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석사학위 논문

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조선대학교 대학원

치의공학과

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2008 년 11 월

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Abstract

Epigallocatechin-3-gallate Inhibits Tumor Growth through the Suppression of HSP70 and HSP90

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Epigallocatechin-3-gallate (EGCG), a catechin found in green tea, has been recognized as a potential therapeutic agent against human cancer. However, mechanism of cytotoxic effect of EGCG on cancer cells has been still unclear. In this study, we assessed the ability of EGCG to regulation of heat shock response signaling in the breast carcinoma cell. EGCG inhibited cell growth in a dose dependent manner and specifically suppressed the expression of HSP70 and HSP90 but other HSPs were not affected. We also found that EGCG inhibited the transcriptional activity of HSP70 and HSP90 promoter through blocking of HSF1 and HSF2. Interestingly, pretreatment of EGCG (50 or 100 μM) in cells synergistically decreased the cell viability by heat shock (44 °C for 1 h) or oxidative stress (H_2O_2, 500 μM for 24 h) compared with untreated

cell. In xenograft mouse model, treatment of EGCG (10 mg/kg) also significantly reduce the tumor size through inhibiting the expression of HSP70 and HSP90. Our findings suggested that EGCG was a potent tumor suppressor that targeted HSP70 and HSP90 expression in breast cancer cells.

국문초록

HSP70 와 HSP90 의 억제를 통한 Epigallocatechin-3-gallate 의 종양 성장 억제효과

Phan Le Cong Huyen Bao Tran

지도교수: 안상건

치의공학과

조선대학교 대학원

Epigallocatechin-3-gallate (EGCG)는 녹차에서 추출한 catechin성분으로 함암 효과를 가지고 있다. 그러나 EGCG에 의한 암세포 억제 기전은 명확하지 않다. 이 연구에서는 유방암 세포주인 MCF-7 cell에서 EGCG와 Heat Shock response와의 연관성을 조사했다. 그 결과 EGCG는 암세포의 성장을 농도 의존적으로 억제하고 HSP70 와 HSP90 를 특이적으로 억제하는 결과를 보였다. 또한 HSP의 전사 인자인 HSF1 와 HSF2 를 억제함으로서 HSP70 와 HSP90 의 전사적인 활성화를 억제하였다. 흥미롭게도, EGCG의 전처리는 heat shock (44 °C로 1 시간) 또는 oxidative stress (H_2O_2, 500 μM 로 24 시간)를 처리한 후 암세포의 성장이 상승적으로 억제됨을 확인하였다. 암세포를 이식한 동물모델에서 EGCG (10 mg/kg)의 주사 7 일후 종양성장의 억제와 함께 면역조직화학적 및 weatern blot 결과로 HSP70 와 HSP90 의 억제됨을 보였다. 결론적으로 EGCG는 HSP70 와 HSP90 의 분자기전 및 활성저해기전 억제를 통해 유방암세포의 성장을 억제하는 것으로 생각된다.

Ⅰ. Introduction

Green tea, a popular beverage consumed worldwide, is known to have a cancer chemopreventive effect against various type of cancers [1]. Catechins are the key components of teas that exert antiproliferative properties. Since green tea is non-toxic and it is effective in a wide range of organs, the worldwide interest in green tea as a cancer preventive agent for humans has been increased.

Epigallocatechin-3-gallate (EGCG), a type of catechins in green tea, has been shown to inhibit cell proliferation [2] and induce apoptosis [3 and 4] in tumour cells. EGCG has been associated with the cellular mechanism including mitogen-activated protein kinases (MAPKs) activation [5], lipooxygenase and cyclooxygenase activities [6], and arrest of the cell cycle [7 and 8] in tumour cells. Gupta et al. reported that oral infusion of green tea inhibited prostate cancer by inhibiting serum insulin-like growth factor-1, restorating of insulin-like growth factor binding protein-3 levels and reducing the proliferating cell nuclear antigen (PCNA) [9]. EGCG administered intraperitoneally (i.p.) and the green tea polyphenol fraction infused orally caused the regression of experimentally induced skin papillomas in mice [10].

Heat shock protein (HSP) was first discovered as a stress protein that are powerfully induced by environmental and physiological stresses in a wide range of species [11 and 12]. The HSPs have been subsequently characterized

as molecular chaperones, proteins which have in common the property of modifying the structures and interactions of other proteins [12, 13 and 14].

Recent studies have shown that HSPs are often found to be overexpressed in a wide range of cancers. It has been increased resistance to apoptosis induced by diverse anticancer agents [15]. Some studies showed that expression of HSP70 and HSP40 are enhanced in gastric tumor tissue, relative to the surrounding normal tissue [16]. Furthermore, overproduction of recombinant HSP70 in MCF-7 cells led to a strong acceleration of cell growth by shortening of G0/G1 phases [17] and depletion of HSP70 led to an apoptosis-like death of a variety of tumor cell types, including human oral carcinoma cells, HSC-2, MCF-7, Molt-4, PC-3, and others [18 and 19]. On the other hand, HSP90 is a molecular chaperone whose association is required for the stability and function of multiple mutated, chimeric and overexpressed signalling proteins that promote cancer cell growth and/or survival. The increase of HSPs in tumors also presents an opportunity for cancer immunotherapy through the innate ability of many HSPs to function as biological adjuvants and to chaperone tumor antigens. Recently, the ATPase domain of HSP90 has been effectively targeted and a very active and unique family of anti-cancer drugs has been produced [20]. On the other hand, it has been revealed HSP90 inhibitors as 17-AAG, 17-DMAG are effective as anti-cancer drug [21]. In general, elevated HSP expression in malignant cells plays a key role in protection from spontaneous apoptosis associated with malignancy as well as

the apoptosis generated by therapy, mechanisms which may underlie the role of HSP in tumor progression and resistance to treatment [22, 23 and 24].

As mentioned above, HSPs are elevated in cancer cells to protect cells from apoptosis. Beside that, EGCG has antiproliferative property and induces apoptosis in cancer cells. It has been reported that EGCG inhibits arylhydrocarbon receptor gene transcription through an indirect mechanism involving binding to HSP90 [25], so whether EGCG could inhibit HSPs activities to induce apoptosis in cancer cells.

Therefore, in this study, we examined the pathway that EGCG inhibited cell proliferation on human breast cancer MCF-7 cells *in vitro* and on xenograft mouse model *in vivo*.

Ⅱ. Materials and Methods

1. Cell culture

The human breast cancer MCF-7 cells and mouse colon adenocarcinoma cells (CT26) were cultured in a humidified 5 % CO_2 atmosphere at 37 ℃, using Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10 % fetal bovine serum (FBS, Sigma), 100 units/ml of penicillin, and 100 mg/ml of streptomycin (Penicillin- Streptomycin, Gibco).

