Progress in Nutrition 2021; Vol. 23, N. 1: e2021018 DOI: 10.23751/pn.v23i1.9571

Original article

Resveratrol triggers apoptosis in colon cancer cells rather than senescence

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Abstract. *Objective:* Resveratrol is a phenolic compound that classified in stilbenoid and used as anticancer agent in many cancer types. The purpose of the study is to determine apoptotic and senescence inducing effects in primary (Colo-320) and metastatic (Colo-741) colon cancer cells. *Methods:* Cell growth and cytotoxicity were detected by MTT method in both Colo-320 and Colo-741 cell lines. Apoptotic and senescence inducing activities were tested with TUNEL staining, X-gal staining and immunocytochemistry using antibodies directed against to Bax, Bcl-2, caspase-3, Hsp27, Lamin B1, p16, cyclin B1. *Results:* According to MTT results, 25 μg/mL and 10 μg/mL concentrations of resveratrol were found more effective for Colo-320 and Colo-741 cell lines, respectively. As a result of immunocytochemical staining, Bax immunoreactivity was significantly increased while Bcl-2 immunoreactivity significantly decreased in Colo-320 cell line. Increased Hsp27, lamin B1 and p16 immunoreactivities on Colo-320 cells were not significant. In Colo-741 cells, Bcl-2 immunoreactivity was significantly increased. Hsp27 immunoreactivity in Colo-320 cell line was significantly higher than Colo-741 cell line. In addition, after resveratrol administration, while the TUNEL positive cells significantly increased in Colo-320 cells, X-gal positive cells was detected in Colo-741 than Colo-320 cells. *Conclusion:* Resveratrol can inhibit cell viability both in primer and metastatic colon cancer cells. However, resveratrol might be more effective triggering mitochondrial-mediated apoptosis in primary colon cancer cells. Apoptotic and cell cycle inhibiting effects of resveratrol may differ by cell type. Therefore, resveratrol may be a potential phytotherapeutic agent for colon cancer according to the cell origin.

Keywords: Resveratrol, cell death, Colo-320, Colo-741

Introduction

Colorectal cancer is the third most common cancer worldwide and the second leading cause of cancer mortality (1). Colon cancer cells have the ability to metastasize and avoid apoptosis via several mechanisms, therefore, treatment should be based on tumor-specific molecular features (2).

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a stilbenoid, a type of natural phenol and it has potential antioxidant, anti-inflammatory, anti-diabetic, anti-aging, cardioprotective, neuroprotective and anticancer effects (3,4).

Apoptosis is programmed cell death in normal and pathological conditions (5). Apoptosis can be triggered for anticancer therapy. Apoptosis is carried

out by caspases and elevated caspase-3 levels have been shown in several colon carcinoma cell lines (e.g. Caco-2, HT-29, and Colo-201) after resveratrol treatment through increased ROS production (6,7). Resveratrol combined with grape seeds induced Bax expression and decreased Bcl-2 levels in HCT-116 colorectal carcinoma cell lines after 24 h incubation (8). Therefore, the levels of caspase-3 (a common executor protein involved in both extrinsic and intrinsic apoptotic pathways), Bcl-2 and Bax (survival and death activator mitochondrial proteins) may be used to determine response to anti-cancer treatment (9).

Senescence is defined as permanent cell cycle arrest in different cell types induced by depletion of the cell pool (10). Cyclin B1, one of the senescence regulators, p16 ^{INK4a}, a cyclin-dependent kinase (CDK) inhibitor and lamin B1 could be used for the detection of senescent cells in the tissue (10). While p16 ^{INK4a} is expected to increase in senescent cells, lamin B1 level is decreased (10,11). Colin et al. reported that resveratrol increased p16 levels to trigger senescence in SW 620 colon cells (12). Cancer cells are immortal and may avoid senescence. During cancer therapy, some treatments either cause cell death or lead to senescence. Therefore, therapy-induced apoptosis or senescence has a crucial role in controlling proliferation, spreading and/or transition of the cancer cells (13). Hsp27 is a potentially oncogenic inhibitor of apoptosis that also suppresses senescence, which may promote cell survival in normal and pathological conditions (14).

