Thesis for the Master of Biomedical Sciences

Development of cell derived nanovesicle from pancreatic stellate cells as a novel drug delivery system

The Graduate School of the University of Ulsan

Department of Biomedical Sciences

Min Woo Kim

Development of cell derived nanovesicle from pancreatic stellate cells as a novel drug delivery system

Supervisor : Prof. Suhwan Chang

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by Min Woo Kim

Department of Biomedical Sciences

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This certifies that the master's thesis of

Min Woo Kim is approved.

Committee Member **Prof. Sun Cheol Choi** **(signature)**

Committee Member **Prof. Min Ji Kang** **(signature)**

Committee Member **Prof. Suhwan Chang** **(signature)**

Department of Biomedical Sciences

University of Ulsan, Korea

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Abstracts

Pancreatic ductal adenocarcinoma is one of the major subtypes of pancreatic cancer and is a common type, accounting for approximately 90% of pancreatic cancer patients. The 5-year survival rate for pancreatic ductal adenocarcinoma is very low, approximately 5%. One of the main problems with this type of cancer is a phenomenon known as desmoplasia. This means that the extracellular matrix (ECM) and fibroblasts form abnormal tissue around the tumor site. This phenomenon causes many treatment difficulties and drug resistance in pancreatic ductal adenocarcinoma. Cancer cells are surrounded by extracellular matrix and fibroblasts, so anticancer drugs cannot be delivered to the cancer cells, resulting in drug resistance. To overcome these problems, efforts are being made to develop new drug delivery systems utilizing cell-derived nanovesicles. This system utilizes cell-derived nanovesicles derived from pancreatic stellate cells, the main constituent cells of desmoplasia, and utilizes the blast cell homing reaction, which is one of the characteristics of exosomes. And this regression reaction has similar characteristics to cell-derived nanovesicles, which are artificial exosomes. We created a nanovesicle made by encapsulating a drug in a cell-derived nanovesicle and confirmed its drug delivery ability through experiments, but additional research on the drug encapsulation efficiency is needed. If these limitations are overcome, drug encapsulated cell-derived nanovesicles can be used in patients with pancreatic ductal adenocarcinoma to increase drug targeting efficiency, reduce concerns about drug side effects with less drug usage than before, and better treatment effects can be expected. In addition, cell-derived nanovesicles, similar to exosomes, have low immunogenicity, high targeting efficiency, and drug delivery ability, so it is expected that they will present new possibilities for the treatment of pancreatic ductal adenocarcinoma through the development of new drug carriers.

Keywords: Exosome, Pancreatic ductal adenocarcinoma, Desmoplasia, Cell derived nanovesicle, drug delivery

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Introduction

Pancreatic cancer is one of the most mortality disease of all cancer types[1]. Pancreatic ductal adenocarcinoma (PDAC) is most common type of pancreatic cancer and it take up almost 90% of all pancreatic cancer[2]. PDAC is an epithelial neoplasm that originates from the pancreatic ductal system[3]. Until now PDAC therapy methods are just surgical approach, and combined with chemotherapy. PDAC patient prognosis are also poor and 5-year overall survival rates are just 5 ~ 8%[3-5].

One of the remarkable features of PDAC is desmoplasia and the site of desmoplasia are composed of extracellular matrix (ECM) proteins, fibroblastic cells such as pancreatic stellate cells (PSC), and immune cells which have several cytokines[6]. In normal tissues, this fibrosis mechanisms are required for aid in inflammation or wound healing process. But in tumor site the desmoplasia is unusual growth of the fibrous tissue in tumor site and this causes tumor proliferation and inhibits tumor to drug uptake[7,8]. And the mechanism of desmoplasia is not clearly studied.

PSC is composition of desmoplasia formation. PSC have two different phenotypes one is quiescent PSC and the other is activated PSC form[9]. In the normal pancreas, PSCs are featured by the presence of vitamin A-containing lipid droplets, called quiescent PSC, and only occupied about 4 ~ 7% of the pancreas[10,11]. In contrast, in PDAC states quiescent PSCs becomes activated PSC and contribute to desmoplasia[11]. For this reason, PSC is important mediators of PDAC desmoplasia and it causes accumulation of ECM. ECM accumulations are block the drug approach to cancer cells, finally it leads to drug resistance[12].

Several nano sized particles are developed and ranged with 10 ~ 1000 nm in diameter, such as liposomes[13], exosomes[14], and Polymeric nanoparticles[15] can loaded some compounds or drugs. Especially, biological nano sized particles, also called extracellular vesicles (EVs), can classify three groups according to their size and biogenesis. Exosomes are in 30 – 200 nm, microvesicles (MVs) are in 100 – 1000 nm, and apoptotic bodies are more than 1000 nm[16]. Explain more about exosomes, it can be secreted many eukaryotic cells and have many advantages for biologically active cargos[17]. Recent studies suggest that EVs, also contain exosomes, can be drug delivery vesicles and can use next generation therapeutics[18]. Not exosomes but Abraxane (*nab*-paclitaxel) is one example of a cancer treatment drug using nanoparticles[19]. Exosomes, as a new generation of therapeutic agents, have the ability to transfer proteins, mRNAs, miRNAs, and such exogenous substances. These naturally sourced

nanocarriers are being explored as drug delivery systems and used in cancer for targeted therapy[20]. However, despite their promise as future drugs, exosomes currently have several limitations. These include challenges related to the total secretion of exosomes and the time-consuming and expensive methods used for their isolation[21].