2. Cell proliferation assay

A total of 3×10^5 MCF-7 cells were cultured in the growth medium in the absence or presence of 10 μM, 50 μM, 100 μM or 200 μM EGCG (Sigma) for 24 h. Each medium was removed and then incubated with 50 μl of MTT solution (5 mg/ml MTT in PBS) for 3 h and then absorbance was determined using an auto reader at 595 nm.

3. Cell transformation assay

MCF-7 cells (2.4×10^4 cells/ml) were cultured in Basal medium eagle (BME, Sigma) containing 1.25 % bacto agar. EGCG (0, 100 μM or 200 μM) was treated into cells. After 10 days incubation, cell transformation was observed.

4. Western blotting

After MCF-7 cells were treated in the absence or the presence of 50 μM, 100 μM or 200 μM EGCG for 24 h, cells were washed with phosphate-buffered saline (PBS, Amresco) and lysed by liquid nitrogen and sonicator. Samples were loaded 40 μg per well and electrophoresed on 10 % or 12 % SDS polyacrylamide gels. The proteins were blotted onto Polyvinylidene Fluoride transfer membranes (PVDF, Bio-Rad). After electroblotting, the membranes were blocked by 5 % skim milk in Tris buffer saline (TBS, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl) at room temperature for 3 h and then incubated with primary antibodies (HSP27, HSP40, HSP60, HSP70, HSP90, HSP110, HSF1, HSF2, β-actin from Santa Cruz Biotechnology) in 5 % milk (1:1000 dilution) at 4 °C overnight. Then, the membranes were washed by Tris buffer saline containing 0.1 % Tween-20 (TTBS) and incubated with secondary antibody (1:2000) in 5 % milk for 1 - 2 h at room temperature, and washed by TTBS again. The bands were visualized using an enhanced chemiluminescence (iNtRON).

5. Luciferase assay

pGL3-HSP70 promoter plasmid or pXP2-HSP90 promoter plasmid was transfected into MCF-7 cells using Fugene 6 reagent (Roche) according to the manufacturer's instructions. After 24 h, cells were shocked by heat at 42 °C for

1 h. Finally, cells were treated in the absence or the presence of EGCG (200 μM) for 24 h. Luciferase activity were assessed.

6. EGCG-Sepharose 4B generation and *in vitro* EGCG pull-down assay

EGCG was conjugated to cyanogens bromide (CNBr)-activated Sepharose (Sigma). EGCG (2.5 mg) was dissolved in 500 μl of coupling buffer [0.1 M $NaHCO_3$ and 0.5 M NaCl (pH 6.0)]. CNBr-activated Sepharose was swelled and washed in 1 mM HCl on a sintered glass filter followed by a wash with coupling buffer. CNBr-activated Sepharose beads were added to the EGCG in coupling buffer at a final concentration of 5 mg of EGCG/ml of wet gel. The coupling solution containing EGCG and Sepharose was mixed end over end at 4 ℃ overnight. Remaining active groups were blocked for 2 h at room temperature in Tris-HCl (0.1 M, pH 8). EGCG-conjugated Sepharose was washed with three cycles of alternating pH washing buffers [buffer 1: 0.1 M acetate and 0.5 M NaCl (pH 4.0), buffer 2: 0.1 M Tris-HCl and 0.5 M NaCl (pH 8.0)]. EGCG-conjugated beads were then equilibrated in binding buffer [0.05 M Tris-HCl and 0.15 M sodium chloride (pH 7.5)]. The control unconjugated CNBr-activated Sepharose beads were prepared as described above in the absence of EGCG.

MCF-7 cells were lysed by RIPA buffer (with SDS) on ice for 1 h. Sample was centrifuged at 12000 rpm 5 minutes. Supernatant mixed gently with unconjugated CNBr-activated sepharose 4B or EGCG-sepharose 4B in the

presence or the absence of 1 μM or 10 μM ATP (Amresco) for 3 h at 4 °C. The beads were washed three times with binding buffer. The bound protein was eluted with SDS loading buffer [0.125 M Tris, 4 % SDS (w/v), 20 % glycerol (v/v), 200 mM dithiothreitol, and 0.01 % bromophenol blue (w/v) (pH 6.8)]. The samples were boiled for 5 min, and the bound protein was separated by SDS-PAGE. The protein was transferred to a PVDF membrane (Millipore). HSP70 and HSP90 were detected by immunoblotting using HSP70 or HSP90 monoclonal antibodies (Santa Cruz) followed by secondary antibody.

7. Tumour cell inoculation and EGCG treatment

Six weeks old male BALB/c mice (Samtako, Daejeon, Korea) were used *in vitro* study. Eight mice were inoculated subcutaneously into the right flank with mouse colon carcinoma cells (CT26) (5×10^6 cells/200 μl DMEM per mouse). 4 days later, when tumor appeared, 8 mice were divided into two group. The first group is control (3 mice), the second group is EGCG group (5 mice). Mice were given daily intraperitoneal injections of 10 mg/kg EGCG or water (control). Tumor growth was measured daily by caliper. Tumor volume was calculated as (length x width^2)/2 [9].

8. Histology and Immunohistochemitry

Mice were killed by chloroform at the seventh day after EGCG or water injection. The tumours were excised, and sectioned. The tumour sections were frozen at −80 °C or fixed in 4 % paraformadehyde.

Tumor was crushed with liquid nitrogen, then incubated with RIPA buffer (added inhibitors as PMSF, aprotinin and sodium orthovanadate) on ice for 1 h. After that centrifuged 12000 rpm 10 minutes at 4 °C. Tranfer supernatant to new tube. The proteins were used for HSP70 and HSP90 immunoblotting.

Immunohistochemical staining for HSP70, HSP90 and proliferating cell nuclear antigen (PCNA) was done. Paraformadehyde-fixed and paraffin-embedded sections were treated by standard deparaffinization. Briefly, endogenous peroxidases were blocked with 1 % H_2O_2 10 minutes, slides were washed in PBS, incubated for 1 h with protein-blocking solution (PBS supplemented with 1.5 % normal goat serum or normal horse serum), incubated overnight at 4 °C with primary antibodies directed against HSP70, HSP90 or PCNA. Then the slides were washed by PBS again, incubated for 1 h at room temperature with peroxidase-conjugated secondary antibodies, washed, incubated with ABC reagent, washed by PBS and then incubated with DAB, washed, counterstained with haematoxylin, washed, dehydrated sections, mounted with Universal Mount. PCNA positive cells were counted in 5 random areas under X400 field.

Ⅲ. Results

1. EGCG inhibits cell proliferation of MCF-7 cells

It has beeen reported that EGCG inhibits cell proliferation in tumor cells [2]. In this study, we investigated the effect of EGCG on cell viability of MCF-7 cells. Cells were treated with various concentrations of EGCG (0, 10, 50, 100, 200 μM) for 24 h, and then cell viability was measured by MTT assay. As shown in Fig. 1, EGCG induced growth inhibition of MCF-7 cells in a dose dependent manner. The concentration caused 50 % cell death (IC50) was approximately 150 μM.

