International Journal of Oral Biology, Vol. 40, No. 2 June 30 2015, p. 63-69

http://dx.doi.org/10.11620/IJOB.2015.40.2.063

International Journal of Oral Biology

Apoptotic Activity of Curcumin and EF-24 in HTB-41 Human Salivary Gland Epidermoid Carcinoma Cells

Ji-Won Kim[1], Seul Ah Lee[1], Dae-San Go[1], Byung-Sun Park[1], Su-Gwan Kim[1], Sun-Kyoung Yu[1], Ji-Su Oh[1], Chun Sung Kim[1], Jeongsun Kim[2], Jong-Tae Park[3], and Do Kyung Kim[1*]

[1]*Oral Biology Research Institute, Chosun University School of Dentistry, Gwangju 501-759, Republic of Korea*
[2]*Department of Dental Hygiene, Gwangju Health University, Gwangju 506-701, Republic of Korea*
[3]*Department of Oral Anatomy, Dankook University School of Dentistry, Cheonan 330-714, Republic of Korea*

(received March 22, 2015; revised Jun 09, 2015; accepted Jun 10, 2015)

Curcumin (diferuloylmethane), a constituent of turmeric powder derived from the rhizome of *Curcuma longa*, has been shown to inhibit the growth of various types of cancer cells by regulating cell proliferation and apoptosis. However, a need exists to design more effective analogs because of curcumin's poor intestinal absorption. EF-24 (diphenyl difluoroketone), the monoketone analog of curcumin, has shown good efficacy in anticancer screens. However, the effects of curcumin and EF-24 on salivary gland epidermoid carcinoma cells are not clearly established. The main goal of this study was to investigate the effects of curcumin and EF-24 on cell growth and induction of apoptosis in human salivary gland epidermoid carcinoma cells. Our studies showed that curcumin and EF-24 inhibited the growth of HTB-41 cells in a dose- and time-dependent manner, and the potency of EF-24 was > 34-fold that of curcumin. Treatment with curcumin or EF-24 resulted in nuclear condensation and fragmentation in HTB-41 cells, whereas the control HTB-41 cell nuclei retained their normal regular and oval shape. Curcumin and EF-24 promoted proteolytic cleavages of procaspase-3/-7/-9, resulting in an increase in the amount of cleaved caspase-3/-7/-9 in the HTB-41 cells. Caspase-3 and -7 activities were detected in viable HTB-41 cells treated with curcumin or EF-24. These results suggest that the curcumin and EF-24 inhibit cell proliferation and induce apoptosis in HTB-41 human salivary gland epidermoid carcinoma cells, and that they may have potential properties as an anti-cancer drug therapy.

Key words: EF-24, curcumin, cell death, apoptosis, salivary gland epidermoid carcinoma cells

*Correspondence to: Do Kyung Kim, Department of Oral Physiology, School of Dentistry, Chosun University, 375 Seosuk-dong, Dong-gu, Gwangju 501-759, Republic of Korea.
Tel.: +82-62-230-6893, Fax: +82-62-232-6896
E-mail: kdk@chosun.ac.kr

Introduction

Salivary gland tumors are a morphologically and clinically diverse group of neoplasms, which may present considerable diagnostic and management challenges to the pathologist or surgeon [1]. Salivary gland tumors are rare with an overall incidence in the Western world of about 2.5 - 3.0 per 100,000 per year [2]. Among salivary gland tumors, the salivary gland epidermoid cell carcinomas are more rare [2]. However, although this tumor has an extremely poor prognosis, unlike most other salivary gland malignancies, survival at 5 years is prognostically significant [2]. Therefore, an understanding of the molecular mechanisms of salivary gland epidermal carcinoma is one of the most important issues for treatment.

New therapeutic strategies are necessary to increase survival rates in patients with salivary gland epidermal carcinomas.

Recent studies have shown that such chemicals derived from natural materials have been identified to elicit chemopreventive and therapeutic effects [3-5]. It has been reported that this effect alters various factors associated with the cell cycle and thereby induce the apoptotic cell death [3-7]. Therefore, induction of apoptosis in cancer cells has become an important indicator of the cancer treatment response in employing a bioactive substance to reduce and control human mortality due to cancer [8,9]. There is a need to find new anti-cancer agents that can kill cancerous cells with minimal toxicity.

Apoptosis, which is a major way of programmed cell death, plays an important role in the regulation of tissue development and homeostasis in eukaryotes [10-12]. Apoptosis may occur via a death receptor-dependent extrinsic or a mitochondria-dependent intrinsic pathway and apoptosis is induced by treatment of chemotherapeutic agents [13,14].

