의학석사 학위논문

Synthesis of ultrasound contrast agent containing siRNA and chemotherapeutic drug for theragnosis and *in vitro* evaluation in prostate cancer model

siRNA 및 항암제를 포함한 테라그노시스용 초음파 조영제의 합성과 생체 외 전립선암 모델에서의 평가

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ABSTRACT

Introduction: Prostate cancer is the leading cause of mortality in male population. For androgen-refractory prostate cancer, targeted gene therapy using RNA interference as well as chemotherapy such as doxorubicin is being highlighted. Using small interfering RNA, specific proteins for tumor cell growth can be inhibited and cell apoptosis can be induced. For effective intracellular delivery of small interfering RNA and chemotherapeutic drug for prostate cancer cell, we synthesized nanocomplex in combination of ultrasound contrast agent, microbubble, and liposome to work as a carrier. We aimed to enhance intracellular delivery of small interfering RNA and chemotherapeutic drug via ultrasound-guided delivery of "microbubble liposome complex".

Methods: The human prostate cancer cell lines PC-3 and LNCaP were obtained from the manufacturer and incubated. Predesigned survivin-targeted small interfering RNA and doxorubicin were purchased. By mixing lipids and sulfur hexafluoride gas core, microbubbles and liposomes were made, and were shaken together to form the complex. The complex was conjugated with anti-Her2 antibody to target prostate cancer cells. The complex was fluorescent-labeled, incubated with prostate cancer cells, and then evaluated under confocal laser scanning

microscopy for intracellular delivery. Next, small interfering RNA, doxorubicin and both were conjugated with the complexes, respectively, and incubated with prostate cancer cells to assess cell apoptosis induced by therapeutic agents. Concurrently, ultrasound exposure was additionally performed in selected cell lines to investigate the effect of ultrasound-guided delivery of the complex.

Results: Under microscopy, significant intracellular uptake of the complex into LNCaP cells which expressed high level of Her2 was confirmed, suggesting effective target-specific delivery of the complex. In LNCaP cells with effective uptake of complex, small interfering RNA and doxorubicin conjugated complex showed significant increase in cell death after the exposure of ultrasound. Subgroup analysis of complexes with different conjugated therapeutics revealed that small interfering RNA plus doxorubicin conjugated complex showed the highest level of cell death after ultrasound guiding, followed by doxorubicin, and siRNA conjugated complexes in order. No significant cell death was confirmed without ultrasound exposure.

Conclusions: Microbubble liposome complex can effectively target prostate cancer cell lines by the conjugation of anti-Her2 antibody. It can also effectively transfer small interfering RNA and doxorubicin into target cell via ultrasound guidance, resulting in significant cancer cell death. Therefore, it can provide promising method of targeted gene

and drug delivery using microbubbles.

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Keywords: prostate cancer

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LIST OF ABBREVIATIONS

RNAi – RNA interference

dsRNA – double-strand RNA

siRNA – small interfering RNA

RISC – RNA-induced silencing complex

MLC – microbubble liposome complex (MLC)

Her2 – human epidermal growth factor receptor type 2

FITC – fluorescein isothiocynate

Dox – doxorubicin

INTRODUCTION

Prostate cancer is the most common non-skin cancer and the second leading cause of cancer mortality in male population (1). For hormone-dependent prostate cancer, androgen deprivation therapy with radiation has been widely accepted as an effective medical treatment (2). However, most of them eventually recur as aggressive androgen-refractory prostate cancer (2, 3). Chemotherapeutic drugs such as doxorubicin have been tried to improve survival rate in androgen-refractory prostate cancer (4), but there is no established therapeutic method yet.

Recently, new targeted gene therapy using RNA interference (RNAi) is being highlighted for the treatment of advanced prostate cancer. RNAi refers to the mechanism of post-transcriptional gene silencing induced by double-strand RNA (dsRNA) processed to 21 – 25 nucleotide length, which is known as small interfering RNA (siRNA) (5). When siRNA is incorporated into RNA-induced silencing complex (RISC) in the cytoplasm of the cell, it promotes sequence-specific gene silencing via degradation of sequence-specific messenger RNA (mRNA) (5, 6). Thereby, siRNA can be a promising therapeutic agent for the cancer treatment by preventing the production of specific proteins that are essential for the growth of tumor cells.

Survivin is a member of the inhibitors of apoptosis protein family (7, 8), which is strongly expressed in prostate cancer cells (9). It is associated with proliferation of prostate cancer cells (8), with resistance to androgen deprivation therapy, and even with metastasis from prostate cancer (10, 11). Consequently, siRNA-mediated down-regulation of survivin expression can reduce prostate cancer cell proliferation and increase apoptosis (7, 8).

In this study, we aimed to enhance intracellular delivery of survivin-targeted siRNA into prostate cancer cell by using microbubble contrast agents designed for ultrasound. We developed “microbubble liposome complex (MLC)”, carrying survivin-siRNA and doxorubicin, to improve delivery into target cells. The main purpose of our study was to evaluate the efficacy of intracellular delivery of siRNA and chemotherapeutic drug by using microbubble with therapeutic ultrasound, and to estimate the possibility of clinical application of ultrasound-guided therapy for advanced prostate cancer using siRNA-containing MLC.