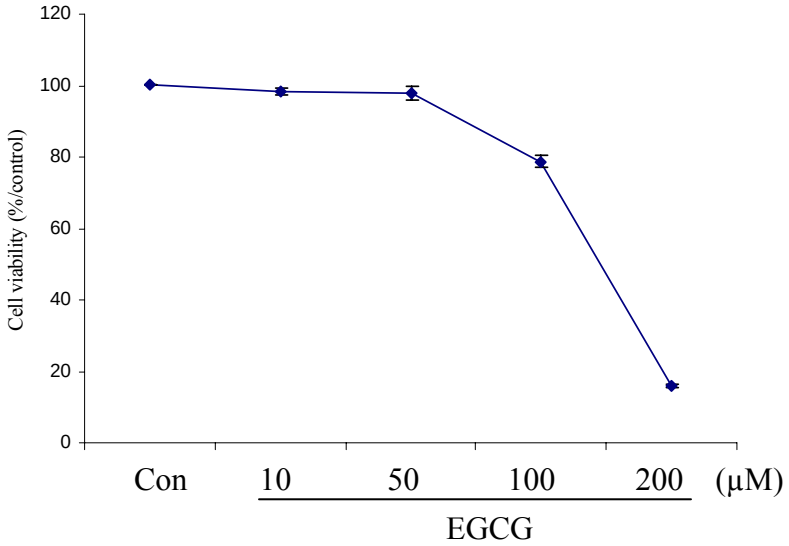

Figure 1. The cytotoxicity effect of EGCG on MCF-7 cells. The data were presented as the mean ± SD.

2. EGCG inhibits adhesion-independent cell transformation

We examined the cell transformation after EGCG treatment. We cultured MCF-7 cells in Basal medium eagle containing 1.25 % bacto agar. After 10 days, colony formation was observed using microscopy. As shown in Fig. 2, cells formed many colonies in control. However, in the presence of EGCG, colony formation was inhibited in a dose-dependent manner. These results demonstrated that EGCG suppressed the proliferation of MCF-7 cells.

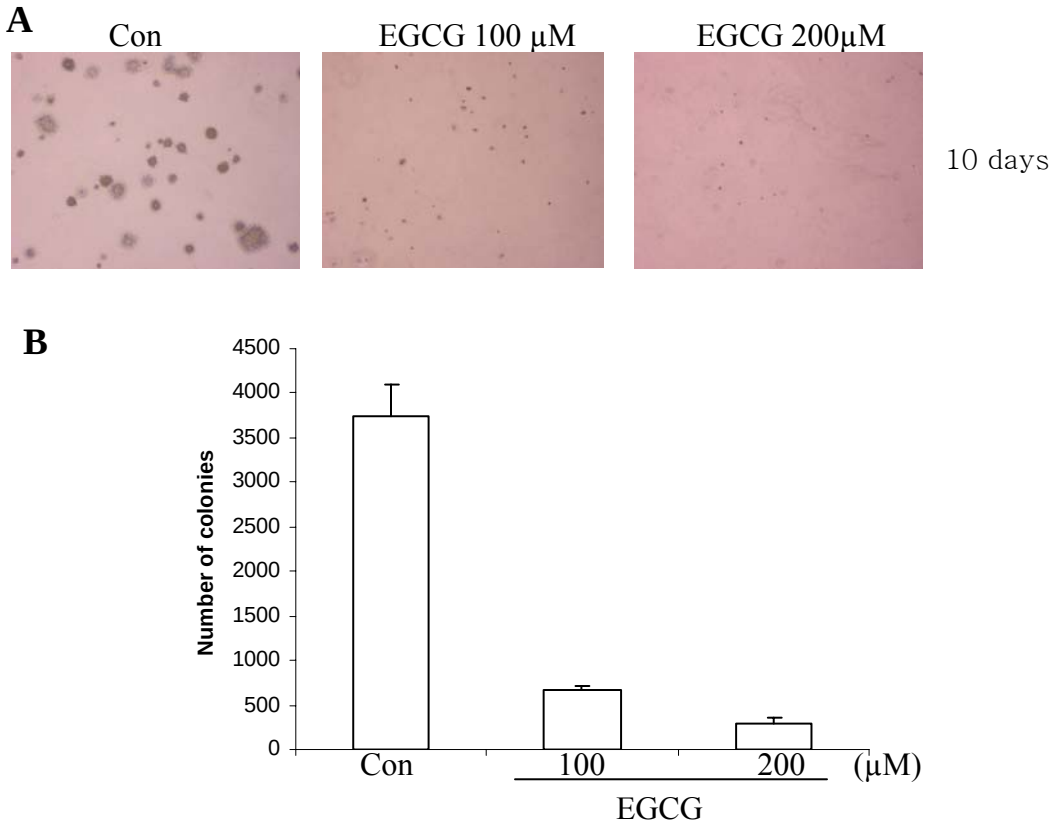

Figure 2. EGCG inhibited transformation of MCF-7 cells. [A] Images were observed by microscopy, X100. [B] The number of colonies from 9 samples were averaged. Error bars indicate SD.

3. EGCG inhibits the expression of HSP70 and HSP90 in MCF-7 cells

To determine the pathway that anticancer effect of EGCG, we examined the effect of EGCG on the expression of HSPs. MCF-7 cells were treated with EGCG (0, 50, 100, 200 μM) for 24 h. Cells were harvested and proteins were extracted. The expression of HSPs were detected by western blot. Treatment of cells with EGCG (200 μM) specifically inhibited the expression of HSP70 and HSP90 while other HSPs were not affected under the same condition (Fig. 3). β- actin was used to confirm the equal amount of proteins loaded in each well.