To overcome the exosomes limitation, cell derived nanovesicles (CDVs)[22] are proposed. CDVs are similar with exosomes structure and its function[23]. Similar to exosomes, CDV have intrinsic stability, biocompatible feature, and inherent homing capability[24]. However, CDV can be produced in much larger quantities than exosomes for the same number of cells, and the yield is approximately 100-fold increased[22,25]. For this reason, we are trying to develop a new drug delivery system using CDV.

Materials and Methods

Cell culture

Human pancreatic stellate cells (PSC) are cultured in SteCM (Stellate Cell Medium; SteCM, ScienCell) supplemented with 2% fetal bovine serum, 1% stellate cell growth supplement and 1% penicillin/streptomycin.

115026, 110621 (Primary pancreatic cancer cell) cells are cultured in RPMI 1640 (HyClone) supplemented with 5% fetal bovine serum (HyClone), 1% penicillin/streptomycin (HyClone), 20 ng/ml EGF, 4 μg/ml hydrocortisone and 4 μg/ml transferrin.

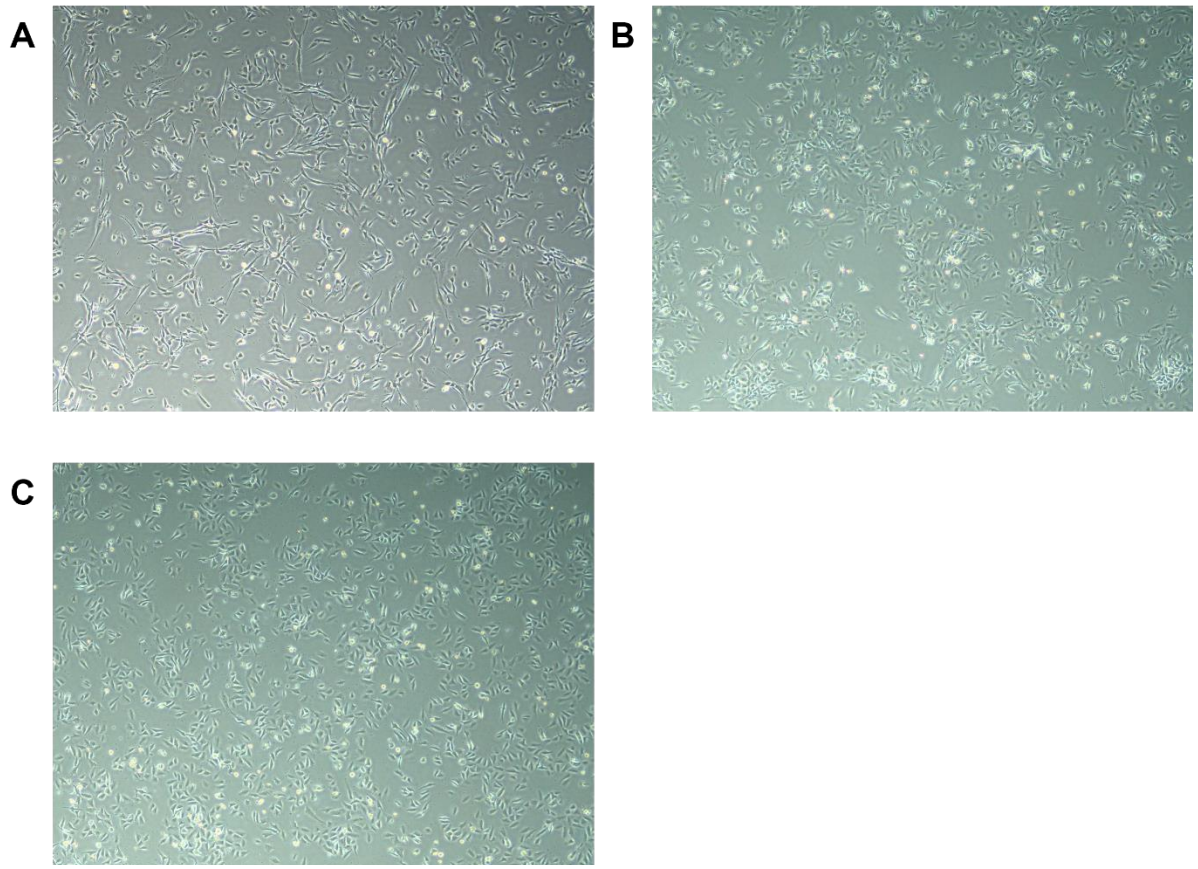

Figure.1 Cells for using this experiments

(A) represents is human pancreatic stellate cells (PSCs) used for CDV production. (B) and (C) represent 115026 and 110621 cells, respectively, which are primary pancreatic cancer cells.

Cell Derived Nanovesicle (CDV) extraction and filtering

Prepare Human Pancreatic Stellate Cell (PSC) and resuspended at 5×10^6 cells/ml in phosphate-buffered saline (PBS, Biowest). Cells were detached by 0.025% Trypsin (Gibco). The resuspended cell was extruded five times through 10 μm, 5 μm and 1 μm polycarbonate membrane filters (Whatman) sequentially using mini extruder (Avanti Polar Lipid). After extrusion, unrefined CDV is purified using a qEV2 70 nm column (IZON) and the purified CDV fractions are collected.

Characterization of CDV

The size and particle count of CDVs were assessed using Nanoparticle Tracking Analysis (NTA) with the Nanosight NS300 (Malvern panalytical). CDVs were diluted in PBS and measured with a camera level 14 and a 405 nm wavelength laser. The target temperature was set to 25℃, and other settings were left on auto. Data were analyzed using NTA 3.4 software, version 3.4.

