

## Quercetin Potentiates TRAIL-induced Apoptosis in Human Colon KM12 Cells

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Received June 15, 2009 / Accepted July 28, 2009

Many cancer cells are sensitive to the TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis. However, some cancer cells show either partial or complete resistance to TRAIL. Human colon carcinoma KM12 cells have been shown to be insensitive to TRAIL-induced apoptosis. To overcome TRAIL resistance in KM12 cells, we targeted key anti-apoptotic molecules involved in the modulation of TRAIL resistance in the cells, and evaluated the effects of quercetin as a TRAIL sensitizer in the cells. We found that quercetin acted in synergy with TRAIL to enhance TRAIL-induced apoptosis in KM12 cells by the down-regulation of c-FLIP and DNA-PKcs/Akt and up-regulation of death receptors (DR4/DR5), which led to the enhancement of TRAIL-mediated activation of caspases and subsequent cleavage of PARP, as well as up-regulation of Bax. These findings suggest that the DNA-PKcs/Akt signaling pathway, as well as c-FLIP, play essential roles in regulating cells in the escape from TRAIL-induced apoptosis. Based on these results, this study provides a potential application of quercetin in combination with TRAIL in the treatment of human colon cancer.

**Key words** : TRAIL, quercetin, DNA-PK/Akt, c-FLIP, KM12 cells, caspase

### Introduction

The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a promising candidate for novel cancer therapies because of its ability to selectively induce apoptosis in cancer cells with no toxicity to normal cells [1,18,41]. TRAIL is a transmembrane protein that functions by binding two closely related receptors, DR4 and DR5. In response to TRAIL, these receptors recruit the adaptor protein FADD (Fas-associated death domain) and the caspase-dependent apoptotic pathway is initiated [24]. Although TRAIL is capable of inducing apoptosis in a wide variety of cancer cells, some cancer cells have shown resistance to the pro-apoptotic effects of TRAIL [5,20,29,35]. Resistance to TRAIL is an important therapeutic problem that may be circumvented by a combination of treatments that act by a variety of mechanisms including a decrease in c-FLIP levels or restoration of caspase-8 expression [34]. Understanding the basis of TRAIL resistance in cancer cells might provide information that would help in the develop-

ment of novel strategies for the clinical application of TRAIL to patients with cancer. TRAIL has shown limited efficacy in the treatment of several colon cancer cell lines due to intrinsic or acquired resistance [11].

The reason for the resistance to TRAIL is not explained solely by the different expression of the receptors. There are many factors contributing to the resistance to TRAIL-induced apoptosis. Another mechanism potentially involved in the regulation of TRAIL sensitivity is expressional modulation of the cellular FADD-like interleukin-1 converting enzyme inhibitory protein (c-FLIP) in the cells [32]. The caspase-8 homologue c-FLIP can inhibit caspase-8 activation and TRAIL-induced apoptosis [27]. This caspase-8 inhibitor is predominantly expressed in two forms (c-FLIP<sub>L</sub> and c-FLIP<sub>S</sub>). The c-FLIP<sub>S</sub> completely inhibits procaspase-8 activation at the death inducing signaling complex, while c-FLIP<sub>L</sub> permits partial cleavage of procaspase-8 to an intermediate p41/p43 form but prevents further processing of procaspase-8 to its active p18/p10 subunits [25]. Elevated expression of c-FLIP has been found in various types of tumor cells such as colorectal carcinoma, gastric carcinoma and pancreatic carcinoma [6,23,39].

Among the cellular signaling pathways that promote cell survival, Akt, a serine/threonine protein kinase, is an im-

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portant survival factor that contributes resistance to TRAIL [3,21,23]. Akt has been shown to inhibit apoptosis and cytochrome c release induced by several pro-apoptotic Bcl-2 family members [13]. A recent report suggested that phosphorylation of Akt at Ser473 may be mediated by DNA-dependent protein kinase (DNA-PK) [7]. DNA-PK, a member of the PI3K-related kinase subfamily of protein kinases, is known to play a central role in the repair of double-stranded DNA breaks and is composed of a large catalytic subunit (DNA-PKcs) and a DNA binding heterodimer (Ku70 and Ku80) [40]. As most agents used in such combinations are inherently toxic, it is imperative to identify nontoxic agents. It has been shown that quercetin (3,3',4',5,7-pentahydroxyflavone), a ubiquitous bioactive plant flavonoid, promotes TRAIL-induced apoptotic death by dephosphorylation of Akt [16]. Quercetin has a wide range of biological activity including induction of apoptosis and inhibition of a variety of enzymes involved in proliferation and in the signal transduction pathways [9].

