A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Dihydrocapsaicin inhibits cell transformation in JB6P+ mouse epidermal cells by regulating mTOR pathway and c-fos

다이하이드로캡사이신의 EGF로 유도되는 세포형질변환 억제 효능 및 분자기전 규명

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석사학위논문

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이 논문을 석사학위 논문으로 제출함

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Abstract

Chili pepper has been reported to possess anti-carcinogenic effects. But there are articles about carcinogenic effect of chili pepper. Capsaicin which is a main component in chili pepper has a controversial point whether it has cancer preventive or promotive effect. Anti-carcinogenic effects of chili pepper components except capsaicin, for example, dihydrocapsaicin, capsiate, and capsanthin, are still unclear. Herein, I examined the inhibition effect of neoplastic transformation on chili pepper components and associated mechanisms of dihydrocapsaicin in JB6 P+ cell line. Epidermal growth factor (EGF)-induced neoplastic transformation was inhibited by 50 μM dihydrocapsaicin in JB6 P+ cells. Dihydrocapsaicin suppressed EGF-induced cyclooxygenase-2 (COX-2) promoter activity and activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) transactivations in JB6 P+ cells. Immunoblot data showed that dihydrocapsaicin inhibited EGF-induced phosphorylation of mTOR signaling pathway and expression of c-fos. Together, these findings suggest that dihydrocapsaicin exerts potent inhibitor of neoplastic transformation against EGF-induced skin inflammation.

Key Words: Dihydrocapsaicin; mTOR pathway; EGF; AP1(activator protein 1);

Student ID: 2011-24109

Contents

Ⅰ. Introduction

Chili peppers are popularly eaten by many people around the world. They are added for flavor and spice to the food. Capsaicin and dihydrocapsaicin occupy the most of the amounts of chili peppers' components. The effect of chili pepper on carcinogenesis is controversial. [1-4]. Previous studies have shown that dihydrocapsaicin which occupies second largest amount possesses anti-carcinogenic properties [5-8]. However, the direct molecular mechanism of dihydrocapsaicin in neoplastic transformation is not fully identified.

Carcinogenesis is divided into three processes including initiation, promotion, and progression. Because the stage of tumor promotion is reversible and lengthy, pre-neoplastic cell can revert to initiated cell in promotion stage. This stage is important to prevent of cancer because of these features [9]. Abnormal growth signaling are involved in process of carcinogenesis [10]. Epidermal growth factor (EGF) in epithelial tumors can stimulate cell growth and lead to abnormal growth signaling. EGF influences cell invasiveness, proliferation, and transformation [11, 12]. Neoplastic transformation is one of the major events in carcinogenic process.

The JB6 mouse epidermal cell system can be divided promotion-sensitive (P+) and promotion-resistant (P-). This model is one of the major

to study the stage of cancer promotion [9]. EGF induced cell transformation in JB6 P+ cells. EGF forms colonies in soft agar because of anchorage-independent cell growth. In JB6 P+ cells, EGF regulate activator protein 1 (AP-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which act as pivotal transcription factors in neoplastic transformation [13, 14]. The AP-1 is a transcription factor which is a heterodimeric or homodimeric protein composed of proteins belonging to the c-fos, c-Jun, ATF and JDP families to regulate neoplastic transformation [15].

AP-1 and NF-κB are regulated by signaling pathways including the mitogen-activated protein kinases (MAPKs) cascades and Akt-mTOR pathway. Akt-mTOR pathway is necessary for EGF–induced cell transformation in mouse epidermal JB6 cells [12]. mTOR which acts downstream of the AKT phosphorylates p70 ribosomal protein S6 kinases (p70S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). p70S6K phosphorylates S6 to regulate protein synthesis and cell proliferation [16].

Herein, I investigated the chempreventive effect of dihydrocapsaicin and its molecular mechanism to support the effect of chili pepper using JB6P+ mouse epidermal cell system. Present study demonstrated that

dihydrocapsicin suppresses EGF-induced cell transformation by inhibiting mTOR pathway and c-fos in JB6 P+ mouse epidermal cells.

Ⅱ. Materials and Methods

2.1. Materials

Dihydrocapsaicin (≥95%) is purchased from cayman (Ann Arbor, MI). Fetal bovine serum (FBS) and the antibody against β-actin were purchased from Sigma-Aldrich (St. Louis, MO). Eagle's MEM, gentamicin, and L-glutamine were obtained from Welgene (Daegu, Korea). Antibodies to detect the phosphorylated forms of JNK1/2, mTOR, AKT(T308), p90RSK, p70S6K, S6, c-fos, and total Akt, p38 and p70S6K were purchased from Cell Signaling Biotechnology (Danvers, MA). Antibodies against phosphorylated ERK1/2 , total ERK1/2, RSK2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). G418 and the luciferase assay substrate were purchased from Promega (Madison, WI).

2.2. Cell culture

The JB6 P+ mouse epidermal cell line was maintained at 37°C in a humidified atmosphere of 5% CO_2 in 5% FBS–MEM. The JB6 P+ mouse epidermal cell line was stably transfected with the AP-1, NF-κB, or COX-2

luciferase reporter plasmid and maintained in 5% FBS–MEM containing 200 μg/mL G418.

2.3. MTS assay

Cell viability was measured by CellTiter 96® Aqueous MTS Reagent (Promega, Madison, WI, USA). JB6P+ cells were seeded in 96-well plates at a density of 5×10^4 cells per well using 5% FBS MEM, then incubated until confluence. After confluence, cell were treated with 0.1% FBS MEM as control and the remaining groups were treated with 0.1% FBS MEM supplemented with 12.5, 25, 50, 100μM on chili pepper components. And the absorbance at 460-560 nm was measured with a microplate reader. The results are expressed here as the percentage MTS reduction relative to the absorbance of control cells.