Curcumin (diferuloylmethane), a constituent of turmeric powder derived from the rhizome of *Curcuma longa*, has antiinflammatory, antimicrobial, antioxidative, immunomodulating and antiatherogenic properties [15-18]. Many studies provide the fact that curcumin has chemopreventive and antiproliferative activity in various types of human cancers [18-23]. Furthermore, curcumin is also pharmacologically safe as it is a naturally occurring compound [24,25]. Unfortunately, natural curcumin has been limited the use because of its poor intestinal absorption [26]. Therefore it remains an excellent compound for the design of more effective analogs. One monoketone analog, EF-24 (diphenyl difluoroketone), is efficacious in anticancer screens [27,28]. Although it is shown to reduce cancer cell viability, its action mechanisms remain to be elucidated. In addition, the effects of EF-24 and curcumin on salivary gland epidermal carcinoma are not clearly established.

In this study, therefore, the effects of EF-24 and curcumin on cell growth and the mechanism of cell death elicited by EF-24 and curcumin were examined in HTB-41 human submaxillary salivary gland epidermoid carcinoma cells.

Materials and Methods

Drugs

EF-24 and curcumin (Fig. 1) were supplied by Sigma

Chemical structures of EF-24 and Curcumin

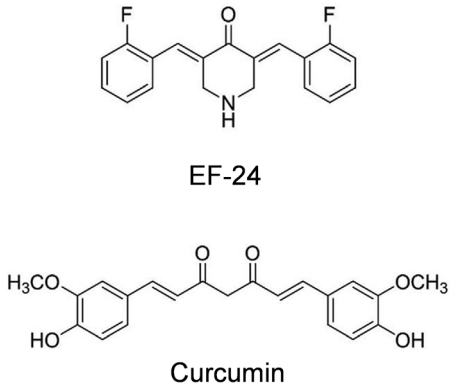

Fig. 1. Chemical structures of EF-24 (diphenyl difluoroketone) and curcumin (diferuloylmethane).

(St Louis, MO, USA). Anti-cleaved caspase-3, anti-cleaved caspase-7 and anti-cleaved caspase-9 antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The cell-permeable fluorogenic substrate PhiPhiLux-G_1D_2 was purchased from OncoImmunin, Inc. (Gaithersburg, MD, USA). Other analytical reagents were purchased based on the analytical grade.

Cell line and cell cultures

HTB-41 human submaxillary salivary gland epidermoid carcinoma cells were provided by American Type Culture Collection (ATCC, Rockville, MD, USA). The HTB-41 cells were grown in modified McCoy's media with 1.5 mM L-glutamine adjusted to contain 2.2 g/L sodium bicarbonate with 10% FBS in accordance with ATCC's instruction. The cells were maintained as monolayers in plastic culture plate at 37°C in a humidified atmosphere containing 5% CO_2.

MTT assay

The cell viability test was performed according to the previously described method with minor modifications [29,30]. The HTB-41 cells were seeded at a concentration of 5 X 10^3 cells/well in a 24-well plate. After 24 hours growth, the cells were treated with EF-24 or curcumin at various concentrations and incubation times. Then, cell viability was assessed using MTT assay. Briefly, the cells were grown in the medium in the absence or presence of EF-24 or curcumin for various incubation times. Following the culture, 0.5 mg/ml of MTT was added to each well. After 4 hours incubation at

37°C, isopropanol with 0.04 M HCl was added to each well to dissolve precipitates. Then, the absorbance was measured at 570 nm using a spectrophotometer (Ultrospec 2000; Amersham Pharmacia Biotech, NJ, USA). Four or five separate experiments were performed for each concentration/ exposure time combination.

Nuclear staining with DAPI

Nuclear staining with DAPI (4',6-diamidino-2-phenylindol) was performed to evaluate apoptosis. The HTB-41 cells were cultured in 24-well plates at a seeding density of 5 X 10^3 cells per well. After 24 hours growth, the cells were treated with 3 μM EF-24 or 100 μM curcumin for 24 hours. The treated HTB-41 cells were fixed with 1% paraformaldehyde for 30 min at room temperature and washed twice with PBS. Permeate the cells with ice-cold ethanol for 5 min at room temperature and washed twice with PBS. The fixed HTB-41 cells were stained with DAPI (300 nM) for 5 min at room temperature in dark, washed twice with PBS and examined by fluorescent inverted microscopy (IX71, Olympus, Japan).