MATERIALS AND METHODS

1. Cell lines and culture

The human prostate cancer cell lines PC-3 and LNCaP were obtained from American Type Culture Collection (Manassas, VA, USA). Cells were routinely maintained in an RPMI-1640 medium supplemented with 10% fetal bovine serum, and were incubated at 37 ºC in a humidified 5% CO_2. Cells were harvested from subcultures, supplemented with 0.01% trypsine EDTA (Sigma-Aldrich Korea Ltd., Seoul, Korea), and re-suspended in fresh medium for experiments.

2. siRNA and chemotherapeutic drug

Predesigned survivin-siRNAs (Human BIRC5 (332), target sequence: CAAAGGAAACCAACAAUAA, GCAAAGGAAACCAACAAUA, CACCGCAUCUCUACAUUCA, CCACUGAGAACGAGCCAGA) were obtained from the siGENOME SMARTpool by Dharmacon (ThermoFisher Scientific, Waltham, MA, USA), and adjusted according to the manufacturer's instructions. Doxorubicin with molecular weight of 579.98 g/mol was purchased from Sigma-Aldrich Korea Ltd.

3. Microbubble liposome complex preparation

First, lipid stocks of 15.4 mg of DPPC (1,2-dipalmitory-sn-glycero-3-phosphatidylcholine), 3.5 mg of cholesterol, 1.0 mg of DCP (dicetyl phosphate), 1.2 mg of DPPE (1,2-dipalmitory-sn-glycero-3-phosphoethanolamine) and 5.0 mg of disulfide were dissolved into 5.0 mL of chloroform to make the lipid mixture (all reagents, Sigma-Aldrich Korea Ltd.). The mixture was lyophilized for 24 hours to remove chloroform and mixed with 2 mL of solvent composed of glycerin, propylene glycol and H_2O at a ratio of 1:2:7. Then, sulfur hexafluoride gas (SF_6) cores were filled in synthesized microbubbles.

Next, freezing and thawing of aforementioned mixtures by utilizing liquid nitrate were repeated in five times to form liposomes, following agitation by sonicator (Sigma Aldrich Korea Ltd.) at 60 ºC for 5 minutes combined with 2 mL of H_2O. Consequent multilamellar vesicles were extruded via polycarbonate filters (filter size, 200 nm) to build liposomes smaller than 200 nm.

Lastly, microbubbles and liposomes were mechanically shaken together at 25 ºC for 2 hours in a dental amalgamator to form MLCs. To target human epidermal growth factor receptor type 2 (Her2) expressed in prostate cancer cell lines (12), MLCs were added with 5 mg of sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sigma Aldrich Korea Ltd.) and then conjugated with anti-Her2 antibodies (Herceptin®, Roche Korea) by additional mechanical

shaking at 4 ºC for 24 hours.

Final synthesized MLCs showed size distribution ranging from 1 to 2 μm.

4. Analysis of intracellular uptake of MLC

MLCs were labeled using fluorescein isothiocynate (FITC, Sigma Aldrich Korea Ltd.) for microbubbles to emit green fluorescence, and Texas red (Sigma Aldrich Korea Ltd.) for liposomes to emit red. PC-3 and LNCaP cells were seeded in 1 well chamber slide at a density of 1 x 10^5 cells per well. Then, the cells were transfected with MLCs and incubated for 3 hours at 37 ºC.

After being washed with cold phosphate buffered saline, the cells were exposed to ultrasound wave using an ultrasound machine (Philips Medical Systems, Bothell, WA, U.S.A.) and a linear probe in a diameter of 3.5 cm. The cells were seeded in a line in chamber slide and submerged in buffered saline. Then, ultrasound wave with the frequency ranging from 5 to 12 MHz was applied over the cell chambers, at intervals of 1 second for 2 minutes. This process was called “US-flashing”.

The transfected cells were additionally incubated for 3 hours at 37 ºC, and the intracellular localization of MLCs were visualized using a confocal laser scanning microscopy with x 400 magnification (Leica

Microsystems, Germany).

5. siRNA and doxorubicin loading to MLC

MLCs were conjugated with survivin-siRNA or doxorubicin in four different methods: (a) MLCs without additional conjugation, (b) MLCs with doxorubicin only (Dox-MLCs), (c) MLCs with siRNA only (siRNA-MLCs), and (d) MLCs with siRNA and doxorubicin (Dox-siRNA-MLCs). To produce these complexes, 5 nmol of siRNA and/or 1 mg of doxorubicin was added to MLCs and dissolved in 2 mL of H_2O. After agitation by sonicator, freezing and thawing were done as previously described, and then final complexes were filtered using 200 nm-sized polycarbonate filters.

In case of doxorubicin, drug loading efficiency was calculated. Before loading, standard curve of ultraviolet (UV) absorbance was obtained from the measured UV intensity using UV-visible (UV-Vis) spectrophotometer (Scinco, Seoul, Korea). After loading, Dox-MLCs were separated from free form of drugs by centrifugation at 13000 rpm, and the supernatant was also evaluated under UV-vis spectrophotometer. Maximal UV detection was at 480 nm. The loading efficiency was calculated using the following rule: (initial drug concentration – drug concentration in the supernatant) / (initial drug concentration) x 100%.

6. Analysis of cell apoptosis via MTT assay

Cell viability according to Dox-siRNA-MLCs delivery and ultrasound exposure

PC-3 and LNCaP cells were seeded 2.0 x 10^3 cells per well in a 96-well chamber slide. The cells were treated with four different methods as follows: (a) no treatment (group 1), (b) Dox-siRNA-MLCs loading (group 2), (c) US-flashing without loading of MLCs (group 3), and (d) Dox-siRNA-MLCs loading with US-flashing (group 4). For group 2 and 4, Dox-siRNA-MLCs were added into each cell well, and the transfected cells were incubated for 3 hours at 37 ºC. The cells in group 3 and 4 underwent US-flashing with the frequency ranging from 1 to 5 MHz for 2 minutes.