2.4. Anchorage-independent cell transformation assay

The effects of dihydrocapsaicin on EGF-induced cell transformation were investigated in JB6 P+ cells. Cells (8×10^3/ml) were treated to EGF with or without dihydrocapsaicin in 1 ml of 0.33% basal medium eagle agar that contained 10% FBS or in 3.5 ml of 0.5% basal medium eagle agar that

contained 10% FBS. The cultures were maintained at 37°C in a 5% CO_2 incubator for 14 days, and the cell colonies were counted under a microscope using the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD), as described by Colburn et al. [17]

2.5. Luciferase assay for transcriptional activity of AP-1, NF-κB, and COX-2

AP-1, NF-κB, and human COX-2 luciferase reporter-transfected JB6 P+ cells were constructed as described earlier [18]. The cells were treated for 1 h with dihydrocapsaicin (12.5, 25 and 50 μM) before exposure to EGF (10ng/ml) and then were incubated for 6 h. Cells were disrupted with lysis buffer and luciferase activity was measured using a luminometer (Luminoskan Ascent; Thermo Electron, Helsinki, Finland).

2.6. Immunoblot assay

Total cell lysates prepared and subjected to western blot as described earlier [18]. After cell lysis, the protein concentration was determined using a dye-binding protein assay kit (Bio-Rad Laboratories, Hercules, CA) as described in the manufacturer's manual.

2.7. Transcription Factor assay

Nuclear protein extracts from JB6P+ cells were obtained in a three-step using the nuclear extract kit (Active Motif, CA, USA). The activation of p-c-Jun, c-fos, and fosB and Jun D were measured using the TransAM™ AP-1 family transcription assay kit (Active Motif, CA, USA). This protocol according to the manufacturer's instructions was indicated to measure the DNA binding activity of AP-1 by ELISA. Cell extract containing AP1 was added in a 96-well plate which is coated with TPA response element (TRE; 5′-TGAGTCA-3′) that binds c-Jun and c-fos and JunD and fosB. After washing, p-c-Jun and c-fos and fosB and JunD antibody were put to these wells for 1 h. And a secondary HRP-conjugated antibody was added. And the absorbance at 450 nm was measured with a microplate reader.

2.8. Statistical analysis

When necessary, data are expressed as means ± S.D., and analysis of variance was used for multiple statistical comparisons. A probability value of $p < 0.05$ was used as the criterion for statistical significance. All analyses were performed using Statistical Analysis Software (SAS, Inc.).

Ⅲ. Results

3.1. Dihydrocapsaicin inhibits EGF-induced neoplastic transformation

MTS assay was performed to investigate the effect of chili pepper components on cell viability in JB6 P+ cells. They did not exhibit toxicity up to 50 mM (Fig. 2A-D). To evaluate the possible chemopreventive activities of chili pepper components, I determined their effects on neoplastic transformation response to EGF stimulation. Treatment with 50 μM dihydrocapsaicin inhibited neoplastic transformation by 67% as compared with EGF-induced transformation. Capsaicin inhibited neoplastic transformation by 50%, capsiate inhibited by 41%, and capsanthin inhibited by 25%. Rapamycin (20 nM) which inhibits neoplastic transformation [19] is used for positive control (Fig. 2E). It indicates that dihydrocapsaicin is a potent inhibitor of neoplastic transformation.

3.2. Dihydrocapsaicin inhibits EGF-induced transactivation of AP-1, NF-κB, and promoter activity of COX-2 in JB6 P+ cells

Neoplastic transformation is regulated by AP-1 and NF-κB activation. To determine whether the transformation suppressed by dihydrocapsaicin involves the inhibition of AP-1 and NF-κB activities, I

measured AP-1 and NF-κB transactivation using JB6 P+ cells that were stably transfected with an AP-1 and an NF-κB luciferase reporter plasmid. Dihydrocapsaicin suppressed EGF-induced AP-1 and NF-κB transactivation (Fig. 3A and B). COX-2 which is one of important inflammatory mediators of cell transformation is mediated by AP-1 and an NF-κB transactivation. COX-2 promoter activity induced by EGF was also attenuated by dihydrocapsaicin (Fig. 3C). These results indicate that dihydrocapsaicin suppresses AP-1 and NF-κB, which may be related to its antitumor activity.

3.3. Dihydrocapsaicin suppresses expression of c-fos induced EGF in JB6 P+ cells

AP-1 is heterodimeric or homodimeric protein composed of proteins belonging to the c-fos, c-Jun, ATF and JDP families. The most important proteins of them are c-fos and c-Jun. To determine how dihydrocapsaicin inhibit AP-1 activity, I investigated whether dihydrocapsaicin suppress c-fos and c-Jun activity using AP-1 transcription assay kit. The result showed that dihydrocapsaicin inhibited EGF-induced c-fos activity and its expression (Fig 4A,C) but not c-Jun activity (Fig 4B).

3.4. Dihydrocapsaicin inhibits EGF-induced mTOR signaling pathway but not phosphorylation of MAPKs.

The MAPKs and mTOR signaling pathway are involved in EGF-induced JB6 P+ cell transformation. mTOR is upstream regulatory protein of NF-κB [20]. I examined whether dihydrocapsaicin had an effect on EGF-induced mTOR signaling pathway. The results showed that dihydrocapsaicin inhibited EGF-induced phosphorylation of p70S6K which is substrate of mTOR (Fig 5B). Dihydrocapsaicin subsequently inhibited phosphorylation of S6 which is substrate of p-p70S6K (Fig 5B). However, dihydrocapsaicin did not inhibit EGF-induced phosphorylation of Akt (S472 and T308) and mTOR (S2481 and S2448) (Fig 5A). The phosphorylations of MAPKs (ERKs, p38, JNKs) induced by EGF were not inhibited by dihydrocapsaicin. dihydrocapsaicin does not inhibit substrates of MAPKs including p90RSK, MSK (Fig 6).