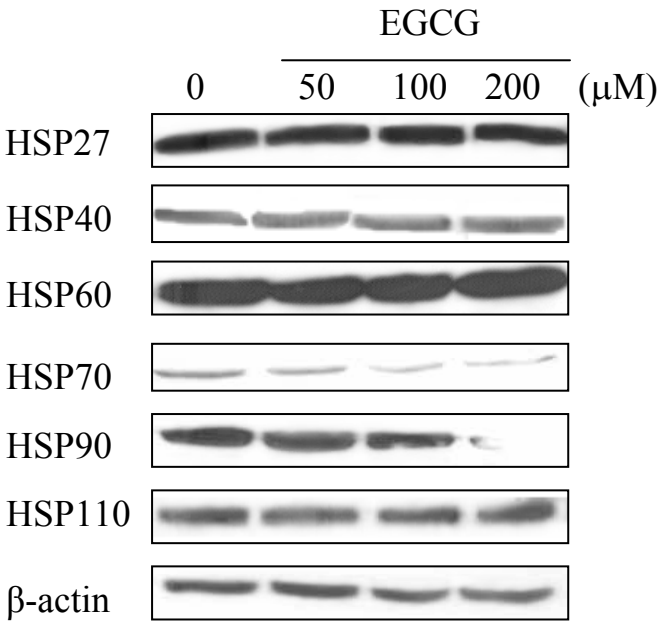

Figure 3. Expression of HSPs after EGCG treatment for 24 h

Futhermore, we wanted to evaluate whether EGCG can inhibit the transcriptional activities of HSP70 and HSP90. pGL3-HSP70 promoter plasmid or pXP2-HSP90 promoter plasmid was transiently tranfected into MCF-7 cells for 24 h, and then cells were shocked by heat at 42 °C for 1 h, finally were treated with 200 μM EGCG. After EGCG treatment for 24 h, luciferase activity was assayed. Reporter assay showed that heat shock (42 °C for 1 h) induced significantly the transcriptional activities of HSP70 and HSP90, consistent with previous report that HSPs is induced by heat shock [12]. However, in the presence of EGCG (200 μM), the transcriptional activities of HSP70 and HSP90 were suppressed (Fig. 4). These results confirmed that EGCG (200 μM) inhibited activities of HSP70 and HSP90 both of transcriptional activity and protein expression in MCF-7 cells. It suggested that EGCG induced the growth inhibition in MCF-7 cells *via* inhibiting HSP70 and HSP90.

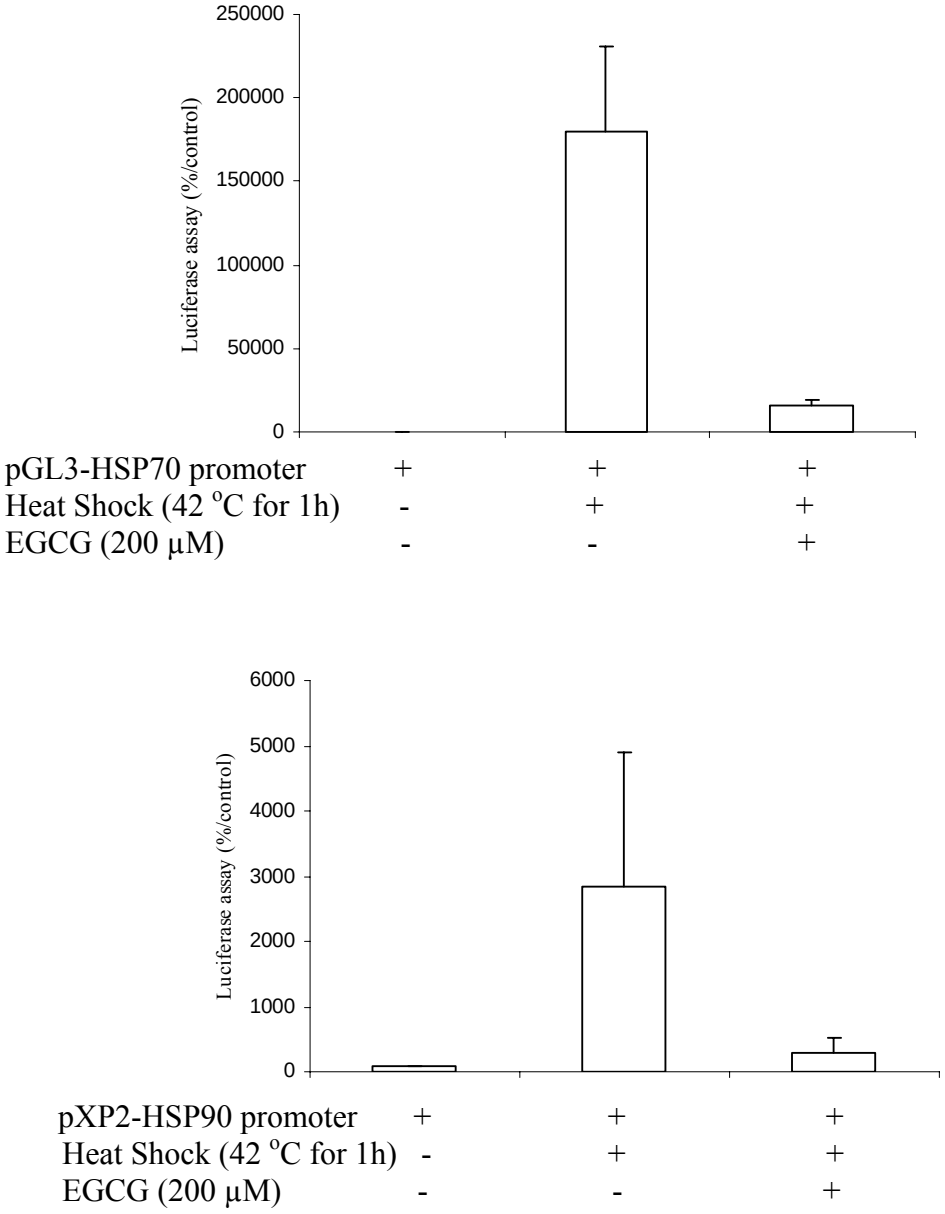

Figure 4. Effect of EGCG on HSP70 and HSP90 promoter activities in MCF-7 cells. The data were presented as the mean ± SD.

4. Inhibition of HSP70 and HSP90 by EGCG caused by the HSF1/HSF2 depletion

The inducible HSP expression is regulated by the heat shock transcription factors (HSFs). Therefore, we wanted to determine whether EGCG inhibits the expression of HSFs. As shown in Fig. 5, EGCG (200 μM) decreased significantly HSF1 and HSF2 expression after treatment for 24 h. This suggested that EGCG targeted HSP70 and HSP90 protein via the inhibition of the HSFs pathway in MCF-7 cells.

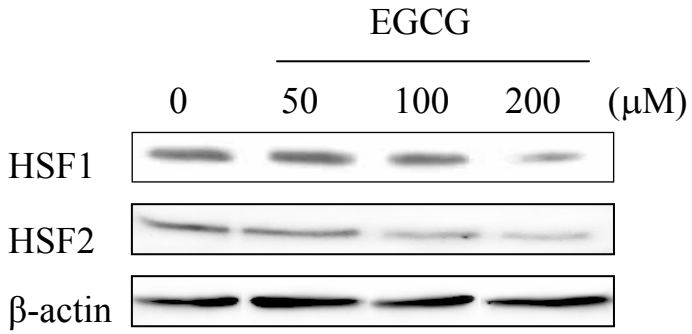

Figure 5. Expression of HSF1 and HSF2 after EGCG treatment for 24 h.