Subsequently, clonogenic MTT assay was performed immediately (day 0) and after 3 days of incubation (day 3). The treated cells were added with 50 μL of MTT reagent (Sigma Aldrich Korea Ltd.) followed by 150 μL of DMSO (Sigma Aldrich Korea Ltd.), and incubated for 3 hours at 37 ºC. The cell plates were shaken for 1 hour to dissolve MTT crystals. Optical densities were read at 450 nm in a spectrophotometer (Scinco), and cell viabilities were calculated in comparison with the control group.

Cell viability according to therapeutic agents and ultrasound guidance

To respectively analyze the effect of each therapeutic agent (survivin-siRNA and doxorubicin) with or without ultrasound exposure, additional MTT assay was performed as follows. PC-3 and LNCaP cells were seeded 2.0 x 10^3 cells per well in a 96-well chamber. The cells were treated with (a) no additives, (b) MLCs without conjugates, (c) Dox-MLCs, (d) siRNA-MLCs and (e) Dox-siRNA-MLCs. Another separate group of cells were treated according to above principles, but they were followed by additional US-flashing. After 3 days of incubation at 37 ºC, the viabilities of cells with different treatments were evaluated clonogenic MTT assay as previously mentioned.

7. Statistical analysis

Data were expressed as means and standard deviations. Differences between experimental groups were evaluated using Mann-Whitney U test and Kruskal-Wallis test. Statistical analyses were performed with commercial statistical software (SPSS, version 18.0; SPSS, Chicago, III). P value less than 0.05 was considered as statistically significant.

RESULTS

1. Efficiency of target-specific intracellular uptake

Figure 1-1 presents the fluorescence microscopic images capturing MLCs in PC-3 cells. Compared with the control images before the incubation with MLCs, no additional green or red fluorescent cell was observed after the transfection of MLCs. Fluorescent cell did not appear after US-flashing either.

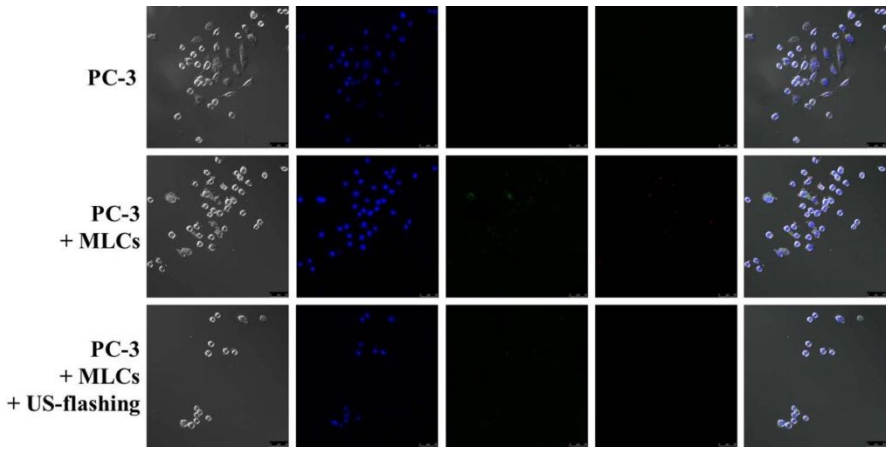


Figure 1-1. Confocal laser scanning microscopy images of PC-3 cells. There is no visible green or red fluorescent cells under microscopy (x400), suggesting poor intracellular uptake of MLCs into PC-3 cells.

On the other hand, LNCaP cells which were known to express higher level of Her2 than PC-3 (12) showed green fluorescence indicating

labeled microbubbles and red fluorescence indicating labeled liposome under microscopy after the transfection of MLCs (Figure 1-2). After US-flashing, LNCaP cells emitting green and red fluorescence signals were also intensely visible (Figure 1-2).

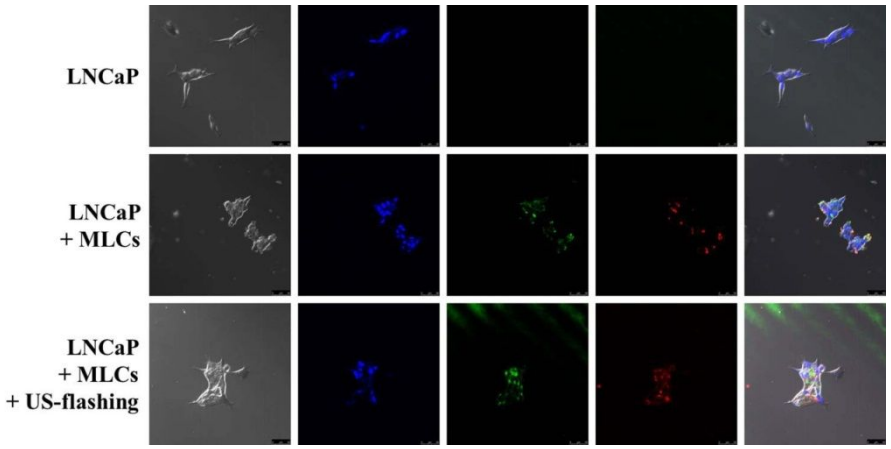


Figure 1-2. Confocal laser scanning microscopy images of LNCaP cells. Green fluorescent cells labeled by FITC and red fluorescent cells labeled by Texas red are observed under microscopy (x400) before and after ultrasound exposure. It shows MLCs bounded with anti-Her2 antibodies can target LNCaP cells efficiently.