Figure 1

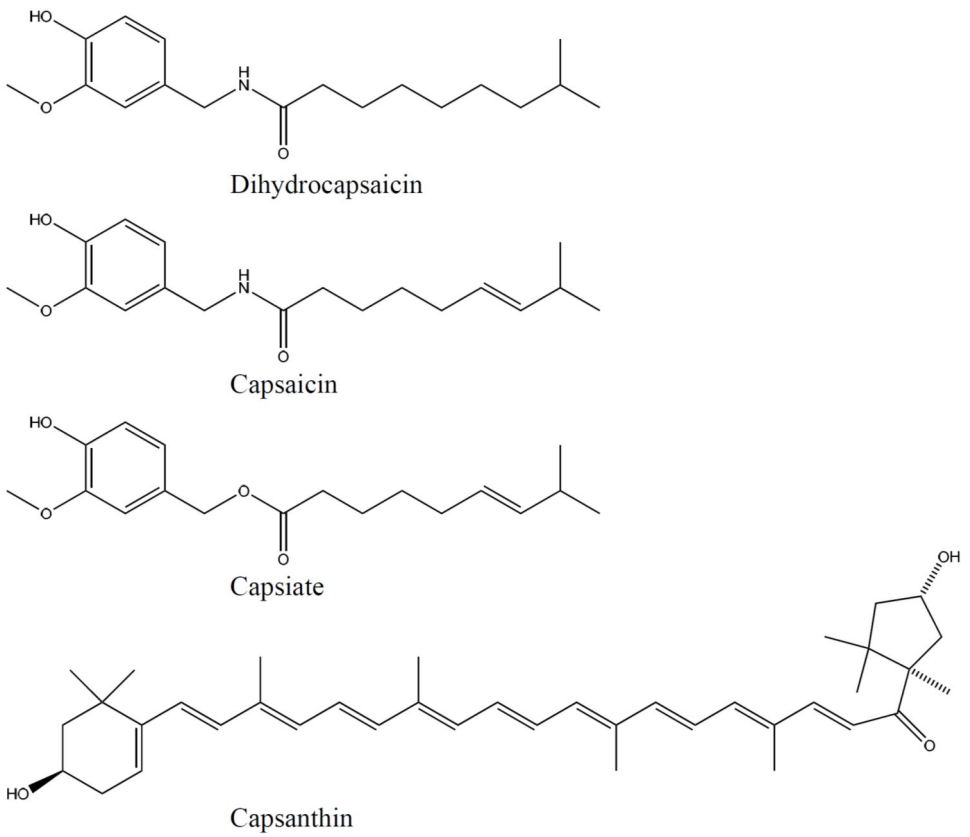

Figure 1. Structure of dihydrocapsaicin, capsaicin, capsiate, capsanthin

Figure 2 (A-D)

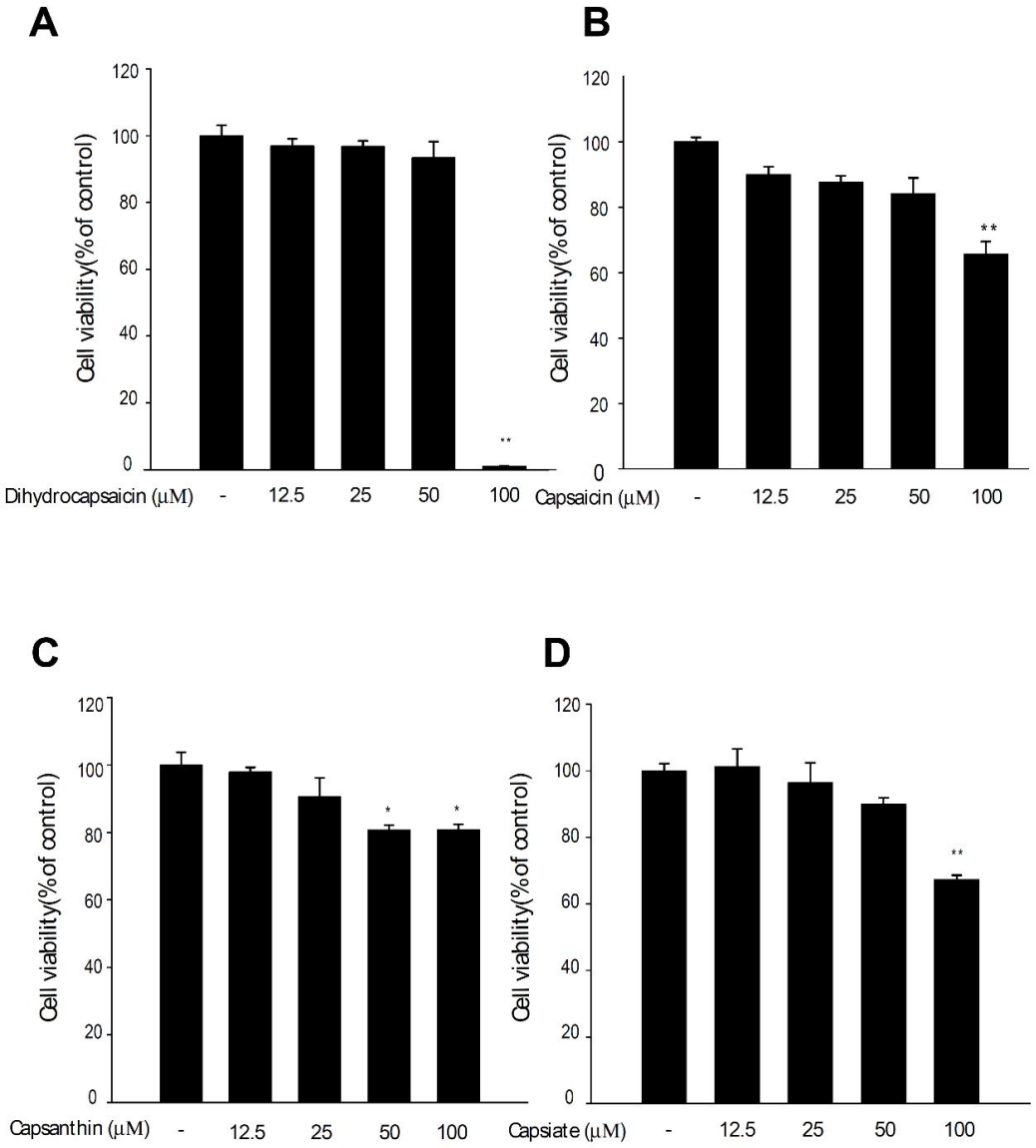
A
Cell viability(%of control)
120
100
80
60
40
20
0
**
Dihydrocapsaicin (μM) - 12.5 25 50 100
B
Cell viability(%of control)
120
100
80
60
40
20
0
**
Capsaicin (μM) - 12.5 25 50 100
C
Cell viability(%of control)
120
100
80
60
40
20
0
*
*
Capsanthin (μM) - 12.5 25 50 100
D
Cell viability(%of control)
120
100
80
60
40
20
0
**
Capsiate (μM) - 12.5 25 50 100

