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The Effects of Apigenin and Curcumin on Autophagy Related Cell Death and Apoptosis †

Sera Kayacan [1], Kaan Yilancioglu [2], Ayse Seda Akdemir [1], Fatma Kaya Dagistanli [1], Gulay Melikoglu [3] and Melek Ozturk [1,*]

[1] Department of Medical Biology, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul 34098, Turkey; serakayacan@gmail.com (S.K.); asedakdemir@gmail.com (A.S.A.); fkayadagistanli@gmail.com (F.K.D.)
[2] Department of Molecular Biology and Genetics, Bioengineering Faculty, Uskudar University, Istanbul 34662, Turkey; kaan.yilancioglu@uskudar.edu.tr
[3] Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, Istanbul 34116, Turkey; gulaymelikoglu@hotmail.com
* Correspondence: mozturk@istanbul.edu.tr
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Abstract: Cervical cancer is one of the frequent types of cancer seen in females. It has been suggested that natural compounds can be used effectively for cancer treatment. Apoptosis and autophagy related cell death play important roles in suppression of tumorigenesis. Apigenin and curcumin are natural products isolated from plant extracts known to have antitumoral, antibacterial and antiviral effects. Varying doses of curcumin and apigenin were applied to HeLa cancer cell lines. The expression of the genes related to apoptosis and/or autophagy related cell death were measured using qRT-PCR and cell viability was measured using MTT assay. Our results showed that curcumin and apigenin are effective on apoptosis and autophagy related cell death in HeLa cells. We suggested that these natural products seem to be a new promising therapeutic approach in cancer.

Keywords: curcumin; apigenin; apoptosis; autophagy related cell death; HeLa cells

1. Introduction

Cancer is a multifactorial disease defined as the uncontrolled division and proliferation of the cells. The drugs derived from plant extracts are being used more frequently for cancer treatment. Apigenin can be used as a chemopreventive agent for its antitumoral, antibacterial and antiviral activities and it has been shown that it can suppress tumor growth. Curcumin is a polyphenol with strong antioxidant and antineoplastic properties that effect cell cycle, apoptosis and differentiation of the cells thus suppressing the proliferation of cancer cells [1,2]. It is of vital importance to investigate the genes effective in cell death regulatory mechanisms to develop new strategies. A break-down in cell death mechanism leads to uncontrolled cell division and proliferation. Apoptosis can be triggered either intrinsically or extrinsically. The intrinsic pathway includes antiapoptotic (e.g., Bcl-2, Bcl-xL, Mcl-1, Bcl-W) and proapoptotic (e.g., Bax, Bak, Bad, Bid, Bif, Bik, Bcl-xS, Noxa, Puma) Bcl-2 family members. The extrinsic pathway includes death receptors such as Fas, TRAILs and TNFR. The major cysteine proteases are caspase-3, -8 and -9. Activation of caspase-3 initiates cell death mechanism finally leading to apoptosis. Autophagy is another major mechanism for cell survival which a deficiency in this system results with uncontrolled cellular proliferation. Depending on the situation, cells can choose autophagy related proteins or cellular death proteins such as Beclin-1, Atg5 and Atg12. Researchers have shown that cells can cross-talk through the interaction of regulatory genes

such as, Beclin-1, Bcl-2, Bcl-xL, Atg5, Bif-1 and BNIP-3 [3]. In this study, considering the newly developed treatment models, we aimed to investigate the effectiveness of curcumin and apigenin separately in HeLa cells.

2. Methods

Cell line growth protocol: Human cervical cancer cell line HeLa (ATCC-CCL-2) (Manassas, VI, USA) was used in this study. The cells were grown at 37 °C and 5% CO_2 in 25cm^2 flasks until 80–85% confluency using Minimum Essential Medium (MEM, Sigma-Aldrich, M2279, St. Louis, MI, USA) containing 10% Fetal Bovine Serum (FBS, Gibco, 10500064, Thermo Fisher Scientific, Waltham, MA, USA) and 50 mg/mL streptomycin and 100 unit/mL penicillin.

The cell viability experiment: The cell viabilities of curcumin (Sigma-Aldrich Company, C1386, St. Louis, MO, USA) (0–100 μM) and apigenin (0–100 μM) were determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.

Gene expression analysis: The mRNA expression of apoptotic regulatory genes (Caspase-3, Fas and TNF), autophagic cell death related genes (Atg5, Beclin-1), and both autophagic and apoptotic cell death regulatory gene (Bif-1) were investigated by qRT-PCR. GAPDH, 18S and β-actin were used as endogenous controls. $2^{-\Delta\Delta Ct}$ method was used to calculate the relative mRNA levels.

Statistical analysis was performed with GraphPad Prism version 7.0 for Windows.