Figure 1-3 shows the comparison of fluorescence intensity of the delivered MLCs on confocal microscopy before and after US-flashing in PC-3 and LNCaP cells, respectively.

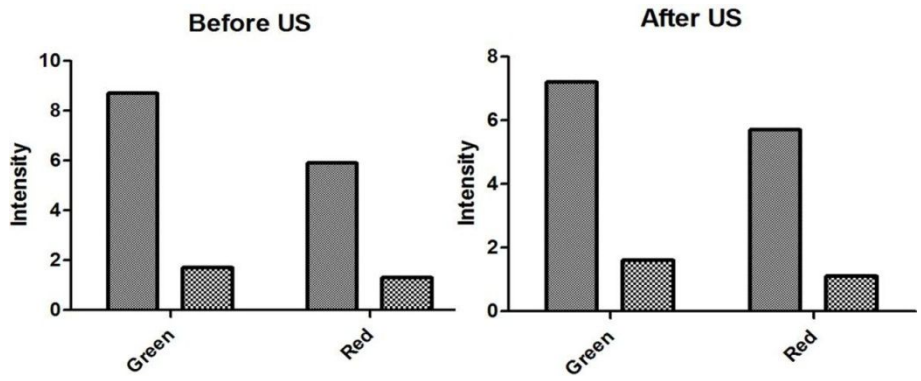


Figure 1-3. Bar graphs depicting signal intensity of fluorescence according to cell lines (solid bar: LNCaP, dashed bar: PC-3). Fluorescence in LNCaP cells is stronger than in PC-3 cells before and after ultrasound exposure. The uptake of MLCs is more effective in LNCaP cells.

2. Efficiency of doxorubicin loading

The efficiency of doxorubicin loading was 61.9%. The concentration of doxorubicin loading was 213.6 μM. The concentration of loaded doxorubicin per treated cell well was 21.4 μM. Figure 2 shows the curve of signal intensity before and after loading of doxorubicin according to UV wavelength.

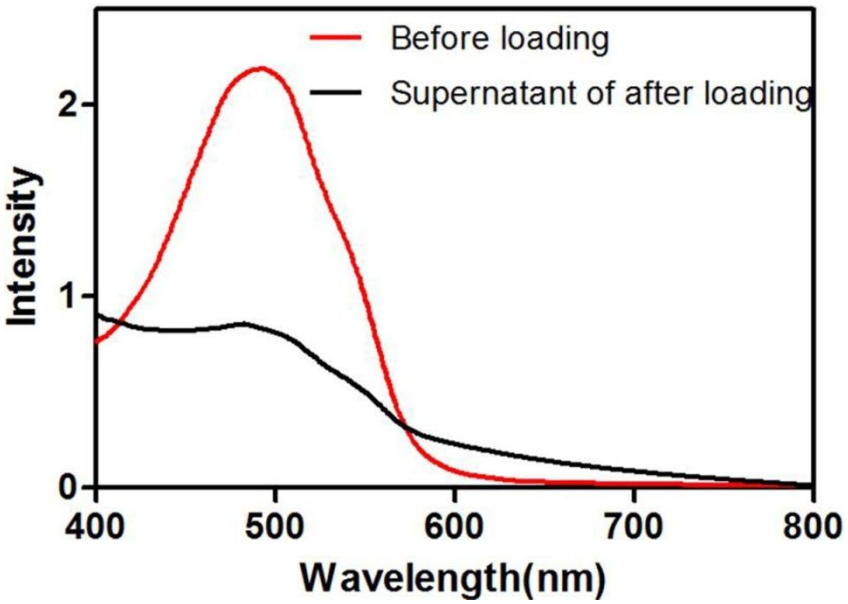


Figure 2. Doxorubicin loading efficiency. The curve presents the difference of ultraviolet (UV) absorptions before and after the loading of doxorubicin. The calculated loading efficiency is 61.9%.

3. Cytotoxicity induced by MLC

Cell viability according to Dox-siRNA-MLCs delivery and ultrasound exposure

In PC-3 cells (Figure 3-1), cell survival rate was more than 90% in all four groups on day 0. On day 3, cell survival rate was reduced by 4% in group 4, but with no statistical significance ($p = 0.12$). Other groups showed no decrease in cell survival rate at all.