Figure 2 E

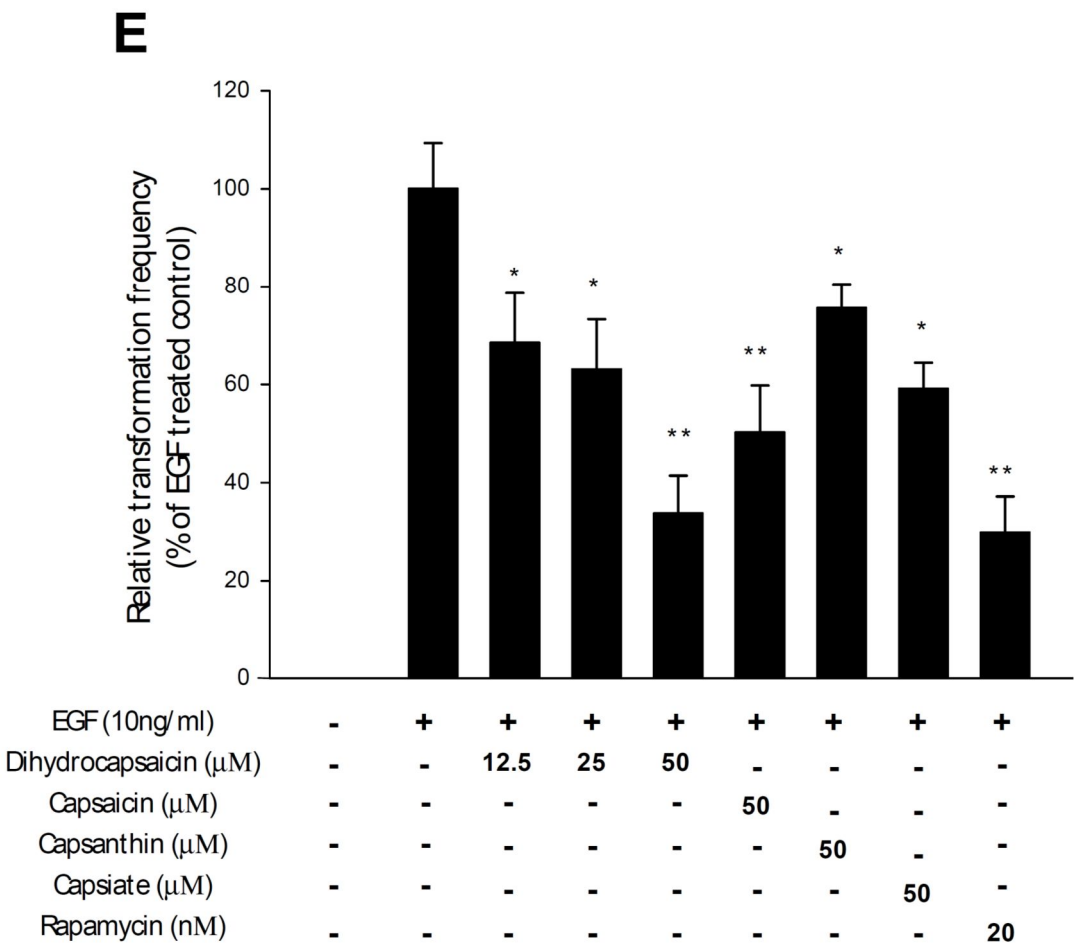
E
Relative transformation frequency
(% of EGF treated control)
120
100
80
60
40
20
0
*
*
**
**
*
*
**
EGF (10ng/ml) - + + + + + + + +
Dihydrocapsaicin (μM) - - 12.5 25 50 - - - -
Capsaicin (μM) - - - - - 50 - - -
Capsanthin (μM) - - - - - - 50 - -
Capsiate (μM) - - - - - - - 50 -
Rapamycin (nM) - - - - - - - - 20

Figure 2. Effect of chili pepper components on EGF-induced cell transformation in JB6 P+ cells.

(A-D) These results are effects of chili pepper components in JB6 P+ cells viability. Dihydrocapsaicin, capsaicin, and capsanthin and capsiate did not affect cell viability, up to 50mM. JB6 P+ cells were treated with chili pepper components for 24hr after reaching 90% confluency. (E) Chili pepper components have the inhibitory effects of chili pepper components on EGF-induced neoplastic transformation of JB6 P+ cells. Dihydrocapsaicin from among these is most effective to inhibit cell transformation. 50 μM dihydrocapsaicin significantly inhibited by 64% neoplastic transformation as compared with EGF-induced transformation. Cell transformation is measured by Materials and Methods and EGF induced colonies were counted after 14 days. Cell colonies were counted by Image-Pro Plus (ver. 4) software program. Values from three independent experiments is measured. Significant differences were indicated using the Student's *t*-test (** $P < 0.01$).

