Differential biological responses of epigallocatechin-3-gallate, green tea polyphenol component, in neonatal human dermal fibroblast cell versus HT-1080 fibrosarcoma cell

Mi Hee Lee

Department of Medical Science

The Graduate School, Yonsei University

Differential biological responses of epigallocatechin-3-gallate, green tea polyphenol component, in neonatal human dermal fibroblast cell versus HT-1080 fibrosarcoma cell

Directed by Professor Jong-Chul Park

The Master's Thesis
submitted to the Department of Medicine Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Master of Medical Science

Mi Hee Lee

June 2006

This certifies that the Master's Thesis of Mi Hee Lee is approved.

Thesis Supervisor : Jong-Chul Park

WoongChon Mar

Hyun Cheol Chung

The Graduate School
Yonsei University

June 2006

ACKNOWLEDGEMENTS

대학원이라는 또 다른 작은 사회에 발을 딛고 어느덧 2년이라는 시간이 지난 시점, 처음 시작의 초심과 다짐들을 되짚어 생각해 봅니다. 내가 과연 과학이라는 학문을 잘 해낼 수 있을까라는 의문과 두려움으로 시작했던 처음의 모습과 지금의 나를 생각해보면 나의 힘, 지식만으로는 할 수 없다는 생각을 다시금 느끼게 됩니다. 아쉬움과 함께 석사과정 동안 많은 것을 느끼고, 많은 사람들을 만나고, 한 단계 성장하는 시간이었던 것 같습니다. 그 결과 부족하지만 작은 결실로 이 논문을 완성하였고, 많은 도움을 주신 분들께 이 작은 지면을 통하여 감사의 말씀을 전합니다.

부족한 저를 연구자의 길로 이끌어 주시고, 많은 가르침을 주신 박종철 교수님께 가장 먼저 감사의 마음을 전하고 싶습니다. 앞으로 시작되는 더 어려운 길에서 연구자의 뜻에 어긋나지 않는 참된 과학자가 되도록 노력하겠습니다. 바쁘신 데도 불구하고 실험에 대한 조언과 더불어, 논문의 지도를 맡아주신 서울대학교 천연물 연구소의 마응천 교수님과 내과학교실의 정현철 교수님께 깊이 감사드립니다. 세미나를 통해 많은 조언을 아끼지 않으시며, 항상 많은 관심과 사랑으로 가르침을 주시는 나노사업단의 이인섭교수님과 세종대학교의 이권용 교수님께도 감사드립니다. 학위과정동안 많은 보살핌을 주신 의학공학교실의 서활 교수님, 김덕원 교수님, 김남현 교수님, 유선국 교수님께도 감사의 마음을 전합니다.

2년 반이라는 시간동안 의학공학교실 일원으로 항상 함께 해준 우리 실험실원 모두에게 감사의 마음을 전하고 싶습니다. 많은 시간 함께 하진 못했지만 많은 격려와 도움을 주시는 이동희 선생님, 일본에서 박사학위 과정 중이신 박봉주 선생님께 감사드립니다. 실험실 생활동안 세세한 것부터 큰 것까지 많은 도움과 조언을 주신 한동욱 선생님, 힘들 땐 상담가로 실험에 어려움이 있을 땐 조언가로 항상 옆에 있어준 현숙 언니, 지금은 다른 곳에서 열심이 생활 중이지만 미희에겐 영원히 오빠인(?) 현정언니 모두 정말 감사드립니다. 같은 학생이라고 서로 위안(?)삼으며 티격태격했던 지금은 SK woman으로 생

활 중인 현주언니, 점점 예뻐지고 있는 혜련언니, 동갑이라고 함께하고 시시콜콜한 이야기까지 서로 나눈 연이 (앞으로도 우리 잘해 보자구!), 군대에서 고생하고 있는 진현이, 더 넓은 사회로 나갈 택형씨, 그리고 일본에서 공부 중인 학희 언니에게도 감사의 마음을 전합니다. 세미나 시간 잠깐 보지만 학문에 열의가 멋진 한인호 선생님, 최재혁 선생님께도 감사드립니다.

학부시절 함께 붙어 다니며, 인생(?)을 논하던 나의 친구들, 지금은 다른 길로 들어갔지만 열심인 옥경이, 우리의 영원한 홍양 은정이, 똘똘이 설주, 먼저 연락하지 못해서 매일 미안한 진희, 실로암이란 동아리서 만나 정든 00학번들, 슬아, 선희, 지수, 미진, 세은, 석영, 경표, 정호와 그 외 많은 친구들, 중학교 시절부터 함께해온 준경이와 인형이, 정말 오랜만에 다시 만나게 되서 더욱 기쁜 경숙이에게도 고마움과 미안함의 마음을 전합니다. 기도의 후원자이신 목사님, 바쁘다는 핑계로 도와주지 못하는 나를 이해해주는 원진언니, 남순언니, 지금은 주부가 된 나의 소중한 친구 남희, 주일학교 선생님들과 친구들, 엘림교회 가족들에게도 감사드립니다.

부족하고 무뚝뚝한 딸을 언제나 믿어주시며 기도의 후원자로 사랑은 주시는 부모님, 어떤 말로도 표현 못할 정도로 감사드리고 사랑합니다. 부모님의 기대 저버리지 않은 맏딸이 되도록 항상 노력하고 보답하는 딸 되겠습니다. 멋진 어린이집 선생님으로 자리 잡고 있는 나의 동생 계희, 예비 간호사로 열심히 공부중인 막둥이 계정이에게도 고마운 마음을 전하며 앞으로 멋진 언니로 기도의 후원자 되겠습니다. 마지막으로 힘들때나 기쁠때 언제나 사랑으로 보살펴 주시며 위로해주시는 나의 하나님 당신의 큰 사랑에 감사드립니다. 앞으로의 길에도 당신의 예비하심을 위해 기도하며 노력하는 참된 크리스챤이 되도록 노력하겠습니다.

석사과정의 끝 시간 지금이 끝이 아닌 더 큰 세계로 나갈 시작이라고 생각합니다. 다시 시작하는 이 순간 염려와 사랑으로 도와주시는 모든 분들을 위해 노력하는 자 되겠습니다.

2006년 6월

이미희 드림

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Differential biological responses of epigallocatechin-3-gallate, green tea polyphenol component, in neonatal human dermal fibroblast cell versus HT-1080 fibrosarcoma cell

Mi Hee Lee

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Jong-Chul Park)

Green tea is one of the most widely consumed beverages in the world today, its medicinal properties have been widely explored. The tea polyphenols are known to be strong antioxidants in food chemistry. Among these polyphenols, EGCG accounts for 50-80% of the catechins and is the major component of green tea and mediate much of biological effects. EGCG possesses antioxidant, anti-inflammatory, anti-proliferative, and anti-cancer activities.

Serveral studies have demonstrated that EGCG have cancer chemopreventive effects in many animal tumor bioassays, and can protect to cell damages against ROS in normal cells.

In this study, we investigated the involvement of growth inhibition, induction of apoptosis, cell cycle distribution and NF-κB modulation as a mechanism of the differential biological response of EGCG in nHDF vs HT-1080 by EGCG. EGCG treatment resulted in a dose-dependent (i) inhibition of cell growth, (ii) G0/G1-phase arrest of the cell cycle, and (iii)

induction of apoptosis in both nHDFs and HT-1080 at different concentration. Western blot analysis revealed that EGCG treatment results in lowering of phospho NF-kB levels in a dose-dependent manner in both nHDF cells and HT-1080, albeit at different concentrations. Briefly, EGCG-mediated inhibition of cell growth and phospho NF-kB expression and induction of apoptosis was found to occur at much higher dose of EGCG in nHDFs as compared to HT-1080. The results of oligo microarray were shown that EGCG regulated gene related cell cycle in nHDFs.

In conclusion, EGCG was found to impart differential dose-based inhibitory response in HT-1080 vs nHDF and EGCG-caused cell cycle deregulation and apoptosis and growth inhibition of cells may be mediated through inhibition of NF-κB activation.