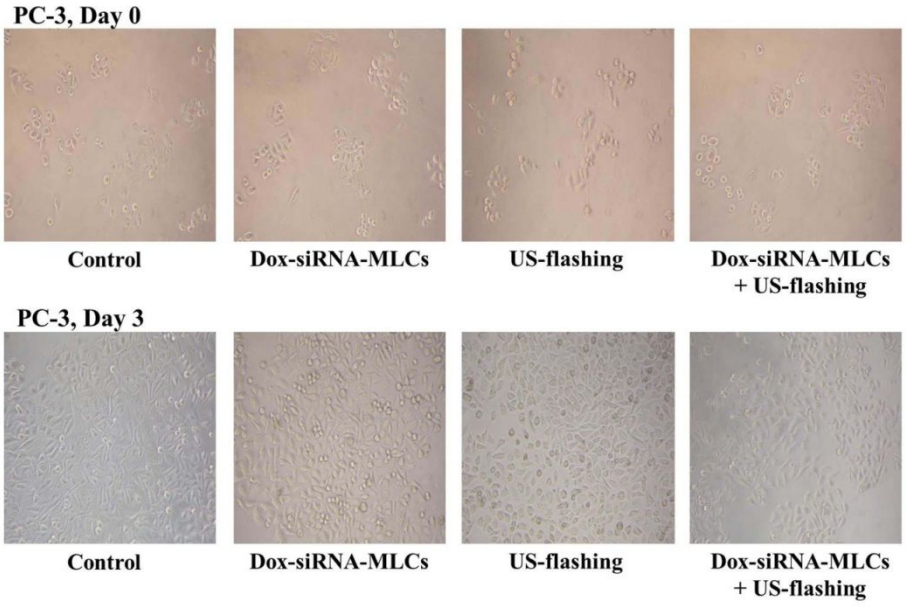


Figure 3-1. Clonogenic MTT assay of PC-3 cells on day 0 and day 3. No significant difference in cell viability between non-treated and treated groups is noted.

In LNCaP cells (Figure 3-2), cell viability on day 0 was also more than 90% in all four groups without significant difference. After 3 days of incubation, group 1, 2 and 3 did not show decrease in cell survival rate. However, group 4 where the cells were treated with Dox-siRNA-MLCs and US-flashing, cell viability was significantly reduced on day 3 than on day 0, meaning significant increase in cell death (day 0, 94.2 ± 2.8% VS. day 3; 41.8 ± 3.2%, $p = 0.01$). On day 3, four groups of cells with different treatments showed significant difference in resultant cell viabilities on day 3 ($p = 0.01$); Especially, cell viability of group 4

was significantly decreased when compared with that of group 1 ($p < 0.01$).

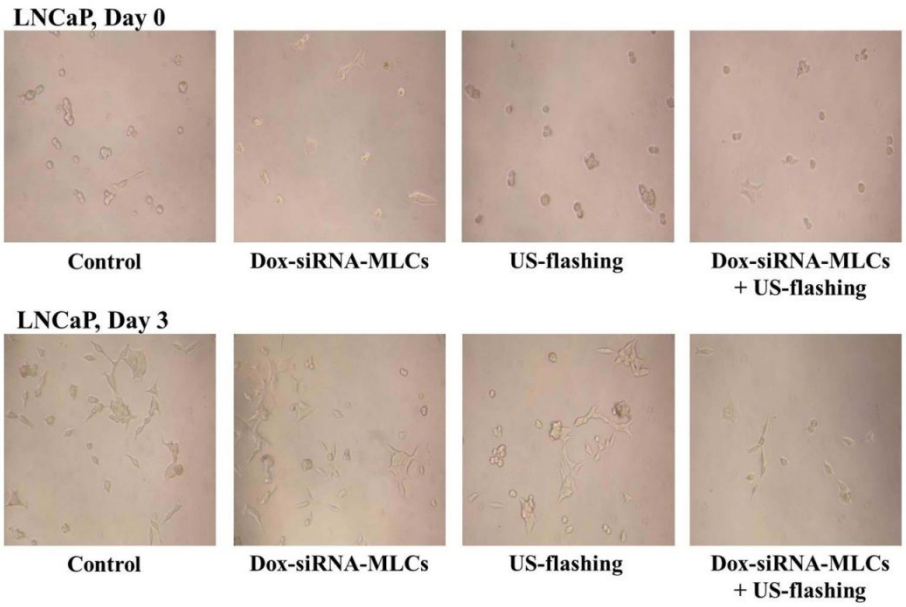


Figure 3-2. Clonogenic MTT assay of LNCaP cells on day 0 and day 3. Cell viability of the group treated with Dox-siRNA-MLCs and following ultrasound exposure shows significant reduction on day 3.

Figure 3-3 summarizes cell survival data with or without treatment.

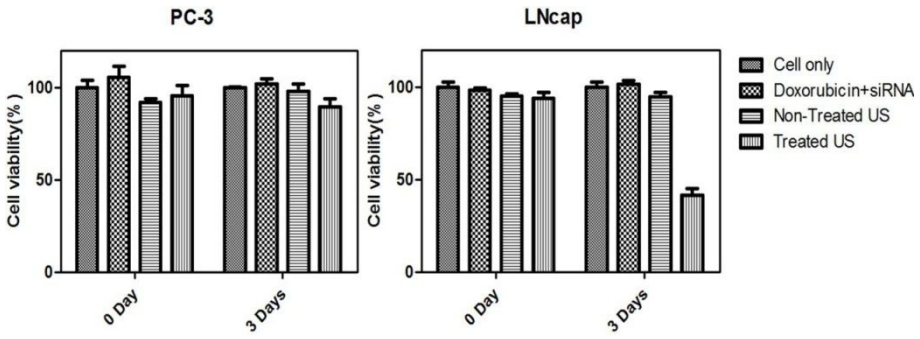
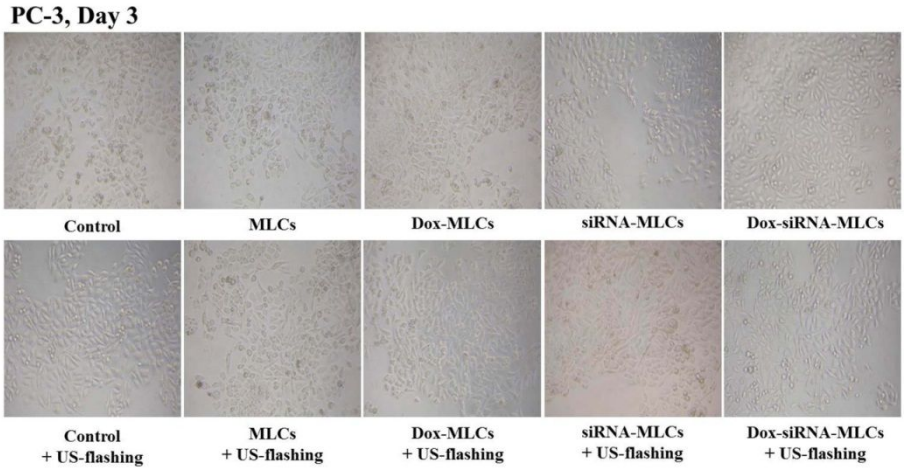