5. Levels of stress damage are increased in EGCG-treated cells

In order to study the effect of EGCG on cell viability of stress-treated cell, MCF-7 cells were treated with EGCG (50 or 100 μM) for 24 h before heat shock (44 ^{o}C for 1 h) or H_2O_2 (500 μM for 24 h) and then cell viability was measured by MTT assay. Interestingly, pretreatment of EGCG in cells significantly decreased the cell viability which induced by heat shock or oxidative stress compared with untreated cell (Fig. 6).

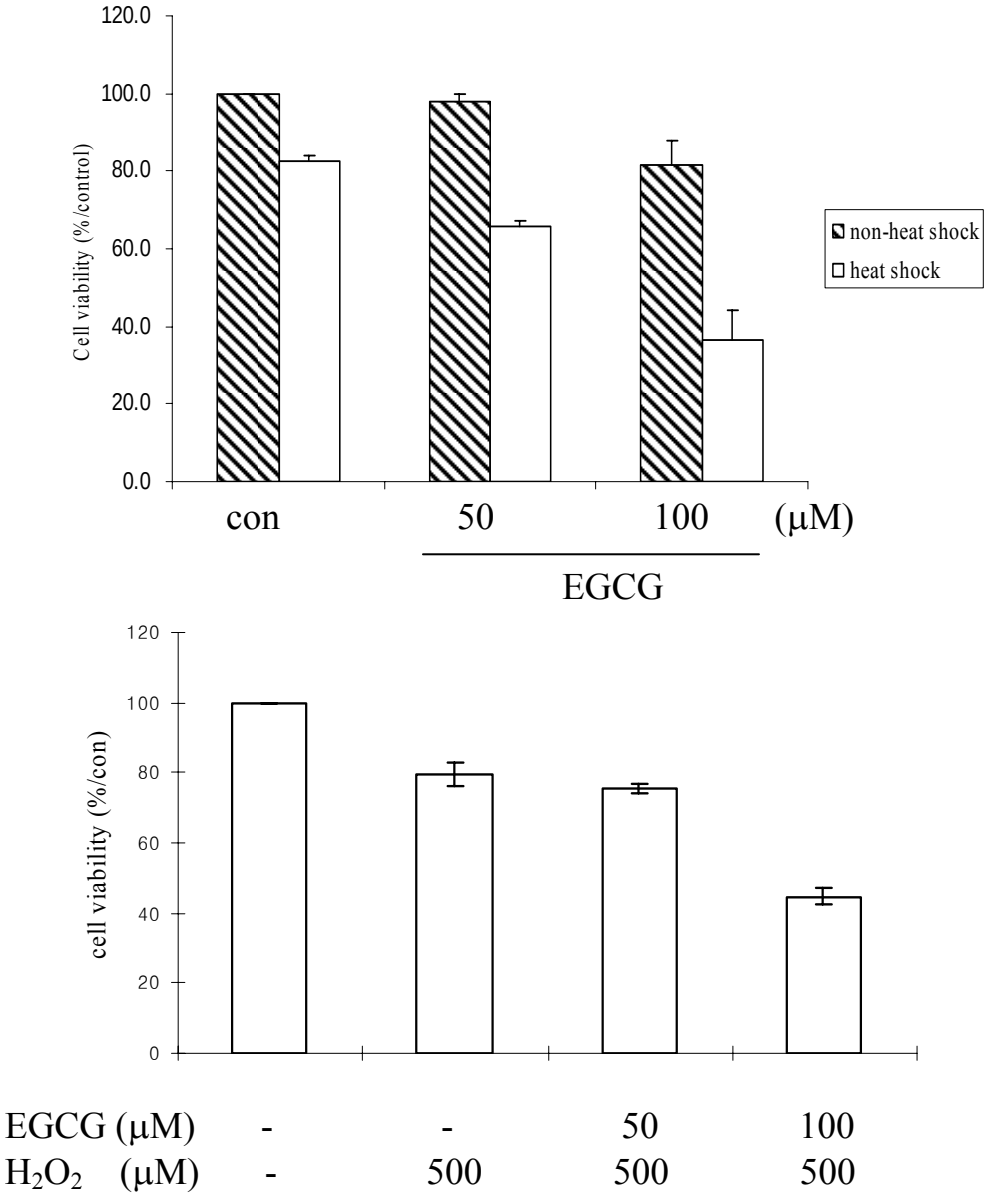

Figure 6. The cytotoxicity effect of EGCG combined with heat shock or H_2O_2 on MCF-7 cells. Cell viability was measured by MTT assay. The data were presented as the mean ± SD.

Next, we determined if EGCG can suppress the overexpression of HSP70 and HSP90 induced by heat shock or oxidative stress. As expect, EGCG decreased significantly the overexpression of HSP70 and HSP90 (Fig. 7). It suggested that EGCG actually had an ability to suppress the expression of HSP70 and HSP90 even these proteins were overexpressed by heat shock or H_2O_2.

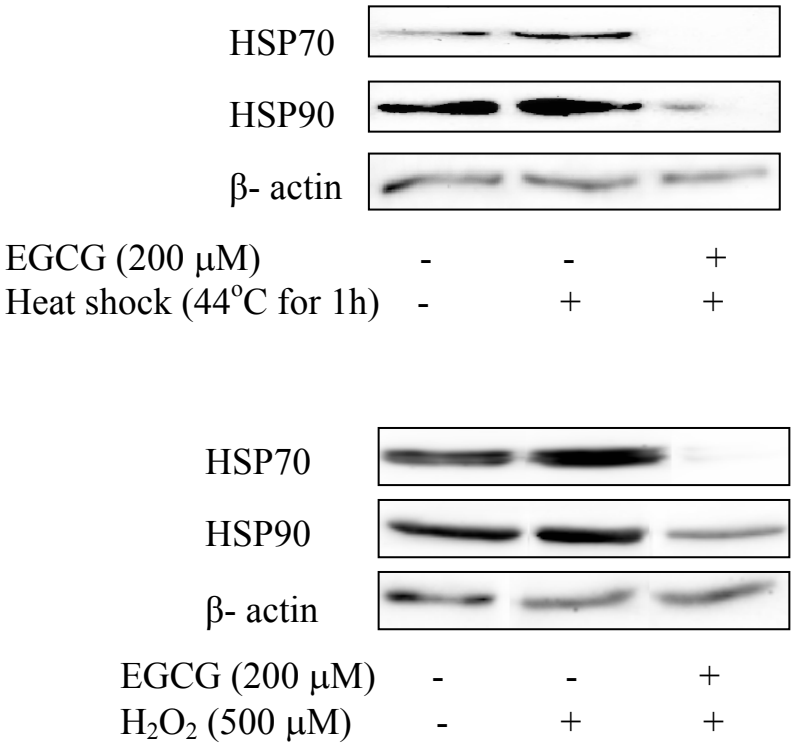

Figure 7. EGCG suppressed the overexpression of HSP70 and HSP90 which induced by heat shock (44 °C for 1 h) or H_2O_2 (500 μM).