The cancer cells proliferate indefinitely and may avoid senescence to get the ability to survive. If the cancer cells bypass senescence in the course of their immortalization, the therapy-induced senescence in cancer cells may loss replicative capacity in virtually cancer cells (10). In addition, some cancer cells are unable to undergo apoptosis, maintain their propensity to senesce (15). Therefore, the balance between apoptosis and senescence is important during curative treatment of cancer cells. The protective effects of resveratrol on cancer may be related to modulating of the cell cycle. In addition, its effect may vary depending on the cell, tissue and primary and metastatic cancers (15). This study aimed to determine the potential effects of resveratrol on the mechanisms of cell death and senescence in primary (Colo-320) and metastatic (Colo-741) colon cancer cell lines.

Material and methods

Cell lines and cell culture

Colo-320 (ATCC: CCL-220.1) and Colo-741 (ECACC: 93052621) cell lines were cultured in the RPMI-1640 medium (Biochrom, FG 1215) supplied with 10% heat inactivated fetal bovine serum (FBS) (Capricorn Scientific, FBS-11B), 1% penicillin-streptomycin (Biochrom, A2213) and 1% glutamine (EMD Millipore, K0282). Cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Confluent cultured cells were sub-cultured using 0.25% trypsin EDTA solution (Biochrom, L 2143).

Cell viability and growth assay

Cytotoxicity was assessed using the MTT assay. MTT assay is based on colorimetric measurement of the reduction of MTT (3-(4,5-di-methylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide) (Biotium, #30006) by living cells to yield a purple formazan product. Colo-320 and Colo-741 cells were collected, suspended in medium and seeded in 96-well culture plates at a density of 5×10^4/ml cells in each well with 100 µl culture medium. The negative control contained neither cells nor resveratrol (Sigma, R5010) and positive control only had the seeded cells. 5 µg/ml, 10 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml concentrations of resveratrol were loaded in triplicate and both cell lines were incubated for 24 h and 48 h. After incubation, MTT solution was heated to 37°C and then 10 µl was added to each well. Following incubation at 37°C in 5% CO_2 for 4 h, 200 µl DMSO (dimethylsulfoxide) was added to dissolve the formazan salts. The absorbance was measured at 570 nm with a spectrophotometer (Versa Max, Molecular Devices, Sunnyvale, USA). All experiments were performed in triplicate.

Immunocytochemistry

Cultured Colo-320 and Colo-741 cells were assessed immunocytochemically for binding of antibodies against Bax, Bcl-2, caspase-3, Hsp27, lamin B1, p16 and cyclin B1. Colo-320 and Colo-741 cells were fixed with paraformaldehyde in PBS (phosphate buffered saline) at 4°C for 30 min. Tween 20 (Sigma-Aldrich) was added for permeabilization for 15 min.

The cells were washed with PBS and endogenous peroxidase activity was quenched by incubation with H_2O_2 for 5 min at room temperature. After washing cells with PBS three times for 5 min, primary antibodies Bax, Bcl-2, caspase-3, Hsp27, lamin B1, p16, and cyclin B1 were added and incubated overnight at 4°C. Biotinylated secondary antibody (Histostain-Plus, IHC Kit, HRP, 859043, Thermo Fischer) was added and incubated for 30 min followed by PBS wash (×3) for 5 min. Streptavidin-peroxidase complex (100 µl) was added to cultured cells which were then washed with PBS. DAB (diaminobenzidine) was added and incubated for 5 min for the enhancement of immuno-labeling. DAB was washed with distilled water. Cells were counterstained with Mayer's hematoxylin for 5 min and mounted using the mounting medium (Merck Millipore, 107961, Germany). All specimens were examined under a light microscope (Olympus BX40, Tokyo, Japan).

Staining of Bax, Bcl-2, caspase-3, Hsp27, lamin B1, p16 and cyclin B1 was also graded semi-quantitatively using the H-SCORE that was calculated with the following equation: H-SCORE=$\Sigma\pi$ (i+1), where i is the intensity of staining with a value of 1, 2 or 3 (mild, moderate, or strong, respectively) and π is the percentage of cells stained at each intensity, varying between 0 and 100%.