We examined the cytotoxic effects of quercetin, in combination with TRAIL, in human colon carcinoma KM12 cells. We demonstrate here that combined treatment with quercetin and TRAIL effectively induces apoptotic cell death in TRAIL-resistant KM12 cells. The results of this study suggest that the down-regulation of c-FLIP and DNA-PKcs/Akt signaling pathway by treatment with quercetin might be involved in the sensitization of KM12 cells to TRAIL-induced apoptosis.

## Materials and Methods

### Cell culture

The human colon carcinoma cell line KM12 was provided by Dr. Isaih J. Fidler (University of Texas M.D. Anderson Cancer Center, Houston, TX, USA). The KM12 cells were maintained in culture (5% CO<sub>2</sub> and 95% air at 37°C) as adherent monolayers in DMEM supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and vitamin solution.

### MTT assay for determining drug sensitivity

Cell proliferation was measured by the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Company, St. Louis, MO., USA) colorimetric dye-reduction method. Exponentially growing cells ( $1 \times 10^4$  cells/well) were plated in a 96 well plate and incubated in

growth medium treated under the indicated condition with TRAIL and/or quercetin at 37°C. After 96 hr, the medium was aspirated using centrifugation and MTT-formazan crystals solubilized in 100  $\mu$ l DMSO. The optical density of each sample at 570 nm was measured using an ELISA reader. The optical density of the media was proportional to the number of viable cells. Inhibition of proliferation was evaluated as a percentage of growth control (no drug in the sample). All experiments were repeated at least two experiments in triplicate.

### Flow cytometric analysis of TRAIL receptors

KM12 cells ( $5 \times 10^5$  cells/well) treated with TRAIL in the presence or absence of quercetin were spun down at  $500 \times g$ , washed with phosphate-buffered saline (PBS) and resuspended in 500  $\mu$ l PBS. The cells were then incubated with 5  $\mu$ l of goat IgG2a, or anti-DR4 or anti-DR5 polyclonal goat antibody (1:100, R&D, Minneapolis, MN) for 1 hr, respectively. After washing with PBS, FITC-conjugated rabbit anti-goat polyclonal antibody (1:200, Sigma Chemical Co., St. Louis, MO., USA) was added to the cell suspension and incubated for 1 hr on ice followed by washing with PBS. After rinsing, the samples were analyzed by flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The data were analyzed using the CellQuest program.

### Western blot analysis

Protein samples were separated by SDS-PAGE and blotted to a nitrocellulose membrane (Hybond-ECL, GE Healthcare). The membrane was incubated with antibody as specified, followed by secondary antibody conjugated with horseradish peroxidase. Specific antigen-antibody complexes were detected by enhanced chemiluminescence (PerkinElmer, Life science). Western blot analysis was performed with the following antibodies: Caspase-3, PARP, phospho-Bad and Bax antibodies (Santa Cruz Biotechnology, CA., USA), anti-Akt, phospho-Akt (Ser-473), Caspase-8 and Caspase-9 antibodies (Cell Signaling Technology, MA., USA), and  $\beta$ -actin antibodies (Sigma-Aldrich, St. Louis, MO., USA), anti-DNA-PKcs antibody (Thermo Fisher Scientific, CA., USA). Secondary antibodies were obtained from GE Healthcare.

### Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Endogenous mRNA of total cellular RNA from KM12 cells

treated with TRAIL was isolated using a RNeasy Mini Kit (Qiagen, Hilden Germany) according to the manufacturer's protocol and assessed by RT-PCR with each dNTP and 1 µg oligo dT. Amplification of 1 µl of these cDNA by PCR was performed using the following gene-specific primers:

DR4 (forward), 5'-CTGAGCAACGCAGACTCGCTGTCCAC-3' and (reverse), 5'-AAGGACACGGCAGAGCCTGTGCAT-3'; DR5 (forward), 5'-CTGAAAGGCATCTGCTCAGGTG-3' and (reverse), 5'-CAGAGTCTGCATTACCTTCTAG-3'; FLIP<sub>L</sub> (forward), 5'-GCTGAAGTCATCCATCAGGT-3' and (reverse), 5'-CATACTGAGATGCAAGAATT-3'; FLIP<sub>S</sub> (forward), 5'-GCTGAAGTCATCCATCAGGT-3' and (reverse), 5'-GATCAGGACAATGGGCATAG-3'; β-actin (forward), 5'-CAGAGCAAGAGAGGCATCCT-3' and (reverse), 5'-TTGAAGGTCTCAAACATGAT-3'. The resulting total cDNA was used in the PCR performed in total volume of 25 µl using the TaKaRa Taq™ Kit (Takara) at 94°C for denaturation of 60 sec, 60°C for annealing of 60 sec, and 72°C for amplification for 90 sec for 30 cycles, followed by a final extension at 72°C for 12 min. The amplified fragments were separated on a 1.5% agarose gel and visualized with ethidium bromide staining.

#### Apoptosis assessment by Annexin V staining

The cells ( $2 \times 10^5$  cells/ml) were treated with or without TRAIL and/or each agent indicated for 8 hr and the cells were then centrifuged and resuspended in 500 µl of the staining solution containing Annexin V fluorescein (FITC Apoptosis detection kit; BD ParMingen San Diego, CA, USA) and propidium iodide in PBS. After incubation at room temperature for 15 min, cells were analyzed by flow cytometry. Annexin V binds to the cells that express phosphatidyl serine on the outer layer of the cell membrane, and propidium iodide stains the cellular DNA of the cells with a compromised cell membrane. These procedures allow for the discrimination of live cells (unstained with either fluorochrome) from apoptotic cells (stained only with Annexin V) and necrotic cells (stained with both Annexin V and propidium iodide).

#### Statistical analysis

The results obtained were expressed as the mean ± S.E. of at least three independent experiments. The statistical significance of differences assessed using the Student's *t*-test and two-way ANOVA (GraphPad software; GraphPad, Santiago, CA) with Bonferroni posttests.  $p < 0.05$  was consid-

ered statistically significant in all experiments.

## Results

### Sensitivity of KM12 cells to TRAIL-induced cytotoxicity and apoptosis

We first examined the susceptibility of KM12 cells to TRAIL-mediated cytotoxicity. The KM12 cells were treated with graded single doses of TRAIL (1~100 ng/ml) for 96 hr, and then the cell viability was measured using a MTT assay. As shown in Fig. 1A, KM12 cells were insensitive to TRAIL-induced cytotoxicity. To more confirm the TRAIL susceptibility of KM12 cells, we examined the binding of Annexin-V by flow cytometry after 8 h of treatment with TRAIL (5- or 25 ng/ml). As expected, the cells also exhibited resistance to TRAIL-induced apoptosis (about 4% at 25 ng/ml of TRAIL) (Fig. 1B). Therefore, these results demonstrate that KM12 cells were insensitive to the effects of TRAIL.

Since TRAIL is known to trigger apoptotic signals via two types of death receptors, DR4 and DR5, we determined whether the mRNA levels and cell surface expression of DR4/DR5 were modulated by TRAIL in the KM12 cells. The DR4 and DR5 mRNAs have been found to be expressed in these cells. The mRNA level of DR5 was slightly increased after treatment with TRAIL, whereas the level of DR4 mRNA was not altered by treatment with TRAIL (Fig. 2A).

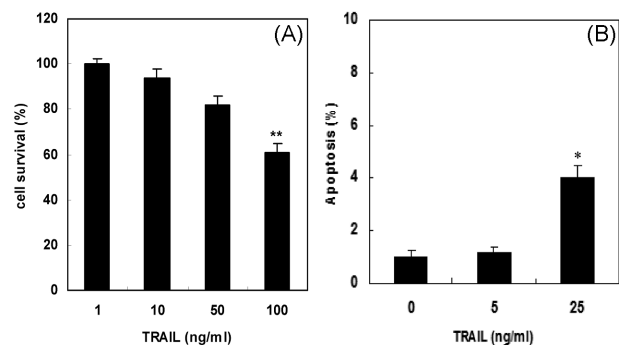


Fig. 1. The susceptibility to TRAIL-mediated cytotoxicity and apoptosis in KM12 cells. (A) The cells were treated with graded single doses of TRAIL (1- to 100 ng/ml), and the percent of cell survival was determined 96 hr after incubation using the MTT assay. (B) Cells were treated with TRAIL (5- and 25 ng/ml) for 8 hr and, the percent of apoptotic cells in each cell population was determined by Annexin V staining and flow cytometry. Each value represents the mean ± S.E. of triplicate determinants. \* $p < 0.05$ , \*\* $p < 0.005$  versus untreated cells.

