석사학위논문

비만 유래 대사증후군 치료용 유전자 치료법에 관한 연구

Studies on the gene therapy for the treatment of obesity-related metabolic syndrome

김 형 진

한양대학교 대학원

2013 년 8 월

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이 논문을 공학 석사학위논문으로 제출합니다.

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Contents

Contents i

List of figures iii

Abstract iv

1. Introduction 1

2. Materials and Methods 4

2.1 Materials

2.2 Mature adipocyte differentiation and 3T3-L1 culture

2.3 Mature adipocyte confirmation using Oil Red O staining

2.4 Gel retardation Assay

2.5 Cell culture

2.6 Cytotoxicity assay

2.7 *In vitro* Gene expression by confocal microscopy

2.8 *In vivo* Gene expression by confocal microscopy

2.9 Insulin tolerance test and glucose tolerance test

3. Results and Discussion 8

3.1 Production of 3T3-L1 mature adipocyte

3.2 Characterization of polyplex

3.3 Cytotoxicity assay

3.4 *In vitro* Gene expression analysis

3.5 *In vitro* luciferase assay

3.6 *In vivo* gene expression analysis

3.7 Immunohistochemistry of *in vivo* fat tissue

3.8 Therapeutic effect in *in vivo* mouse model

4. Conclusion 21

5. References 22

국문요지 25

List of Figures

Figure 1. 3T3-L1 adipocyte differentiation 9

Figure 2. Confirmation of mature adipocyte by Oil Red O staining 10

Figure 3. Polyplex Characterization 11

Figure 4. *In vitro* Gene expression by confocal microscopy 12

Figure 5. Transfection efficiency comparison in 3T3-L1 preadipocyte 13

Figure 6. Transfection efficiency comparison in 3T3-L1 mature adipocyte 14

Figure 7. In vivo Gene expression by confocal microscopy 17

Figure 8. Immunohistochemistry of *in vivo* fat tissue 18

Figure 9. Insulin tolerance test 19

Abstract

Studies on the gene therapy for the treatment of obesity-related metabolic syndrome

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Obesity is occurring at epidemic proportions in worldwide. Obesity develops when energy intake exceeds energy expenditure. Adipocyte are the main fat storage facilities in our body and are associated with development of obesity, type 2 diabetes and obesity induced metabolic syndrome. Currently the adipocyte targeted non viral gene delivery carrier does not exist. Non viral gene delivery carriers are known to be safer than viral vector. Protein transduction domain (PTDs) or cell penetrating peptides (CPPs) are short peptides that facilitate cellular uptake of various nano size particles to small chemical molecules and large fragments of DNA. We have shown an adipocyte targeted non viral gene delivery system by attaching a nona arginine peptide to an adipocyte targeting sequence (ATS). This carrier was designed as C-ATS-9R-C. It was confirmed that the carrier was very efficiently to condense plasmid DNA and bound with and internalized into adipocyte cells in vitro and in vivo mouse model. And there is no cytotoxicity in differentiated adipocyte. This C-ATS-9R-C showed that transfection and gene expression in differentiated adipocyte cell are possible. RNA interference (RNAi) is known that expression of a targeted

gene can be knocked down with high specificity and selectivitiy. This study was to develop an adipocyte targeted gene delivery carrier: which will be able to shRNA. Finally, we aimed for confirmation in the clinically relevant obese mouse model to improve insulin resistance. Therefore, C-ATS-9R-C is possible to deliver therapeutic gene to treat obesity.

Key words: Adipocyte cell, obesity, non viral gene delivery, protein transduction domain(PTD), adipocyte targeting sequence(ATS), shRNA therapy, obesity therapy

1. Introduction

According to recent studies, one of the Korean people is overweight. Obesity people who body mass index (BMI) is greater than 25 (kg / m²), in 2008, accounted for 30.7%. In particular, the ratio of extremely obese population whose BMI is over 30 has increased nearly double from 2.3% in 1998 to 4.1% in 2008. Obesity leads a lot of obesity-related disease, such as diabetes, hyperlipidemia, and atherosclerosis. [1]. But until now for treatment of obesity such as prevent the absorption of the fat itself, suppress appetite and promotes the body's heat production secondary to reduce fat cells [2, 3].Reductil, the most famous obesity drug suppressing appetite, was withdrawn from the market because it was reported in 2010 that Reductil has a serious side-effect increasing the rate of cardiovascular disease [4]. For this reason, in order to treat of obesity, the carrier has been focused on the research that can deliver drugs specifically to the non-local self-secondary access. In case of obesity, excess amount of fat or lipids are accumulated into the lipid droplets of adipocyte cells[5-8].The obesity is promoted when energy intake is much more than consumption. A small proportion of the nutrient intake is excreted in the urine [1].

There are two types of obese tissue. They are the white adipocyte tissue and the brown adipocyte. Brown adipocyte tissue burns lipid to produce heat using expression of mitochondrial uncoupling protein-1 gene [9]. The brown adipose tissue is involved in the regulation of energy balance to the activation of the heat generated by excessive energy intake [10]. The white adipocyte tissue is made up of cells capable storing lipids. The white adipocyte tissue store energy for long periods of time, and secrets other types of

inflammatory cytokine. This tissue which offers the primary therapeutic target for the treatment of type 2 diabetes, induced obesity metabolic syndrome and obesity[11-13]. To targeting adipocyte tissue, adipocyte targeting moiety was required. Currently many researchers proceed with the obesity therapy using viral vector because of high gene expression efficiency[4, 14, 15].

However, due to safety problem, the researchers began looking for non-viral gene delivery system. Kolonin et al. found a small peptide sequence CKGGRAKDC. This sequence can bind Prohibitin that is found adipocyte vasculature receptor [16]. We named this sequence adipocyte targeting sequence (ATS). Prohibitin has 30~32kDa molecular weight and found in the mitochondria [6]. But changes the expression of prohibitin in differentiated adipocyte cell [17]. ATS has only three positively charged. Therefore it is not enough to condense plasmid DNA and shRNA. So we attached protein transduction domain (PTD) behind the adipocyte target sequece. Nona arginine(9R) is well known for protein transduction domain for intracellular delivery.[18-20] So the final peptide sequence is C-KGGRAKD-RRRRRRRRR-C (C-ATS-9R-C). In this study, It was confirmed that the C-ATS-9R-C is a carrier to deliver gene in *in vitro* adipocyte. For confirming gene expression in adipocyte, Red Fluorescent protein (RFP) plasmid was used. For make sure the drug in animal models adipocyte tissue specifically we used the FITC labeled C-ATS-9R-C and the RFP plasmid polyplex. By subcutaneous injection and was confirmed confocal laser scanning microscopy.