Immunoblotting

The HTB-41 cells were treated with 3 μM EF-24 or 100 μM curcumin for 24 or 48 hours. Immunoblotting was performed according to the previously described method with minor modifications [31,32]. The anti-cleaved caspase-3, -7 or -9 antibodies (1:1000 dilution, Cell Signaling Technology, Inc., Danvers, MA, USA) were used as the primary antibodies.

Determination of caspase activation

The activity of caspase-3/-7 was determined using the cell-permeable fluorogenic substrate PhiPhiLux-G_1D_2 (OncoImmunin, Inc. Gaithersburg, MD, USA), which was used according to the manufacturer's instructions. The HTB-41 cells were treated with 3 μM EF-24 or 100 μM curcumin for 24 hours and incubated with PhiPhiLux-G_1D_2. The activity of caspase-3/-7 was visualized by fluorescence microscopy (IX71, Olympus, Japan).

Data analysis

All experiments were performed at least four times. The results were presented as the mean ± SEM. The statistical significance was analyzed by using Student's *t*-test for the two group comparison and one way analysis of variance for the multi-group comparisons. A p value <0.05 was considered statistically significant. All statistical analyses were performed using Excel and SPSS software.

Results

Growth inhibition of HTB-41 cells by EF-24 and curcumin

To analyze and compare the effect of EF-24 and curcumin on the viability of HTB-41 cells, the cells were treated with EF-24 and curcumin at various concentrations for 24 and 48 hours, and then the MTT assay was performed. As shown in Fig. 2, treatment of curcumin from 0.1 to 30 μM for 24 hours or from 0.1 to 3 μM for 48 hours did not significantly affect the cell viability of HTB-41 cells, but curcumin 100 μM for 24 hours or curcumin 10, 30 and 100 μM for 48 hours reduced HTB-41 cell viability. When the HTB-41 cells were treated with EF-24 from 0.1 to 10 μM for 24 or 48 hours, EF-24 inhibited the growth of HTB-41 cells in the concentration- and time-dependent manners, suggesting that curcumin and EF-24 induce HTB-41 cell death (Fig. 2). The IC_{50} values of curcumin and EF-24 on the cell viability after 24 or 48 hours treatments are shown in Table 1. The apparent

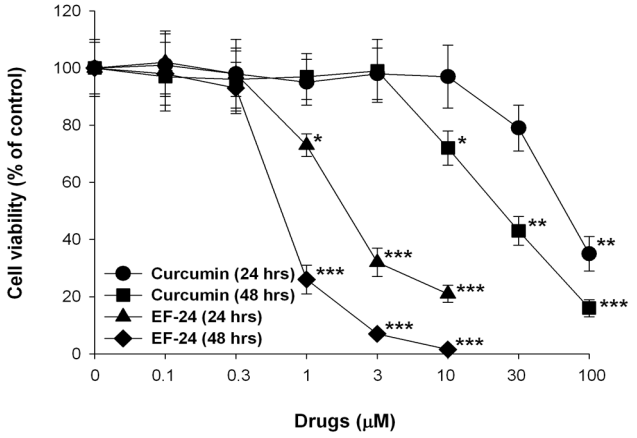

Fig. 2. Concentration- and time-dependent effects of EF-24 and curcumin on the cell viability in HTB-41 cells. The HTB-41 cells were treated with various concentrations of EF-24 and curcumin or without EF-24 and curcumin for 24 and 48 hours. The cell viabilities were determined by the MTT assays. The percentage of cell viability was calculated as a ratio of A570 nm of EF-24 or curcumin treated cells and untreated control cells. Each data point represents the mean ± SEM of four experiments. $^*P$<0.05 vs. control, $^{**}P$<0.01 vs. control and $^{***}P$<0.001 vs. control (the control cells measured in the absence of EF-24 or curcumin).

Table 1. Anti-proliferative effects of curcumin and EF-24 in HTB-41 cells

Time (hours)	IC_{50} (μM)	
	Curcumin	EF-24
24	75.24 ± 9.75	2.18 ± 0.37
48	25.62 ± 4.26	0.75 ± 0.06

The IC_{50} values represent the mean ± SEM for four experiments.