6. EGCG competes with ATP for binding to HSP70 and HSP90 ATPase domain

We found that EGCG inhibited the expression of HSP70 and HSP90 in MCF-7 cells, may due to EGCG could bind to these heat shock proteins. To investigate this prediction, we did EGCG-Sepharose 4B pull-down assay. Mixing EGCG-Sepharose 4B and cell total protein extract for 3 h and then did immunoblotting with HSP70 and HSP90 antibodies. In the fraction containing proteins bound with EGCG after pull-down, the band of HSP70 and HSP90 were detected (Fig. 8). It supported that EGCG bound to HSP70 and HSP90.

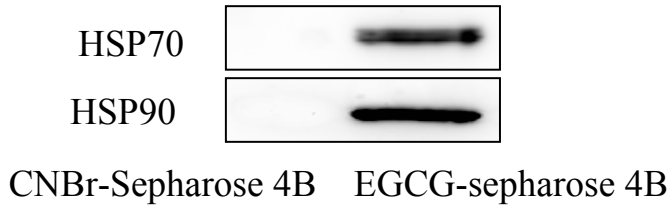

Figure 8. Binding assay of EGCG and HSP70 or HSP90. MCF-7 cells were lyzed by RIPA buffer (with SDS), and then were mixed with CNBr-activated™ sepharose 4B or EGCG-CNBr activated™ sepharose 4B, after that beads were washed by RIPA buffer (without SDS) and added with 2X SDS loading buffer. Samples were resolved by western blot with anti-HSP70 and anti-HSP90 bodies.

We wanted to know the reason why EGCG inhibited the expression of HSP70 and HSP90. We hypothesized that EGCG might compete with ATP for binding to ATPase domain in HSP70/ HSP90 and inhibit the chaperone activity of these proteins. We revealed that the binding of EGCG and HSP70 or HSP90 was decreased in the presence of ATP in a dose dependent manner (Fig. 9). This result demonstrated that EGCG inhibited the activities of HSP70 and HSP90 by competing with ATP in ATPase domain.

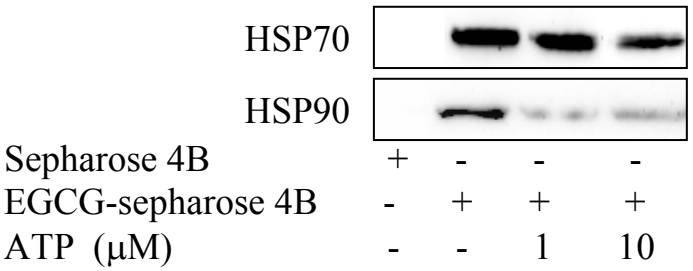

Figure 9. EGCG competed with ATP for binding to ATPase domain of HSP70 and HSP90.

7. EGCG suppresses the tumor growth *in vivo*

Next, we determined the effect of EGCG on cancer growth in animal model. There were two groups of mice. The group received intraperitoneally (i.p) water injection served as control. EGCG solution (10 mg/kg) was injected intraperitoneally into the second group. All of the animals appeared healthy with no loss of body weight (Fig. 10). Interestingly, treatment of EGCG

(10 mg/kg) on BALB/c mice inoculated with mouse colon carcinoma cell (CT26) reduced the tumor size compared with water-injected mice for 7 days (Fig. 11).

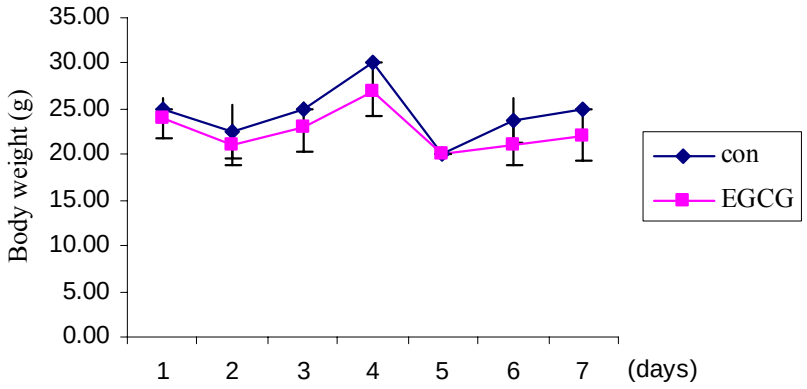

Figure 10. Body weight of mice after EGCG or water injection for 7 days. The data were presented as the mean ± SD.