TUNEL assay

TUNEL assay was performed to determine DNA fragmentation in apoptotic cells. *In situ* Apoptosis Detection Kit (Apoptag Plus Peroxidase *In Situ* detection kit, S7101, Millipore, USA) was used labeling the apoptotic cells. Cultured cells fixed with 4% paraformaldehyde used in fixation of the cells at 4 °C for 30 minutes. After fixation cultured cells washed three times with PBS and incubated with H_2O_2 for 5 min at room temperature. Then TdT enzyme added and the cells incubated for 1 h at 37 °C. After that, the cells washed with stop wash buffer for 10 min at room temperature and then washed with PBS three times for 5 min. And then they were incubated with streptavidin-peroxidase for 45 min and washed with PBS three times for 5 min again. Cultured cells were incubated with DAB for 5 min to make staining visible. After this, the cells were rewashed with distilled water and mounted with mounting medium.

X-gal assay

The cultured cells were fixed with 4% paraformaldehyde in 2 mM MgCl twice in PBS for 30 min at room temperature. After this the cells were labelling with -gal staining buffer containing 40 mM citric acid (Likitkimya., 77-92-9), 150 mM sodium chloride (Surechem, S0102/R6), 5 mM potassium ferro cyanide (K4Fe(CN)6 , Labshop41, 13746-66-2), 5 mM potassium ferri cyanide(K3Fe(CN)6, Zag Chem., 14459-95-1), 2 mM MgCl (Fluka,63063), monobasic sodium phosphate 2 (NaH2PO4 (Merck, 10634), and 1 mg/ml X-gal (C14H15BrClNO6, NZYtech, MB020501), then incubated overnight at 37 °C. After this, the cells washed three times with aspirated staining and buffer solutions. After washing the cells, they were stained with Mayer's hematoxyline and washed with distilled water once and closed with mounting medium.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). The study data were analyzed using the GraphPad Prism 8 statistical software. Statistical differences among groups were analyzed using the two-way ANOVA and Mann Whitney U where appropriate. A *P* value of less than 0.05 was considered statistically significant.

Results

Cell viability and cytotoxicity

MTT analyses showed that resveratrol led to greater inhibition of cell growth at 25 µg/ml concentration for Colo-320 cells (Figure 1A) and 10 µg/ml concentration for Colo-741 cells (Figure 1B) in comparison to other concentrations after 48 h incubation.

Immunocytochemical evaluation

Bax immunoreactivity was detected in all groups but Colo-320 control group showed less immunostaining intensity for Bax (Figure 2A,B). After H-SCORE analyses, Bax immunoreactivity was only significantly higher

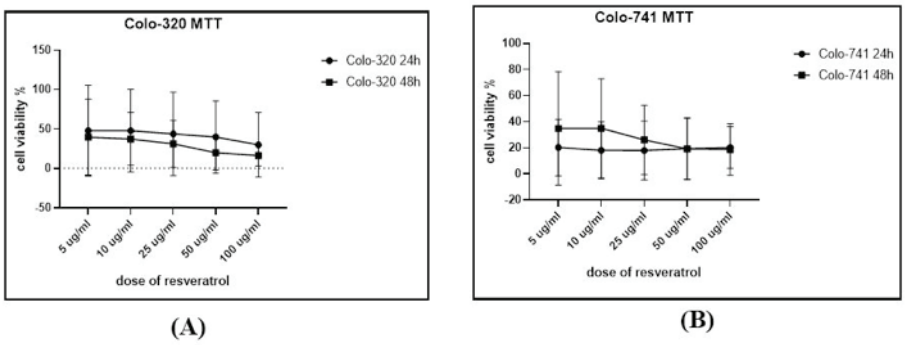

Figure 1. Dose-response curves and IC50 values of resveratrol Colo-320 (A) and Colo-741 (B) cells treated with different concentrations of resveratrol for 24 and 48 hrs respectively