Figure 3

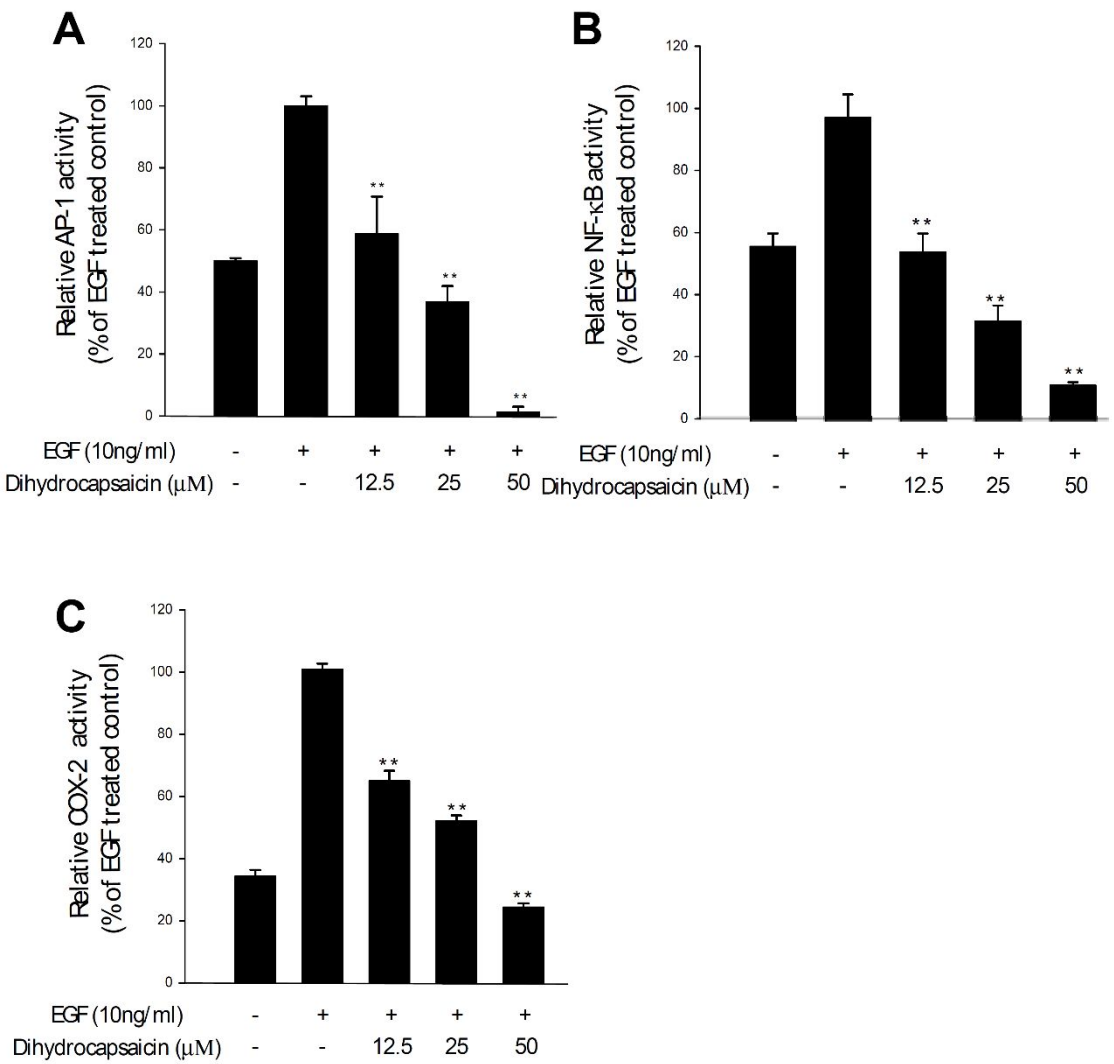
A
Relative AP-1 activity
(% of EGF treated control)
120
100
80
60
40
20
0
**
**
**
EGF (10ng/ml) - + + + +
Dihydrocapsaicin (μM) - - 12.5 25 50
B
Relative NF-κB activity
(% of EGF treated control)
120
100
80
60
40
20
0
**
**
**
EGF (10ng/ml) - + + + +
Dihydrocapsaicin (μM) - - 12.5 25 50
C
Relative COX-2 activity
(% of EGF treated control)
120
100
80
60
40
20
0
**
**
**
EGF (10ng/ml) - + + + +
Dihydrocapsaicin (μM) - - 12.5 25 50

Figure 3. Effect of dihydrocapsaicin on EGF-induced AP-1, NF-κB and COX-2 luciferase activity in JB6 P+ cells.

(A) Dihydrocapsaicin inhibits EGF-induced AP-1 transactivation in JB6 P+ cells. Data are representative of 3 independent experiments that gave similar results. (B) Dihydrocapsaicin inhibits EGF-induced NF-κB transactivation. Data are representative of 3 independent experiments that gave similar results. (C) Dihydrocapsaicin suppresses COX-2 promoter activity. A luciferase reporter plasmid including AP-1, NF-κB and COX-2 stably were transfected in JB6 P+ cells. JB6 P+ cells were cultured as described in the Materials and Methods (** $P < 0.01$). There are significant differences in group treated with EGF alone and co-treated dihydrocapsacin and EGF).

Figure 4

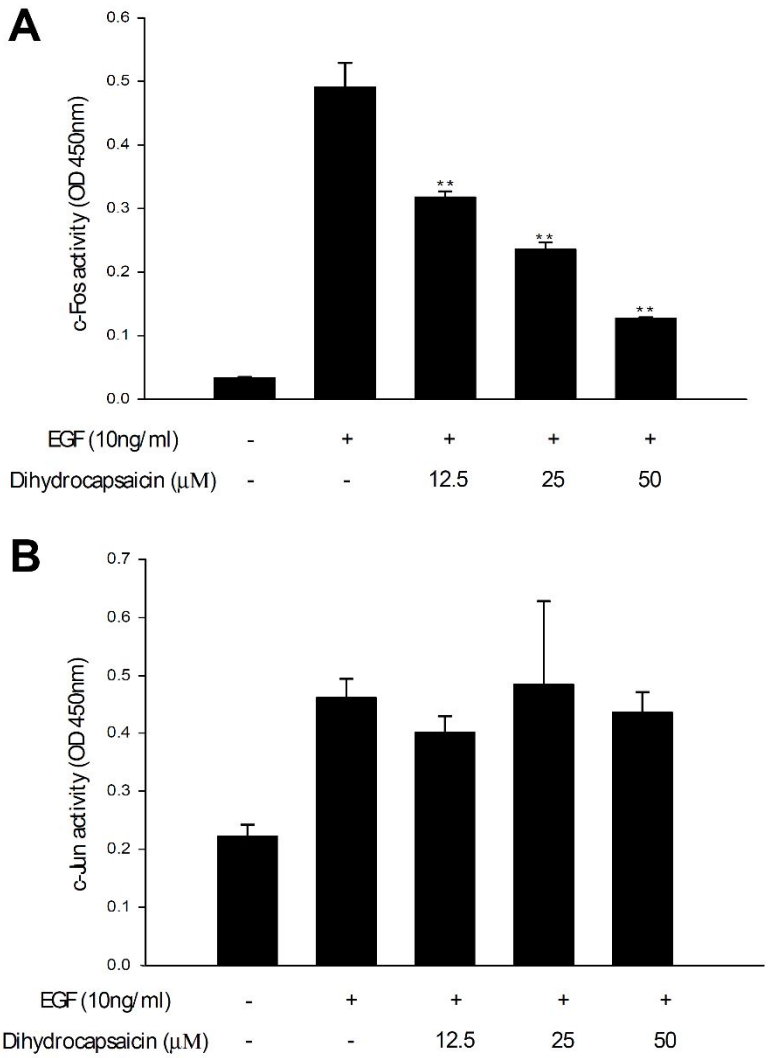
A
c-Fos activity (OD 450nm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
**
**
**
EGF (10ng/ml) - + + + +
Dihydrocapsaicin (μM) - - 12.5 25 50
B
c-Jun activity (OD 450nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
EGF (10ng/ml) - + + + +
Dihydrocapsaicin (μM) - - 12.5 25 50
C
EGF (10ng/ml) - + + + +
Dihydrocapsaicin(μM) - - 12.5 25 50
c-fos