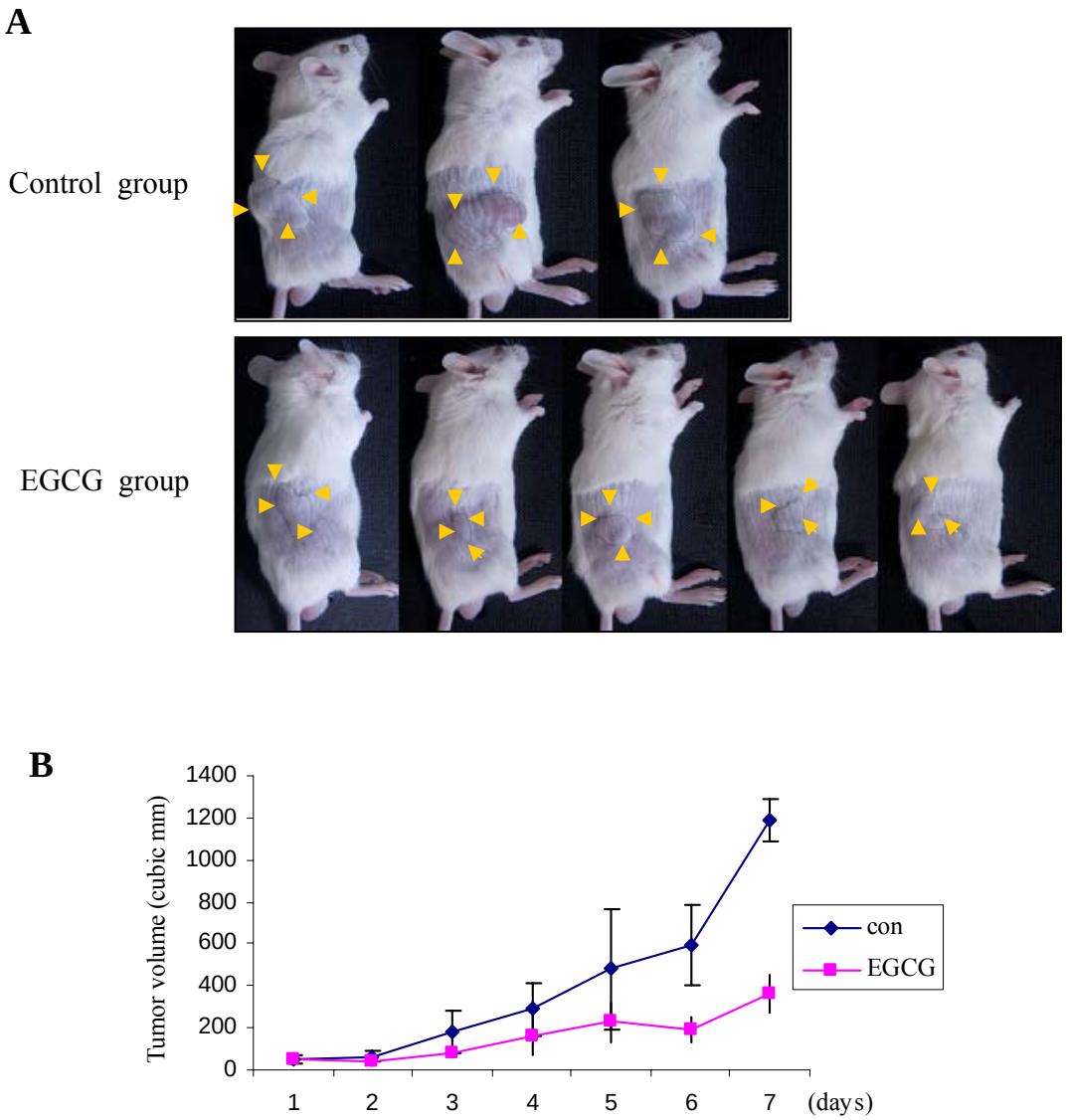

Figure 11. Effect of EGCG (10mg/kg) on tumor growth *in vivo*. [A] Image of tumor xenograft in mice at the seventh day after injection. [B] Effect of EGCG on tumor volume after injection for 7 days. The data were presented as the mean ± SD.

At the seventh day after injection, tumors were excised from mice. PCNA (Proliferating Cell Nuclear Antigen) was originally identified as an antigen that is expressed in the nuclei of cells during the DNA synthesis phase of the cell cycle [26]. Therefore, it was used as a marker of proliferating cells. To test the effect of EGCG on cell proliferation in tissue, immunohistochemistry for PCNA was performed. As shown in Fig. 12, the number cells of PCNA positive nuclei in mice treated with EGCG were statistically lower than those in control mice. This result supported that EGCG reduced evidently the cell proliferation in tumors.

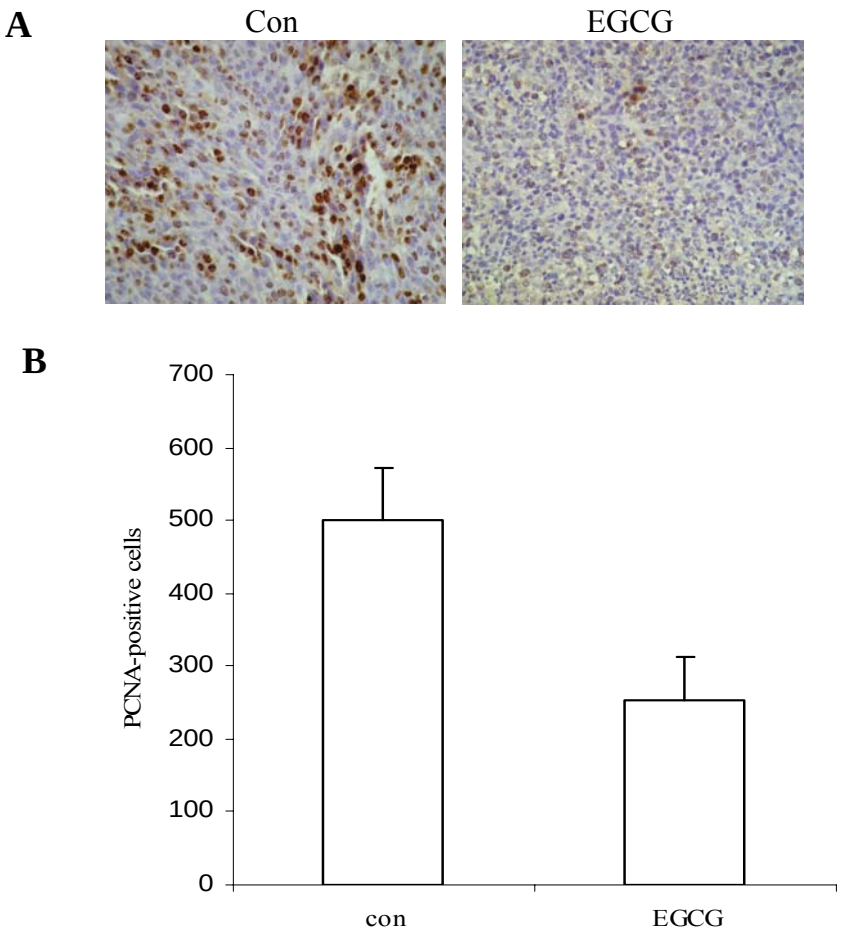

Figure 12. Effect of EGCG (10mg/kg) on cell proliferation *in vivo*. [A] Immunohistochemistry staining for PCNA in CT26 xenograft tumor masses, X400. [B] The number of PCNA positive cells in microscopic (X400) fields from 5 samples were averaged. Error bars indicate SD.

Next, to determine whether EGCG suppressed the level of HSP70 and HSP90 in tumor tissues, immunoblotting or immunohistochemistry of HSP70 and HSP90 were carried on. Fig. 13 has shown that expression of HSP70 and HSP90 were suppressed dramatically in tumor tissues which injected with

EGCG compared with tissues of control mice. These results showed that EGCG inhibited the proliferation of cancer cells via suppressing HSP70 and HSP90 both *in vitro* and *in vivo*.