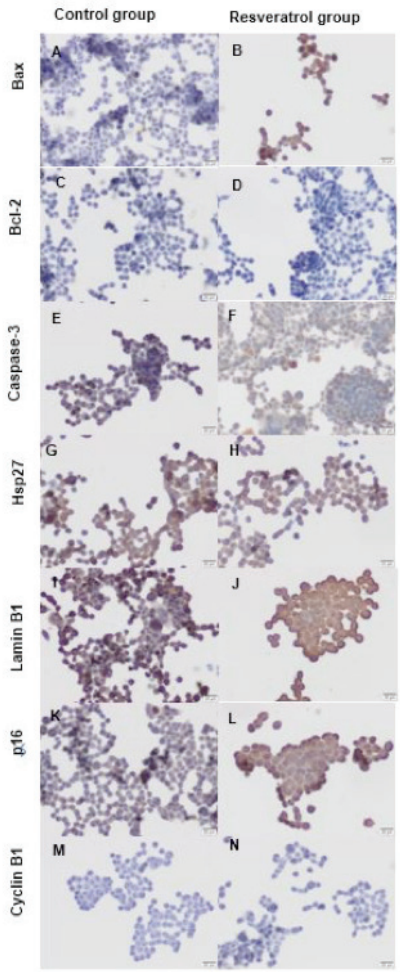

Figure 2. Immunoreactivity of Bax (A,B), Bcl-2 (C,D), Caspase-3 (E,F), Hsp27 (G,H), Lamin B1 (I,J), p16 (K,L), Cyclin B1 (M,N) in control (A,C,E,G,I,K,M) and 25μg/ml resveratrol-treated (B,D,F,H,J,L,N) Colo-320 cells. Scale bars= 20 μm.

in resveratrol-treated Colo-320 cells than in Colo-320 control group ($p < 0.05$, Table 1). Additionally, there was no difference between either resveratrol-treated Colo-741 or Colo-741 control (Figure 3A,B) or resveratrol-treated Colo-320 groups (Table 1) for Bax intensity.

Bcl-2 immunoreactivity was weak or negative in resveratrol-treated Colo-741 group and other groups, respectively (Figure 2C,D and 3C,D). While the H-SCORE value of Bcl-2 was decreased after resveratrol administration in Colo-320 group, it was higher in resveratrol-treated Colo-741 group, and these immunoreactivities were significant in comparison to control groups ($p < 0.05$, Table 1).

Moderate or strong caspase-3 immunoreactivities were detected in resveratrol-treated or control groups of Colo-320 and Colo-741 cells, respectively (Figure 2E,F and 3E,F). Resveratrol-treated Colo-320 cells showed lowest H-SCORE value versus control group, but the difference was not significant ($p > 0.05$, Table 1). In addition, H-SCORE value of caspase-3 was not significant in resveratrol-treated or control groups ($p > 0.05$, Table 1).

Immunoreactivity of Hsp27 was strong in control Colo-320 cells (Figure 2G) and decreased to moderate level after resveratrol administration (Figure 2H). However, this reduction was not significant ($p > 0.05$, Table 1). In Colo-741 cells, Hsp27 intensity was very weak or negative (Figure 3G,H). The H-SCORE value of Hsp27 was significantly higher in resveratrol-treated Colo-320 cells in comparison to resveratrol-treated Colo-741 cells ($p < 0.05$, Table 1).

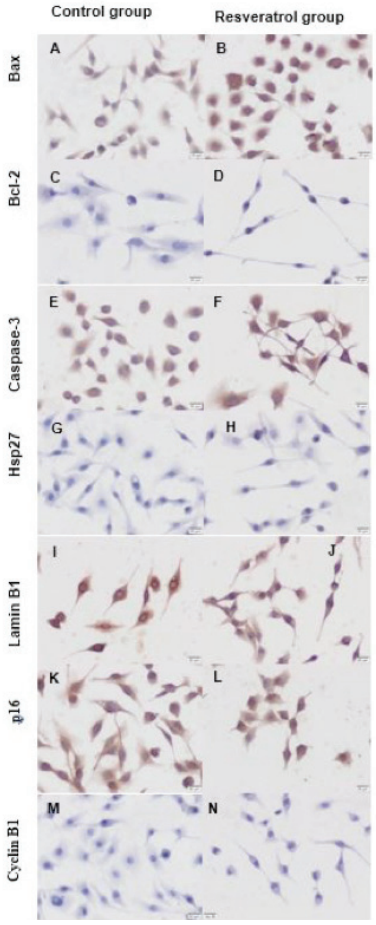

Figure 3. Immunoreactivity of Bax (A,B), Bcl-2 (C,D), Caspase-3 (E,F), Hsp27 (G,H), Lamin B1 (I,J), p16 (K,L), Cyclin B1 (M,N) in control (A,C,E,G,I,K,M) and 10μg/ml resveratrol-treated (B,D,F,H,J,L,N) Colo-741 cells. Scale bars= 20 μm.