Doxorubicin loaded CDV extraction

When resuspending the cells in PBS, mix them with doxorubicin (Sigma-Aldrich). The concentration of doxorubicin used is 1mg/ml, which is pre-prepared and mix with PBS in a 1:1 ratio to achieve a concentration of 500 μg/ml for resuspension. Subsequently, the resuspended cells were extruded using the same method and passed through a size exclusion column to collect the main fraction.

Measurement of the concentration of doxorubicin encapsulated in CDVs

Doxorubicin fluorescence was detected using a Victor X3 Multilabel Micro plate reader (Perkin Elmer) with excitation/emission wavelengths at 485 nm/579 nm. To measure the concentration of doxorubicin encapsulated in CDVs, first calculated a standard curve using free doxorubicin. The concentrations of free doxorubicin used for the standard curve were 10, 5, 3, 1, 0.8, 0.6, 0.4, 0.2, and 0 μg/ml, which were measured and calculated. Next, the concentration of Dox-loaded CDV was measured for each fraction, and the concentration of doxorubicin in each fraction was calculated using previously established standard curve.

***In vitro* Doxorubicin loaded CDVs Cytotoxicity of cells**

115026, 110621 (primary pancreatic cancer cell) cells are seeding in 96well cell culture plate. Doxorubicin-loaded CDVs (Dox-CDVs) were suspended in PBS and prepared in three concentrations, which were mixed with RPMI 1640 media at ratios of 1:10, 1:100, and 1:1000. These specific concentrations of Dox-CDVs were then added to individual wells.

After 48 and 72 hours, Quanti-Max (Biomax) was treated in a 1:10 ratio of each well's volume and incubated at 37℃ for a minimum of 30min to a maximum of 2 hours. Each plate was measured using the Synergy HT multimode microplate reader (Biotek).

In vivo Targeting of CDVs to the pancreas

CDVs were injected intravenously to NSG mouse. After injection, mice were sacrificed at 6 and 12 hours to harvest liver, pancreas, spleen, and kidney tissues for RNA extraction. Human-specific gene primers were designed to confirm CDV targeting in mouse organs. (Table 1)

Table 1. RT-PCR primer sequence

Target	Sequence
Human α-SMA	Forward 5'-GCTTCTAAAACACTTTCCTGCT-3'
	Reverse 5'-GAAGTTACCAGTAGCCTATTTCA-3'
Human RPL13a	Forward 5'-CGAAGATGGCGGAGGTGCAG-3'
	Reverse 5'-GGTTTTGTGGGGCAGCATAC-3'
Mouse GAPDH	Forward 5'-AACTTTGGCATTGTGGAAGG-3'
	Reverse 5'-ACACATTGGGGGTAGGAACA-3'
Mouse RPL13a	Forward 5'-GAGGTCCGGTGGAAGTACCA-3'
	Reverse 5'-TGCATCTTGGCCTTTTCCTT-3'

As an alternative approach, CDVs were labeled with Cy7 to verify their targeting. Extruded CDVs were incubated with Cy7 mono NHS ester (5 μM, Amersham Biosciences) for 2hr at 37℃ and then Cy7-labeled CDVs were isolated using size exclusion column. Then, each pancreatic cancer orthotopic mouse should be intravenously injected with 100 μl of the main fraction. After several hours, Cy7-labeled CDV injection mice are measured Cy7 fluorescence by IVIS spectrum (PerkinElmer) for confirming pancreas targeting.

Result

CDVs production and characterization

Several methods have already been developed in various studies for creating CDVs. In this study, we employed a mini extruder to produce CDVs. Initially, PSCs were harvested, and the cells were suspended in PBS at a concentration of 5×10^6 cells/ml. Subsequently, the prepared cells were sequentially passed through 10 μm, 5 μm, 1 μm polycarbonate membrane filters using mini extruder to generate CDVs.

The extruded CDVs are filtered through a size exclusion column. After filtration, the main fraction containing purified CDVs is obtained. When measured using Nanosight, the results showed an average size within 200 nm, and a particle count of $1 \sim 5 \times 10^{10}$ particles/ml. (Figure.2 A - D)