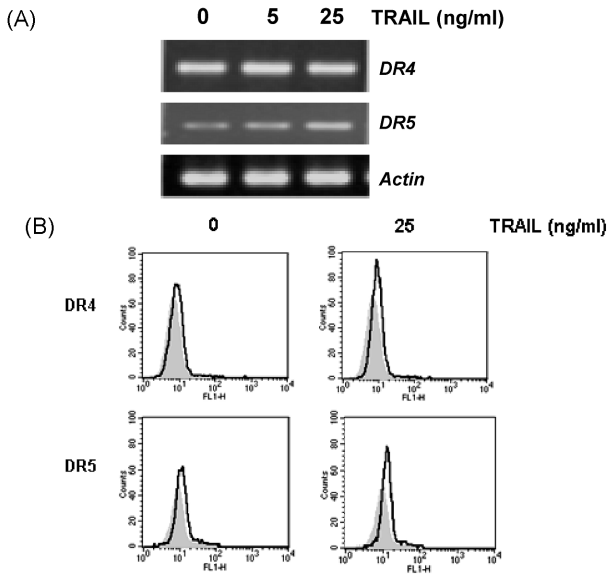


Fig. 2. Effect of TRAIL on mRNA and cell surface expression levels of DR4/DR5 in KM12 cells. (A) The mRNA levels of DR4 and DR5 in the cells treated with or without TRAIL (5- and 25 ng/ml for 8 hr) were determined by RT-PCR. (B) Cells were treated with 25 ng/ml TRAIL for 8 hr. Thereafter, cells were stained with control IgG or anti-DR4 (or -DR5) antibody (1:100), and subsequently labeled with FITC-conjugated secondary antibodies (1:200) to determine the surface expression of DR4 and DR5. The cell surface expression was measured by a flow cytometer. Shaded and unshaded peaks correspond to control and specific stainings, respectively

Moreover, the cell surface expression of DR4/DR5 was not significantly altered by TRAIL treatment (Fig. 2B). These data suggest that low expression of death receptors on the cell surface in KM12 cells was associated with a poor response to TRAIL.

### Cooperation of quercetin and TRAIL to induce apoptosis in KM12 cells

In search of an effective treatment strategy for TRAIL-resistant KM12 cells, we evaluated whether polyphenol quercetin could act as a potent sensitizer of TRAIL in the KM12 cells. When KM12 cells were treated with TRAIL (1- to 50 ng/ml) in the absence or presence of quercetin (10- or 100 uM) for 96 hr, the susceptibility to TRAIL-induced cytotoxicity was measured by the MTT assay. As shown in Fig. 3A, the KM12 cells were insensitive to TRAIL alone. However, the combination of TRAIL and quercetin significantly increased the cytotoxicity in the cells. These findings suggest that quercetin in combination with TRAIL promoted TRAIL-induced cytotoxicity in KM12 cells in a

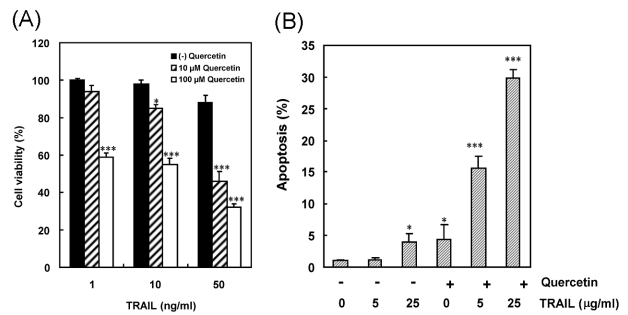


Fig. 3. Effect of quercetin on TRAIL-mediated cytotoxicity and apoptosis in KM12 cells. (A) The cells were treated with the indicated doses of TRAIL in the presence (10- or 100 uM), or absence of quercetin for 96 hr, and the percent of cell survival was determined using the MTT assay. (B) Cells were exposed to TRAIL (5- or 25 ng/ml) in the presence or absence of 50uM quercetin for 8 hr, and thereafter, the percent of apoptotic cells in each cell population was determined by Annexin V staining and flow cytometry. Each value represents the mean±S.E. of triplicate determinants. \* $p < 0.05$ , \*\*\* $p < 0.001$  versus TRAIL alone treated cells at the same dose point.