Function of fatty acids both as signals for metabolic regulation and as an energy, conducting through enzymatic and transcriptional network to modulate gene expression, growth and survival pathway, and inflammatory and metabolic responses. Fatty acid binding protein (FABPs) are group of molecules that control lipid responses in cells are

also strongly linked to inflammatory and metabolic pathways [17]. Cytoplasmic Fatty acid binding protein are small protein that bind to fatty acids and are expressed in a highly tissue specific process. Adipocyte fatty acid binding protein, also known FABP4, was highly expressed in mature adipocyte and adipose tissue. Fabp4 regulated the differentiation of adipocyte. In resent studies, lack of FABP4 provides significant protection from insulin resistance and hyperinsulinemia associated with genetic or dietary obesity [18].

The target delivery of short hairpin RNA is one of the most major challenges in gene therapy[21-24]. However due to high negative charge and their large size, do not readily penetrate cells [21, 25]. Approaches for enhancing delivery of shFABP4 to adipocyte cells have condensed our carrier and confirmed that adipocyte tissues improve insulin resistance using insulin tolerance test.

2. Materials and Methods

2.1 Materials

3T3-L1 Preadipocyte cells were purchased from Korean cell bank. Fetal bovine serum (FBS), Penicillin streptomycin and Dubecco's Modified Eagle's Medium (DMEM) high glucose were purchased from WelGENE (Seoul, Korea). Dc protein assay kit was purchased from Bio-Rad Laboratories (Hercules, CA) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Aldrich (St. Louis, MO). Luciferase Assay Kit was purchased from Promega (USA). Dexanethasone was purchased from Sigma-Aldrich (St. Louis, MO). Insulin and 3-Isobutyl-1-methylxanthine (IBMX) were purchased from Wako (Japan). C-ATS-9R-C and FITC-ATS-9R peptides were purchased from Peptron Inc (Korea).

2.2 Mature adipocyte differentiation and 3T3-L1 culture.

3T3-L1 Preadipocyte cells were cultured and induced differentiate as per previously stated method. Preadipocyte cells were cultured in complete media (CM) containing Dubecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum supplemented with 100U/ml penicillin and 100mg/ml streptomycin at 37°C under humidified conditions (5% CO2 atmosphere). For adipocyte differentiation, 4days after cell seeding, cells were treated with differentiation induction medium containing

complete media, 10μg/ml Insulin, 1μM Dexamethasone and 0.5ml 3-Isobutyl-1-methylxanthine (IBMX) for 72hours. After that, differentiation media were replaced with adipocyte maintenance medias containing complete media and 10μg/ml insulin. Media were changed in every two days.

2.3 Mature adipocyte confirmation using Oil Red O staining

Adipocyte differentiation was confirmed by Oil Red O staining method described previously [26]. For preparation of Oil Red O staining 0.7g Oil Red O powder dissolved in 200ml isopropanol. After Oil red O powder melts, filtered using a 0.22um syringe filter. Cultures were fixed for at least 1 h with PBS containing 3.7% formaldehyde, then washed with 60% Isopropanol and dried in the clean bench, stained for 15minutes by a working solution of Oil red O. And then staining cells were washed four times by deionized water. For quantitative analysis of mature adipocyte accumulated Oil Red O was eluted from cells by 100% Isopropanol and absorbance was taken at 520nm

2.4 Gel retardation Assay

Polyplex was made by 1μg RFP(Takara Japan), Deionized water and C-ATS-9R-C (Weight ratio ,3,4,5). Incubation are done for 30 minutes at room temperature and run in 0.8% agarose gel(Lonza USA) by electrophoresis for 25 minutes in 100 V.

2.5 Cytotoxicity assay

For cytotoxicity assay of polyplex, 3T3-L1 cells were seeded $2x10^4$ cell in 24well culture plate and incubated for 24hours at 37°C under humidified conditions (5% CO2 atmosphere). After incubation time culture plate treated polyplex made by RFP plasmid gene, ATS-9R and lipofectamine with different weight ratio. After transfection incubated for 24 hours at 37°C under humidified conditions (5% CO_2 atmosphere). Then 10% of MTT reagent was added into total media in each well and incubate for 4 hours at 37°C. After incubation added 500µl DMSO solution and incubate for 15 minutes at 37°C. After incubation the optical density was measured by 570nm.

2.6 *in vitro* Gene expression by confocal microscopy

Polyplex was made by 2µg RFP, deionized water and C-ATS-9R-C at weight ratio 3 and incubated for 30 minutes in room temperature. 3T3-L1 preadipocyte cells were grown and differentiated on 6 well plate. At day 10 of differentiation, cells were treated polyplex and incubated for 48 hours. After incubation, cells were fixed in 3.7% formaldehyde. Cells were washed with PBS and mounted by DAPI staining nucleus. Images were obtained in Carl Zeiss confocal microscopy.

2.7 *in vivo* Gene expression by confocal microscopy

Polyplex was made by 30µg RFP, deionized water and FITC-C-ATS-9R-C at weight ratio 3 and incubated for 30 minutes in room temperature. Mice were anesthetized by ketamine at dose of 100mg/kg and xylazine at a dose of 100mg/kg intraperitonealy. After

mice were anesthetized, polypex was injected in subcutaneous C57BL/6J obese mouse model. After 48hours of transfection, mice were sacrificed and isolated fat tissue. RFP expression in fat tissue was observed in Carl Zeiss confocal microscopy.

2.8 Immunohistochemistry of *in vivo* fat tissue

Immunofluorescence costaining of representative fat tissue sections at 2 days after FITC-ATS-9R delivery. After 48 hours inject FITC-C-ATS-9R-C, the mice were sacrificed, fat tissue were isolated and E-cadherin antibody alexa 555(cell signaling) was added to the fixed cryo-sectioned adipose tissue and incubated for 1 hour at 37°C in dark. After incubation tissue was washed with PBS and then observed in Carl Zeiss confocal microscopy.

2.9 Insulin tolerance test and glucose tolerance test

For Glucose tolerance test(GTT) and Insulin tolerance test(ITT), blood glucose level was measured after 16 h (for GTT) or 6 h (for ITT) of fasting, using a Accu-Chek Active model GC kit (Roche Diagnostics GmbH, Geramany). After this measure, glucose (3 g/kg body weight) or insulin (0.75U/kg body weight) was dosed by intraperitoneal injection, respectively. Blood was collected from the tail vein at 0, 30, 60, 90, and 120 min post-dose for glucose determination.