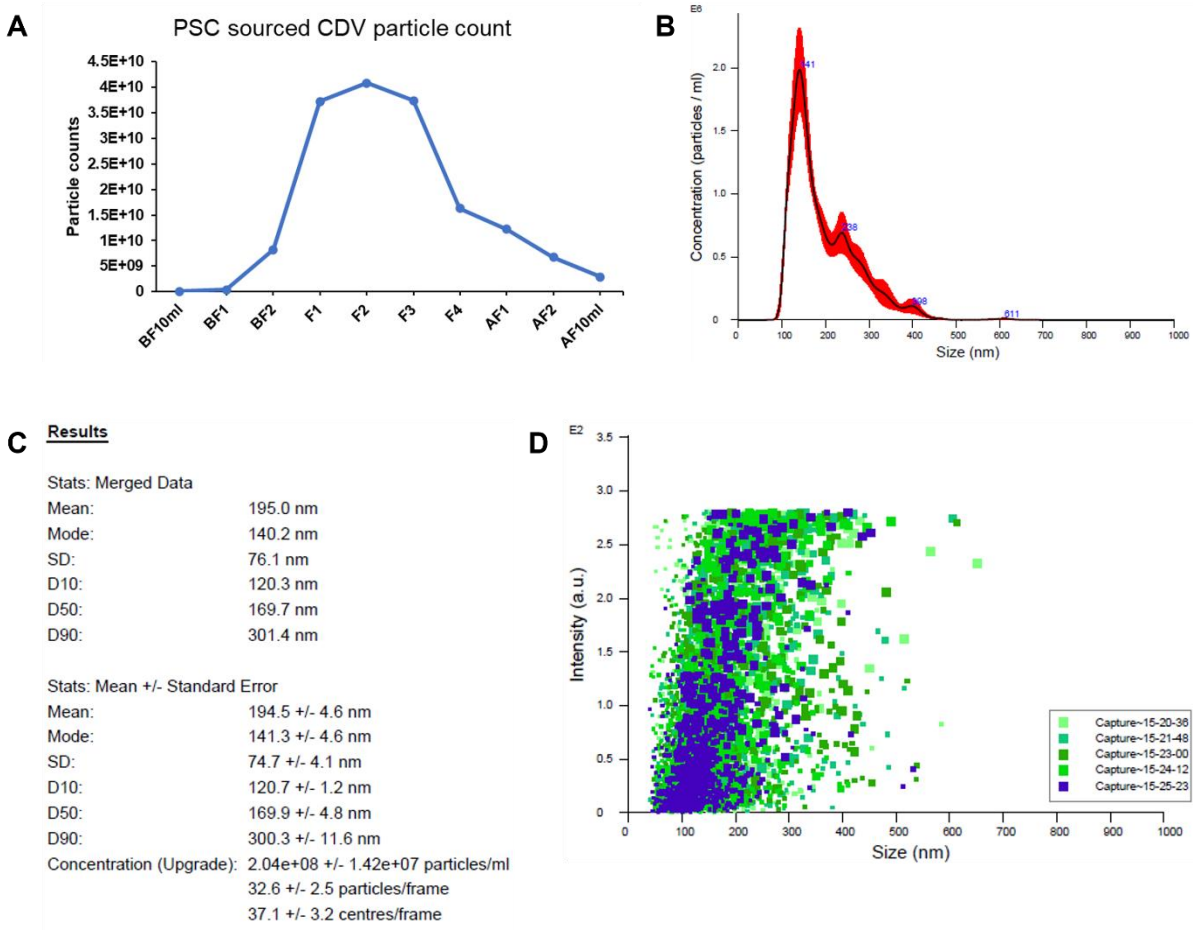

Figure.2 CDV production result

A represents the results of particle count verification using Nanosight after producing CDV. (BF/AF 10ml means Before/After Fraction 10 ml, F1 ~ 4 means Main fraction1 ~ 4, and BF/AF1 ~ 2 means Before/After Fraction 1 ~ 2) **B** shows a graph illustrating the average size distribution of captured particles in the form of a size distribution of captured particles in the form of a size distribution graph. **C** is the result obtained through Nanosight analysis, which provides information about the size and particle count (concentration) of CDVs in each fraction. **D** represents a plot depicting the captured size and relative light intensity of individual CDVs.

Doxorubicin loaded CDV and measurement of Doxorubicin concentration

The method for loading doxorubicin into CDVs involves mixing doxorubicin with a suspension of cells in PBS and extruding them a mini extruder. The extrusion process is carried out in the same manner, and the final concentration of doxorubicin in the PBS is adjusted to 500 μg/ml before extrusion. After sequentially extruding the cell suspension (PBS + doxorubicin), it is loaded onto a size exclusion column to obtain the main fraction containing purified CDVs. Then, the amount of Doxorubicin in each fraction was measured. (Figure.3B) When measuring the amount of doxorubicin encapsulated in the samples, a standard solution for standard curve was concurrently established to improve the accuracy of doxorubicin quantification. (Figure.3A)

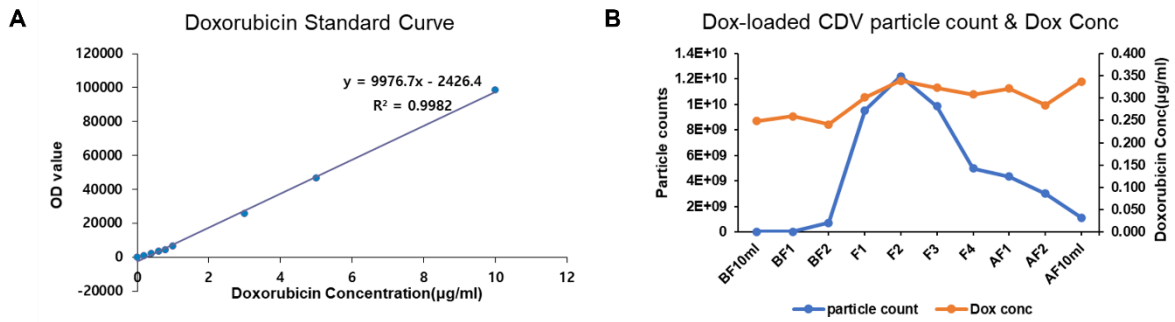

Figure.3 Doxorubicin standard curve and Dox-loaded CDVs production result

A is an example of a doxorubicin standard curve used for quantifying the doxorubicin content in Dox-loaded CDVs. Using this standard curve, we measured the concentration of the drug in future experiments. **B** represents the particle counts Dox-loaded CDVs and the Doxorubicin concentration for each fraction. (BF/AF10 ml means Before/After Fraction 10 ml, F1 ~ 4 means Main fraction1 ~ 4, and BF/AF1 ~ 2 means Before/After Fraction 1 ~ 2)