Key words : epigallocatechin gallate(EGCG), fibrosarcoma cell(HT-1080), neonatal human dermal fibroblast(nHDF), cell cycle, apoptosis, nuclear factor κB

Differential biological responses of epigallocatechin-3-gallate, green tea polyphenol component, in neomatal human dermal fibroblast cell versus HT-1080 fibrosarcoma cell

Mi Hee Lee

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Jong-Chul Park)

I. INTRODUCTION

1. Green tea Polyphenol

Green tea is one of the most widely consumed beverages in the world today, its medicinal properties have been widely explored. Tea derived from the leaves of the plant *Camellia sinensis* of the family Theaceae has been consumed in Southeast Asia for thousands of years. In ancient China, tea was lauded for various beneficial health-promoting effects, e.g., as medicinal remedies for headaches, body aches, depression, immune enhancement, digestion, and detoxification, as an energizer, as an antioxidant, and to prolong life[1]. Green tea contains many polyphenols known as catechins. The tea polyphenols are known to be strong antioxidants in food chemistry. However, the bioavailability of the catechins is limited because of their polyphenolic structure and their rapid

methylation and conjugation upon absorption[2]. The polyphenols, present in green tea, possess high antioxidant activities, which, in turn, protect cells against the adverse effects of damaging reactive oxygen species (ROS) that are constantly produced in the body. ROS, such as superoxide radical, hydroxyl radical, singlet oxygen, hydrogen peroxide, peroxynitrite, and alkoxyradicals, damage lipids, protein, and nucleic acids, and cellular components, such as ion channels, membranes, and chromatin, lead to cellular injury and cellular dysfunctions. These ROS are known to contribute to the etiology of many chronic health problems, including cardiovascular diseases, inflammatory diseases, diabetes, obesity, and cancer[3,4]. The polyphenolic constituents of tea can act as scavengers of ROS and thereby prevent damage to cellular macromolecules. The scavenging activity of the specific catechin molecules is related to the number of o-dihydroxy and o-hydroxyketo groups, solubility, concentration, the accessibility of the active group to the oxidant, and the stability of the reaction product[5]. The major catechins in green tea are epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epicatechin (EC), and (+)-catechin (C). Among these polyphenols, EGCG (Figure 1) accounts for 50-80% of the catechins and is the major component of green tea and mediate much of biological effects[6]. EGCG possesses antioxidant[7], anti-inflammatory[8], anti-proliferative[9], and anti-cancer activities[10-13].

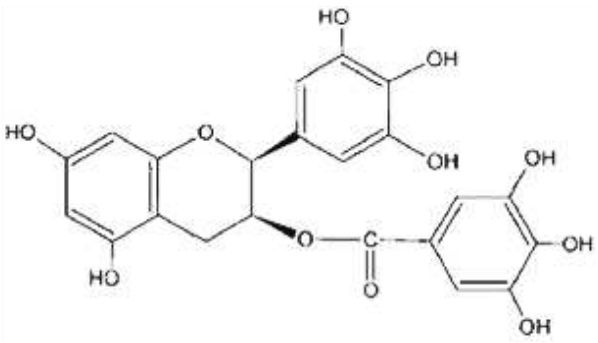

Figure 1. Structure of epigallocatechin 3-gallate (EGCG), the major polyphenolic constituent of green tea

2. Activity of EGCG in cancer cell

Green tea has potentially preventive effects against cancer. Several studies have demonstrated that EGCG have inhibitory effects against tumor formation and growth and anti-proliferative effects against many cancer cell lines and different animal model. Expression of the metastasis-associated 67-kDa laminin receptor confers EGCG responsiveness to cancer cells at physiologically relevant concerntrations[14]. In vitro studies have shown that EGCG can inhibit growth of human mammary and lung cancer cells, prostate cancer cells, lymphoma cells, leukemic cells, and lung and colon cancer cell lines by directly affecting the signaling pathways involved the activity of many protein kinases, blockage of the activation of transcription factors, such as activation protein-1 (AP-1) and nuclear factor κB (NF-κB), inhibition of cell proliferation, induction of apoptosis, modulation of cell cycle regulation as arrest of the cell cycle in G0/G1 phase, interference of receptor binding, and suppression of invasiveness and of angiogenesisin cell growth[15,16]. Epidermiological studies suggest also that EGCG has both antimatrix metalloproteinase and antiangiogenesis activities that can prevent the formation solid tumor and may be chemopreventative or inhibitory toward lung, skin, and liver cancer[17-19], bladder and ovarian tumors[20,21].

3. Activity of EGCG in normal cells

Cancer cells were more sensitive than normal cells to the anti-proliferative activity of EGCG and EGCG induced apoptosis in cancer, but not in normal cells. Although many experiments suggests chemopreventive effects of EGCG, activity of EGCG in normal cell was not mainly focus. Upon non-frozen preservation of vascular tissues, GTP pretreatment resulted in significantly maintained cellular viabilities and eNOS expressions, enhanced mechanical properties & arterialized vessels, histologically well-preserved vascular structures[22]. When the osteoblastic cells were subject to R-F/T, the synergistically protective effect of GTP addition to freezing solution on attachment, proliferation, ALP activity and morphology was evaluated[23]. Also, EGCG was proved to be effective in protecting cold-preserved hepatocytes from cold-induced injury[24,25]. The studies suggest that EGCG can protect to cell damages against ROS on normal cells and maintain lower metabolic activity in cells without freezing.

4. Nuclear factor κB (NF-κB) pathway

Chemopreventive agents regulate the molecules in the cell signal transduction pathways including NF-κB, Akt, MAPK, p53, AR, and ER pathways. By modulating cell signaling pathways, these components, among other mechanisms, activate cell death signals and induce apoptosis in precancerous or cancer cells, resulting in the inhibition of cancer development and/or progression[26]. Among many signaling pathways, various study indicate that EGCG can inhibit both AP-1 and NF-κB activities, and provide evidence that these effects play a role in the inhibition of both cell growth and malignant cell transformation by this compound[27-30]. EGCG mediated activation of caspases is critical, at least in part, for inhibition of NF-κB and subsequent apoptosis[31]. It has been well accepted that NF-κB of transcription factors signaling pathway plays important roles in the control of cell growth, apoptosis, inflammation, stress response, and many other physiological processes[32-36]. NF-kB consists of multiple members of the Rel family of proteins that include NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB and c-Rel. The prototypical NF-κB complex is a p50/RelA heterodimer. The NF-κB dimers are largely sequestered in the cytoplasm in an inactive form through its association with an IκB inhibitor[37]. The activation of NF-B can be divided into two phases. The first phase involves cytoplasmic events culminating in the activation of the IκB kinases (IKK1 and IKK2). These kinases promote N-terminal phosphorylation of serines 32 and 36 in IκB, leading to its polyubiquitylation and proteasome- mediated degradation. The liberated NF-κB complex rapidly translocates to the nucleus, ending the first phase[38]. The second phase occurs primarily in the nucleus and involves posttranslational modification of the NF-κB

transcription factor complex or relevant histones surrounding NF-κB target genes[39]. Both phosphorylation of RelA on serine 536 are required for NF-κB to achieve its full transcriptional activity and translocate to the nucleus following activation[40,41]. This process allows translocation of active NF-κB complexes into the nucleus, where they bind to specfic DNA motifs in the promoter/enhancer regions of target genes and activate their transcription. Modulation in the transactivation domains and/or nuclear translocation of NF-kB by a variety of stimuli leads to cell cycle arrest and apoptosis (Figure 2)[42,43].

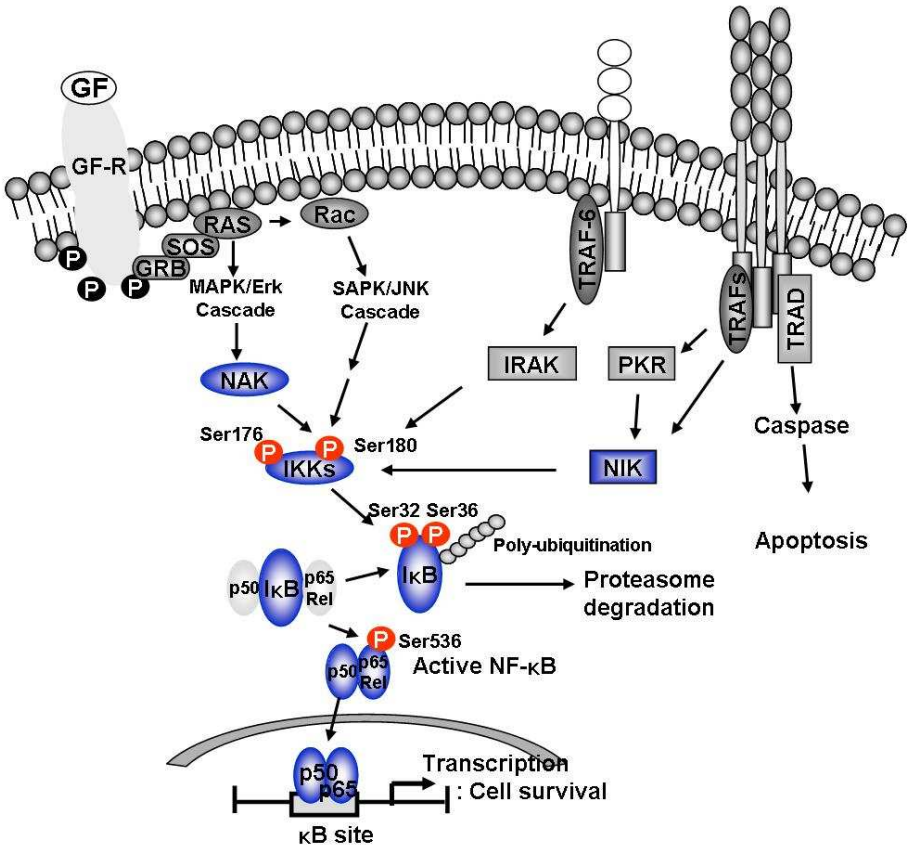

Figure 2. Nuclear factor κB (NF-κB) pathway

5. Objective of this study

The purpose of this study is compared whether EGCG, green tea polyphenol component possesses to differential biological responses in normal neonatal human dermal fibroblast versus HT-1080, human fibrosarcoma cell line, according to dose control.