dose-dependent manner. In addition, we examined whether TRAIL-induced apoptosis was increased by treatment with quercetin when the KM12 cells were treated with TRAIL in the presence or absence of quercetin (Fig. 3B). As expected, TRAIL and quercetin synergistically induced apoptosis in the KM12 cells, whereas treatment of the cells with TRAIL or quercetin alone resulted in a low level of apoptosis induction in the cells. Therefore, our data suggest that quercetin enhanced the cytotoxic and apoptotic effects of TRAIL in KM12 cells.

### Correlation between sensitization of KM12 cells by quercetin to TRAIL-induced apoptosis with down-regulation of c-FLIP

Since the expression of cellular FLICE-inhibitory protein (c-FLIP<sub>L</sub> and c-FLIP<sub>S</sub>) as well as DR4/DR5 potentially controls TRAIL responses and plays an important role in the increased susceptibility to TRAIL-induced apoptosis [17], we investigated the change in the mRNA and protein levels of c-FLIP<sub>L</sub> and c-FLIP<sub>S</sub> in KM12 cells by co-treatment with TRAIL and quercetin using RT-PCR and western blot analysis, respectively. The RT-PCR analysis confirmed that the mRNA level of c-FLIP<sub>L</sub> and c-FLIP<sub>S</sub>, especially c-FLIP<sub>S</sub>, were more significantly reduced in the cells co-treated with TRAIL and quercetin than with either agent alone (Fig. 4A), suggesting that the combined treatment with TRAIL and quercetin was effective against the inhibition of c-FLIP<sub>L/S</sub>

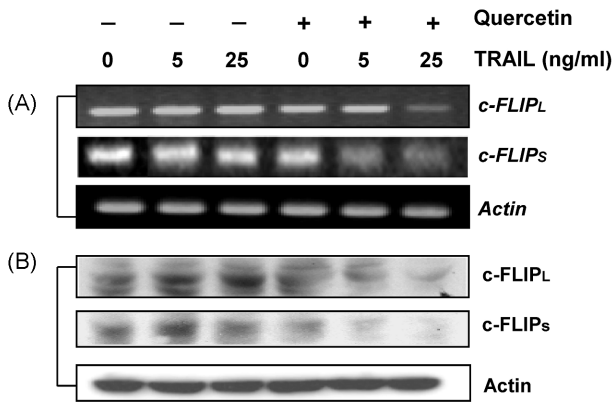


Fig. 4. The combination effect of quercetin and TRAIL on the expression of c-FLIP. The cells were treated with TRAIL (5 or 25 ng/ml) in the presence or absence of quercetin (50 mM) for 8 hr and RT-PCR was performed to monitor the changes in the mRNA level (A) and protein level (B) of c-FLIPL and c-FLIPs were determined by RT-PCR and western blot analysis, respectively. b-Actin (Actin) was used as an internal control for comparing the samples.

transcription. Consistent with results of c-FLIP<sub>L/S</sub> mRNAs, the western blot analysis showed that the protein levels of c-FLIP<sub>L</sub> and c-FLIP<sub>S</sub> in KM12 cells co-treated with TRAIL and quercetin were significantly decreased compared to the cells treated with each agent alone (Fig. 4B). These results indicated that sensitization of KM12 cells by quercetin to TRAIL-induced apoptosis might be closely associated with down-regulation of c-FLIP.