Immunostaining intensity of lamin B1 was very strong and similar in all groups (Figure 2I,J and 3I,J). The H-SCORE values of lamin B1 in all groups were similar and non-significant (Table 1).

A strong immunoreactivity for p16 was detected in both resveratrol-treated Colo-320 (Figure 2L) and Colo-741 cells (Figure 3L) these intensities were similar ($p > 0.05$, Table 1). In addition, greatest intensity of p16 was only detected in control group of Colo-741 cells (Figure 3K), but H-SCORE value for this intensity was not significant when compared to resveratrol-treated Colo-741 cells ($p > 0.05$, Table 1).

Very weak immunoreactivity (Figure 2M,N and 3M,N) and similar H-SCORE values (Table 1) for cyclin B1 were detected in all groups but there was no significant difference between resveratrol-treated Colo-320 cells and control group ($p > 0.05$) or between resveratrol-treated Colo-741 cells and control group ($p > 0.05$).

TUNEL Assay

TUNEL assay was used both Colo-320 and Colo-741 cells that were incubated with 25 μg/mL and 10 μg/mL doses of resveratrol for 48 h, respectively. TUNEL positive cells increased in resveratrol-treated Colo-320 cells (Figure 4A,B) when compared with control group ($p < 0.001$, Table 2.) On the other hand, TUNEL positive cells were found in resveratrol-treated Colo-741 cells, but the difference was not significant when

Table 1. H-SCORE analysis of Bax, Bcl-2, Caspase-3, Hsp27, Lamin B1, p16 and Cyclin B1 for control and resveratrol groups of Colo-320 and Colo-741 cells.

	Colo-320 cells		Colo-741 cells	
	Control group	**Resveratrol group**	**Control group**	**Resveratrol group**
Bax	227.763±8.217	317.929±58.84[8]a	293.750±14.930	287.289±10.666
Bcl-2	247.115±20.038	105.463±5.78[7a,]b	100.000±0.000	289.583±35.59[9a,]b
Caspase-3	293.808±16.584	242.445±11.318	285.972±10.974	290.416±68.019
Hsp27	266.915±23.396	290.873±4.09[5]b	100.000±0.000	144.047±66.34[0]b
Lamin B1	290.059±14.510	310.742±30.516	281.250±37.500	257.916±61.242
p16	286.356±15.351	287.196±17.897	313.125±33.112	278.125±25.769
Cyclin B1	100.000±0.000	101.408±2.816	100.000±0.000	100.000±0.000

Data are expressed as means ±SD and were compared by 2 way-ANOVA

[a]Significant difference between resveratrol-treated group and control group both in Colo-320 and Colo-741 cells ($P < 0.05$)

[b]Significant difference between resveratrol-treated Colo-320 cells and resveratrol-treated Colo-741 cells ($P < 0.01$)

compared with control group (Figure 4C,D, Table 2). The number of TUNEL positive cells in Colo-741 (Figure 4D) cell line was significantly greater than Colo-320 cells ($p < 0.05$, Figure 4B, Table 2).

X-gal Staining

Decreased X-gal positive cells was detected in resveratrol-treated Colo-320 cells versus control group but the difference was not significant (Figure 5A,B, Table 3). Senescent cells were similar in resveratrol-treated Colo-741 cells and control group (Figure 5 C,D, Table 3). On the other hand greater X-gal positive cells was detected in resveratrol-treated Colo-741 than resveratrol-treated Colo-320 cells ($p < 0.05$, Figure 5B,D Table 3).

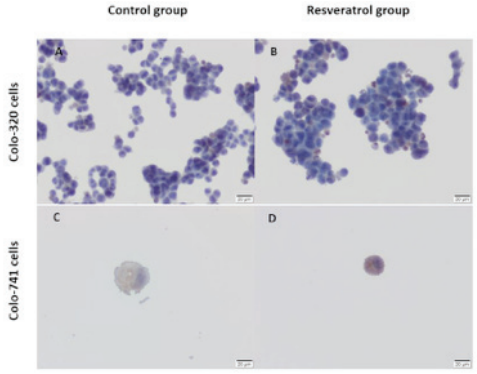

Figure 4. Evaluation of TUNEL staining: (A) Colo-320 control cells, (B) resveratrol-treated Colo-320 cells, (C) Colo-741 control cells, (D) resveratrol-treated Colo-741 cells.