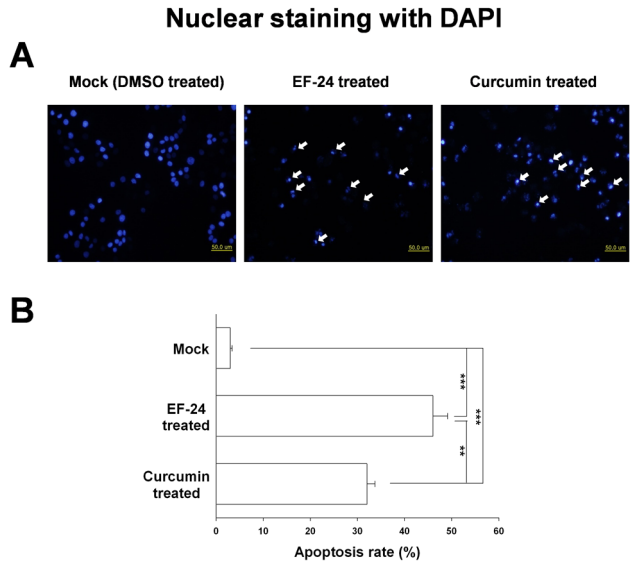

Fig. 3. Apoptotic nuclear changes induced by EF-24 and curcumin. The cells were treated with 3 μM EF-24 or 100 μM curcumin for 24 hours. (A) Representative DAPI-stained fluorescence photomicrographs show the nuclei morphology of HTB-41 cells. Arrows indicate chromatin condensation, reduced nuclear size and nuclear fragmentation typically observed in apoptotic cells. (B) The percentage of apoptotic cells was calculated as the ratio of apoptotic cells to total cells. $^{**}P < 0.001$ vs. the curcumin treated cells and $^{***}P<0.001$ vs. control (the control cells measured in the absence of EF-24 or curcumin).

potency of EF-24 was > 34 times that of curcumin. More importantly, the effects were observed at an EF-24 concentration < 3 μM, a concentration at which curcumin had no significant effect on cell proliferation, indicating the enhanced potency of EF-24.

Changes in nuclear morphology by EF−24 and curcumin

The nuclear morphological changes were assessed by DAPI staining. As shown in Fig. 3A, the nuclei of the control HTB-41 cells (Mock) had a normal regular and oval shape. Treatment with 3 μM EF-24 or 100 μM curcumin for 24 hours resulted in nuclear condensation and fragmentation, which are characteristics of apoptosis. As quantified in Fig. 3B, EF-24 and curcumin significantly increased the apoptotic rate of HTB-41 cells to 45.9 ± 3.1% and 31.8 ± 1.7%, respectively.

Activation of caspases by EF−24 and curcumin

The levels of cleaved caspase-3, -7 and -9 were examined by immunoblotting and the activity of caspase-3/-7 was detected by fluorescence microscopy using a selective fluorogenic substrate since caspase-3, -7 and -9 are effector caspases of apoptotic cell death [33-36]. Treatment with 3 μM EF-24 or 100 μM curcumin for 24 or 48 hours significantly promoted the proteolytic cleavages of procaspase-3 in the HTB-41 cells, with the increases in the amount of cleaved

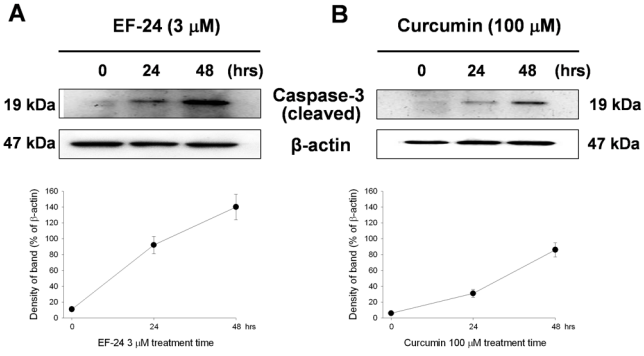

Fig. 4. Proteolytic cleavage of caspase-3 by EF-24 or curcumin treatment in HTB-41 cells. Activity of cleaved caspase-3 by EF-24 (A) or curcumin (B) was measured in HTB-41 cells. The cells were treated with 3 μM EF-24 or 100 μM curcumin for 24 or 48 hours. The cell lysate was prepared and analyzed by immunoblotting as described in "MATERIALS AND METHODS". The lower panels show the quantitative data for upper panels analyzed by using Imagegauge 3.12 software after β-actin normalization.