**Association with down-regulation of DNA-PKcs/Akt and the enhanced TRAIL susceptibility in KM12 cells treated with quercetin**

It has been reported that constitutively active Akt is an important regulator of TRAIL sensitivity, and that activation of Akt inhibits TRAIL-induced apoptosis [21]. In addition, a high level of phosphorylated Akt (p-Akt) is closely correlated with TRAIL resistance. The results of recent studies have suggested that DNA-PKcs acts upstream to Akt and directly phosphorylates and activates Akt [4]. Since it has been reported that quercetin inhibits DNA-PK activity [12], we investigated whether sensitization to TRAIL-induced apoptosis through quercetin-mediated down-regulation of DNA-PKcs/Akt was occurred in the KM12 cells. When the cells were treated with TRAIL in the presence or absence of quercetin for 8 hr, the changes in the levels of DNA-PKcs, pAkt and total Akt (tAkt) were determined (Fig. 5A). The levels of both DNA-PKcs and pAkt were significantly

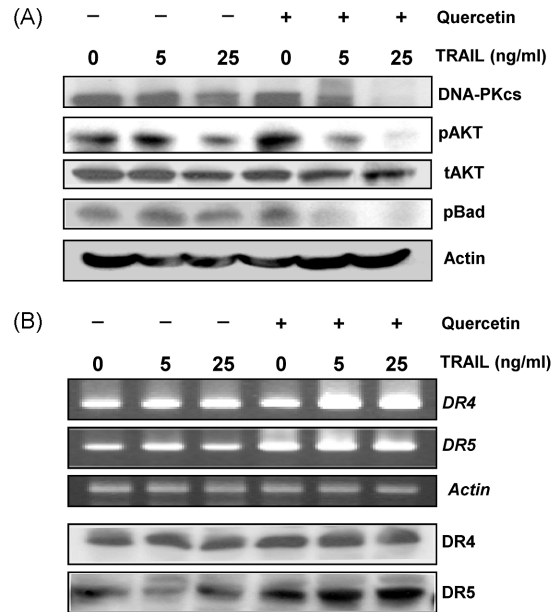


Fig. 5. Effect of quercetin on TRAIL-induced modulation of DNA-PKcs/Akt signaling molecules and expression of death receptors in KM12 cells. (A) The cells were treated with TRAIL (5- or 25 ng/ml) in the presence or absence of quercetin (50 mM) for 8 hr. Thereafter, western blot analysis was performed to monitor the changes in the levels of DNA-PKcs, phosphorylated Akt (pAkt), total Akt (tAkt) and phosphorylated Bad (pBad). Actin was used as an internal control for comparing the samples. (B) The changes in the mRNA level (upper panels) and protein level (lower panels) of DR4/DR5 in the cells co-treated with TRAIL and quercetin were determined by RT-PCR and western blot analysis, respectively.

reduced in the cells co-treated with quercetin and TRAIL, when compared to the cells treated with either agent alone. In addition, phosphorylation of Bad, a target molecule of Akt, was significantly decreased in the cells co-treated with quercetin and TRAIL. However, the total Akt level was not modulated by the combined treatment with TRAIL and quercetin. These results suggest that suppression of DNA-PKcs by quercetin leads to the inhibition of phosphorylation and activation of Akt, and are closely associated with enhanced TRAIL susceptibility in KM12 cells treated with quercetin. In addition, we examined whether the modulation of DR4/DR5 mRNA and protein levels by quercetin might be involved in its sensitizing effect on TRAIL-induced apoptosis in KM12 cells using RT-PCR and western blot analysis, respectively (Fig. 5B). We found that co-treatment of KM12 cells with TRAIL and quercetin induced an increase in the mRNA and protein levels of DR5, suggesting functional role of DR5 in the quercetin-mediated stimulation of TRAIL-in-

duced apoptosis.

### Involvement of activation of caspase cascade in the enhancement of TRAIL-induced apoptosis by quercetin treatment

Apoptosis follows a well-coordinated activation of cysteine proteases, known as caspases, which require proteolytic cleavage for activation. TRAIL-induced apoptosis requires the activation of caspases and poly-(ADP-ribose) polymerases (PARP) cleavage is a hallmark of caspase activation. TRAIL-induced activation of caspase-8 leads to activation of downstream caspases including caspase-9 and -3. To measure both the pro-forms and activated forms of initiator caspases (caspases-8) and the executioner caspases (caspases-3 and caspases-9) in the KM12 cells co-treated with TRAIL and quercetin, the cells were treated with quercetin (50  $\mu$ M) in the presence or absence of TRAIL (5- or 25 ng/ml for 8 hr). Western blotting analysis of using antibodies capable of recognizing both the pro-form and activated form of these caspases was performed (Fig. 6). Consistent with the more vigorous apoptotic response of