Table 2. The percentage of TUNEL positive Colo-320 and Colo-741 cells treated with resveratrol at 25 μg/ml and 10 μg/ml concentration for 48h, respectively.

	Control group	Resveratrol group
Colo-320 cells	1.30±1.56	18.12±10.52[a]
Colo-741 cells	25.00±50.00	100.00±0.00[b]

Data are expressed as means ±SD and were compared by Mann Whitney U

[a]Significant difference between resveratrol-treated group and control group both in Colo-320 and Colo-741 cells ($P < 0.05$)

[b]Significant difference between resveratrol-treated Colo-320 cells and resveratrol-treated Colo-741 cells ($P < 0.05$)

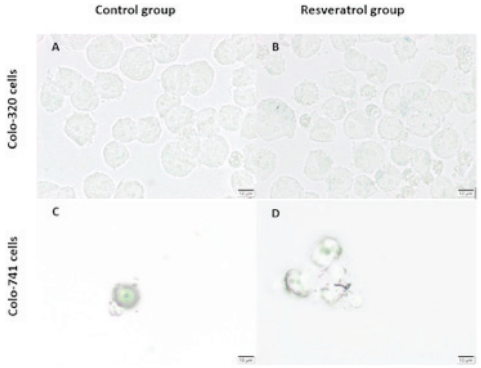

Figure 5. Evaluation of X-gal staining: (A) Colo-320 control cells, (B) resveratrol-treated Colo-320 cells, (C) Colo-741 control cells, (D) resveratrol-treated Colo-741 cells.

Table 3. Quantitative analysis of the percentage of X-Gal positive Colo-320 and Colo-741 cells treated with resveratrol at 25 μg/ml and 10 μg/ml concentration for 48h, respectively.

	Control group	Resveratrol group
Colo-320 cells	17.33±21.17	9.63±2.77[b]
Colo-741 cells	100.00±0.00	100,00±0,00[b]

Data are expressed as means ±SD and were compared by Mann Whitney U

[a]Significant difference between resveratrol-treated group and control group both in Colo-320 and Colo-741 cells ($P < 0.05$)

[b]Significant difference between resveratrol-treated Colo-320 cells and resveratrol-treated Colo-741 cells ($P < 0.05$)

Discussion

Resveratrol is a bioactive compound that has shown preventive and therapeutic effects against many cancer types (3). Based on recent studies, resveratrol induces apoptosis and cell cycle arrest and suppresses several cancer cell lines to induce cell proliferation and growth (16). Resveratrol and its metabolites can also show inhibitory effects on metastasis of cancer cells (17). Several studies have demonstrated that the anti-cancer mechanism of resveratrol differs according to the type and origin of cancer cells (18).

The biological effect of resveratrol on cellular death or senescence may be controlled in various molecular pathways, however, its role is unknown in primary or metastatic cancer cells (15). In addition, induction of cell senescence may be an important mechanism

preventing the differentiation and proliferation of tumor cells and may induce apoptosis (15). Therefore, the balance of the cellular senescence and apoptosis post-cancer therapy should be investigated further.

Bax and Bcl-2 are important regulatory proteins for mitochondria-mediated apoptosis and the Bax/Bcl-2 ratio is considered as a marker for cell apoptosis or survival (9). Resveratrol triggers apoptosis by increasing Bax and caspase-3 expression and decreasing Bcl-2 and Bcl-2/Bax ratio in a dose- and time-dependent manner in lung and colon cancer cell lines (19,20). Also, Baek et al. showed that resveratrol suppressed the expression of anti-apoptotic Bcl-2 protein and promoted apoptosis in squamous cell carcinoma of the head and neck cells (21). In this current study, resveratrol increased the level of the pro-apoptotic protein Bax. In addition, because of reduced Bcl-2, the Bax/Bcl-2 ratio was greater in Colo-320 cells than in Colo-741 cells. These results show that resveratrol triggers the mitochondria-mediated apoptotic pathway in primary colon cancer cell lines. On the other hand, our results suggest that metastatic colon cancer cells might increase Bcl-2 expression to protect cell survival to overcome resveratrol action or maybe the dose of resveratrol and incubation time were not enough to trigger Bax expression in Colo-741 cells in this study. Using resveratrol at a higher dose or in combination with another phenolic compound may facilitate apoptosis in metastatic colon cancer cells.