3. Results

We found that curcumin and apigenin had cytotoxic effects on HeLa cells (Figure 1) and determined that curcumin and apigenin induced apoptosis and autophagy in a dose dependent manner with an IC50 value of 0.05 μg/μL ($p < 0.001$). In curcumin treated group the expression levels of Fas, TNF, caspase-3, ATG5, Beclin-1 and Bif-1 genes were significantly increased as 4.85, 4.28, 2.05, 1.31, 1.53 and 4.6 folds for (Figure 2). In apigenin treated group the expression levels of the relevant genes were found as 0.52, 0.26, 0.13, 0.4, 0.07 folds, respectively (Figure 3). In addition, we showed that curcumin induced the cross-connection between apoptotic and autophagy related cell death in HeLa cells by increasing the expression of Bif-1 gene (Figure 2). When curcumin or apigenin were applied individually, we saw that both drugs affected the expression of genes in varying ranges. However, the curcumin effect was stronger than the effect of apigenin.

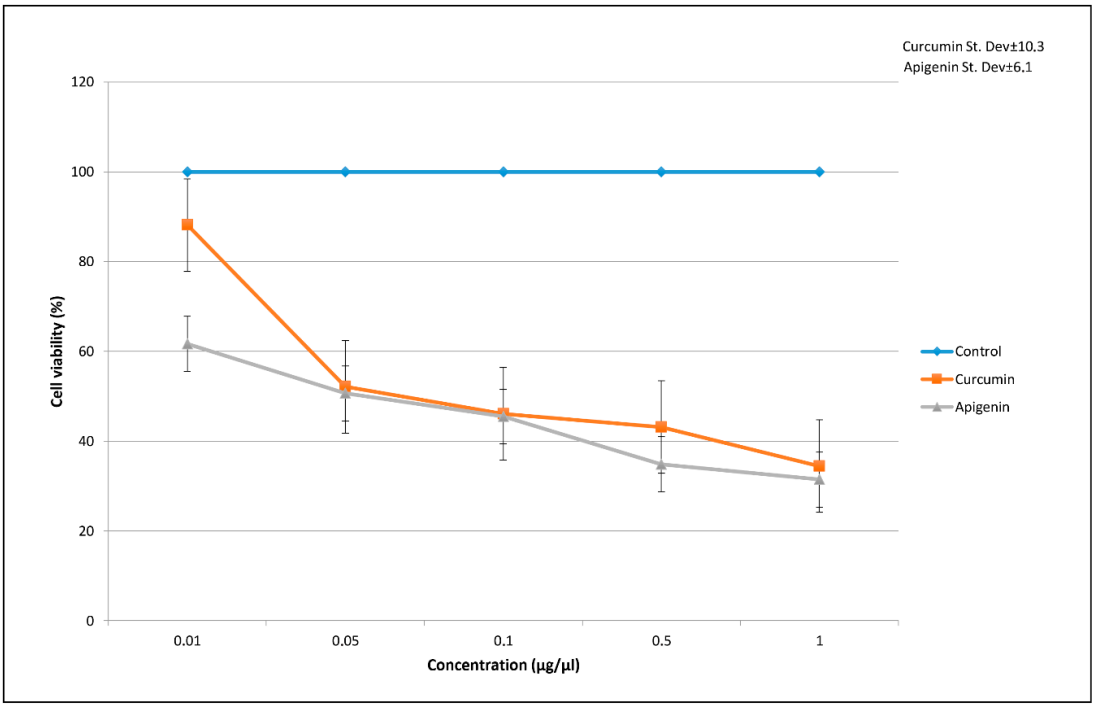

Figure 1. Cell viability of the HeLa cells depending on drug concentrations.

