receptor alpha ligand, inhibits growth of human ovarian cancer. Mol Cancer Ther. 2007;6:1379-86.

8) Wang H, Yokoyama Y, Tsuchida S, Mizunuma H. Malignant ovarian tumors with induced expression of carbonyl reductase show spontaneous regression. Clin Med Insights Oncol. 2012;6:107-15.

9) Osawa Y, Yokoyama Y, Shigeto T, Futagami M, Mizunuma H. Decreased expression of carbonyl reductase 1 promotes ovarian cancer growth and proliferation. Int J Oncol. 2015;46:1252-8.

10) Kobayashi A, Yokoyama Y, Osawa Y, Miura R, Mizunuma H. Gene therapy for ovarian cancer using carbonyl reductase 1 DNA with a polyamidoamine dendrimer in mouse models. Cancer Gene Ther. 2016;23:24-8.

11) Simpson RJ, Jensen SS, Lim JWE. Proteomic profiling of exosomes: current perspectives. Proteomics. 2008;8:4083-99.

12) Antimisiaris SG, Mourtas S, Marazioti A. Exosomes and exosome-inspired vesicles for targeted drug delivery. Pharmaceutics. 2018;10:218.

13) Aryani A, Denecke B. Exosomes as a nanodelivery system: a key to the future of neuromedicine? Mol Neurobiol. 2016;53:818-34.

14) Alvarez-Erviti L, Seow Y, Schapira AH, Gardiner C, Sargent IL, Wood MJA, Cooper JM. Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission. Neurobiol Dis. 2011;42:360-7.

15) Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Mol Ther. 2013;21:185-91.

16) Krikun G, Mor G, Alvero A, Guller S, Schatz F, Sapi E, Rahman M, et al. A novel immortalized human endometrial stromal cell line with normal progestational response. Endocrinology. 2004;145:2291-6.

17) Lu T, Tang J, Shrestha B, Heath BR, Hong L, Lei YL, Ljungman M, et al. Up-regulation of hypoxia-inducible factor antisense as a novel approach to treat ovarian cancer. Theranostics. 2020;10:6959-76.

18) Wakui M, Yokoyama Y, Wang H, Shigeto T, Futagami M, Mizunuma H. Efficacy of a methyl ester of 5-aminolevulinic acid in photodynamic therapy for ovarian cancers. J Cancer Res Clin Oncol. 2010;136:1143-50.

19) Miura R, Yokoyama Y, Shigeto T, Futagami M, Mizunuma H. Inhibitory effect of carbonyl reductase 1 on ovarian cancer growth via tumor necrosis factor receptor signaling. Int J Oncol. 2015;47:2173-80.

20) Oikiri H, Asano Y, Matsusaki M, Akashi M, Shimoda H, Yokoyama Y. Inhibitory effect of carbonyl reductase 1 against peritoneal progression of ovarian cancer: evaluation by ex vivo 3D-human peritoneal model. Mol Biol Rep. 2019;46:4685-97.

21) Rosenblum D, Joshi N, Tao W, Karp JM, Peer D. Progress and challenges towards targeted delivery of cancer therapeutics. Nat Commun. 2018;9: 1410.

22) Lässer C. Exosomes in diagnostic and therapeutic applications: biomarker, vaccine and RNA interference delivery vehicle. Expert Opin Biol Ther. 2015;15:103-17.

23) Kajiyama H. A new strategy for overcoming peritoneal metastasis and drug resistance in epithelial ovarian cancer by inhibition of EMT (epithelial-mesenchymal transition). Acta Obst Gynaec Jpn. 2008;60:1629-40.

24) Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles-endogenous nanocarriers for targeted cancer therapy. Biochim Biophys Acta. 2014;1846:75-87.

25) Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharmacol Sin. 2017;38:754-63.

26) Reza AMMT, Choi YJ, Yasuda H, Kim JH. Human adipose mesenchymal stem cell-derived exosomal-miRNAs are critical factors for inducing anti-proliferation signaling to A2780 and SKOV-3 ovarian cancer cells. Sci Rep. 2016;6:38498.

27) Gu Z, Da Silva CG, van der Maaden K, Ossendorp F, Cruz LJ. Liposome-based drug delivery systems in cancer immunotherapy. Pharmaceutics. 2020;12:1054.

28) Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal doxorubicin: review of animal and human studies. Clin Pharmacokinet. 2003;42:419-36.