3. Result and Discussion

3.1 Production of 3T3-L1 mature adipocyte

The 3T3-L1 cell adipocyte the most commonly used for cell line for research adipocyte differentiation[27]. When cell seeding after 2 days put in differentiation media, cell morphology was gradually starting to look and began to change in the shape of round, large lipid droplets in the cytoplasm. Almost 90% of the cells had a lipid droplet **[Figure: 1]**. The lipid droplet size in the mature adipocyte cells continue to grow over the date. Oil Red O staining solution is a widely used for dye to confirm adipocyte differentiation. It lipid droplet membrane selectively in the adipocyte cells can dye. Adipocyte differentiation and that have many lipid droplet, as have a lot of Oil Red O **[Figure 2 A]**. For quantitative analysis, accumulated Oil Red O was eluted and quantified in UV spectrophotometer at 520nm. Came up to a value of 0.4 to 0.7 when Preadipocyte Oil Red O staining. Mature adipocyte OD values began to rise from the fourth day The Day 7 showed a value of about 1.5. Starting on differentiation, we can say the fully differentiated form Day 7 **[Figure 2 B]**.

3.2 Polyplex Characterization

Polyplex was made by RFP plasmid DNA, deionized water and C-ATS-9R-C and lipofectamine with different weight ratio and characterized by gel retardation assay **[Figure 3 A]**. From gel retardation assay, we can check that make polyplex with DNA from the weight ratio 3.

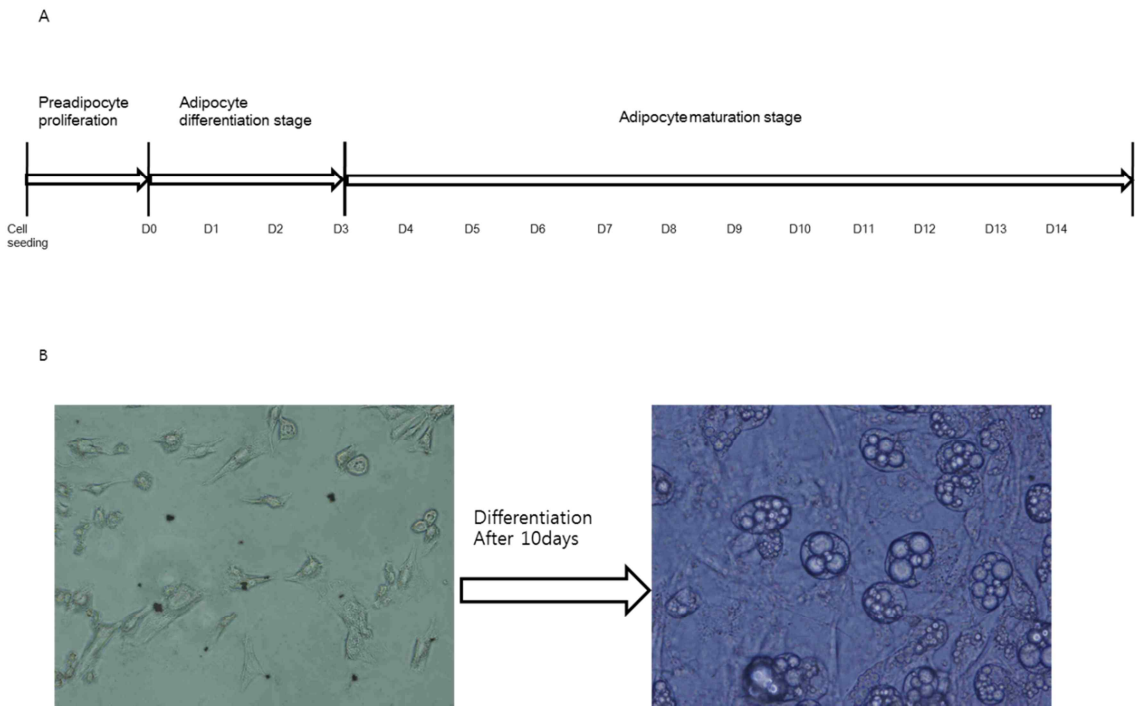

Figure 1: 3T3-L1 adipocyte differentiation

(A) Schematic diagram shows the process to produce the mature adipocyte.

(B) 3T3-L1 will change the media to induce differentiation after 4 days to treat the maturation media. Subsequently the image of the mature adipocyte can get through 14 days after 3T3-L1 cells seeding. All images were captured in Nikon fluorescence microscope at 20X magnification

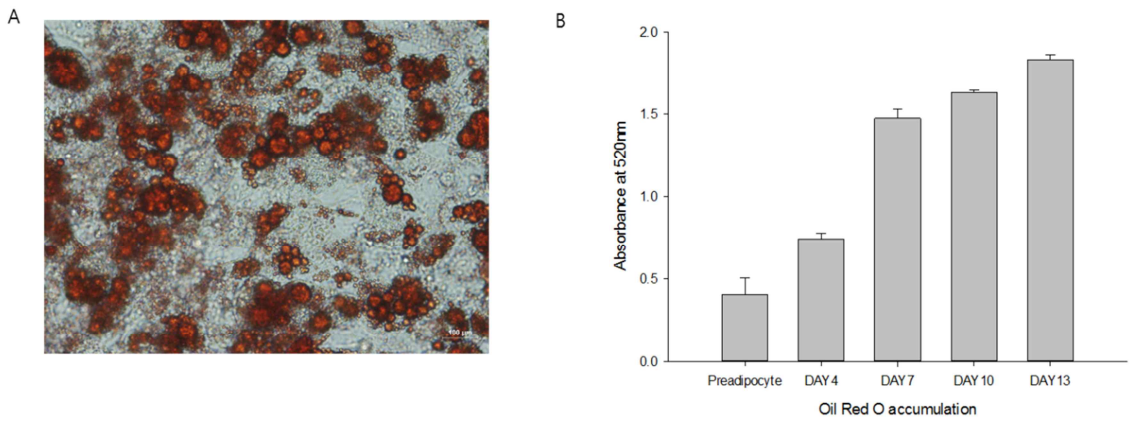

Figure 2: Confirmation of mature adipocyte by Oil Red O staining

(A) Maturation media for the past 10 days after treat and staining by Oil red O staining solution. Image can captured in Nikon Fluorescence microscope at 20X magnification

(B) The mature adipocyte Oil Red O staining using 100% isopropanol to elute oil red o staining solution were taken at 520nm.