Figure 4. The effect of dihydrocapsaicin on EGF-induced AP-1 family's activity in JB6 P+ cells

(A) Dihydrocapsaicin inhibit EGF-induced c-fos activity in a dose dependent manner. After JB6 P+ cells extract nuclear protein, it added 96-well plate which is coated with TPA response element and then p-c-Jun, c-fos and fosB JunD antibody is treated. And then the absorbance was measured with a microplate reader. But fosB and JunD do not induced by EGF. Dihydrocapsaicin inhibits only c-fos activity. (B) Dihydrocapsaicin does not suppresses c-Jun activity. Columns, means determined from three independent experiment; bars, SD (**, $P < 0.01$, significant difference between groups treated with dihydrocapsaicin and groups untreated). (C) When cells were 90% confluency, JB6 P+ cells were starved using 0.1% FBS-MEM for 24hr and then treated dihydrocapsaicin. And they were treated with EGF (10ng/ml). Dihydrocapsaicin inhibits EGF-induced expression of c-fos. Data are three independent experiments which indicated similar results.

Figure 5

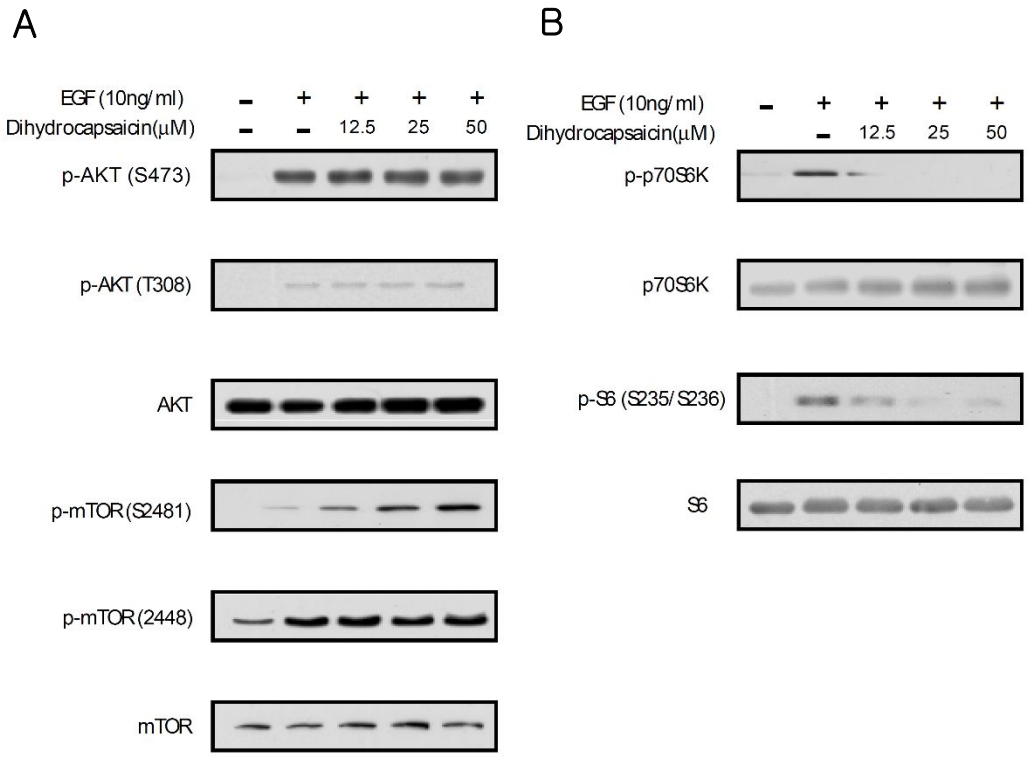
A
EGF (10ng/ml) - + + + +
Dihydrocapsaicin(μM) - - 12.5 25 50
p-AKT (S473)
p-AKT (T308)
AKT
p-mTOR (S2481)
p-mTOR (2448)
mTOR
B
EGF (10ng/ml) - + + + +
Dihydrocapsaicin(μM) - 12.5 25 50
p-p70S6K
p70S6K
p-S6 (S235/S236)
S6

Figure 5. The effect of dihydrocapsaicin on EGF-induced AKT-mTOR pathway in JB6 P+ cells

(A, B) Dihydrocapsaicin inhibits mTOR pathway in JB6 P+ cells. When cells were 90% confluency, JB6 P+ cells were starved using 0.1% FBS-MEM for 24hr and then treated dihydrocapsaicin. And they were treated with EGF (10ng/ml). Dihydrocapsaicin inhibits EGF-induced phosphorylation of p70S6K, pS6. Data are three independent experiments which indicated similar results.