In this study, we investigated the involvement of growth inhibition, induction of apoptosis, cell cycle distribution and NF-κB modulation as a mechanism of the differential biological response of EGCG in nHDF vs HT-1080 by EGCG. Furthermore, we observed change of gene expression in nHDFs with EGCG to compare the mRNA levels response of 3000 genes by using cDNA microarrrays.

II. MATERIALS AND METHODS

1. (-)-Epigallocatechin gallate (EGCG)

EGCG extracted from green tea were kindly supplied by prof. suong-hyu Hyon of PFI Inc., Kyoto, Japan. Its purity exceeded 99%. The EGCG was stocked as 100 mM dissolved in distilled water by filtering with syringe.

2. Cells and cell cultures

Human fibroblast cells, neonatal human dermal fibroblasts (nHDFs) were purchased from Cambrex corporation (East Rutherford, NJ, USA) and used between passages 5 to 15. Human fibrosarcoma cells, HT-1080 were obstained from Amerucan Type Culture Collection (Rockville, MD, USA). nHDFs were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) and a 1% antibiotic antimycotic solution (Sigma). HT-1080 cells were cultured in minimum essential medium (MEM, Gibco ™ Invitrogen Corp., Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Sigma). The cells were routinely maintained at 37℃ and 5% CO2 in a humid environment.

3. Cell growth inhibition assay

The nHDFs and HT-1080 were inoculated in culture wells (24-well culture plate, Falcon, NJ, SUA) with 1㎖ growth medium containing 1×10^5 cells, respectively. The cells were treated with EGCG ranging from 10 to 1000 μM for 24 h. The viability cells were quntified using MTT assay which measures the mitochondrial dehydrogenase activity of living cells, based on the reduction of the yellow tetrazolium salt-3-(4,5-dimethylthiazol-2-yi)-2,5-diphenyltetrazolium bromide (MTT, Sigma) by metabolically activity cells to form insoluble purple formazan crystals and was used to estimate cell viability and proliferation[44]. After cell incubation, the cells were incubated with 0.5 ㎎/㎖ MTT reagent in the last 4 h of the culture period tested at 37 ℃ in the dark. The media were decanted and then washed twice with PBS. Subsequently, the produced formazan salts were dissolved with dimethyl sulphoxid and the solution was transffered to a 96 wells plate (Falcon). The absorbance was measured using an ELISA reader (Spectra Max 340, Molecular Device Inc., CA, USA) at a wavelength of 570 nm.

4. DNA cell cycle analysis

nHDFs were incubated in 6-well plate for 24 h with EGCG at 100, 200, 300 and 400 μM in complete medium. HT-1080 were incubated for 24 h with EGCG at 10, 20, 40, 80, 100 and 200 μM in complete medium. The cells were trypsinized thereafter, washed twice with cold phosphate-buffered saline (PBS, 10 mM, pH 7.2), and centrifuged. The pellet was resuspended in 50 ㎕ of cold PBS and 450 ㎕ of cold ethanol (Sigma) for 1 h at 4 C. The cells were centrifuged at 1100 rpm for 5 min; the pellet was washed twice with cold PBS, suspended in 500 ㎕ of PBS and incubated with RNase (20 Units/ml, final concentration, Sigma) at 37 ℃ for 30 min. The cells were chilled over ice for 10 min, stained with propidium iodide (100 ㎍/㎖ final concentration) for 1 h and analyzed by flow cytometry (FCM, FACSCalibur, Becton Dickinson, San Jose, CA, USA).

5. Apoptosis assay

For quantification of apoptosis, nHDFs and HT-1080 were incubated at density of 5×10^5 cells in 6-well plate for 24 h with EGCG at 100, 200, 300 and 400 μM and 10, 20, 40, 80, 100 and 200 μM, respectively. The cells were trypsinized, washed with phosphate-buffered saline (PBS, 10 mM, pH 7.2), and processed for labeling with fluorescein labeled Annexin V (Annexin-V FITC) and propidium iodide (PI) by use of an ApoTarget apoptosis kit obtained from Biosource International, Inc. (Camarillo, CA, USA) as per product instruction. In brief, the cells were resuspened in 100㎕ of binding buffer. 5㎕ Annexin V-FITC and 10㎕ propidium iodide (50 ㎍/㎖) were added to the samples for 5-15 min in the dark. The labeled cells were then analyzed by flow cytometry (FCM, FACSCalibur, Becton Dickinson, San Jose, CA, USA). The data obtained was analyzed using the dot plot and histogram of CELLQuest software, written by Mac-App (Becton Dickinson).

6. Western blot

For western blotting of NF-κB expression, nHDFs and HT-1080 were incubated at density of 5×10^5 cells in 6-well plate for 24 h with EGCG at 100, 200, 300 and 400 μM and 10, 20, 40, 80, 100 and 200 μM, respectively. The cells were collected using a cell scraper after being washed with cold PBS and lysed with ice-cold RIPA buffer [150mM NaCl, 100mM Tris-HCl (pH 8.0), 1% Triton X-100, 1% Deoxy-cholic acid, 0.1% SDS, 5mM EDTA, 10mM NaF, 5mM DTT, 1mM PMSF, 1mM sodium vandidate, 20μM leupeptin, 100μM aprotinin]. The mixture was then centrifuged at 12,000 x g for 10 min at 4℃. The protein concentration was determined by the DC Bio-Rad assay using the manufacturer's protocol (Bio-Rad Laboratories, Inc., Hercules, CA). The 35 ㎍ of the protein from each extract was separated by 12.5% SDS-PAGE. The separated proteins were electrophoretically transferred to PVDF membrane. After blocking with a buffer containing 5% non-fat milk and 0.1% Tween 20 for 1h at room temperature, the membrane was probed with a rabbit polyclonal NF-κB antibody (Cell signalling technology, Danvers, MA, USA), a rabbit polyclonal phospho NF-κB(Ser 537) antibody (Cell signalling technology) and a mouse monoclonal GAPDH antibody for normalization (Chemicon International, Temecula, CA, USA) for 2 h at room temperature, respectively. After washing three times with TBS-T buffer, the membranes bonded antibody were detected with anti-rabbit secondary antibody HRP conjugated (Santa Cruz Biotechnology) or HRP anti-mouse IgG (BD Biosciences Pharmingen, Franklin Lakes, NJ, USA) for 1 h at room temperature. The membrane was washed again, and the signals were visualized by a reaction with an ECL kit (ECL plus, Amersham, Buckinghamshire, England) for 5 min and exposed to

Hyperfilm ECL (Amersham). The intensity of each band was measured with Scion image.

7. DNA Microarray

In order to investigate to regulation of gene expression on nHDF treated EGCG, DNA microarray was requested to Genocheck (Ansan, Korea). nHDFs were incubated for 24 h with or without EGCG (200 μM). This study used GenoCheck platinum human cancer 3.0K oligo chip of Qiagen (Hilden, Germany). Briefly process schema of this experiment shown Figure 3.

8. Statistical analysis

All experiments were performed in three times. The results are reported as a mean ± standard deviation and analyzed for significance using Student t-test (two tiled) (Excel 2003, Microsoft, WA, USA). The p-value of the effect had to be < 0.05% to be considered significant.