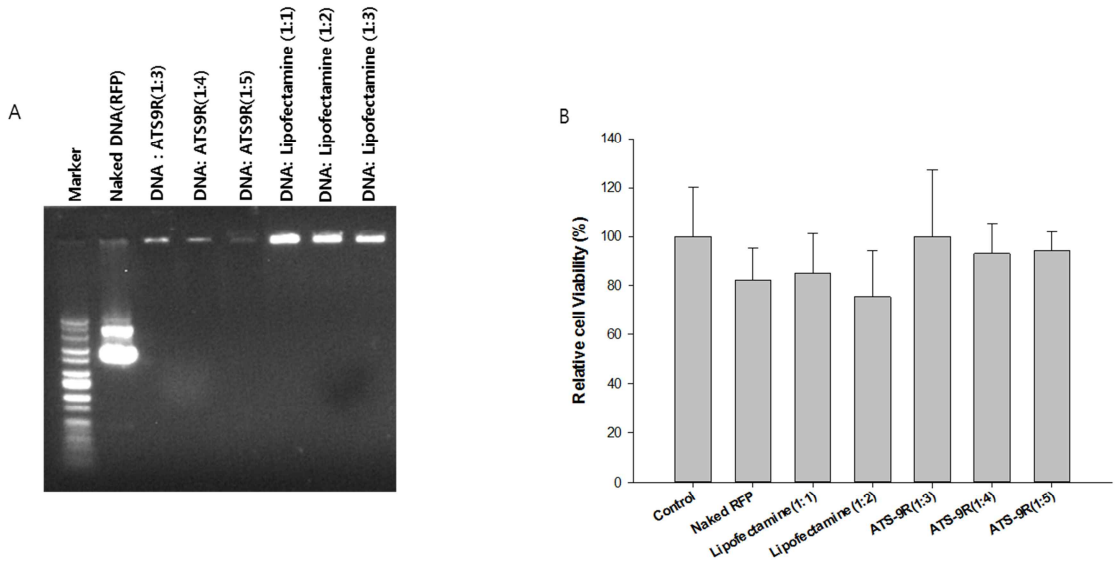

Figure 3: Polyplex Characterization

(A) Gel retardation assay

(B) Cytotoxicity assay of polyplex, 3T3-L1 cells were seeded $2x10^4$ cell in 24well culture plate and incubation 24hours at 37°C under humidified conditions (5% CO2 atmosphere). After incubation time treated polyplex made by RFP plasmid gene, ATS-9R and lipofectamine with different weight ratio. the optical density was measured by 570nm.

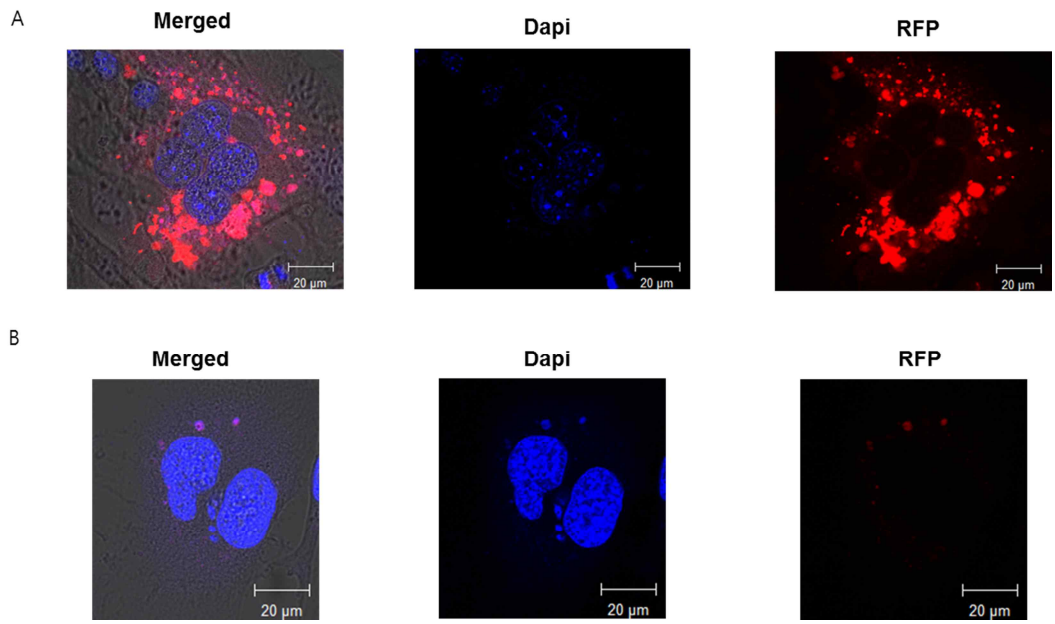

Figure 4: *in vitro* Gene expression by confocal microscopy

Polyplex was made by 2µg RFP, deionized water and C-ATS-9R-C at weight ratio 3 and incubated for 30 minutes in room temperature. 3T3-L1 preadipocyte cells were grown and differentitated on 6 well plate. Cells were mounted by DAPI staining nucleus. Images were obtained in Carl Zeiss confocal microscopy.

(A) ATS-9R/RFP

(B) Control

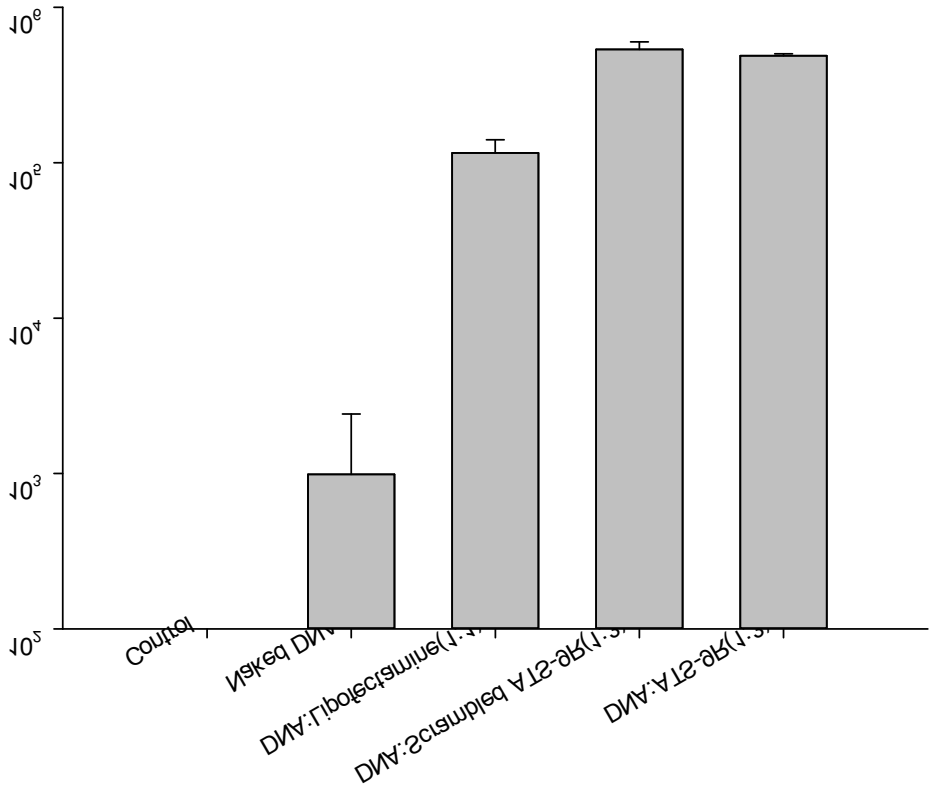

Figure 5: Transfection efficiency comparison in 3T3-L1 preadipocyte.