Figure 6

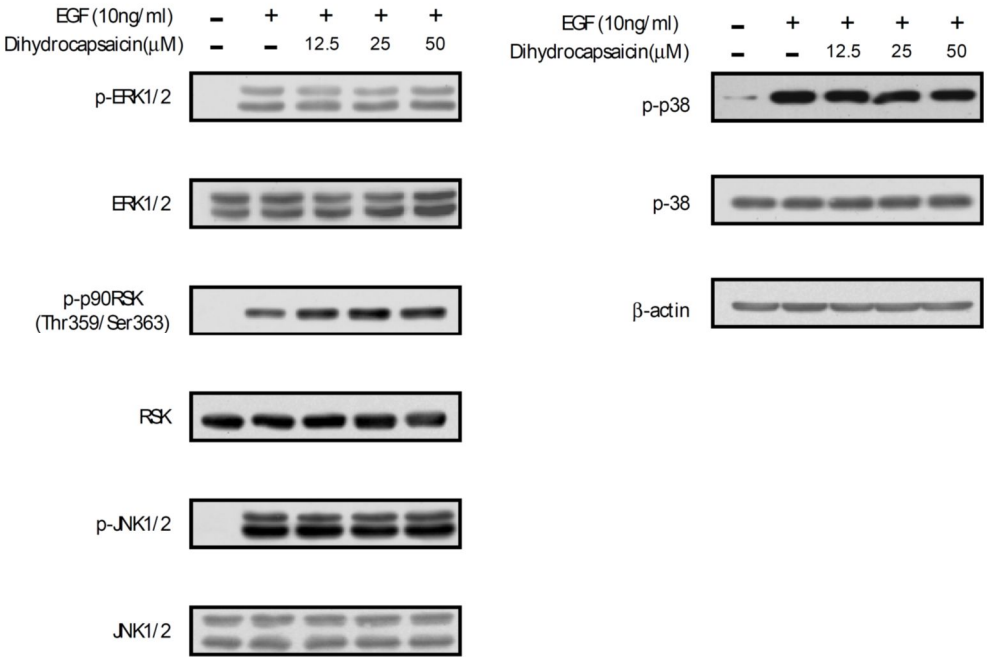
EGF (10ng/ml) - + + + +
Dihydrocapsaicin(μM) - - 12.5 25 50
p-ERK1/2
ERK1/2
p-p90RSK (Thr359/Ser363)
RSK
p-JNK1/2
JNK1/2
EGF (10ng/ml) - + + + +
Dihydrocapsaicin(μM) - - 12.5 25 50
p-p38
p-38
β-actin

Figure 6. The effect of dihydrocapsaicin on EGF-induced phosphorylation of MAPKs in JB6 P+ cells

Dihydrocapsaicin does not inhibit EGF-induced phosphorylation of ERK, RSK, and JNK and p-38 MAPK. When 90% confluence, JB6 P+ cells were starved using 0.1% FBS-MEM for 24hr and then treated dihydrocapsaicin or not for 1 h. Then they were treated with EGF (10ng/ml). Data are three independent experiments.

Figure 7

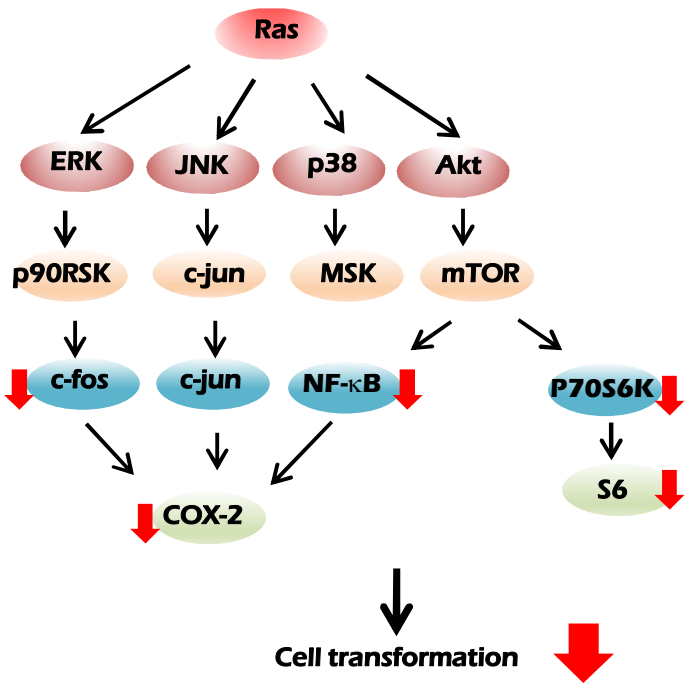

Figure 7. Proposed molecular mechanism of dihydrocapsaicin in preventing cell transformation.

Dihydrocapsaicin might suppresse phosphorylation of p70S6K and S6 and expression of c-fos causing cell transformation.

Ⅳ. Discussion

Chili pepper components can be divide into capsaicinoids and capsinoids [21]. The main components of capsaicinoids which produce a sensation of burning in chili pepper are capsaicin and dihydrocapsaicin. The effect of chili pepper on carcinogenesis is controversial. Some studies reported chili pepper possess anti-carcinogenic effects [3, 4]. Other studies showed the carcinogenic effect of chili pepper [1, 2]. Capsaicin which actively is discussed and studied as regard on cancer has been also a controversial issue whether it promotes cancer or prevents cancer. [22-24]. Capsaicin which actively is discussed and studied as regard on cancer has been also a controversial issue whether it promotes cancer or prevents cancer. [22-24]. The main components of capsinoids which are less pungent is capsiate. Besides, capsnthin is the main colouring components of chili pepper.

Dihydrocapsaicin is a natural flavonoid that has been isolated from chili pepper. Some papers have reported that the differential cytotoxicity in dihydrocapsaicin treated lung cancer cell lines was due to catalase-mediated autophagy [7]. Dihydrocapsaicin has been shown to inhibit an isoform of cytochrome P450 (CYP), CYPs involved in activation of pre-carcinogens

[8]. However, the molecular mechanism on cancer prevention effect of dihydrocapsaicin is still unclear. In present study, I found that dihydrocapsaicin was the most effective chili pepper component in regard of inhibiting EGF-induced cell transformation.

AP-1 and NF-κB regulate neoplastic transformation [25]. AP-1 is important transcription factor involved in neoplastic transformation in both cell culture and animal models. AP-1 upregulates transcription of genes containing the TPA DNA response element (TRE; 5'-TGAG/CTCA-3') [26]. AP-1 and NF-κB have been significantly regulated COX-2, and COX-2 is key gene in the cell transformation [27]. My results show that dihydrocapsaicin inhibited cell transformation by inhibiting AP-1 and NF-κB transactivation, and subsequently inhibiting COX-2 promoter activities.