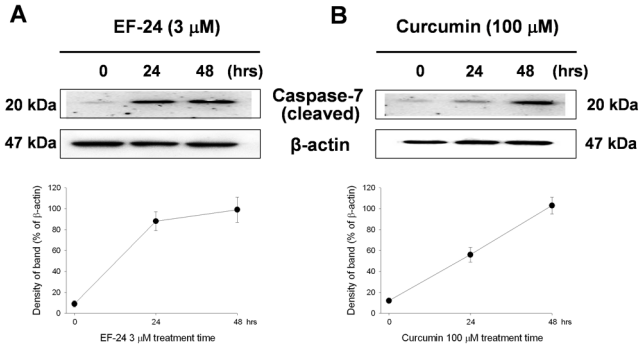

Fig. 5. Proteolytic cleavage of caspase-7 by EF-24 or curcumin treatment in HTB-41 cells. Activity of cleaved caspase-7 by EF-24 (A) or curcumin (B) was measured in HTB-41 cells. Other legends are the same as in Fig. 4.

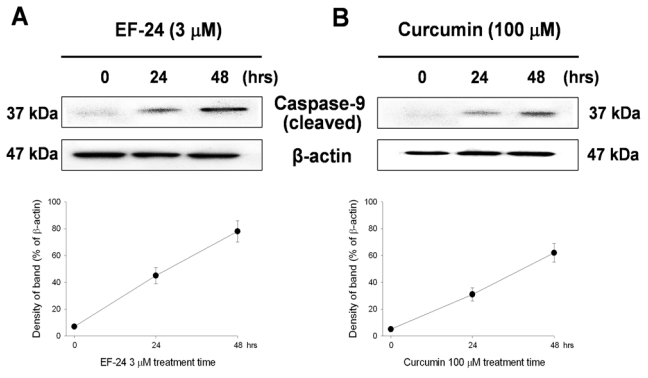

Fig. 6. Proteolytic cleavage of caspase-9 by EF-24 or curcumin treatment in HTB-41 cells. Activity of cleaved caspase-9 by EF-24 (A) or curcumin (B) was measured in HTB-41 cells. Other legends are the same as in Fig. 4.

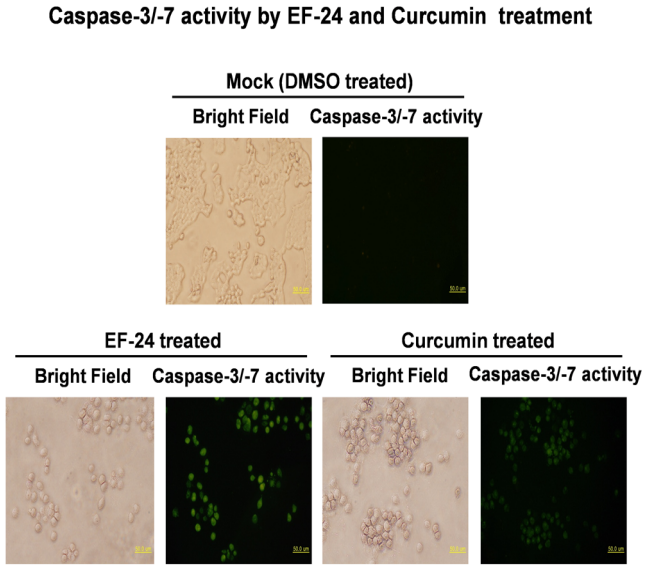

Fig. 7. Activation of caspase-3/-7 by EF-24 or curcumin treatment in living HTB-41 cells. The cells were treated with 3 μM EF-24 or 100 μM curcumin for 24 hours and added specific cell-permeable substrate Phiphilux G1D2. Active of caspase-3/-7 was visualized by fluorescence microscopy.

caspase-3 (Fig. 4). Either EF-24 (3 μM) or curcumin (100 μM) for 24 or 48 hours also promoted the proteolytic cleavages of procaspase-7, with the increases in the amount of cleaved caspase-7 (Fig. 5). Treatment with EF-24 (3 μM) or curcumin (100 μM) promoted the proteolytic cleavages of procaspase-9 in the HTB-41 cells (Fig. 6).

In addition, activation of caspase-3/-7 in EF-24 or curcumin treated HTB-41 cells was confirmed by fluorescence microscopy using a fluorogenic substrate. As shown in Fig. 7, either the EF-24 or curcumin treatment led to activate the caspase-3/-7 in the living HTB-41 cells.

Discussion

In recent years, there has been a global trend toward the importance of naturally occurring phytochemicals in plants for the prevention and treatment of human diseases [37,38]. Several of these phytochemicals are shown to have potential values as cancer chemopreventive or therapeutic agents within the human body [37-40]. Most of these bioactive phytochemicals exert their cancer chemotherapeutic activity by blocking cell cycle progression and triggering apoptotic cell death [37-40].