Caspases are enzymes that control apoptosis (22). Caspases-8 and 9 activate caspase-3 in mitochondria or cell membrane-mediated apoptosis, respectively which is the final step of apoptosis (9,22). El-Readi et al. reported that resveratrol increased caspase-3,8 and caspase-6/9 activity similar to anticancer drug camptothecin in Caco-2 cell lines (7). Chen et al. demonstrated that resveratrol increased caspase-3 and 9 expressions in metastatic colon cancer cell line SW620 (23). In the present study, the caspase-3 level was reduced in resveratrol-treated Colo-320 cells but it was increased in Colo-741 cells versus control groups, albeit non-significantly. Although resveratrol decreased cell survival both in primary and metastatic colon cancer cells, it was unable to trigger caspase-3 activity in a dose- and time-dependent manner in the studied cell lines. Testing caspase-8 and 9 activations may be helpful to determine the apoptotic phase in these cell lines. Moreover, using a higher dose of resveratrol and a combination of resveratrol with an anticancer drug might be more effective in triggering caspase-3 activity in these cells. Also, our results showed that TUNEL positive cells increased in resveratrol-treated Colo-320 cells. It may suggest that resveratrol may induce apoptosis via caspase-independent pathways in primary colon cancer cells.

Senescence drives cells to stop their cell cycle, resulting in a loss of proliferative ability (10). Previous studies have demonstrated that resveratrol and its metabolites promote cellular senescence in different types of cancer by controlling "senescence-associated molecular markers" (24,25). Hsp27 is a heat shock protein that regulates senescence and is overexpressed in different types of cancer cells (14). It was shown that a high dose of resveratrol (250 μM) decreased Hsp27 expression in breast cancer cell line MCF-7 and effectively sensitized cells to doxorubicin at 48 h (26). However, the relationship between decreased Hsp27 and senescence through resveratrol induction is still unclear. In contrast to other studies, Hsp27 expression increased non-significantly in the resveratrol group in both Colo-320 and Colo-741 cell lines. However, greater Hsp27 expression was observed in primary colon cancer cells than in metastatic colon cancer cells in the current study. These results may suggest that resveratrol causes more stress in primary colon cancer cell lines than in metastatic colon cancer cells. The expression of the survival protein Hsp27 was increased to a greater extent in primary cancer cells to escape senescence when compared to metastatic cells.

Bcl-2 proteins, play an important role in the regulation of the intrinsic pathway of apoptosis, include pro-apoptotic members such as Bax and anti-apoptotic members mainly Bcl-2 (27). Bax protein stimulates the intrinsic apoptotic pathway through inducing the efflux of cytochrome c from the mitochondria. Cytochrome c interacts with cytosolic apoptosis protease-activating factor-1 (Apaf-1) and pro-caspase-9 to form apoptosome which activates caspase-3 and apoptosis in the cell (28). On the other hand, Hsp-27 indirectly inhibits Bax protein translocation to mitochondria and prevents apoptosis via obstruction of cytochrome c releasing (29). In our study, resveratrol

induced intrinsic apoptosis with elevated level of Bax protein in primary colon cancer cells. However, Hsp27 protein level increased in colon cancer cells that may be escape apoptosis by this way. Also, our results indicated that metastatic colon cancer cells have resistance to resveratrol. Interestingly, Bcl-2 immunoreactivity increased in resveratrol-treated metastatic colon cancer cells which might provide highly proliferating cancer cells escape death pathways.

In recent years, the number of studies have demonstrated that resveratrol can modulate tumor cell migration and invasion through regulation of epithelial-mesenchymal transition in different cancers types such as glioma and pancreatic cancer (30,31). Matrix metalloproteinase 2, MMP 2, is a Hsp27 activated enzyme and related to metastatic progression (32). Elevated levels of Hsp27 proteins showed that resveratrol might not effective inhibiting metastasis of colon cancer cells in this study. These results also need to be evaluated with further assessment of different signaling pathway molecules that includes all metastasis pathways in colon cancer.