Polyplex was made by 2µg p-β-luci, deionized water and C-ATS-9R-C at weight ratio 3 and incubated for 30 minutes in room temperature. 3T3-L1 preadipocyte cells were grown on 6 well plate

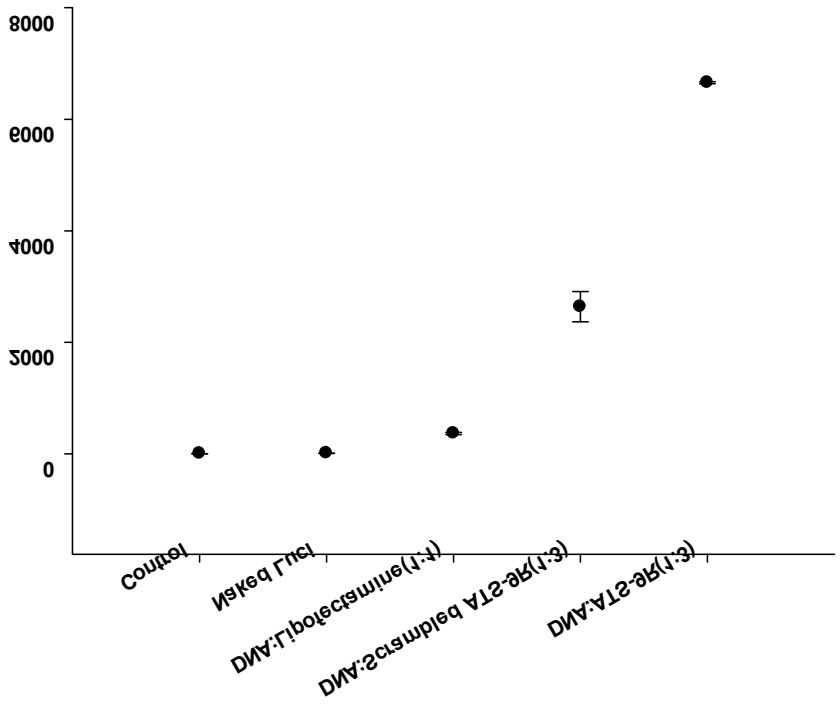

Figure 6: Transfection efficiency comparison in 3T3-L1 mature adipocyte

Polyplex was made by 2μg p-β-luci, deionized water and C-ATS-9R-C at weight ratio 3 and incubated for 30 minutes in room temperature. 3T3-L1 preadipocyte cells were grown and differentiated on 6 well plate.

3.3 Cytotoxicity assay

Condensed polyplex delivery to cells for checking when toxicity tests were carried out. Cell viability test was measured by using MTT assay. Looking at the **[Figure 3 B]**, Cell viability was more than 80% of all C-ATS-9R-C group and cell viability in the weight ratio 1:3 was the most. In Lipofectamine group, the cell viability can be confirmed in weight ratio 1:1.

3.4 *In vitro* Gene expression analysis

In vitro gene expression in all experiments used differentiated adipocyte before transfection. As a cytotoxicity test results **[Figure 3 B]** polyplex was the 1:3 ratio RFP/ATS-9R. Lower gene expression level if we use higher weight ratio, may be polyplex is formed strongly compacked in higher weight ratio and DNA can not be released from its carrier. The role of prohibitin is very important when the differentiation of 3T3-L1.[6] Prohibitin level is upregulated in 3T3-L1 cells during differentiation and increases with time of differentiation and its level decrease after day 12. [28] Polyplex was made by RFP, deionized water and C-ATS-9R-C at weight ratio 3. There is no red fluorescence in control. But compared with the control after expressing the RFP, we can see the red fluorescence **[Figure 4]**. As a result our carrier seems to be a higher gene expression compared with the control.

3.5 *In vitro* luciferase assay

Our transfection assay results showed that C-ATS-9R-C in differentiated adipocyte significantly

expressed the luciferase activity of reporter plasmids. Luciferase acitivity in preadipocyte confirmed in all of the carrier(lipofectamine, scrambled ATS-9R and ATS-9R) **[Figure 5].** Complementary to this carrier transported the luciferase activity of the reporter plasmid but lipofectamine did not transport luciferase plasmid **[Figure 6]**. Furthermore, the finding that C-ATS-9R-C was indicates that deliver efficiently differentiated adipocyte.

3.6 in vivo gene expression analysis

C57BL/6J mouse is a commonly used animal model of obesity research. When the body weight of mouse becomes over 35 gram, is considered as obese. Mice were fed 60% Kcal high fat diets. The experiment was performed more than 35 gram [29]. RFP plasmid DNA was condensed by C-ATS-9R-C and the polyplex was administered into the different fat pads of obese mice by local injection. After 48 hours the mice were sacrificed, fat tissue were isolated and RFP expression was observed in Carl Zeiss confocal microscopy **[Figure 7]**.