Curcumin has been extracted from the dried ground rhizome of the perennial herb *Curcuma longa*. Several studies have suggested that curcumin induces cell cycle arrest and apoptosis in various cancer cells [18-23]. However, it is needed the design of more effective analogs because of curcumin's poor intestinal absorption [26]. EF-24, the monoketone analog of curcumin (Fig. 1), is efficacious in anticancer screens and has been shown to inhibit the growth of human breast tumor xenografts in a mouse model [27,28]. Although EF-24 can reduce cancer cell viability, the mechanisms of action remain to be elucidated, and the effects of EF-24 and curcumin on salivary gland epidermoid cell carcinoma are not clearly established. In this study, therefore, the cytotoxic effect of EF-24 and the mechanism of cell death exhibited by EF-24 were examined in HTB-41 human submaxillary salivary gland epidermoid carcinoma cells. The results of this study indicate that EF24, a novel curcumin analog, possesses profound promise as an anti-salivary gland cancer therapeutic.

In MTT assay, curcumin 100 μM for 24 hours or curcumin 10, 30 and 100 μM for 48 hours reduced HTB-41 cell viability (Fig. 2). EF-24 inhibited the growth of HTB-41 cells in the concentration- and time-dependent manners (Fig. 2). This corresponded with the results of EF-24 and curcumin that have anti-cancer effects via the suppression of cancer cell growth in various types of cancer cells [18-23]. Also, the apparent potency of EF-24 was > 34-fold that of curcumin. These results speculate that EF-24 and curcumin have cytotoxicity to salivary gland epidermoid carcinoma cells with EF-24 having enhanced potency. In addition, these results indicate that it has potential value for anti-cancer drug discovery.

The induction of apoptosis in cancer cells is one of useful strategies for anti-cancer drug development [31]. So, many

studies were performed for screening of apoptosis from plant-derived compounds [37-40]. In this study, treatment with EF-24 and curcumin induced nuclear condensation and fragmentation in HTB-41 cells, suggesting apoptotic cell death (Fig. 3). These results indicate that EF-24 and curcumin inhibit the growth of HTB-41 cells by activating cell apoptosis.

The activation of a family of intracellular cysteine proteases, called caspases, is known to play an important role in the initiation and execution of apoptosis induced by various stimuli [34,35]. Among the caspases identified in mammalian cells, caspase-3, caspase-7 and caspase-9 may serve as effector caspases of apoptotic cell death [33-35]. Caspase-3, caspase-7 and caspase-9 are synthesized as inactive proenzymes (of sizes 32 kDa, 35 kDa and 47 kDa, respectively), which require proteolytic activation to cleaved enzymes (of sizes 19 kDa, 20 kDa and 37 kDa, respectively) [33-35]. This study revealed that low levels of cleaved capase-3, -7 and -9 were present in EF-24- or curcumin-untreated HTB-41 cells, and the amount of cleaved enzymes was increased after the EF-24 or curcumin treatment in HTB-41 cells (Fig. 4, 5 and 6). In addition, the activity of caspase-3/-7 was increased by EF-24 or curcumin treatment in living HTB-41 cells compared to dimethyl sulfoxide (DMSO) treatment as a control (Fig. 7). These results suggested that EF-24 and curcumin induce apoptotic cell death through caspase-3-, -7- and -9-dependent processing in the HTB-41 cells. However, the mechanisms of apoptosis induced by EF-24 and curcumin in HTB-41 cells are not yet completely understood. Further studies will be needed to reveal the precise cellular and molecular mechanisms of apoptosis induced by EF-24 and curcumin.

In conclusion, these results indicate that the EF-24 and curcumin inhibit cell proliferation and induce apoptosis in HTB-41 human salivary gland epidermoid carcinoma cells. Moreover, the potency of EF-24 was > 34 times that of curcumin. Therefore, EF-24 could be a model compound for the further development of natural product-derived anti salivary gland epidermoid carcinoma agents. However, to elaborate this nascent possibility, further investigation of its activity including *in vivo* and purification of bioactive compounds is now in progress.

Acknowledgements

This study was supported by research fund from Chosun University, 2015.

Conflict of interest

The authors declare that they have no conflicting interest.