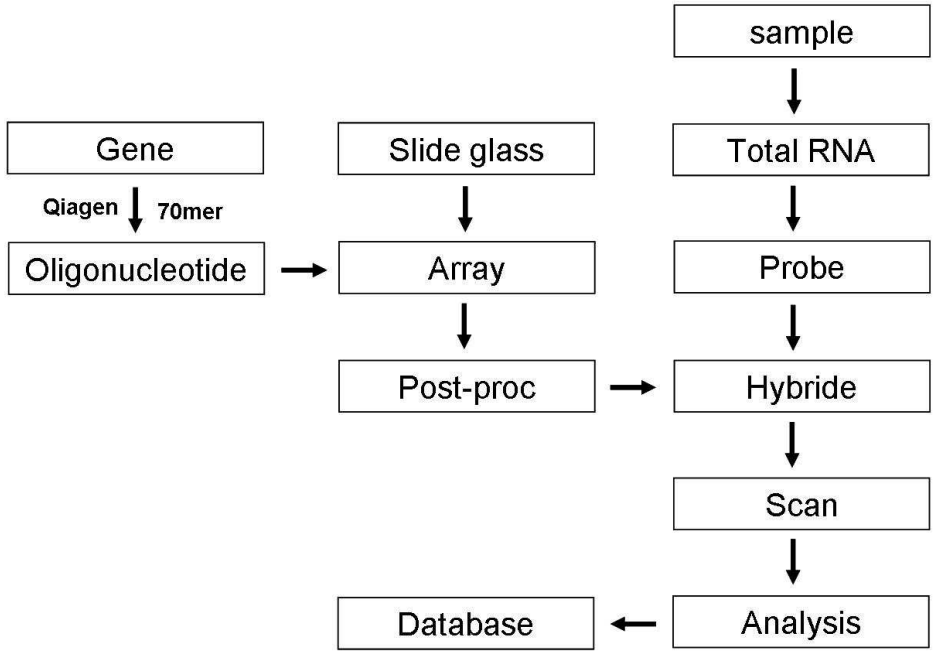

Figure 3. DNA microarray schema

III. RESULTS

1. The comparison of cells growth inhibition by EGCG in nHDF vs HT-1080

We examined effect of increasing concentration of EGCG on growth of the nHDFs and HT-1080 using MTT assay. EGCG treatment inhibited the growth in a dose-dependent manner. As shown in Figure 4, treatment with EGCG (10- 200 μM) during 24 h on HT-1080 resulted significantly inhibition of cell growth with concentration of upper 40 μM, inhibition of cell growth in a dose-dependent, which was more pronounced in upper 80 μM of EGCG treatment. However, nHDFs did impart significant inhibitory response at the highest dose of EGCG, about 10 folds of concentration for growth inhibition on HT-1080 (Figure 5).

2. Effect of cell cycle progression by EGCG

We were examined the effects of EGCG on cell cycle progression in nHDFs and HT-1080 cells. Cell cycle distribution analysis of nHDFs treated with varying concentration of EGCG (100-400 μM) showed a appreciable G0/G1 arrest (Figure 6). EGCG treatments increased the percentage of cells in G0/G1 phase at increasing treatment concentration of EGCG and the percentage of cells in S-phase was significantly reduced by EGCG on nHDFs. The data showed that cell cycle distribution of HT-1080 treated with 10-40 μM EGCG showed to don't any significant change (Figure 7).

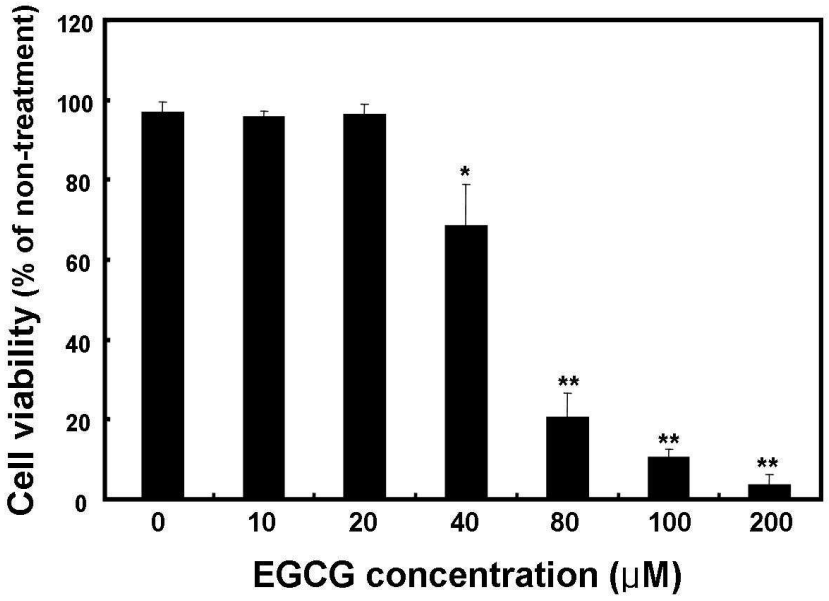

Figure 4. The effect of growth inhibition by EGCG in HT-1080. The data are represented as percent of control, where the control, non-treated cells, represent 100%. The results are reported as a mean ± SD (n = 3). The data is analyzed by a students' t-test, and the values are significantly different from the non-treated control (* $p < 0.05$, ** $p < 0.01$).

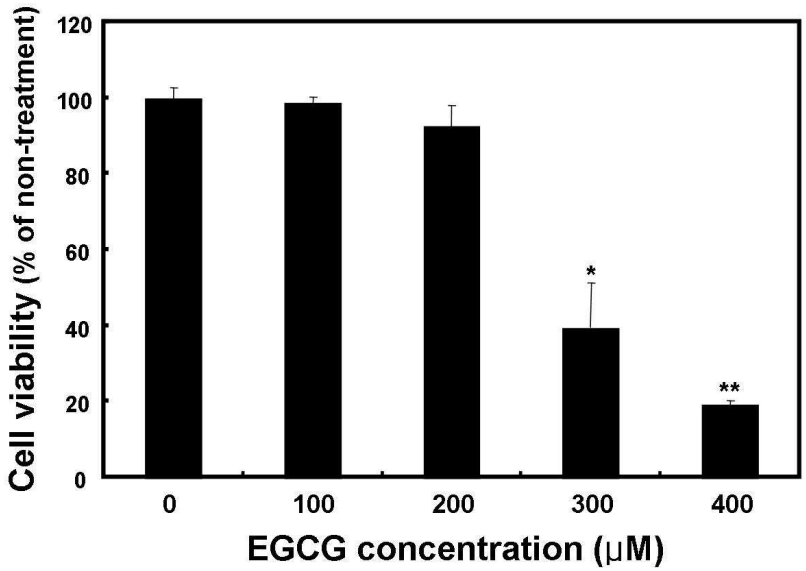

Figure 5. The effect of growth inhibition by EGCG in nHDFs. The data are represented as percent of control, where the control, non-treated cells, represent 100%. The results are reported as a mean ± SD (n = 3). The data is analyzed by a students' t-test, and the values are significantly different from the non-treated control (* $p < 0.05$, ** $p < 0.01$).

EGCG	% Cell Population		
(μM)	G0/G1	S	G2/M
0	44.5	28.4	27.1
100	66.1	28.8	5.1
200	78.4	21.1	0.5
300	86.3	7.6	5.1
400	89.5	6.2	4.4

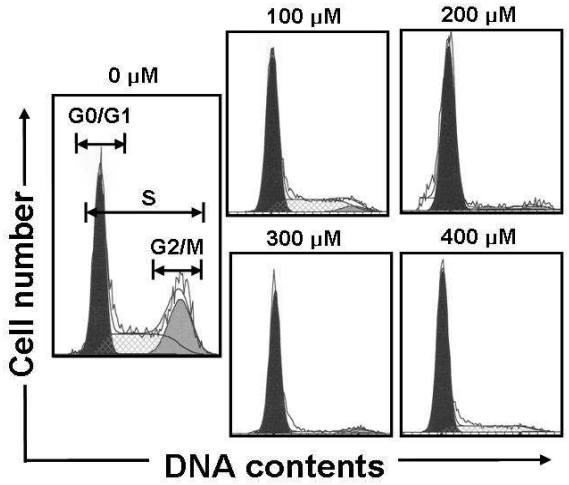

Figure 6. Effects of EGCG on cell cycle distribution in nHDF. The cells were treated with or without EGCG (100, 200, 300 and 400 μM for 24 h) and analyzed by flow cytometry. The percentages of cells in the G0/G1, S, and G2/M phases are calculated using Modifit computer software and represented within the histograms. The data shown at the upper panel are from a representative experiment repeated three times with similar results.

EGCG	% Cell Population		
(μM)	G0/G1	S	G2/M
0	58.1	30.3	11.6
10	51.7	35.3	13.0
20	46.4	39.3	14.3
40	47.9	37.6	15.5
80	67.1	24.6	8.3
100	68.9	20.5	10.6
200	69.2	20.9	9.9

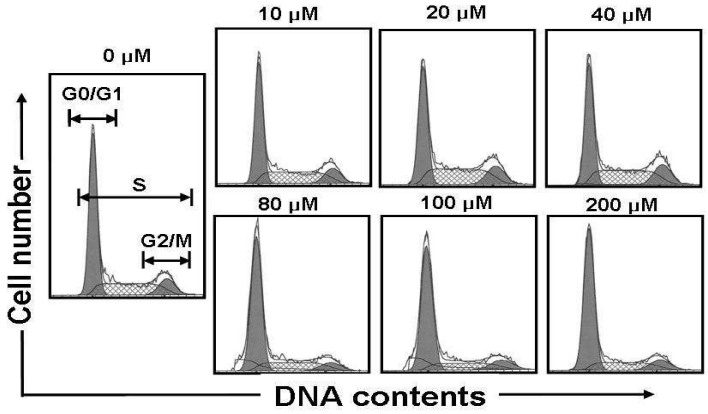

Figure 7. Effects of EGCG on cell cycle distribution in HT-1080. The cells were treated with or without EGCG (10, 20, 40, 80, 100 and 200 μM for 24 h) and analyzed by flow cytometry. The percentages of cells in the G0/G1, S, and G2/M phases are calculated using Modifit computer software and represented within the histograms. The data shown at the upper panel are from a representative experiment repeated three times with similar results.