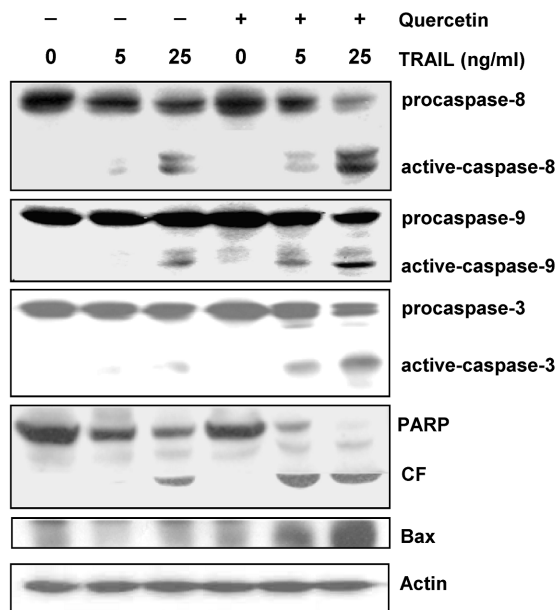


Fig. 6. Effect of quercetin on TRAIL-induced activation of caspases and PARP cleavage in KM12 cells. The cells were treated with quercetin (50 mM) in the presence or absence of TRAIL (5- or 25 ng/ml) for 8 hr and the levels of both total caspase (caspases-3, -8 or -9) and its active form and the levels of PARP cleavage and Bax were determined by western blot analysis. Actin could be used as an internal control for comparing the samples. CF: cleaved fragment.

KM12 cells to the combined treatment of TRAIL and quercetin, the cleavage and processing of procaspase-8 to its active forms occurred to a greater degree in the cells co-treated with TRAIL and quercetin compared to the cells treated with quercetin or TRAIL alone. Similar results were observed for caspase-9 and caspase-3 activation in KM12 cells co-treated with quercetin and TRAIL. Moreover, the combined treatment with quercetin and TRAIL in KM12 cells caused severe cleavage of PARP, a well known endogenous substrate of caspase-3, compared to the cells treated with quercetin or TRAIL alone. Furthermore, treatment with the TRAIL/quercetin combination led to the up-regulation of pro-apoptotic Bax. Therefore, these results demonstrate that the apoptotic potential of the combination of quercetin-TRAIL occurs through caspase-dependent pathways.

## Discussion

Recently, it has been reported that flavonoids sensitized *in vitro* cancer cells to apoptosis induced by anticancer agents for example the members of the TNF family: TNF- $\alpha$ , Fas or TRAIL [19,33]. Moreover, one strategy to overcome resistance to TRAIL in TRAIL-resistant cancer cells is to combine TRAIL with other agents that activate apoptotic pathways, and natural products that facilitate apoptosis in TRAIL-resistant cancer cells [8,37]. It has been reported that dietary polyphenols, commonly known as flavonoids, may act as chemopreventers by reducing the incidence of many types of cancers, especially in colon epithelia [10]. Indeed, it has been reported that combined treatment with TRAIL and dietary flavonoid quercetin enhanced TRAIL-mediated apoptosis in some cancer cells through various mechanisms depending on a wide range of biological activities of quercetin [15,28,30,31].

In this study, we found that quercetin acted synergistically with TRAIL to induce apoptosis in human colon carcinoma KM12 cells, and it occurs through new mechanism of the potentiation by quercetin of TRAIL. We revealed that the down-regulation of c-FLIP and DNA-PKcs/Akt signaling pathway by quercetin treatment, which overcame the TRAIL-resistance in the KM12 cells. Previous studies have shown that Akt is implicated in mediating a variety of biological responses and plays an important role in survival when cells are exposed to various kinds of apoptotic stimuli [2,38]. In addition, our finding suggest a new mechanism of TRAIL resistance that the expression of DNA-PKcs as well

as c-FLIP interfered with TRAIL-induced apoptotic signaling in KM12 cells through activation of the Akt signaling pathway.