The quantitative comparison of free doxorubicin and Dox-loaded CDVs in cells

An experiment was conducted to determine the difference in efficiency between Free-Dox and Dox-loaded CDVs at the same drug concentration. 115026, 110621 cells (primary pancreatic cancer cells) were seeded at 1×10^3 cells/well in 96-well plates, respectively. After 12 hours of seeding, three different concentrations were prepared by diluting the main fraction at ratios of 1:10, 1:100, and 1:1000 in the media, and these dilutions were added to each well. Similarly, Free-Dox was diluted in the media at the same concentrations and added to the wells. After treatment with Free-Dox and Dox-loaded CDVs, a Quanti-Max (BIOMAX) was added in appropriate amounts after 48 and 72 hours to conduct a cell viability assay. As evident from the results, a reduction in proliferation was observed in cells treated at a 1:10 ratio, with no such effect observed in other treatment ratios. The cells were subjected to concentrations ranging from 0.00034 μg/ml to 0.034 μg/ml in the experiments. The results indicated a decrease in cell proliferation only at the highest concentration when compared to the control (Figure.4 A - D).

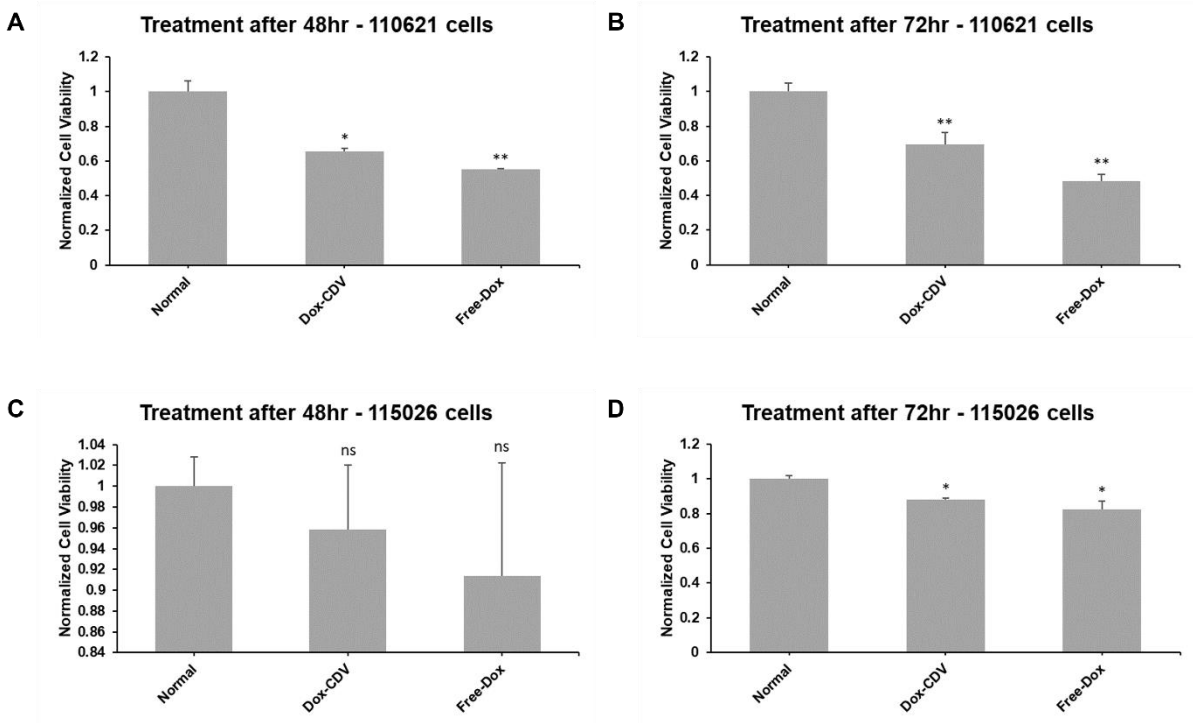

Figure.4 Comparison of drug efficacy between free doxorubicin and Dox-loaded CDVs

A and **B** represent graphs showing the relative proliferation measured 48 hour and 72 hour after drug treatment in 110621 cells. **C** and **D** represent graphs showing the relative proliferation measured 48 hour and 72 hour after drug treatment in 115026 cells (*: $p < 0.05$, **: $p < 0.001$).

Confirmation of CDV targeting to the pancreas using RT-PCR

To confirm the targeting of CDVs to specific organs, experiments were conducted by intravenously injecting CDVs into NSG mice. Since these CDVs were created using human PSCs, it was anticipated that CDVs would accumulate in several organs within the mouse, allowing for the extraction of human RNA for analysis. To verify this, after administering CDVs to the mice, we extracted RNA from the liver, pancreas, spleen, and kidney respectively. Subsequently, RT-PCR was performed using primers specifically designed for human-specific sequences. After synthesizing cDNA from mouse organ RNA and conducting RT-PCR using human-specific designed α-SMA primers, no significant differences between human and mouse were observed (Figure.5 A - D). In liver sample exhibited an increasing tendency, but it was not statistically significant (Figure.5A). And the other samples showed similar values to the normal control (Figure.5 B - D). As the human-specific primers were not performing as expected, so alternative methods were decided to explore for confirming targeting.