Figure 3-3. Bar graph depicting cell viability in PC-3 and LNCaP cells. Decreased viability of LNCaP cells treated with Dox-siRNA-MLCs and US-flashing is noted.

Cell viability according to therapeutic agents and ultrasound guidance

Figure 4-1A and B demonstrated clonogenic assay according to therapeutic agents and ultrasound guidance after 3 days of incubation.

(A)



(B)

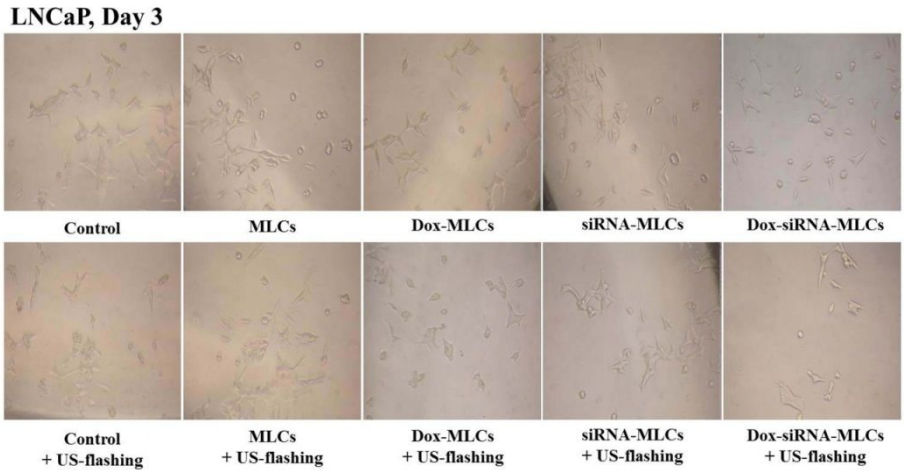


Figure 4-1. Clonogenic MTT assay of PC-3 and LNCaP cells, respectively.

(A) PC-3 cells show no significant difference in cell viability between the groups.

(B) Decrease in LNCaP cell viability is demonstrated in the cells

treated with Dox-MLCs and ultrasound (bottom, middle), siRNA-MLCs and ultrasound (bottom, second from right), and Dox-siRNA-MLCs and ultrasound (bottom, farthest to the right).

Cell survival in PC-3 cells was over 90% in all subgroups without significant difference, regardless of therapeutic agents used. Additional US-flashing did not increase the extent of cell death in PC-3 cells either (all groups, $p > 0.05$).

In LNCaP cells, however, the viabilities of cells treated with Dox-MLCs, siRNA-MLCs and Dox-siRNA-MLCs showed significant decrease after additional US-flashing (Dox-MLCs, 88.0 ± 3.4% VS. 63.0 ± 1.8%; siRNA-MLCs, 87.0 ± 4.1% VS. 73.0 ± 3.8%; Dox-siRNA-MLCs, 85.0 ± 2.9% VS. 55.0 ± 3.5%, all $p < 0.01$). Among them, the cells treated with ultrasound-guided Dox-siRNA-MLCs showed the highest reduction in cell viability, followed by the cells treated with ultrasound-guided Dox-MLCs and ultrasound-guided siRNA-MLCs. Figure 4-3 summarizes the overall rate of cell viability in subgroups of PC-3 and LNCaP cells, respectively.

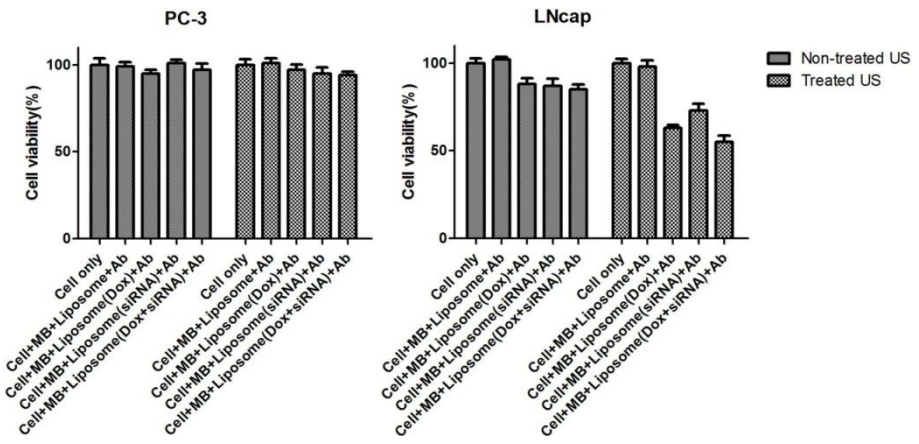


Figure 4-2. Bar graph depicting cell viability in PC-3 and LNCaP cells. After exposure of ultrasound, LNCaP cell viabilities are decreased in the cells treated with Dox-MLCs, siRNA-MLCs and Dox-siRNA-MLCs.