References

1. Speight PM, Barrett AW. Salivary gland tumours. Oral Dis. 2002;8:229-240. doi: 10.1034/j.1601-0825.2002.02870.x.
2. Ellis GL, Auclair PL, Gnepp DR. Surgical pathology of the salivary glands. In: Primary squamous cell carcinoma (Auclair PL and Ellis GL, eds.). Philadelphia, W. B. Saunders Company, 1991. pp. 369-378.
3. Cheng YL, Lee SC, Lin SZ, Chang WL, Chen YL, Tsai NM, Liu YC, Tzao C, Yu DS, Harn HJ. Anti-proliferative activity of *Bupleurum scrozonerifolium* in A549 human lung cancer cells *in vitro* and *in vivo*. Cancer Lett. 2005;222:183-193. doi: 10.1016/j.canlet.2004.10.015.
4. Park DI, Lee JH, Moon SK, Kim CH, Lee YT, Cheong J, Choi BT, Choi YH. Induction of apoptosis and inhibition of telomerase activity by aqueous extract from *Platycodon grandiforum* in human lung carcinama cells. Pharmacol Res. 2005;51:437-443. doi: 10.1016/j.phrs.2004.11.003.
5. Tan ML, Suaiman SF, Najimuddin N, Smian MR, Tengku Muhammad TS. Methanolic extract of *Pereskia bleo* (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinama, T47-D cell line. J Ethnopharmacol. 2005;96:287-294. doi: 10.1016/j.jep.2004.09.025.
6. Hoshino T, Hara A, Inoue M, Honda J, Imai Y, Oizumi K, Yokoyama MM. Flow cytometric measurement of NK cell cytotoxicity. J Clin Lab Immunol. 1991;36:39-43.
7. Tian Z, Chen S, Zhang Y, Huang M, Shi L, Huang F, Fong C, Yang M, Xiao P. The cytotoxicity of naturally occurring styryl lactones. Phytomedicine. 2006;13:181-186. doi: 10.1016/j.phymed.2004.07.010.
8. Smets LA. Programmed cell death (apoptosis) and response to anti-cancer drugs. Anti-Cancer Drug. 1994;5:3-9.
9. Paschka AG, Butler R, Young CYF. Induction of apoptosis in prostate cancer cell lines by the green tea component, (−)-epigallocatechin-3-gallate. Cancer Lett. 1998;130:1-7. doi: 10.1016/S0304-3835(98)00084-6.
10. Green DR, Reed JC. Mitochondria and apoptosis. Science. 1998;281:1308-1312. doi: 10.1126/science.281.5381.1309.
11. Hengartner MO. The biochemistry of apoptosis. Nature. 2000;407:770-776. doi: 10.1038/35037710.
12. Kaufmann SH, Hengartner MO. Programmed cell death: alive and well in the new millennium. Trends Cell Biol. 2001;11:526-534. doi: 10.1016/S0962-8924(01)02173-0.
13. Kaufmann SH, Earnshaw WC. Induction of apoptosis by cancer chemotherapy. Exp Cell Res. 2000;256:42-49. doi: 10.1006/excr.2000.4838.
14. Reed JC. Apoptosis-regulating proteins as targets for drug