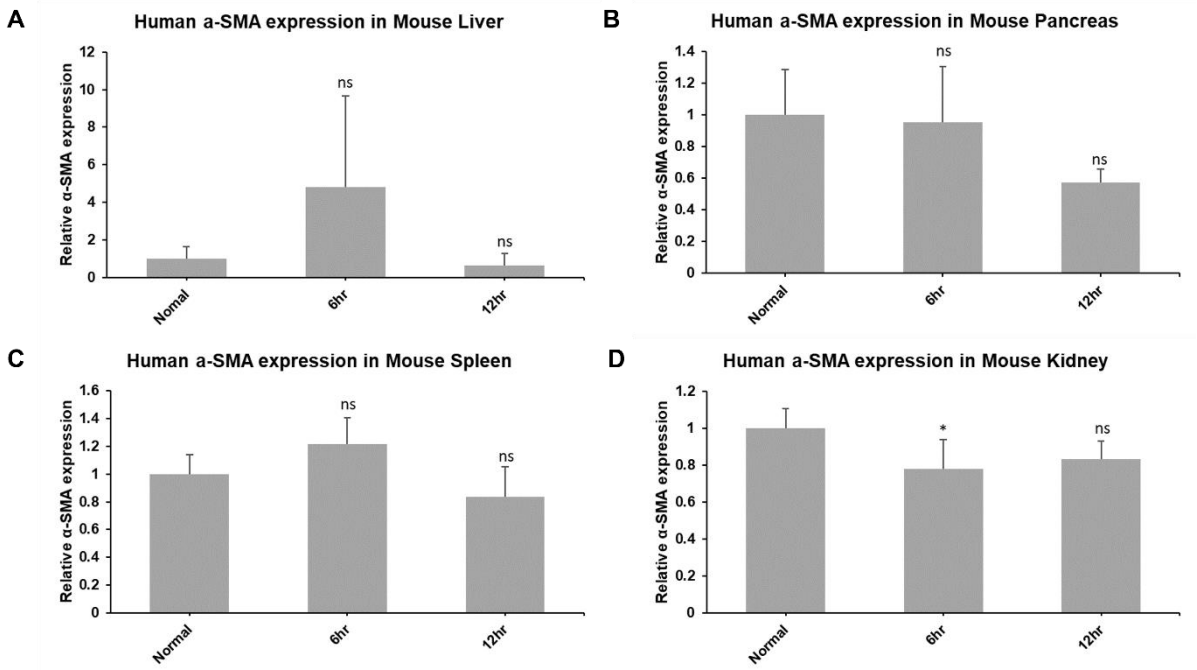

Figure.5 Detection of human α-SMA in mouse organs following CDV injection

A to **D** represent the liver, pancreas, spleen, and kidney respectively. In the liver, there seemed to be increase in the 6hr samples, but it did not reach statistical significance based on the *p-value*. In other samples as well, significant results could not be obtained.

Confirmation of CDV targeting to the pancreas using Cy7 dye labeling

The other way to confirm pancreas targeting following CDV injection, Cy7 mono NHS ester were used. After extruding CDV and treating it with Cy7 at a concentration of 5 μM, Cy7-labeled CDVs were obtained through size exclusion column filtration. The peak particle count in the main fraction was 3.19×10^{10} particle/ml, so 3.19×10^{9} particle/100μl CDVs were intravenously injected.

In this experiment, a pancreatic cancer orthotopic model was used. After several time points, Cy7 fluorescence was assessed using IVIS spectrum. Since Cy7 could not be confirmed in mice without tumor, Cy7-labeled CDV was injected into orthotopic model mice and confirmed *in vivo* and *ex vivo*. As a result of IVIS image measurement, Cy7 could not be observed in *in vivo*. While Cy7 measurements were not feasible *in vivo* in mice, but they were detectable in *ex vivo* measurements (Figure.6 A - D).