However, under similar treatment condition treated on nHDFs, cell population in the G0/G1 phase of HT-1080 slightly increased. In this experiment, the G0/G1 cell population of controls at the specified doses did not change and ranged between 44 and 58%, probably because growing (unsynchronized) cells were employed in these experiments.

3. Induction of apoptosis

The effect of EGCG to observe the cell death inductionwas evaluated by using annexin V-FITC and PI double staining. Annexin V-FITC detects externalized cell membrane phosphatidylserine, which is one characteristic of apoptosis; and PI, normally impenetrable through the intact cell membrane, is able to stain the nucleus during middle or late stage apoptosis when membrane integrity is lost. With the used of an Annexin V-propidium iodide double staining regime, four populations of cells are distinguishable: (a) viable: annexin-V negative and PI negative; (b) early apoptotic cell : annexin-V positive and PI negative; (c) late apoptotic cells: annexin-V positive and PI positive; (d) necrotic cells: annexin-V negative and PI positive. We characterized the induction of apoptosis in nHDFs and in HT-1080 following EGCG treatment of 100-400 μM and 10-200 μM, respectively. As evident by the distribution of annexin V and PI, compared to non-treated control, EGCG treatment of nHDF cells resulted in significant apoptosis at 300 μM. The apoptosis was not seen at 100, 200 μM dose whereas at upper 300 μM dose, a slight indication of apoptosis was evident (Figure 8). Therefore, EGCG of high dose accompanied with necrosis of cells . nHDF treated EGCG (100-400 μM) induced apoptosis in a dose-dependent manner. EGCG treatment of HT-1080 was observed to induction of apoptosis at upper 40

μM. High dose as 200 μM, concentration to doesn't induce cell death on nHDF cells, HT-1080 was induced completely to apoptosis (Figure 9).

4. NF-κB inhibition of EGCG

Employing western blotting analysis, we investigated the effect of co-treatment of EGCG on activation of NF-κB. EGCG did not affect the protein level of the NF-kB p65 subunit in the cytoplasm both nHDF cells and HT1080 (Figure 10, 11). However, it decreased the active form, phospho-NF-kB p65(Ser536). As shown in Figure 12, EGCG treatment resulted in a remarkably decrease of NF-kB activity in comparison to non-treated and EGCG (100, 200, 300 and 400μM) alone treated groups. Also, we were found that treatment of HT-1080 with EGCG (from 10 to 200μM) inhibit NF-κB activation in a dose-dependent manner (Figure 13).

EGCG (μM)	survival[a)]	apoptosis		necrosis[d)]
		early[b)]	late[c)]	
0	96.96	0.38	0.61	2.05
100	96.75	0.35	0.32	2.58
200	97.07	0.13	0.15	2.66
300	60.57	18.82	13.5	7.11
400	32.02	3.71	5.32	58.95

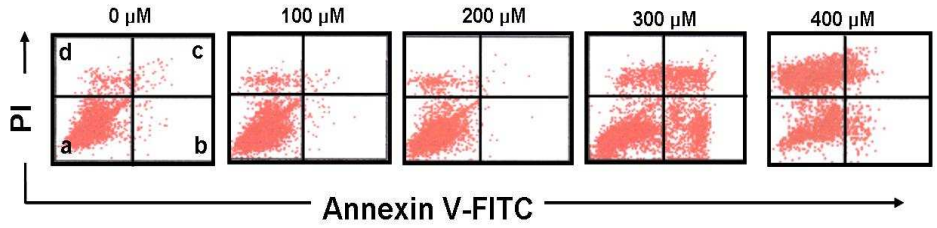

Figure 8. Induction of apoptosis by treated EGCG in nHDFs. The cells were treated with or without EGCG (100, 200, 300 and 400 μM for 24 h) and analyzed by flow cytometry. The percentages of cells in the viable, early apoptosis, late apoptosis and necrosis phase are calculated using the dot plot and histogram of CELLQuest software. The data shown at the upper panel are from a representative experiment repeated three times with similar results.

EGCG (μM)	survival[a)]	apoptosis		necrosis[d)]
		early[b)]	late[c)]	
0	86.17	12.40	1.32	0.10
10	83.02	15.92	1.05	0.01
20	81.80	17.51	0.62	0.07
40	79.68	17.14	3.05	0.13
80	21.95	9.08	68.37	0.61
100	12.05	6.30	80.09	1.56
200	0.96	1.76	95.07	2.01

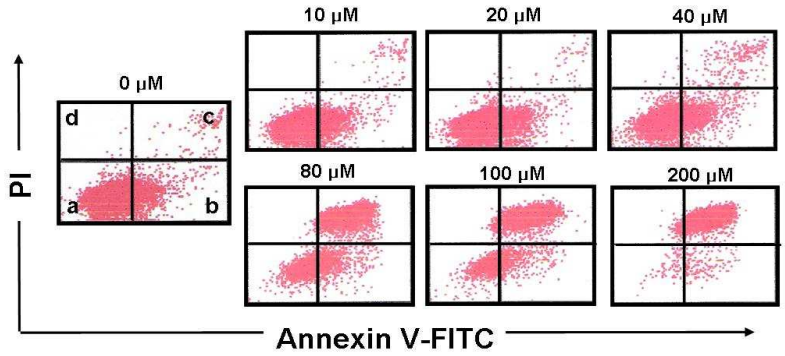

Figure 9. Induction of apoptosis by treated EGCG in HT-1080. The cells were treated with or without EGCG (10, 20, 40, 80, 100 and 200 μM for 24 h) and analyzed by flow cytometry. The percentages of cells in the viable, early apoptosis, late apoptosis and necrosis phase are calculated using the dot plot and histogram of CELLQuest software. The data shown at the upper panel are from a representative experiment repeated three times with similar results.

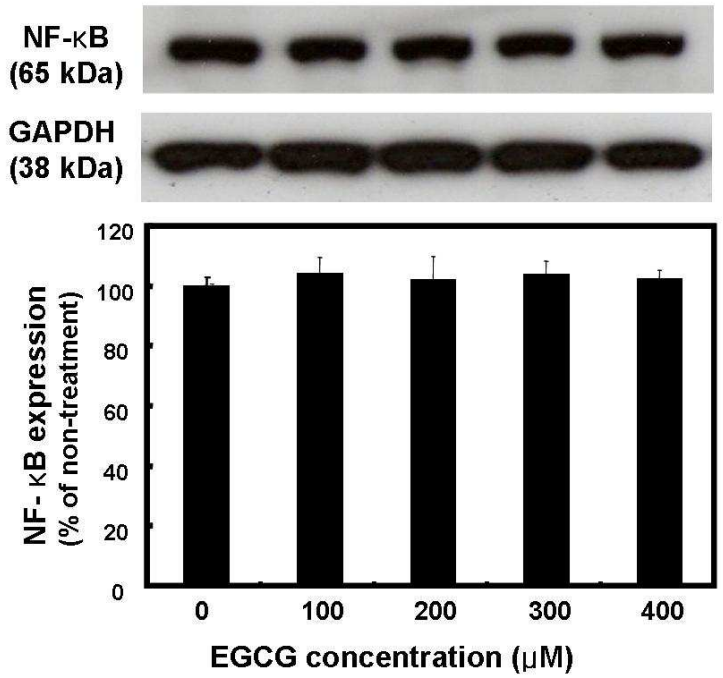

Figure 10. Effect of EGCG on the expression of NF-κB in nHDF. The blot shown in the upper panel is representative of 3 independent experiments showing similar results. The down panel shows the densitometric analyses of the 3 different blots. The data is analyzed by a students' t-test, and the results are not significantly different from the non-treated group ($p < 0.05$).