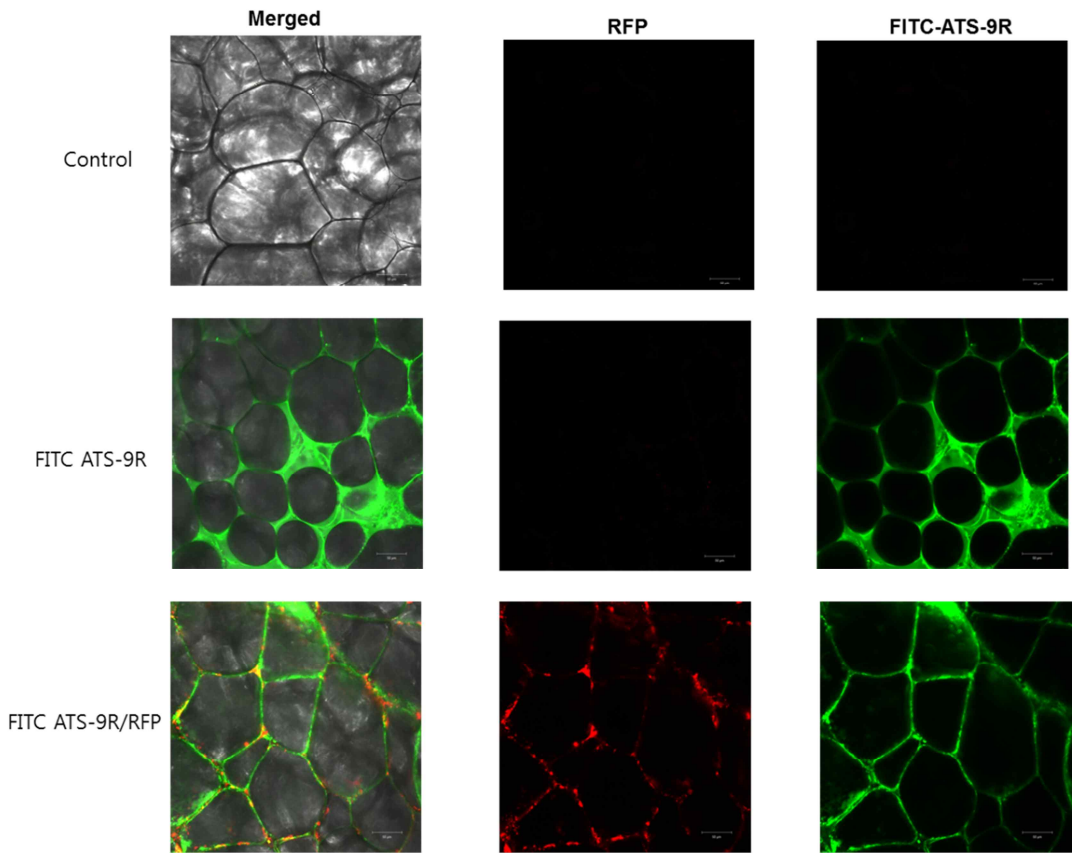

Figure 7: *in vivo* Gene expression by confocal microscopy

Polyplex was made by 30μg RFP, deionized water and FITC-C-ATS-9R-C at weight ratio 3 and incubated 30 minutes in room temperature. Mice were sacrificed and isolated fat tissue. RFP expression in fat tissue was observed in Carl Zeiss confocal microscopy.

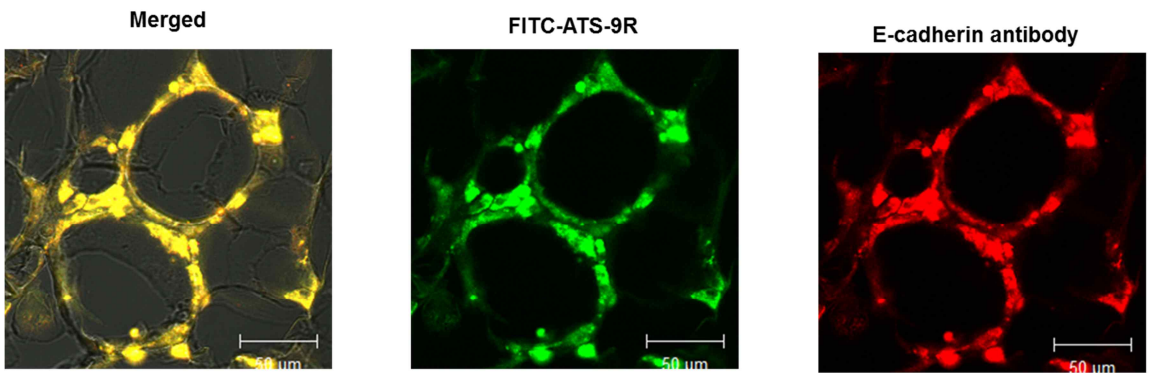

Figure 8: Immunohistochemistry of *in vivo* fat tissue

Immunofluorescence costaining of representative fat tissue sections at 2 days after FITC-ATS-9R delivery. Mature adipocyte cell plasma membrane display FITC-ATS-9R accumulation and coexpress E-cadherin antibody (red).

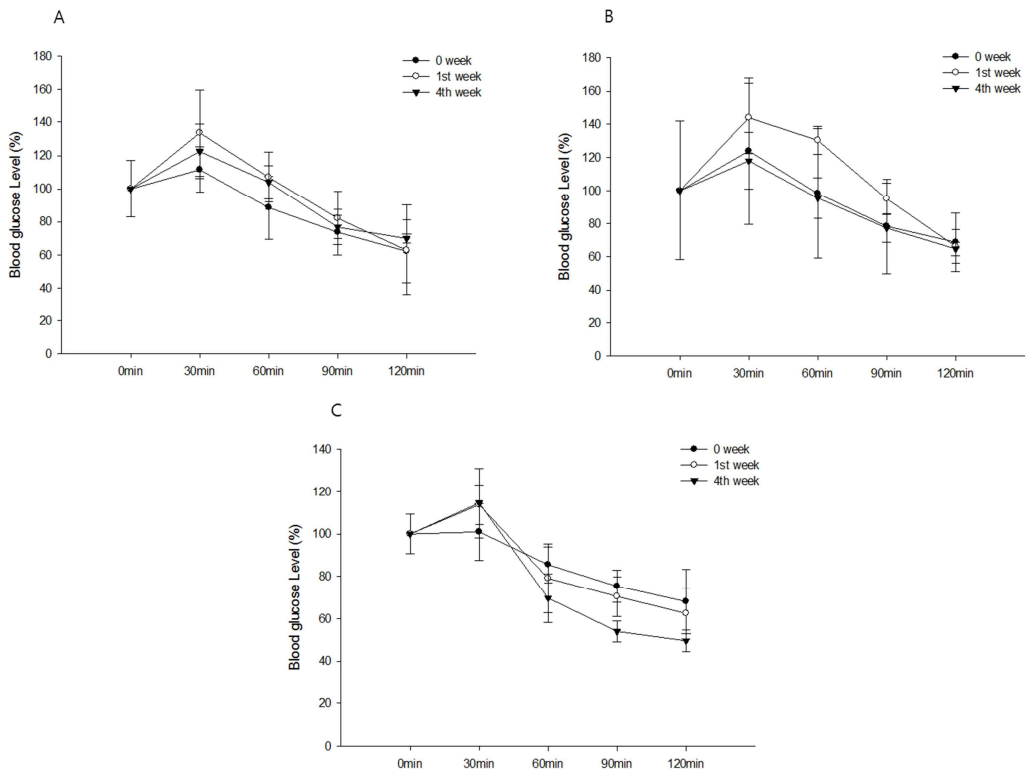

Figure 9: Insulin tolerance test

Mice were fed 60%Kcal high fat diet. For Insulin tolerance test(ITT), blood glucose level was mesured 6 h (for ITT) of fasting. After this measure insulin (0.75U/kg body weight) was dosed by intraperitoneal injection, respectively. Blood was collected from the tail vein at 0, 30, 60, 90, and 120 min post-dose for glucose determination.