DISCUSSION

RNAi is a biological mechanism that dsRNA interferes with and blocks the expression of a particular gene that has a homologous sequence with it (13). This dsRNA is known as a "siRNA", a small RNA with 21 – 25 nucleotides length which is generated from the cleavage of long dsRNA by the act of ribonuclease named as dicer (13). siRNAs are incorporated into RISC that promotes endonucleolytic cleavage of complementary targeted mRNA (14), resulting in sequence-targeted suppression of specific transcription – from mRNA to target protein. Therefore, siRNAs are emerging as new therapeutic molecules using gene silencing for the treatment of cancer (13).

Therapeutic siRNAs for prostate cancer have been actively investigated over years. Survivin, the member of the inhibitor of apoptosis family expressed in prostate cancer cell, has been identified as an attractive therapeutic target, since it not only maintains cancer cell viability, but also gives resistance to androgen deprivation therapy and mediates distant metastasis (8, 9, 11, 15). Recently, Paduano et al. (7) demonstrated that silencing of survivin gene by siRNAs induced apoptosis in prostate cancer cells and increased sensitivity to chemoagent. Several other studies also proved that down-regulation of survivin by siRNAs could increase chemosensitivity of prostate cancer

cells (16, 17).

However, intracellular delivery of siRNAs is challenging because siRNAs are easily degraded in the physiologic environment and cannot cross cell membranes efficiently due to anionic characteristics (18, 19). Thus, additive “carriers” must be attached to siRNAs for effective intracellular delivery; the carriers need to be able to protect siRNAs from degradation, transport siRNAs to target cells and release them into cytoplasm with minimum toxicity (18, 20). Among many different carriers that have been proposed including viruses (21), peptide conjugated (22) and/or lipid conjugated nanoparticles (18, 23), polymeric nanoparticles are considered to be advantageous in silencing applications.

In our study, we aimed to enhance intracellular delivery of siRNA-based therapeutic agents by using microbubbles. Microbubble is basically a gas bubble with 3 μm of diameter, originally developed as a contrast agent to improve ultrasound imaging (24). However, it can also work as an effective carrier of transgene and target specific tissue guided by incorporating target-specific ligands (24). The delivery of siRNAs using microbubbles occurs with the destruction of microbubbles by ultrasound irradiation (sonoporation); therefore, more

selective targeting of tissues by focusing ultrasonic field can be achieved (25).

We constructed MLCs conjugated with anti-Her2 antibody to target prostate cancer cell lines known to express Her2 (12). We determined the efficacy of intracellular uptake of MLCs with the use of ultrasound, on the basis of confocal microscopic images. The result revealed that intracellular delivery of MLCs was effective in LNCaP cells which had higher expression of Her2 than PC-3 cells. Thus, we could assume that MLCs could effectively target prostate cancer cell lines, guided by conjugated target-specific antibodies.

We also put together survivin-siRNA and chemotherapeutic drug, doxorubicin, with MLCs. What was noteworthy in our study was that doxorubicin was conjugated with MLCs, not injected in cell wells as in most of previous studies. In LNCaP cells with effective uptake of MLCs, Dox-siRNA-MLCs produced significant decrease in cell viability after the exposure of ultrasound. On MTT assay, LNCaP cells treated with Dox-siRNA-MLCs and US-flashing showed significant reduction in cell viability than other cells treated with either Dox-siRNA-MLCs or US flashing only. Considering that targeting effect of MLCs to LNCaP cells was superb due to anti-Her2 antibodies, this result verified that survivin-siRNA and doxorubicin could be

effectively transferred to prostate cancer cells with the use of ultrasound, and induce cell deaths by presenting cell toxicity.

Also notable is the fact that we proved ultrasound exposure after intracellular delivery of survivin-siRNA and doxorubicin could significantly increase the cytotoxic effect of the therapeutic agents. Intracellular delivery of molecules using microbubbles has already known to be effective since it could use ultrasound energy deposition in tissue (25). Previous studies suggested that ultrasound energy disrupting microbubbles could even increase *in vivo* cell permeability (26).

Taken altogether, our study could support the fact that ultrasound-guided delivery of microbubbles could be a strong and effective tool of intracellular delivery of therapeutic agents including siRNA and chemotherapeutic drugs and increase their cytotoxic effects. A schematic drawing of ultrasound-guided intracellular delivery of siRNA and doxorubicin using MLCs into PC-3 and LNCaP cells is presented in Figure 5.

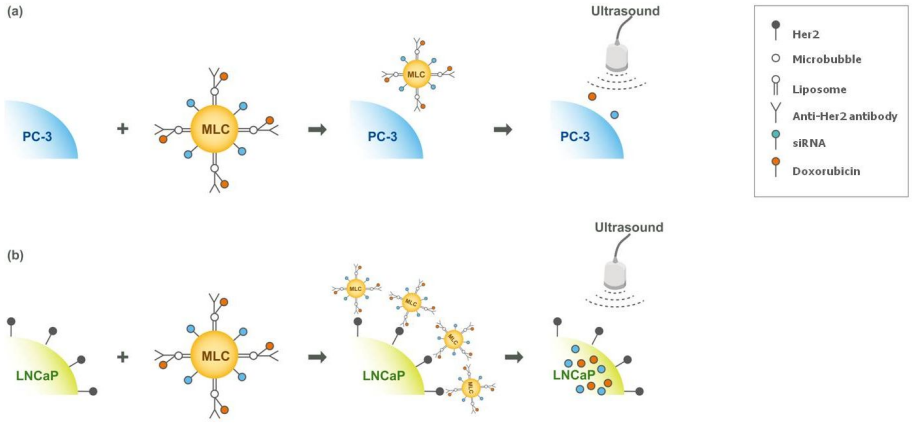


Figure 5. A schematic drawing of ultrasound-guided intracellular delivery of siRNA and doxorubicin using MLCs.