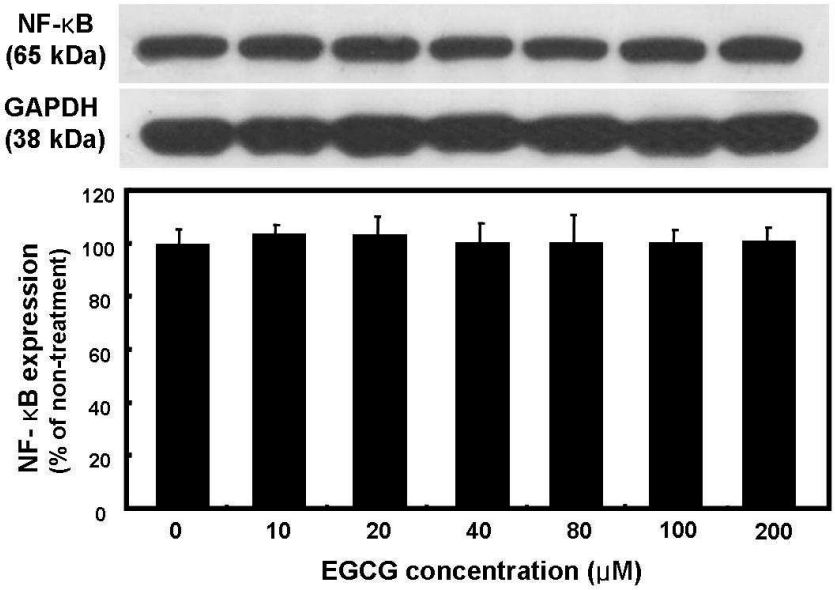

Figure 11.Effect of EGCG on the expression of NF-κB in HT-1080. The blot shown in the upper panel is representative of 3 independent experiments showing similar results. The down panel shows the densitometric analyses of the 3 different blots. The data is analyzed by a students' t-test, and the results are not significantly different from the non-treated group ($p < 0.05$).

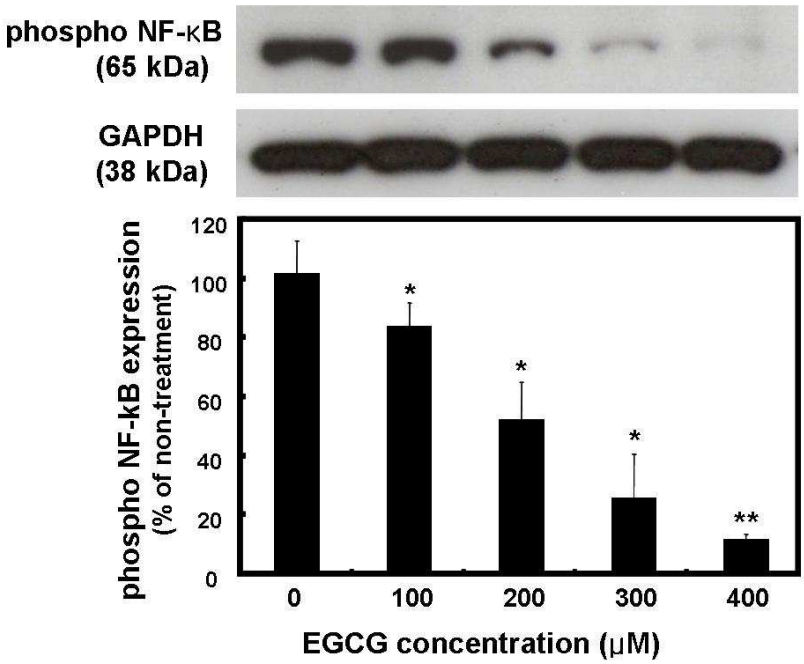

Figure 12. Effect of EGCG on the expression of phospho NF-κB in nHDFs. The blot shown in the upper panel is representative of 3 independent experiments showing similar results. The down panel shows the densitometric analyses of the 3 different blots. The data is analyzed by a students' t-test, and the results are significantly different from the non-treated group (*$p < 0.05$, ** $p < 0.01$).

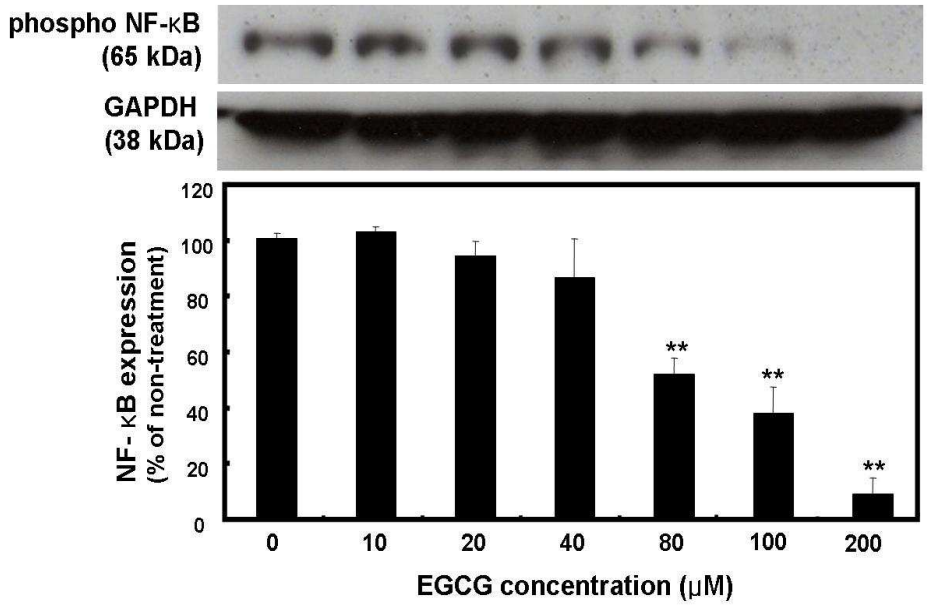

Figure 13. Effect of EGCG on the expression of phospho NF-κB in HT-1080. The blot shown in the upper panel is representative of 3 independent experiments showing similar results. The down panel shows the densitometric analyses of the 3 different blots. The data is analyzed by a students' t-test, and the results are significantly different from the non-treated group (*$p < 0.05$, ** $p < 0.01$).

5. Regulation of gene expression on nHDF cells by EGCG

We used DNA microarray system to determine whether the gene expression of normal cells, nHDF could be influenced by the treatment with EGCG 200μM, not affecting concentration to cell growth, during the 24 h treatment. The results demonstrated that the variation of gene expression from human cancer DNA chip shown that the expressions of 16 gene/protein were over-regulated more than at 2-fold and expressions of 47 gene/protein were down-regulated more than at 2-fold among 3000 gene/protein. in response to 200 μM EGCG during the 24-h treatment (data not shown), comparison with non-treted cells. Several patterns of change were observed as gene and protein related signal transduction, cell metabolism and cell communication etc. Regulation of genes related cell growth, death and cell cycle were mainly observed (Table 1). The roles of these genes in cell were described table 2 and 3.

Table 1. Oligo microarray determination of cell death and cycle related gene in nHDF with 200μM EGCG

Gene	Gene symbol	Fold
up-regulation		
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	3.3
DNA-damage-inducible transcript 3	DDIT3	2.2
Protein tyrosine phosphatase, receptor type, U	PTPRU	2.0
down -regulation		
Cell division cycle 2, G1 to S and G2 to M	CDC2	5.6
Cyclin A2	CCNA2	5.1
MAD2 mitotic arrest deficient-like 1 (yeast)	MAD2L1	3.7
Cyclin B2	CCNB2	3.5
Protein kinase, membrane associated tyrosine/threonine 1	PKMYT1	3.4
Cyclin B1	CCNB1	3.1
MCM 5 minichromosome maintenance deficient 5, cell division cycle 46 (*S. cerevisiae*)	MCM5	2.8

Table 2. The roles summary of over expressed gene in nHDF with 200μM EGCG

Gene	***Summary***
CDKN1A	○ A potent cyclin-dependent kinase inhibitor ○ Functions as a regulator of cell cycle progression at G1 ○ Can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair.
PTPRU	○ A member of the protein tyrosine phosphatase (PTP) family. ○PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation.
TNFRSF10C	○ Is not capable of inducing apoptosis ○ Function as an antagonistic receptor that protects cells from TRAIL-induced apoptosis. ○ The expression of this gene was detected in many normal tissues but not in most cancer cell lines, which may explain the specific sensitivity of cancer cells to the apoptosis-inducing activity of TRAIL.

Table 3. The roles summary of down expressed gene in nHDF with 200μM EGCG

Gene	***Summary***
CDC2	○ Encoded by this gene is a member of the Ser/Thr protein kinase family. ○ A catalytic subunit of the highly conserved protein kinase complex known as M-phase promoting factor (MPF), which is essential for G1/S and G2/M phase transitions of eukaryotic cell cycle. ○ The phosphorylation and dephosphorylation of this protein also play important regulatory roles in cell cycle control.
CCNA2	○ Function as regulators of CDK kinases. ○ This cyclin binds and activates CDC2 or CDK2 kinases, and thus promotes both cell cycle G1/S and G2/M transitions.
MAD2L1	○ Acomponent of the mitotic spindle assembly checkpoint that prevents the onset of anaphase until all chromosomes are properly aligned at the metaphase plate.
CCNB2	○ Play a key role in transforming growth factor beta-mediated cell cycle control.