discovery. Trends Mol Med. 2001;7:314-319. doi: 10.1016/S1471-4914(01)02026-3.
15. Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. Oncogene. 2001;20:7597-7609.
16. Miquel J, Bernd A, Sempere JM, Diaz-Alperi J, Ramirez A. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Arch Gerontol Geriatr. 2002;34:37-46. doi: 10.1016/S0167-4943(01)00194-7.
17. Banerjee M, Tripathi LM, Srivastava VM, Puri A, Shukla R. Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. Immunopharmacol Immunotoxicol. 2003;25:213-224.
18. Sahu RP, Batra1 S, Srivastava SK. Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. Br J Cancer. 2009;100:1425-1433. doi: 10.1038/sj.bjc.6605039.
19. Aggarwal BB, Banerjee S, Bharadwaj U, Sung B, Shishodia S, Sethi G. Curcumin induces the degradation of cyclin E expression through ubiquitin-dependent pathway and up-regulates cyclin-dependent kinase inhibitors p21 and p27 in multiple human tumor cell lines. Biochem Pharmacol. 2007;73:1024-1032. doi: 10.1016/j.bcp.2006.12.010.
20. Bachmeier B, Nerlich AG, Iancu CM, Cilli M, Schleicher E, Vene R, Dell''Eva R, Jochum M, Albini A, Pfeffer U. The chemopreventive polyphenol Curcumin prevents hematogenous breast cancer metastases in immunodeficient mice. Cell Physiol Biochem. 2007;19:137-152. doi: 10.1159/000099202.
21. Hauser PJ, Han Z, Sindhwani P, Hurst RE. Sensitivity of bladder cancer cells to curcumin and its derivatives depends on the extracellular matrix. Anticancer Res. 2007;27: 737-740.
22. Shankar S, Srivastava RK. Involvement of Bcl-2 family members, phosphatidylinositol 30-kinase/AKT and mitochondrial p53 in curcumin (diferulolylmethane)-induced apoptosis in prostate cancer. Int J Oncol. 2007;30:905-918. doi: 10.3892/ijo.30.4.905.
23. Wahl H, Tan L, Griffith K, Choi M, Liu JR. Curcumin enhances Apo2L/TRAIL-induced apoptosis in chemoresistant ovarian cancer cells. Gynecol Oncol. 2007;105:104-112. doi: 10.1016/j.ygyno.2006.10.050.
24. Ammon HP, Wahl MA. Pharmacol Curcuma longa. Planta Med. 1991;57:1-7. doi: 10.1055/s-2006-960004.
25. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as 'Curecumin': from kitchen to clinic. Biochem Pharmacol. 2008;75:787-809. doi: 10.1016/j.bcp.2007.08.016.
26. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4:807-818. doi: 10.1021/mp700113r.
27. Adams BK, Ferstl EM, Davis MC, Herold M, Kurtkaya S, Camalier RF, Hollingshead MG, Kaur G, Sausville EA, Rickles FR, Snyder JP, Liotta DC, Shoji M. Synthesis and biological evaluation of novel curcumin analogs as anti-cancer and antiangiogenesis agents. Bioorg Med Chem. 2004;12:3871-3883. doi: 10.1016/j.bmc.2004.05.006.
28. Adams BK, Cai J, Armstrong J, Herold M, Lu YJ, Sun A, Snyder JP, Liotta DC, Jones DP, Shoji M. EF24, a novel synthetic curcumin analog, induces apoptosis in cancer cells via a redoxdependent mechanism. Anticancer Drugs. 2005;16:263-275.
29. Lee MH, Kim MM, Kook JK, Kim DK, Kim HR, Kim HJ, Kim CS. Ethanol extracts of *Angelica decursiva* induces apoptosis in human oral cancer cells. Int J Oral Biol. 2010;35:215-220.
30. Shin WC, Kim CS, Kim HJ, Lee MH, Kim HR, Kim DK. Growth inhibition of human head and neck squamous cell carcinomas by *Angelica decursiva* extracts. Int J Oral Biol. 2010;35:153-158.
31. Kim JY, Kim HS, Kang HS, Choi JS, Yokozawa T, Chung HY. Antioxidant potential of dimethyl lithospermate isolated from *Salvia miltiorrhiza* (red sage) against peroxynitrite. J Med Food. 2008;11:21-28. doi: 10.1089/jmf.2007.040.
32. Kim CS, Moon IS, Park JH, Shin WC, Chun HS, Lee SY, Kook JK, Kim HJ, Park JC, Endou H, Kanai Y, Lee BK, Kim DK. Inhibition of L-type amino acid transporter modulates the expression of cell cycle regulatory factors in KB oral cancer cells. Biol Pharm Bull. 2010;33:1117-1121. http://doi.org/10.1248/bpb.33.1117.
33. Cohen GM. Caspases: the executioners of apoptosis. Biochem J. 1997;326:1-16.
34. Datta R, Kojima H, Yoshida K, Kufe D. Caspase-3-mediated cleavage of protein kinase C theta in induction of apoptosis. J Biol Chem. 1997;272:20317-20320. doi: 10.1074/jbc. 272.33.20317.
35. Liu X, Zou H, Slaughter C, Wang X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell. 1997;89: 175-184. doi: 10.1016/S0092-8674(00)80197-X.
36. Hu W, Kavanagh JJ. Anticancer therapy targeting the apoptotic pathway. Lancet Oncol. 2003;4:721-729. doi: 10.1016/S1470-2045(03)01277-4.
37. Christou L, Hatzimichael E, Chaidos A, Tsiara S, Bourantas KL. Treatment of plasma cell leukemia with vincristine, liposomal doxorubicin and dexamethasone. Eur J Haematol. 2001;67:51-53. doi: 10.1034/j.1600-0609.2001.067001051.x.
38. Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in cancer therapy with plant based natural products. Curr Med Chem. 2001;8:1467-1486. doi: 10.2174/0929867013372094.
39. Pezutto JM. Plant-derived anticancer agents. Biochem Pharmacol. 1997;53:121-133. doi: 10.1016/S0006-2952(96) 00654-5.
40. van Poppel G, van den Berg H. Vitamins and cancer. Cancer Lett. 1997;114:195-202. doi: 10.1016/S0304-3835(97)04662-4.