There have been a few studies describing apoptosis of cancer cells via intracellular delivery of siRNA with microbubbles. Wang et al. (27) transfected survivin-siRNA using microbubbles and ultrasound to ovarian cancer cells, and proved increase in apoptosis of cancer cells. He et al. (28) also developed lipid microbubble containing siRNA that could target yolk sac carcinoma cell lines, resulting in significant decrease in cell viability. However, our current study was first to demonstrate effective intracellular delivery and cytotoxicity of Dox-siRNA-MLCs in prostate cancer cell lines.

Despite these promising findings, there are some limitations in our study. First, in assessing intracellular uptake of MLCs, quantitative flow cytometry was not performed along with confocal microscopic examination. Second, fluorescent imaging was performed after 3 hours from transfection. Accordingly, total uptake of MLCs might be underestimated since delayed uptake could be eliminated from our analysis. Further studies will be needed including delayed analysis after sonoporation to strengthen our result. Lastly, the result of current study was only based on *in vitro* study. True effect of siRNA and doxorubicin delivery by ultrasound-guided microbubbles *in vivo* cannot be precisely estimated. Therefore, *in vivo* studies of MLCs will be needed to expand our result to clinical fields.

In conclusion, MLCs with the use of ultrasound could effectively target prostate cancer cell lines by the conjugation of anti-Her2 antibody. They can effectively transfer siRNAs and doxorubicin into target cell via ultrasound guidance, resulting in significant cancer cell death. Therefore, we could provide promising methods for delivering siRNAs and chemotherapeutic drugs to prostate cancer cell in current study. This result can open up the possibility of clinically available targeted gene therapy and may increase the prospects for therapeutic application *in vivo* in the near future.

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국문 초록

서론: 전립선암은 남성에서 높은 암 사망률을 나타내는 질병이다. 안드로겐 불응성 전립선암의 경우, doxorubicin과 같은 항암제와 더불어 RNA 간섭을 이용하는 유전자 치료가 새롭게 각광받고 있다. 짧은 간섭 RNA를 이용하면, 암세포 성장에 기여하는 특정 단백질을 억제함으로써, 세포 사멸을 도모할 수 있다. 본 연구에서는 짧은 간섭 RNA의 세포 내 전달을 위해서 마이크로버블과 리포좀을 이용한 일종의 “매개전달체” 를 합성하였다. 초음파 유도 하에 이 매개체를 이용하여, 짧은 간섭 RNA와 항암제를 효과적으로 세포 내로 전달하고자 하였다.

방법: 인간 전립선암 세포 숙주인 PC-3와 LNCaP, 그리고 survivin 특정 짧은 간섭 RNA와 doxorubicin을 구입하였다. 지질과 sulfur hexafluoride 가스를 혼합하여, 마이크로버블과 리포좀을 합성하였고, 이들을 혼합하여 매개체를 완성하였다. 이 매개체에 추가적으로 Her2 항체를 연결하여, 효과적으로 전립선암 세포들을 타겟하도록 하였다. 매개체는 형광물질을 혼합하여 공초점 현미경으로 세포 내 전달을 확인하였다. 다음으로, 짧은 간섭 RNA와 doxorubicin, 혹은 둘 모두를 각각 매개체에 접합시키고, 전립선암 세포와 함께 배양하여 세포 독성을 알아보았다. 일부 선택된 세포군에는 추가적인 초음파 노출을 시행하여, 초음파 유도하의 매개체 전달의 효과를 알고자 하였다.

결과: 공초점 현미경 하에서, Her2 발현이 높은 LNCaP 세포군에서의 매개체 흡수가 유의하게 높아, Her2 항체를 이용한 특정 세포 타겟팅의 효과를 확인하였다. 매개체의 흡수가 높은 LNCaP 세포군에서는, 짧은 간섭 RNA와 doxorubicin을 합성한 매개체의 첨가와 추가적인 초음파 노출로 세포 사멸이 유의하게 증가함을 역시 확인하였다. 그 중에서도, 짧은 간섭 RNA와 doxorubicin을 함께 합성한 매개체 전달 시 세포 사멸도가 가장 높았고, doxorubicin 합성 매개체, 짧은 간섭 RNA 합성 매개체의 차례로 높은 세포 독성을 나타내었다. 초음파 노출이 없는 경우, 유의한 세포 사멸은 보이지 않았다.

결론: 마이크로버블 리포좀 합성 매개체는 Her2 항체를 접합함으로써 효과적으로 전립선암 세포를 타겟한다. 또한, 초음파 유도 하에 효과적으로 짧은 간섭 RNA와 doxorubicin을 세포 내로 전달하여, 유의한 세포 독성을 나타내게 한다. 따라서, 이 매개체가 앞으로의 유전자 치료에 크게 기여할 수 있을 것으로 예측된다.

주요어: 전립선암
초음파
마이크로버블
RNA 간섭
독소루비신

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