(A) Control

(B) ATS-9R/sh-Luci

(C) ATS-9R/sh-FABP4

3.7 Immunohistochemistry of *in vivo* fat tissue

Having established the target specificity of C-ATS-9R-C in differentiated adipocytes, we administered the FITC-C-ATS-9R-C into a genetic model of obesity and immunohistochemistry , the leptin-deficient ob/ob mouse, and investigated bind affinity of prohibitin and FITC-C-ATS-9R-C. After 48 hours inject FITC-C-ATS-9R-C, the mice were sacrificed, fat tissue were isolated and E-cadherin antibody alexa 555 was added to the fixed cryo-sectioned adipose tissue. It could be observed that prohibitin in cell membrane attached to the C-ATS-9R-C.

3.8 Therapeutic effect in *in vivo* mouse model

When delivery of the obese mouse model injected into the ATS-9R, we observed gene expression in fat tissue. In order to reduce the presence of FABP4 protein in fat cells, we used the short hairpin RNA(sh-FABP4), polyplex was made by sh-FABP4, deionized water and C-ATS-9R-C at weight ratio 3. Feeding a high fat diet to C57BL/6 mice promotes the development of obesity and lnsulin resistance. To analyze the role of C-ATS-9R-C/sh-FABP4 in fat tissue we conducted insulin tolerance test. In Insulin tolerance test, we can be seen that the due to sh-fabp4 improves insulin sensitivity.

4. Conclusion

In this study, we confirmed a safe and efficient non viral gene delivery carrier both *in vitro* and *in vivo* condition. We have tried to optimize the polyplex weight ratio. In differentiated adipocyte stage, prohibitin moves to the adipocyte plasma membrane. Through Immunohistochemistry we showed that the C-ATS-9R-C can be attach to the *in vivo* adipocyte prohibitin. When toxicity tests were carried out to check, condensed polyplex is delivered in cells. And there is no cytotoxicity in differentiated adipocyte. We used fluorescent gene to determine whether gene which is delivered into obese tissue by gene carrier is expressed or not expressed. We have also confirmed that adipocyte tissues improve insulin resistance due to targeted gene delivery. This study offers important insight and indicates that the Function of the C-ATS-9R-C as a gene delivery in adipocyte tissue plays a same role in *in vitro* and *in vivo*. Therefore, it is possible that delivery of our carrier in adipocyte tissue might also show beneficial effects against diabetes and obese inducing metabolic syndrome.

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국문요지

최근 연구에 따르면, 한국인 3명 가운데 1명은 비만이라고 한다. 체질량 지수(BMI)가 25(kg/m²) 이상인 비만 인구는 2008년 30.7%를 차지했다. 특히 BMI가 30 이상인 고도비만 인구 비율이 1998년 2.3%에서 2008년 4.1%로 2배 가까이 증가했다. 비만인구가 점차적으로 늘어나면서 비만 관련 질병들인 당뇨병, 고지혈증 그리고 동맥경화 같은 병들이 많이 발생 하고 있다. 하지만 현재까지 나온 약들은 지방에 특이적으로 반응하여 지방세포를 줄이는 것이 아니라 2차적으로 식욕을 억제하거나, 몸의 열 생성을 촉진시켜 지방을 분해하는 방법, 지방자체의 흡수를 막는 방법 뿐이다. 그나마도 비만 치료제로 가장 유명했던 식욕을 억제시키는 약인 리덕틸이 2010년에 심혈관 질병 발생비율을 높이는 심각한 부작용이 보고되면서 시장에서 퇴출되었다. 이런 이유로 비만을 줄이기 위해서는 2차적인 접근이 아닌 지방 자체에 특이적으로 약물을 전달할 수 있는 전달체 연구에 초점을 맞추게 되었다. 지방세포는 크게 갈색지방세포와 백색지방세포로 이루어져 있다. 갈색지방세포는 우리가 알고 있는 미토콘드리아가 많으며 음식물을 섭취하면 열을 생성하는 기관이다. 백색지방은 우리가 잘 알고 있는 음식물을 많이 섭취할 때 남는 에너지를 지방소립이라는 형태로 저장하며 다양한 형태의 사이토카인이 분비되는 세포이다. 백색지방도 또한 우리가 건강을 유지할 때 꼭 필요한 세포지만, 과도하게 영양 섭취를 하면 백색지방에 지방소립이 많아지면서 비만이 오게 된다. 이렇게

많은 양의 지방소립이 백색지방에 들어오게 되면 지방세포에 만성염증질환이 나타나게 되고 지방산을 지방소립형태로 저장하지 못하게 된다. 그렇게 되면 지방산이 다른 기관에 저장된다. 이 증상이 심해지면 2형 당뇨병, 고혈압, 고지혈증, 동맥경화증이 생긴다. 이러한 비만을 치료하기 위해 많은 연구자들이 전달효율이 높은 전달체로서 바이러스를 많이 사용한다. 하지만 바이러스는 체내에 들어가서 우리가 원하는 치료 효과가 아닌 다양한 부작용을 만들기 때문에 안전하지가 않다는 큰 단점이 있다. 그렇기 때문에 바이러스가 아닌 비바이러스성 전달체를 찾게 되었다. Kolonin 이 CKGGRAKDC이라는 지방혈관세포에 달라붙는 작은 펩타이드 서열을 찾았다. 이 서열을 우리가 지방세포 특이적인 서열 (ATS)라고 부르고 이 서열 끝에다가 단백질전달체로 잘 알려진 9개의 아르기닌을 붙였다. 그렇게 만들어진 C-KGGRAKD-RRRRRRRRR-C을 ATS-9R이라고 명명했다. 이 지방세포 특이적인 서열은 지방혈관세포의 프로히비틴이라는 수용기관에 붙는다. 프로히비틴이라는 수용기관은 미토콘드리아에서 많이 발견되는데 지방세포가 백색지방세포로 분화하는 과정에서 발현양이 변화한다. 우리가 한 연구로는 백색지방세포로 분화를 하면 프로히비틴이 백색지방세포 세포막으로 이동하게 되고 그 수용기관을 인식하여 지방세포 특이적인 서열이 붙어서 우리가 원하는 유전자를 전달한다. 이렇게 연구실에서 독자적으로 만든 ATS-9R을 가지고 세포 실험을 통하여 유전자와 복합체를 만들었을 때 세포독성이 없고 유전자와 잘 복합체를 이루는 것을 확인할 수 있었다. 또한 세포 내로 전달체가 유전자를 잘 전달 하여 발현을 하는지 보기 위해서 형광물질 발현 유전자를 이용하여 공초점현미경으로 확인을