Table 3. ~continued

Gene	***Summary***
PKMYT1	○ This kinase preferentially phosphorylates and inactivates cell division cycle 2 protein (CDC2), and thus negatively regulates cell cycle G2/M transition.
CCNB1	○ Encoded by this gene is a regulatory protein involved in mitosis. ○ Two alternative transcripts have been found, a constitutively expressed transcript and a cell cycle-regulated transcript, that is expressed predominantly during G2/M phase.
MCM5	○ The encoded protein is upregulated in the transition from the G0 to G1/S phase of the cell cycle and may actively participate in cell cycle regulation.

IV. DISCUSSION

Studies over the past 20 years have demonstrated that green tea polyphenol possess chemoprevention activities and antioxidant, anti-inflammatory, and antiapoptotic properties[45] EGCG have been shown to induce differential responses in normal versus tumor cells at similar concentration[46]. Normal cells are protected; e.g., EGCG protects human salivary acinar and duct cells from radiation and chemotherapeutic drug-induced apoptosis[47] and induced terminal differentiation in NHEK[48,49]. In contrast, tumor cells are induced to undergo apoptosis[50,51]. GTPP potently inhibited TNF-α, a proapoptotic cytokine[52], and EGCG inhibited TNF-α mediated activation of the NF-κB pathway, thereby protecting normal cells from TNF-α induced apoptosis[53]

We designed this study to investigate the involvement of nuclear transcription factor NF-kB as an important contributor for the differential growth inhibitory, cell cycle deregulatory and apoptotic effects of EGCG. For this study, we employed HT-1080 as the model cancer cell type and nHDF as the model normal cell type. The data obtained from cell growth and apoptosis assay as well as revealed that EGCG treatment caused a inhibition of cell growth and induced finally apoptosis at dose-dependent manner. Apoptosis is closely related to growth not only because its outcome is the opposite, but also because growth cells are more apoptosis prone. The striking observation of cell growth was that EGCG treatment was able to inhibit growth and induce apoptosis in nHDFs at the highest dose of upper 10 fold more than concentration employed for the cancer cells. Futhermore, nHDFs treated EGCG of high dose were undergone to necrosis, accomplished with apoptosis. These findings suggested that

EGCG could be developed as an agent for specifically eliminating cancer cells without affecting the normal cells at below 200 μM, but use of excessive concentration can cause to cell death in normal cells.

In cell cycle distribution, EGCG treatment induces G0/G1 arrest of slight fluctuation. However, striking results of G0/G1 arrest in nHDF observed at EGCG concentration not inhibition of cell growth and induction of apoptosis. It is interesting to observe that at these doses, in the nHDF, the cell cycle distribution results Go/G1 phase arrest. These phenomena might be due to the cytostatic effect of EGCG in nHDFs. Employing similar experimental conditions, it was established that EGCG treatment results in a significant inhibition of cell growth and function, a G0/G1-phase arrest of the cells in the cell cycle, and induction of apoptosis on justly differential dose level in both nHDF and HT-1080. Next, it was assessed the involvement of NF-κB during the biological responses to EGCG. The data obtained from western blot analysis revealed that EGCG treatment did not affect in the constitutive levels of NF-κB, in both nHDFs and HT-1080. These observations suggest the existence of possibilities for the mechanism of EGCG-mediated NF-κB inactivation and apoptosis: EGCG may cause phosphorylative degradation of NF-κB, thereby inhibiting the translocation of NF-κB. Recent studies have suggested an antiapoptotic function and the cell cycle regulation of NF-κB. It is also possible that EGCG-mediated downmodulation of NF-κB might, at least in part, be responsible for deregulation of the cell cycle machinery and apoptotic of cells[46] The mechanism of this differential response by different of EGCG concentraion is not known.

Since in the normal cells the responses are observed at very high doses, strategies may be crafted to use EGCG for chemoprevention and/or therapy against cancer. DNA microarray system to determine the gene expression of nHDF was observed mainly regulation of gene related cell growth,

death and cell cycle. To extend the DNA microarray data, the protein levels of cyclin B1 and cell division cycle 2 (cdc-2), down-regulated gene, in nHDFs treated EGCG (100, 200, 300 and 400 μM) should be further determined by Western analysis.

V. CONCLUSION

In this study, we evaluated whether EGCG, green tea polyphenol component possesses to differential biological responses in normal neonatal human dermal fibroblast versus HT-1080, human fibrodarcoma cell line, according to dose control.

Our results show that EGCG treatment inhibited the growth and activation of NF-κB, induced G0/G1 arrest and apoptosis in HT-1080 at dose dependent manner, but not in nHDF treated similarly EGCG dose. However, EGCG treatment of high dose inhibited the growth and activation of NF-κB, induced G0/G1 arrest and not only apoptosis but also necrosis in nHDFs. EGCG-caused cell cycle deregulation and apoptosis of cells may be mediated through NF-kB inhibition. In the results of DNA microarray, EGCG may be able to modulate the cell cycle arrest .

In conclusion, EGCG was acted more sensitive in HT-1080 than nHDFs, through observed the responses at very high dose and regulated expression of gene related cell cycle pathway and induced growth inhibition.

REFERENCES

1. Siddiqui IA, Afaq F, Adhami VM, Ahmad N, Mukhtar H. Antioxidants of the Beverage Tea in Promotion of Human Health. Antioxid Redox Signal. 2004;6(3):572-582.
2. Yang CS, Sang S, Lambert JD, Hou Z, Ju J, and Lu G. Possible mechanisms of the cancer-preventive activities of green tea. Mol Nutr Food Res. 2006;50(2):170-175.
3. Katiyar SK and Mukhtar H. Tea in chemoprevention of cancer: epidemiological and experimental studies. Int J Oncol 1996;8:221-238.
4. Wei H, Zhang X, Zhao JF, Wang ZY, Bickers D, and LebwohlM. Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. Free Radic Biol Med 1*996;* 26:1427-1435.
5. Liao S, Kao YH, and Hiipakka RA. Green tea: biochemical and biological basis for health benefits. Vitam Horm 2001;62: 1-94.
6. Green tea. Altern Med Rev 2000;5(2):372-375.
7. Katiyar SK, Elmets CA. Green tea polyphenolic antioxidants and skin photoprotection. Int J Oncol 2001; 18: 1307-1313
8. Lin YL, Lin JK. (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide- induced activity of transcription factor nuclear factor-kappaB. Mol Pharmacol 1997; 52: 465-472
9. Sachinidis A, Skach RA, Seul C, Ko Y, Hescheler J, Ahn HY, et al. Inhibition of the PDGF beta-receptor tyrosine phosphorylation and its downstream intracellular signal transduction pathway in rat and human vascular smooth muscle cells by different catechins. *FASEB J* 2002; 16: 893-895

10. Mukhtar H, Ahmad N. Tea Polyphenols: prevention of cancer and optimizing health. *Am J Clin Nutr* 2000; 71(6 Suppl): 1698S-1702S
11. Wang ZY, Huang MT, Ho CT, Chang R, Ma W, Ferraro T, Reuhl KR, Yang CS, Conney AH. Inhibitory effect of green tea on the growth of established skin papillomas in mice. *Cancer Res* 1992; 52: 6657-6665
12. Sazuka M, Murakami S, Isemura M, Satoh K, Nukiwa T. Inhibitory effects of green tea infusion on *in vitro* invasion and *in vivo* metastasis of mouse lung carcinoma cells. *Cancer Lett* 1995; 98: 27-31
13. Cao Y, Cao R. Angiogenesis inhibited by drinking tea. Nature 1999; 398: 381
14. Tachibana H, Koga K, Fujimura Y, Yamada K. A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol 2004;11:380-381.
15. Komori A, Yatsunami J, Okabe S, Abe S, Hara K, Sugamura M, et al. Anti-carcinogenic activity of green tea polyphenols. J Cancer Res Clin Oncol 1993;23:186-90.
16. Yang CS, Maliakal P, and Meng X.Inhibition of carcinogenesis by tea. Annual Review of Pharmacology and Toxicology 2002;42:25-052.
17. Lee IP, Kim YH, Kang MH, et al. Chemopreventative effect of green tea (*Camellia sinensis*) against cigarette smoke-induced mutations (SCE) in humans. J Cell Biochem 1997;27:S68-S75.
18. Picard D. The biochemistry of green tea polyphenols and their potential application in human skin cancer. Altern Med Rev 1996;1:31-42.
19. Hirose M, Hoshiya T, Akagi K, et al. Effects of green tea catechins in a rat multi-organ carcinogenesis model. Carcinogenesis 1993;14:1549-1553.
20. Sato D. Inhibition of urinary bladder tumors induced by N-butyl-N-(4-hydroxybutyl)-nitrosamine in rats by green tea. Int J Urol 1999;6:93-99.
21. Sugiyama T, Sadzuka Y. Enhancing effects of green tea components on the

antitumor activity of adriamycin against M5076 ovarian sarcoma. Cancer Lett 1998;133:19-26.