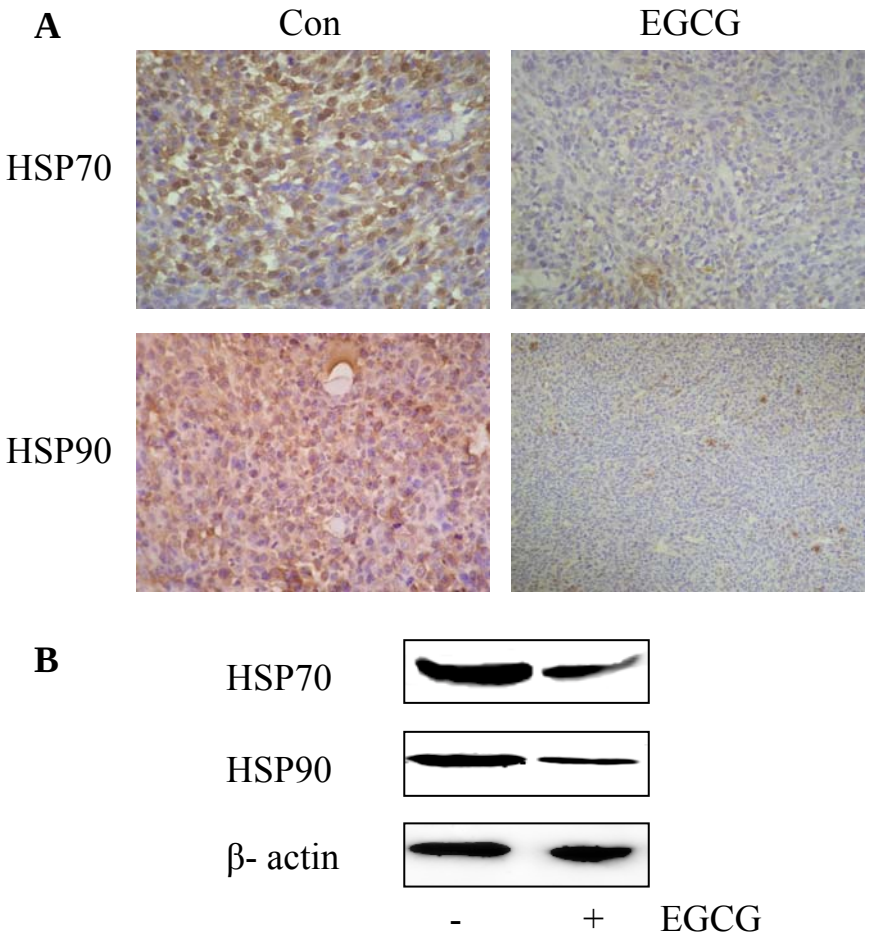

Figure 13. Expression of HSP70 and HSP90 on tumor tissues. [A] Immunohistochemistry for HSP70 and HSP90, X400. [B] Suppression of HSP70 and HSP90 expression in tumor tissues caused by EGCG.

IV. Discussion

It has been indicated that EGCG, a predominant compound of green tea, has an ability to suppress cancer development such as stomach cancer [3], prostate cancer [4]. Previous studies observed that EGCG caused cell proliferative inhibition by G1/S phase arrest through down-regulation of cyclin D1, cdk4, cdk6 [27, 28]. However, we observed the antiproliferative effect through arresting cell cycle at G2/M phase (data not shown). It is necessary to investigate more the effect of EGCG on expression of regulating proteins in G2/M phase of cell cycle.

Heat shock proteins have been subsequently characterized as molecular chaperones which have in common the property of modifying the structures and interactions of other proteins [12, 13 and 14]. Level of HSPs become elevated in many cancers, HSPs were considered as target for cancer drug development. In the present study, using western blot and reporter assay, we showed evidences that EGCG repressed the cell growth through inhibiting HSP70 and HSP90 expression (Fig. 3 and 4).

It has been reported that HSFs bind to HSPs promoter, and modulate their expression. The HSF gene family includes heat shock transcription factor 1 (HSF1), the molecular coordinator of the heat shock response, as well as two less well-characterized genes, heat shock transcription factor 2 (HSF2) and heat shock transcription factor 4 (HSF4) [29, 30, and 31]. In normal condition, HSF presents in the cytoplasm and the nucleus in a monomeric form that has

no DNA binding activity through its interactions with HSPs. In response to environmental and physiological stresses, the monomeric HSFs combine into trimers and accumulate within the nucleus. In the nucleus, the trimers bind to the heat shock elements (HSE), that is specific DNA sequences in the heat shock gene promoters. When attached to DNA, HSF will be phosphorylated. The transcriptional activation of the heat shock genes leads to elevated levels of HSPs [32]. This study examined the effect of EGCG on HSFs expression. We demonstrated that the level of HSF1 and HSF2 were reduced by EGCG treatment after 24 h (Fig. 5). It suggested that EGCG suppressed the expression of HSP70 and HSP90 *via* the inhibition of the HSFs pathway. Studies for HSFs activation after EGCG treatment as HSF phosphorylation, DNA binding activity, HSF trimerization or HSF localization will be further investigated.

Next, we observed the effect of combined treatment with EGCG and heat shock or oxidative stress (H_2O_2) on the cell growth using MTT assay. Pretreatment of EGCG strongly inhibited the cell growth compared with heat shock or H_2O_2 treatment alone (Fig. 6). In immunoblotting assay, heat shock (44 ^{o}C for 1 h) or oxidative stress stimulated the expression of HSP70 and HSP90, but EGCG (200 μM) suppressed the expression of HSP70 and HSP90 induced by heat shock or H_2O_2 (Fig. 7). We concluded that EGCG inhibited the proliferation of MCF-7 cells, and EGCG combined with heat shock or oxidative

stress strengthened the effects. EGCG conbined with heat shock or oxidative stress may enhance the sensitivity of stimuli to tumor.

We also observed EGCG bind to HSP70 and HSP90 using CNBr-sepharose pull down assay (Fig. 8). Futhermore, we wanted to test the pathway that EGCG inhibited the activities of HSP70 and HSP90. The chaperone activity of the HSPs is controlled by a reaction cycle of ATP binding, hydrolysis and nucleotide exchange to mediate a series of rapid association-dissociation cycles between the HSP and its target polypeptide [33, 34 and 35]. We hypothesized that EGCG may compete with ATP for binding to ATPase domain in HSP70 / HSP90 and inhibit the chaperone activity of these proteins. As expect, we showed evidence that EGCG competed with ATP in HSP70/HSP90 ATPase domain (Fig. 9), offering an opportunity to propose mechanisms which may contribute, at least partially, to explain the well-recognized anti-apoptotic effect of EGCG in cancer cells.

Like *in vitro* experiment, we investigated whether EGCG suppressed the cancer cell growth *in vivo via* the inhibition of HSP70 and HSP90. First, PCNA staining indicated that EGCG repressed significantly the cell proliferation in tissues which were treated with EGCG (10 mg/kg) compared with control tissues (Fig. 11). Then, by western blot or immunohistochemistry also displayed that the expression of HSP70 and HSP90 were suppressed in EGCG-treated tumor tissues compared with control tissues (Fig. 12).

In conclusion, our results indicated that EGCG inhibited cancer growth through the suppression of HSP70 and HSP90 both of *in vitro* and *in vivo*. This observation showed that EGCG may a potential chemopreventive agent against tumors.

V. References

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논문제목	한글 : HSP70 와 HSP90 의 억제를 통한 Epigallocatechin-3-gallate 의 종양 성장 억제효과 영문: Epigallocatechin-3-gallate Inhibits Tumor Growth through the Suppression of HSP70 and HSP90.				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 -조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함

2. 위의 목적을 위하여 필요한 범위 내에서의 편집・형식상의 변경을 허락함.
다만, 저작물의 내용변경은 금지함.

3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.

4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.

5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.

6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음

7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

2009 년 2 월

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