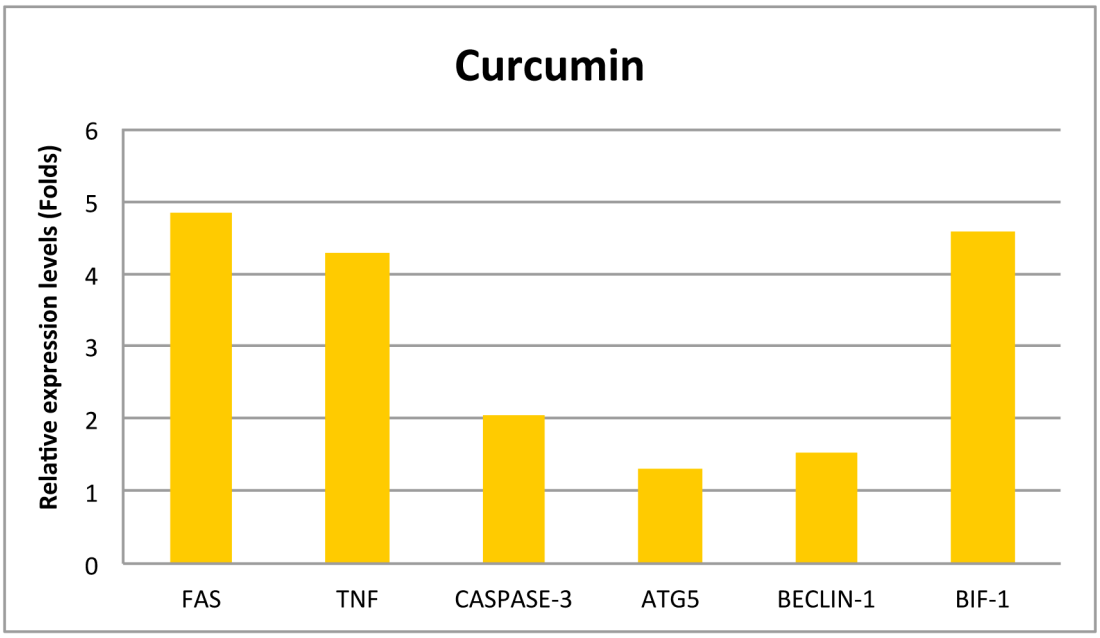

Figure 2. Expression levels of the genes after Curcumin treatment in HeLa cells.

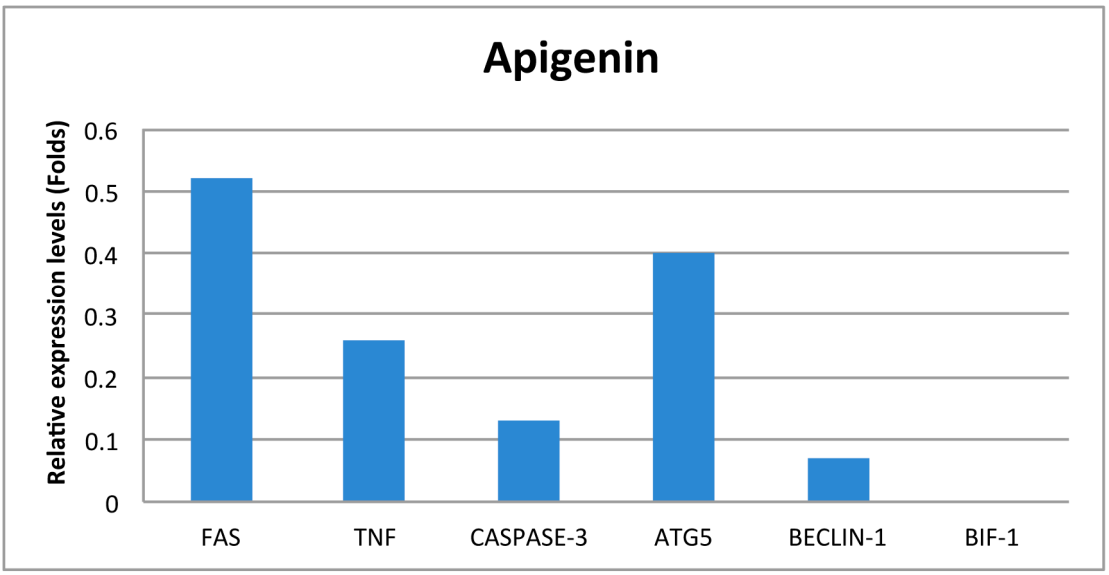

Figure 3. Expression levels of the genes after Apigenin treatment in Hela cells.

4. Discussion

In our study, we investigated the cytotoxic and antiproliferative effects of curcumin and apigenin on relevant genes known to be associated with types of cell death such as apoptosis and autophagy. We showed that single use of apigenin and curcumin affected the expression of the genes in a dose dependent manner. Caspases are essential actors of apoptotic intrinsic or extrinsic pathways. Activation of caspase-3 triggers caspase-8 and caspase-9 mediated apoptosis. In our study, we observed that an increase in expression level of caspase-3 increased apoptosis. Also, TNF and Fas expressions were increased leading to apoptosis. If the autophagy mechanism is disabled or impaired, cytotoxicity may occur. It has been shown that TNF can induce autophagy [4]. Recent studies have shown the cross-talk between autophagy and apoptosis [5–7]. Bif-1 acts as a tumor suppressor leading to cellular growth in HeLa cells by activating apoptosis and providing cross-talk between apoptosis and autophagy [8]. It can interact with Beclin-1, an overexpressed gene in many types of cancer, and inhibit tumorigenesis. Recent studies have also shown that Atg5 and Beclin-1 expression result with autophagy related cell death [5]. According to our results, single use of

curcumin and apigenin decreased the tumor growth by increasing the expression of apoptotic and autophagy related cell death genes.

5. Conclusions

Our results indicated that curcumin and apigenin inhibit HeLa cell proliferation, regulate the genes of apoptosis and autophagy, and curcumin also provides the crosstalk between autophagy related cell death and apoptosis in HeLa cells. We suggest that use of these natural products seem to be a new promising therapeutic approach in treatment of cervix cancer.

Author Contributions: S.K., K.Y., F.K.D. and M.Ö. conceived and designed the experiments, S.K. and S.A. performed the experiments, S.K., M.Ö. and K.Y. analyzed the data, S.K., K.Y. and M.Ö. wrote the paper.

Conflict of interest: The authors declare no conflict of interest.

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