22.Han DW, Park YH, Kim JK, Lee KY, Hyon SH, and Suh H et al. Effects of green tea polyphenol on preservation of human saphenous vein. Biotech 2004;10:109-117

23. Han DW, Kim HH, Lee MH, Baek HS, Lee KY, and Hyon SH, et al. Protection of osteoblastic cells from freeze/thaw cycle-induced oxidative stress by green tea polyphenol. Biotech Let 2005;27:655-600.

24. Kagaya N, Tagawa YI, Nagashima H, Saijo R, Kawase M, and Yagi K. Suppression of cytotoxin-induced cell death in isolated hepatocytes by tea catechins. European Journal of Pharmacology 2002;450:231- 236

25.Kagaya N, Hara Y, Saijo R, Kamiyoshi A, Tagawa YI, Kawase M, et al. Novel function of rare catechin, epigallocatechin-3-(3″-O-methyl)gallate, against cold injury in primary rat hepatocytes. J Bioscie Bioengin 2003;96:559-563.

26. Sarkar FH, Li Y. Cell signaling pathways altered by natural chemopreventive agents. Mutation Research 2004;555: 53-64

27. Dong Z , Ma W, Huang C, Yang CS. Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (-.)-epigallocatechin gallate, and theaflavins. Cancer Res. 1997;57:4414-4419.

28. Nomura M, Ma W, Chen N, Bode AM, Dong Z. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced NFkappaB activation by tea polyphenols, (.-)-epigallocatechin gallate and theaflavins. Carcinogenesis 2000;21:1885-1890.

29.Afaq F, Adhami VM, Ahmad N, Mukhtar H, Inhibition of ultraviolet B-mediated activation of nuclear factor kappaB in normal human epidermal keratinocytes by green tea constituent (-.)-epigallocatechin-3-gallate.

Oncogene 2003;22:1035-1044.

30.Chung JY, Huang C, Meng X, Dong Z, Yang CS. Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-*ras*transformed cells: structure-activity relationship and mechanisms involved, Cancer Res.1999;59: 4610-4617.

31. Gupta S, Hastak K, Afaq F, Ahmad N, Mukhtar H. Essential role of caspases in epigallocatechin-3-gallate-mediated inhibition of nuclear factor kappaB and induction of apoptosis. Oncogene 2003; 1-16

32. Storz P, Toker A. NF-kappaB signalling-an alternate pathway for oxidative stress responses. Cell Cycle 2003;2:9-10.

33. Lin A. , Karin M. NF-kappaB in cancer: a marked target, Semin. Cancer Biol. 2003;13:107-114.

34. Li Q, Verma IM . NF-kappaB regulation in the immune system.Nat. Rev. Immunol. 2002;2:725-734.

35. Yamamoto Y, Gaynor RB, Role of the NF-kappaB pathway in the pathogenesis of human disease states. Curr. Mol. Med. 2001;1:287-296.

36. Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer:from innocent bystander to major culprit, Nat Rev Cancer. 2002;2:301-310.

37. Bours V, Bentires-Alj M, Hellin AC, Viatour P, Robe P,Delhalle S, Benoit V and Merville MP. (2000). Biochem.

38. Ghosh S, Karin M. Missing pieces in the NF-B puzzle. Cell 2002;109:S81-S96.

39. Chen LF, Greene WC. Shaping the nuclear action of NF-B.

Nat. Rev. Mol. Cell Biol. 2004;5:392-401.

40.Chen LF, Williams SA, Mu Y, Nakano H, Duerr JM, Buckbinder L, Greene WC. NF-κB RelA phosphorylation regulates RelA acetylation. molecular and biology 2005; 25(18): 7966-7975.

41.Sasaki CY, Barberi TJ, Ghosh P, Longo DL. Phosphorylation of RelA/p65

on serine 536 defines an IκB-independent NF-κB pathway. The Journal of biological cemistry 2005;280(41);34538-34547.

42. Joyce D, Albanese C, Steer J, Fu M, Bouzahzah B , Pestell RG. NF-kappaB and cell-cycle regulation: the cyclin connection. Cytokine Growth Factor Rev., 2001;12:73-90.

43. Karin M and Lin A. NF-kappaB at the crossroads of life and death.Nat. Immunol., 2002;3: 221-227.

44. Berridge V, Tan AS. Characterization of the cellular reduction of 3-(4,5 -dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. Arch Biochem Biophys 1993;303:474 482.

45. Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K et al. A new function of green tea: prevention of lifestyle-related diseases. Ann NY Acad Sci 2001;928:274-280.

46.Ahmad N, Gupta S, Mukhtar H. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kB in cancer cells versus normal cells. Arch Biochem Biophys 2000;376:338-346.

47. Yamamoto T, Staples J, Wataha J, Lewis J, Lockwood P, Schoenlein P, Rao S, Osaki T, Dickinson D, Kamatani T, et al. Protective effects of EGCG on salivary gland cells treated with gamma-radiation or cis-platinum(II) diammine dichloride. Anticancer Res 2004;24:3065-3073.

48. Hsu S, Bollag W, Lewis J, Huang Q, Singh B, Sharawy M et al. Green tea polyphenols induce differentiation and proliferation in epidermal keratinocytes. J Pharmacol Exp Ther 2003;306:29-3.,

49. Balasubramanian S, Sturniolo MT, Dubyak GR and Eckert RL. Human epidermal keratinocytesundergo(-)-epigallocatechin-3-gallate-dependent differentiation but not apoptosis. Carcinogenesis 2005;26(6):1100-1108 .

50. Ahmad N, Feyes DK, Nieminen AL, Agarwal R, Mukhtar H. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 1997;89:1881-1886.,

51. Yamamoto T, Hsu S, Lewis J, Wataha J, Dickinson D, Singh B et al. Green tea polyphenol causes differential oxidative environments in tumor versus normal epithelial cells. J Pharmacol Exp Ther 2003;307:230-236.

52. Yang F, de Villiers WJ, McClain CJ, Varilek GW. Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. J Nutr 1998;128:2334-2340.

53. Wheeler DS, Catravas JD, Odoms K, Denenberg A, Malhotra V, Wong HR. Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1- dependent proinflammatory signal transduction in cultured respiratory epithelial cells. J Nutr 2004; 134:1039-1044.

국문초록

섬유아세포와 섬유종양세포에서 녹차 폴리페놀 EGCG의 차별적인 생물학적 반응성 비교

<지도교수 박 종 철>

연세대학교 대학원 의과학과

이미희

녹차는 오늘날 세계에서 가장 많이 소비되는 음료중 하나로 약용의 성질은 널리 조사되어지고 있다. 녹차 폴리페놀은 이미 식품화학에서 강한 항산화제로 알려져있다. EGCG는 피러한 폴리페놀 중 하나로 카테킨의 약 50-80%으로 이루어져 있으며, 생물학적 효과를 매개하는 주된 물질중 하나이다. 이미 EGCG는 항산화, 항염증, 항증식, 항함활성을 가지고 있는 것으로 알려져있다.

여러 연구에서 EGCG는 암세포의 화학적 예방효과를 가지며 정상세포의 손상을 예방한다고 증명하였다.

이 연구에서 우리는 정상세포인 nHDF와 암세포인 HT-1080에서 EGCG에 의한 차별적인 생물학적 반응의 메카니즘으로 세포성장, 세포사멸, 세포 주기 분포 및 NF-κB 변화의 관련성을 조사하였다.

EGCG의 처리는 처리농도에 의존하여 서로 다른 농도에서 두가지 세포에서 모두 (i) 세포성장의 억제, (ii) 세포주기의 G0/G1 분열억제 (iii) 세포사멸을 유도한다. Western blot 분석에서 EGCG의 서로 다른 농도에도 불구하고, 두 가지 세포 모두 phospho NF-kB의 발현이 감소되는 것을 확인하였다.

요약하면, EGCG에 매개된 세포성장 억제, NF-kB 발현의 억제, 세포사멸의 유도는 HT-1080 세포와 비교하며 더 높은 농도에서 일어난다. 또한 DNA microarray 결과 nHDF 세포에서 EGCG는 세포주기와 관련된 유전자들을 조절하는 것을 보여주었다.

결론적으로, EGCG는 정상세포와 암세포에서 서로 다른 농도를 기본으로 억제반응을 주는 것으로 확인되었으며 EGCG에 의한 일련의 반응인 세포 주기 통제, 세포사멸 유도, 세포 성장 억제는 NF-kB 활성의 억제를 통하여 일어남을 제시한다.

핵심되는 말 : EGCG, 섬유종양세포 (HT-1080), 정상 섬유아세포 (nHDF), 세포주기, 세포사멸, NF-κB