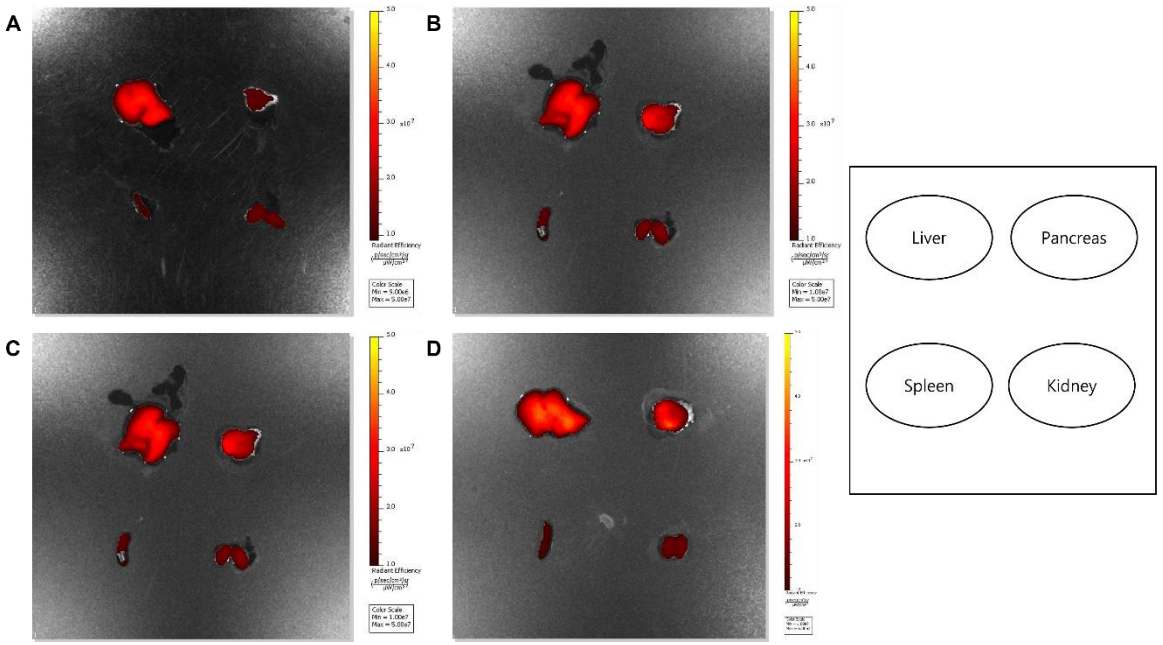

Figure.6 Confirmation of CDV targeting using Cy7 labeled CDV

A to **D** represent time points at 12, 24, 48, and 72 hours after Cy7 labeled CDV injection, respectively. When examining all time points, a tendency was observed for higher fluorescence values in the pancreas compared to the control sample.

When examining the ROI values individually, it can be observed that there is a tendency for higher values in the pancreas compared to the control group injected with PBS (Table.2). The targeting to the pancreas has been confirmed, but further experiments are needed for a more definitive confirmation.

Table 2. ROI value

	12hr	24hr	48hr	72hr	Normal
Liver	1.48E+09	1.25E+09	1.46E+09	1.70E+09	1.85E+09
Pancreas	7.12E+08	3.29E+08	5.18E+08	6.01E+08	1.54E+08
Spleen	6.65E+08	2.90E+08	1.49E+08	1.36E+08	1.27E+08
Kidney	7.79E+08	3.66E+08	2.85E+08	2.14E+08	3.44E+08

Discussion

In this study, I developed a novel drug delivery system using cell-derived nanovesicles. I produced Cell Derived Nanovesicles (CDVs) by mechanically pressing pancreatic stellate cells. Subsequently, loaded a type of anticancer drug, doxorubicin[26,27], during the pressing process to create Dox-loaded CDVs, with the aim of achieving a higher anti-tumor effect with a lower dose. If the targeting is appropriate, using a low dose can ensure that the drug reaches only the desired area, resulting in anticancer effects without adverse effects on other tissues. For these reasons, a new drug delivery system was developed by encapsulating drugs in CDVs.

Started with approximately 1×10^7 cells and suspended PSCs in PBS at a concentration of 1×10^6 cells/ml. Subsequently, produced CDVs by passing them through a polycarbonate filter, resulting in CDVs with sizes within 200nm. The highest peak particle count reached $1 \sim 2 \times 10^{10}$ particles/ml. At first, I attempted the extraction with a cell concentration of 1×10^6 cells/ml. However, after referring to other research papers[22], I increased the cell concentration 5×10^6 cells/ml and found that this higher cell concentration allowed us to obtain CDVs with a yield similar to the previous attempt. As a result, I decided to maintain a fixed cell concentration of 5×10^6 cells/ml for the future extrusions. And then unpurified CDV samples were passed through size exclusion column to collect refined CDV samples. After collecting fractions both before and after the main fraction, it was confirmed that the highest peak was indeed within the main fraction. With these parameters verified for CDV production, proceeded to the step of loading drugs into these CDVs.

The anticancer drug used for CDV drug loading was doxorubicin, and the loading method involved mixing PBS with doxorubicin to achieve a final concentration of 500 μg/ml during cell resuspension in PBS. Subsequently, the same method was applied to sequentially extrude the mixture through a polycarbonate membrane filter. After extrusion, the main fraction of Dox-loaded CDVs was obtained using a size exclusion column. Particle counts and the concentration of doxorubicin were measured to conduct a cell viability assays.

Free doxorubicin at the same concentration as the Dox-loaded CDV was prepared, and 96-well plates were seeded with 115026 and 110621 cells to assess the effectiveness. The results indicated that the proliferation of cancer cells was not reduced, suggesting that the concentration of doxorubicin encapsuled into the CDVs may have been too low. It was anticipated that the low drug concentration in

the CDVs resulted in limited effects on proliferation. To address this, efforts are underway to improve the efficiency of doxorubicin loading into the CDVs.

The primary aim of drug-loaded CDVs is to confirm targeting to the pancreas. Achieving effective targeting to the desired organ is crucial, as it allows for higher efficacy with lower drug quantities and helps mitigate the toxicity associated with anticancer agents. For these reasons, targeting the pancreas was set as the goal.

As the first approach, attempts were made to detect human cell-derived RNA in the organs of mice that had been administered CDVs. Since human RNA is not typically detected in mouse organs, it was hypothesized that human RNA would be detectable in mice infected with CDVs. However, RT-PCR yielded results that were not statistically significant, and the recorded CT value were exceptionally high, making the results less reliable. The difficulty in detecting human RNA in mouse organs was attributed to the high sequence similarity between human and mouse α-SMA sequences. In an attempt to address this challenge, the primers were designed to include a portion of the 3' UTR from the human sequence, but this adjustment did not lead to the detection of human-specific RNA expression.