DNA-PK acts upstream to Akt and is important for both phosphorylation and activation of Akt. This suggests that targeting of DNA-PK as a key modulator of TRAIL responsiveness might help to design TRAIL-based combinations for treatment of KM12 cells. Indeed, it has been reported that tumor cells resistant to anticancer drugs show increases in both DNA-PK expression and its kinase activity [36,42], and the use of inhibitors that have the inhibitory action of DNA-PK activity improved the effectiveness of anticancer drugs [14]. Therefore, TRAIL in combination with agents that down-regulate DNA-PK might have clinical applicability for the treatment of TRAIL-insensitive KM12 cells.

Since quercetin inhibits the expression and activity of both DNA-PK and Akt, we investigated whether quercetin could potentiate TRAIL-induced apoptosis in the KM12 cells- by inhibition of DNA-PK and p-Akt signaling cascade. The combination of TRAIL and quercetin resulted in the inhibition of Akt phosphorylation via down-regulation of DNA-PK in the cells. Moreover, quercetin-induced DR4/DR5 up-regulation might play an important role for the enhancement of TRAIL sensitivity in KM12 cells. In addition, sensitization of KM12 cells by quercetin to TRAIL-induced apoptosis might be closely associated with down-regulation of c-FLIP. Indeed, it has been reported that Akt may control the cytotoxic effect of TRAIL by modulating c-FLIPs expression in human gastric cancer cells [26]. Our results also showed that sensitization of KM12 cells by quercetin to TRAIL-induced apoptosis might be closely associated with down-regulation of the Akt/c-FLIP pathway.

Consistent with the more vigorous apoptotic response of KM12 cells to combined treatment with TRAIL and quercetin, our finding showed that processing of procaspase-8, -3, and -9 to their active forms occurred to a greater degree and subsequent cleavage of PARP and up-regulation of Bax in the cells co-treated with TRAIL and quercetin compared to the cells treated with quercetin or TRAIL alone. These results suggest that the apoptotic potential of combined quercetin-TRAIL might be occur by caspase-dependent pathways.

Therefore, our data suggest that quercetin was a potent sensitizer for TRAIL-induced apoptosis and thus might be a candidate to overcome resistance to TRAIL-insensitive other cancer cells expressing constitutive high levels of

DNA-PKcs/Akt/c-FLIP, and thus inhibition of these molecules by quercetin may be beneficial for anticancer therapy with TRAIL.

In conclusion, the finding of this study provides important insights into how quercetin promotes TRAIL-induced apoptosis in TRAIL-resistant KM12 cells. This model provides a framework for overcoming of TRAIL resistance in other cancer cells.

## Acknowledgements

This study was supported by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare and Family affairs, Korea (0920050) (S. H. Kim) and by Pusan National University Research Grant (C. D. Kang).

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### 초록 : 사람 대장암 KM12세포에서 quercetin 의한 TRAIL이 유도하는 세포사멸의 증가

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많은 암세포에 선택적 세포독성을 나타내는 TNF-related apoptosis-inducing ligand (TRAIL)는 유효한 항암제로서 사용될 수 있지만, TRAIL에 내성을 나타내는 암세포에는 TRAIL-sensitization의 방법이 필요하다. 사람 대장암 세포인 KM12 세포도 TRAIL에 내성을 나타낸다. 본 연구에서는 KM12세포의 TRAIL 내성에 대한 새로운 표적 분자의 발굴과 이를 토대로 한 새로운 내성극복 방법을 연구하였다. 새로운 TRAIL sensitizer로서 quercetin을 발굴하고, 이를 KM12세포에 TRAIL 과 병용 처리하여 TRAIL의 효과증강을 시도하였다. KM12세포에서 quercetin은 c-FLIP의 발현을 감소시키고, DNA-PK/Akt 신호전달경로를 억제함으로써, death receptors (DR4/DR5) 발현을 증강시켰다. 또한 caspases (caspase 3, -8 및 -9)활성 증가와 PARP cleavage, 이에 따른 Bax의 발현을 증강시키는 기전으로 TRAIL에 의한 apoptosis를 증대시키는 활성이 있음을 밝혔다. 즉 quercetin이 KM12세포의 TRAIL-sensitization에 사용될 수 있음을 제시하였다. 이러한 연구 결과는 대장암의 치료 시 TRAIL과 quercetin 병용함으로써 치료 효과를 높일 수 있는 새로운 약제 병용 방법을 제시하였고, 다른 TRAIL 내성 종양에 응용 될 수 있음을 시사하였다.