Lamin B1 is a protein found in the nuclear membrane and regulates cellular proliferation (33). Lamin B1 expression differs according to the type of cancer (33). Moreover, prostate, liver and pancreatic cancers seem to be associated with higher lamin B1 expression, whereas gastric, lung and colon cancers are linked to lower lamin B1 expression (33,34). Additionally, lower lamin B1 expression can be detected in senescent cells and this could help cancer cells to hide in a dormant state (35). Studies showed that different active compounds increase lamin B1 expression in colorectal cancer cell lines and drive cancer cell senescence (36). However, there is not any data that conclusively shows the relationship between resveratrol and senescence through lamin B1. In the current study, lamin B1 levels increased in Colo-320 cells whereas a reduction was observed in Colo-741 cells after resveratrol administration. However, this difference was not significant, suggesting that it may be dose- and time-dependent. Colo-320 cells might have increased their lamin B1 levels to escape senescence. On the other hand, resveratrol was more effective in decreasing lamin B1 in Colo-741 cells. However, decreasing lamin B1 and promoting senescence may be a defense mechanism for metastatic cancer cell line to help the maintenance of cancer cells and escape apoptosis.

CDK inhibitors regulate the cell cycle through cyclins and CDKs. p16 is a tumor suppressor protein from the CDK inhibitor family and has a role in cellular senescence (37). Ji et al. showed that a 100 μM dose of resveratrol led to an increase in p16 and induced premature cell senescence in lung cancer (24). Colin et al. reported that resveratrol induces an increase in p16 levels and promotes cellular senescence in colon cancer cells. However, they also found that as the exposure time increased, the colon cancer cells became resistant to resveratrol (12). Our results showed similar p16 levels between control and resveratrol groups in the Colo-320 cell line but decreased p16 levels were found in the resveratrol-treated Colo-741cell line, with no significant difference compared to control group. It can be suggested that resveratrol may induce senescence in Colo-741 cells line when used at a higher dose via increasing p16 levels.

Cyclin B1 is a key regulator of the cell cycle and its expression is upregulated in cancer cells such as colon cancer (38). Silencing cyclin B1 drives pancreatic cancer cells into senescence (39). The current study is the first to investigate the relationship between resveratrol and senescence through cyclin B1. According to our results, cyclin B1 expression did not change after resveratrol administration in Colo-320 cells or Colo-741 cells. Considering the X-gal staining results, senescent cells decreased in resveratrol-treated Colo-320 cells but it was not significant. It may be suggested that resveratrol may be stimulate apoptosis in primary colon cancer cells rather than senescence. On the other hand, greater senescent cells were found in resveratrol-treated Colo-741 cells when compared to Colo-320 cells. Furthermore, metastatic cancer cells may be avoiding from apoptosis through senescence. It may be considered that higher dose and incubation duration may be helpful to observed significant increased senescent cells and decreased lamin B1 levels after resveratrol administration in metastatic cells.

Resveratrol and its metabolites have several effects on cancer cells but their exact intracellular concentrations associated with their anti-cancer activities have yet to be determined. Primary and metastatic cancer cells display distinctive cell characteristics and therefore, the main limitation of our study might involve a lack of assessment of the intracellular level of the agent. Moreover, it may be useful to test caspases-8

and 9 to determine the phase of resveratrol-induced apoptosis in colon cancer cells. The findings of the current study may serve as a basis for future studies.

Conclusion

The effects of resveratrol on apoptosis and cell survival were evaluated in primary and metastatic colon cancer cells. The results demonstrated that resveratrol induced mitochondria-mediated apoptosis and decreased cell survival in primary colon cancer cells rather than senescence. Moreover, decreased cell viability without the apoptotic pathway further confirmed the effect of resveratrol in metastatic colon cancer cells. Triggering of apoptosis or controlling the balance between survival and death signaling pathways may be crucial to prevent metastasis and for the treatment of cancer patients. Besides, control of senescent cancer cells still poorly understood for therapy effectiveness and patient survival. Resveratrol may affect apoptosis and cell cycle via different mechanisms depending on the specific cell type. Further studies are needed to investigate the pharmacotherapeutic effects of resveratrol.

Conflict of interest

No potential conflict of interest relevant to this article was reported by the authors.

Acknowledgement:

The authors would like to thank Near East University, Research Center of Experimental Health Sciences (DESAM) and Manisa Celal Bayar University, Histology and Embryology Laboratory for their support throughout the study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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