As an alternative method for evaluating the organ-specific targeting of CDVs, Cy7 mono NHS ester was employed as a labeling agent. This labeling process involved treating CDVs with 5 μM of Cy7 prior to passing them through a size exclusion column, followed by a 30 minutes incubation at 37℃. During this process, Cy7 was incorporated into the CDVs. Following the passage through the size exclusion column, Cy7-labeled CDVs were obtained. These labeled CDVs were subsequently imaged using the IVIS spectrum, confirming detection in the main fraction. Additionally, injection of these CDVs into mice aimed to verify their precise targeting to the pancreas. Given the regressive effects on pancreatic stellate cells (PSCs), a major contributor to desmoplasia, it was expected that CDVs produced using PSCs would exhibit effective pancreas targeting. Pancreatic cancer orthotopic mice injected with Cy7 labeled CDV confirmed the targeting towards the pancreas. However, as only one mouse was used for the normal control, additional experiments are needed for a more robust statistical significance.

Through previous experiments, Cell Derived Nanovesicles were generated from Pancreatic Stellate Cell and the possibility of using them as a therapeutic agent was confirmed. It will be possible to overcome the limitations of existing exosome production and use CDV, which is similar to exosomes, as a new drug delivery vehicle. The current problem is that the efficiency of encapsulating drugs in CDV is low, but if this problem is solved, it is expected to show better drug delivery ability. Additionally,

high targeting efficiency can be expected by utilizing the parent cell regeneration characteristics of exosomes. CDV made using PSC was confirmed to target pancreas well, and it is expected that this will solve the problem of drug delivery efficiency caused by desmoplasia in PDAC. It is expected to exhibit better antitumor effects through combination treatment with other drugs while suppressing desmoplasia. Although this study was limited to PDAC, it may be equally applicable to other cancers through additional research.

Conclusion

This study was conducted to develop a type of artificial exosome called cell derived nanovesicle (CDV) as a new drug delivery vehicle. Exosomes require time to be produced and secreted from cells and have a low yield. However, in the case of CDV, it is more efficient than exosomes in terms of time and production because it only requires cell culture and mechanical extrusion. With these advantages, we aim to create a new artificial exosome drug by encapsulating the drug in CDV.

CDVs were successfully produced using PSC. As a result, it was possible to produce approximately $1 \sim 5 \times 10^{10}$ particles/ml in the main fraction. After confirming CDV production, drug encapsulation experiments began and doxorubicin, one of the anticancer drugs, was encapsulated. This resulted in a drug concentration of approximately 0.3 μg/ml in the main fraction. When we attempted to treat primary pancreatic cancer cells using the generated doxorubicin-encapsulated CDV, we were able to confirm the effect of reducing cell proliferation. However, there still remains the problem that the concentration of the drug encapsulated in CDV is too low, and additional experiments are being conducted to resolve this.

After successful encapsulation of the drug into CDVs, to ensure that the drug was targeted to the desired site, RNA expression was examined for targeting of CDVs made from human cells in mouse tissues using RT-PCR. However, because the α-SMA sequences between humans and mice are more than 90% identical, it was difficult to specifically detect human RNA. Because of this, we attempted an alternative method of labeling Cy7, utilizing fluorescence to confirm targeting. We confirmed the targeting of CDV to the pancreas in the orthotopic mouse model using Cy7, yet further experiments need to be conducted.

We developed CDV as a new drug carrier, encapsulated the drug, and confirmed its anticancer effect and targeting to specific organs. CDVs allow for more effective results with lower drug doses while maintaining in vivo safety. Since targeting to the desired organ is possible using CDV, the anti-cancer effect can be maximized while minimizing the side effects of the drug. As a result, we succeeded in developing CDV as a new drug carrier.

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Korean Abstract

췌장 도관 선암종은 췌장암의 주요 아형 중 하나이며, 췌장암 환자에서 약 90%를 차지하는 흔한 유형입니다. 췌장 도관 선암종의 5년 생존율은 약 5%로 매우 낮습니다. 이러한 유형의 암의 주요 문제 중 하나는 결합조직형성증으로 알려진 현상입니다. 이는 세포외기질(ECM)과 섬유아세포가 종양 부위 주변에 비정상적인 조직을 형성한다는 의미입니다. 이러한 현상은 췌장 도관 선암종에서 많은 치료 어려움과 약물 저항성을 유발합니다. 암세포 주변을 세포외기질과 섬유아세포가 둘러싸고 있어 항암제가 암세포까지 전달되지 못해 이러한 약물 저항성이 발생합니다. 이러한 문제를 극복하기 위해 세포 유래 나노베지클을 활용한 새로운 약물 전달 시스템을 개발하려고 노력하고 있습니다. 이 시스템은 결합조직형성증의 주요 구성세포인 췌장 성상 세포에서 유래한 세포 유래 나노베지클을 활용한 시스템으로, 엑소좀의 특징 중 하나인 모세포 회귀 반응을 이용하는데 이는 인공 엑소좀인 세포 유래 나노베지클 또한 이러한 특징을 가지고 있습니다. 세포 유래 나노베지클에 약물을 봉입시켜 만든 나노베지클을 만들어 내었고 실험을 통해 약물 전달 능력을 확인했으나 약물 봉입 효율에 관한 추가적인 연구가 필요합니다. 이러한 한계점이 극복된다면 췌장 도관 선암종 환자에서 약물이 봉입된 세포 유래 나노베지클을 이용하여 약물 표적화 효율을 높이고 기존보다 적은 약물 사용량으로 약물 부작용에 대한 걱정을 덜어내고 더 나은 치료 효과를 기대할 수 있습니다. 또한, 세포 유래 나노베지클은 엑소좀과 유사하게 면역원성이 낮고, 표적화 효율이 높으며, 약물 전달 능력을 갖고 있어 새로운 약물 전달체 개발을 통해 췌장 도관 선암종 치료의 새로운 가능성을 제시할 수 있기를 기대합니다.