The activity of AP-1 is meditated by multiple signaling pathways including MAPKs. Upstream proteins of AP-1 are ERK (Extracellular signal-regulated kinases)-RSK (p-90 ribosomal S6 kinases), p-38 (p38 mitogen-activated protein kinases)-MSK (mitogen- and stress-activated kinases) and JNK (c-Jun N-terminal kinases). In present study, dihydrocapsaicin inhibited AP-1 transactivation by blocking c-fos activity and its expression without affecting MAPK pathways. The activity of NF-κB is regulated by multiple signaling including PI3K-Akt pathway. Akt-

mTOR pathway is important critical role in many biological processes including cell growth, apoptosis, insulin action, cell migration, and integrin function [28-30]. My results showed that dihydrocapsaicin suppressed EGF-induced NF-kB transactivation by inhibiting phosphorylation of p70S6K1 and S6. However, phosphorylation of Akt and mTOR by EGF treatment were not regulated by dihydrocapsaicin. Therefore, further study how dihydrocapsaicin mediate phosphorylation of p70S6K1 remain to be elucidated.

Overall, my study reveals a novel molecular mechanism in which dihydrocapsaicin as the natural phytochemical inhibits EGF-induced cell transformation by suppressing c-fos and mTOR pathway (Fig 7). According to my findings, dihydrocapsaicin exerts a significant anti-transformation effect. Further studies with two-stage tumorigenesis experiment to determine the cancer prevention effects of dihydrocapsaicin may elucidate the chemopreventive effects and the molecular target of dihydrocapsaicin has been to find. In conclusion, dihydrocapsaicin might be useful in the treatment of EGF-associated neoplastic transformation.

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Ⅵ. 국문초록

고추는 한국인이 많이 섭취하는 식품 중에 하나로 매운맛을 내기 위해 사용된다. 이러한 고추에는 캡사이신, 다이하이드로캡사이신, 캡산틴, 캡시에이트 등의 성분으로 구성되어 있다. 고추에 관한 연구논문을 보면 고추가 암을 유발시킨다는 논문이 있는 반면에 암을 억제시킨다는 논문도 있다. 하지만 고추에는 다양한 성분이 존재함에도 불구하고 지금까지 암과 관련된 연구로써는 주로 캡사이신 위주로 연구가 진행 되어왔다. 캡사이신 외에 다이하이드로캡사이신은 비교적 가장 많은 함량이 들어있고 암과

관련된 연구 보고가 적기 때문에 이에 대한 기능성을 연구하고자 실험을 하게 되었다.

정상세포는 개시단계, 촉진단계, 진행단계를 거쳐 암세포가 된다. 그 중에서 촉진단계는 다른 단계와 달리 가역적이고 오랜 시간 거쳐서 발생하기 때문에 암 예방 치료 연구에 있어서 중요하다. 정상 세포에서는 EGF(epidermal growth factor)라고 하는 표피생장인자를 필요한 만큼만 생산하지만 암세포에서는 과하게 생성한다. 표피생장인자가 비정상적으로 많이 발현되면 개시단계에 있는 세포가 촉진단계로 진입하게 된다. 개시단계에서 촉진단계로 들어가는 것을 억제하는 식물을 찾기 위해서는 JB6 P+ mouse epidermal cell 을 주로 사용한다. 개시단계에 있는 JB6 P+ mouse epidermal cell 을 표피생장인자로 유도시킨 후 고추 성분들을 처리하여 세포형질전환(암세포와 유사한 형질로 변환)을 억제하는 성분이 있는지 실험하였다. 그 결과 다이하이드로캡사이신, 캡사이신,

캡산틴, 캡시에이트 중에서 다이하이드로캡사이신이 가장 많이 세포형질전환을 억제시켰다. 세포형질전환을 일으키는 전사인자에는 AP-1 과 NF-κB 가 있다. 이 전사인자의 프로코터결합 능력을 다이하이드로캡사이신이 억제하는지 확인하기 위해 luciferase assay 를 하였다. 그 결과 다이하이드로캡사이신이 AP-1 과 NF-κB 의 프로모터결합능력을 모두 줄였다. 다이하이드로캡사이신이 AP-1 luciferase activity 를 줄였기 때문에 AP-1 의 상위 단백질인 ERK, p38, JNK 또한 줄이는지 웨스턴으로 확인 하였다. 하지만 모두 줄이지 않았기 때문에 다이하이드로캡사이신이 AP-1(c-Jun 과 c-fos 로 구성된 단백질)에 직접 영향을 미치지 않았을까 생각하였고 이를 확인하기 위해 활성을 측정해본 결과 다이하이드로캡사이신은 오직 c-fos 만 줄였다.

앞선 luciferase assay 실험을 통해 다이하이드로캡사이신이 NF-κB를 감소시켰습니다. NF-κB의 상위 단백질인 AKT 신호전달기작에

도 다이하이드로캡사이신이 영향을 미치는지 웨스턴으로 확인하였다. 그 결과, 암세포 증식에 관여하는 단백질인 p70S6K, S6의 인산화를 줄였다. 본 연구 결과를 통해 다이하이드로캡사이신은 세포성장인자에 의해 유도된 세포형질변환에 대한 화학적 예방물질로 제시될 수 있으며, 이는 주로 c-fos 와 mTOR 신호전달체계를 억제하여 일으키는 것으로 확인되었다.

주요어 : 다이하이드로캡사이신; mTOR 신호전달체계; 표피생장인자; AP1;

감사의 글

석사 입학한지 얼마 안된 것 같은데 벌써 2년이 흘러 졸업을 앞두고 있습니다. 많이 어색하기도 했고 실수도 많이 했었던 엊그제 같았던 석사 입학이 벌써 2년이라는 시간이 흘러 석사 4학기가 되어 졸업을 앞두고 있다는 것이 믿기지 않습니다. 그리고 졸업하기 까지 도와주신 이형주교수님과 실험실 선배님들과 동기들에게 감사합니다. 실험에 대해 전반적으로 지도해 주시고 발표능력을 키워주신 교수님께 진심으로 감사 드립니다. 또한 정성근 박사님, 상균오빠, 영진언니, 상권오빠, 조은오빠, 은정언니, 승준오빠, 세미, 민정이 그리고 동기 재각이, 지훈이 모든 분들께 감사합니다. 또한 항상 제 옆에서 응원해주고 격려해준 부모님, 그리고 동생 희진이에게도 감사합니다.