하였다. 동물 모델에서도 약물이 지방세포 특이적으로 가는지 확인 하기 위하여 FITC 표지 전달체와 형광물질 발현 유전자 복합체를 만들어서 피하주사로 넣어주고 공초점현미경으로 확인하였다. 더불어 지방세포 표적 유전자 전달로 인하여 인슐린 저항성이 개선되는 것을 확인했다. ATS-9R의 발견이 획기적인 것이 이제까지 비만관련 유전자 치료 연구를 진행할 때 지방세포 특이적인 전달체가 존재 하지 않기에 유전자의 발현양을 조절 할 수가 없었다. 그렇기 때문에 특히 동물모델 실험에서는 동물 자체가 그 유전자 발현을 안되게 만들거나 더 많이 발현하게 만들어야 하는 어려운 연구가 되었다. 또한 지방세포만이 아니라 모든 세포에 유전자가 발현이 조절되어 연구자가 원하는 양상이 나오지 않은 부작용이 생겼다. 이러한 결과를 바탕으로, 지방세포에 특이적인 유전자 전달 기술을 이용한다면 다양한 유전자를 지방세포만 쉽게 억제시키거나 발현시켜 비만 연구를 치료할 수 있는 가능성을 확인할 수 있었다.

감사의 글

2011년 6월달이었습니다. 한양대학교 일반대학원 생명공학과 석사과정 합격소식을 접한 날이. 그렇게 실험과 인연을 맺게 되었고 2년이 지난 지금 졸업을 앞두게 되었습니다. 연구실에서 보낸 2년 이라는 시간 동안 저에게 공부와 실험을 할 수 있게 열정적으로 지도해주신 김용희 교수님께 진심으로 감사 드립니다. 교수님께서 끊임없이 저에게 열정을 쏟아 주셨기에 지금까지 제가 발전을 할 수가 있었습니다.

연구년이라 수업을 듣진 못했지만 항상 인자하게 인사를 받아주신 이민형 교수님, 제가 부족한 부분을 항상 지적해주시고 유전학에 대해서 체계를 잡아주신 임태연 교수님 두 분 모두 바쁘신 와중에도 제 논문을 심사해 주셔서 감사합니다. 수업시간 뿐만 아니라 연구를 함에 있어 항상 도움을 주신 이근용 교수님, 류성언 교수님, 윤채옥 교수님, 신흥수 교수님, 이동윤 교수님, 이상경 교수님 까지 모두 한 분 한 분께 진심으로 감사의 말씀을 드리며, 매 학기 마다 직접 상담을 통해 동기부여를 해주신 김성완 석좌교수님께도 감사의 말씀 꼭 전하고 싶습니다.

석사기간 동안 정말 가족같이 힘들 때나 기쁠 때나 옆에서 도와준 BRL식구들 너무나 고맙습니다. 여러분이 있기에 제가 정말 즐겁게 2년이라는 시간을 보낼 수 있었습니다. 같이 연구를 한 기간은 짧지만 연구자의 정석을 보여주신 우리 BRL의 가장 큰 형님이신 영욱이형, 제 모든 연구를 꼼꼼히 체크해주시면서 실험에 대해 알려주신 광석이형, 석사기간 내내 제가 연구를

할 수 있게 실험실의 살림을 관리 하시는 두 아이의 아버지 장경이형. 이 3분의 박사님이 계시기에 제가 석사과정을 무사히 보낼 수 있었습니다. 정말 감사드립니다. 실험실 분위기를 편안하게 만들어 주신 용훈이형, 시크하신 창훈이형, 친절하게 실험에 대해 알려준 현린이, 대학원까지 같이 오게 된 7년 친구 소미, 까칠해 보이지만 정이 많은 지원이, 앞으로 2년동안 연구실에서 생활하게 된 SBT 용석범, 생공여신 정지영, 열정이 넘치는 종환이까지 모두 모두 함께 연구해서 행복했습니다. 같이 입학해서 졸업까지 함께한 상우형, 보라, 자히드 모두 감사합니다.

제가 여기까지 올 수 있게 항상 뒤에서 말없이 응원해주시고 지지해주신 우리 부모님 정말 감사드립니다. 매일 힘들다고 투정만 부리는 철 없는 아들을 여기까지 잘 이끌어 주신 우리 아버지, 어머니, 매일 저를 위해 기도해주시는 우리 할머니, 지금은 군대에서 나라를 지키고 있지만 꼬박꼬박 형의 안부를 묻는 우리 귀염둥이 막내 의진이. 소중한 가족들께도 감사하다는 말씀 드립니다. 그리고 7년이라는 시간 동안 언제나 한결같이 옆에 있어준 우리 선영이 그대가 있기에 제가 이렇게 석사라는 시간을 보낼 수 있었습니다.

이제 졸업을 앞두고 더 큰 과정을 나가기 위한 문턱에 서니 설레임과 두려움이 앞섭니다. 하지만 지금까지 도움과 힘이 되어주신 모든 분들께 부끄럽지 않는 사람이 되기 위해 더욱 노력하겠습니다.

2013년 6월

김형진 드림

연구 윤리 서약서

본인은 한양대학교 대학원생으로서 이 학위논문 작성 과정에서 다음과 같이 연구윤리의 기본원칙을 준수하였음을 서약합니다.

첫째, 지도교수의 지도를 받아 정직하고 엄정한 연구를 수행하여 학위논문을 작성한다.

둘째, 논문작성시 위조, 변조, 표절 등 학문적 진실성을 훼손하는 어떤 연구부정행위도 하지 않는다.

2013.06.28

학 위 명 : 공학석사

학 과 : 생명공학과

지도교수 : 김용희

성 명 : 김 형 진 (서명)

한양대학교 대학원장 귀하

Declaration of Ethical Conduct in Research

I, as a graduate student of Hanyang University, hereby declare that I have abided by the following Code of Research Ethics while writing this dissertation thesis, during my degree program.

"First, I have strived to be honest in my conduct, to produce valid and reliable research conforming with the guidance of my thesis supervisor, and I affirm that my thesis contains honest, fair and reasonable conclusions based on my own careful research under the guidance of my thesis supervisor.

Second, I have not committed any acts that may discredit or damage the credibility of my research. These include, but are not limited to: falsification, distortion of research findings or plagiarism."

2013.06.28

Date

Degree : M.S.

Department : DEPARTMENT OF BIOENGINEERING

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