Original article

Anti-Tumorigenic Effect of Resveratrol in HepG2 cells Controlled with Cytochrome-c Dependent Cell Death

Nadire Kıyak[1,2], Eda Becer [2,3], H. Seda Vatansever[2,4], Aysel Kükner[1]

[1]Faculty of Medicine, Department of Histology and Embryology, Near East University, Nicosia, Mersin 10 Turkey; [2]DESAM Institute, Near East University, Nicosia, Mersin 10 Turkey; [3] Faculty of Pharmacy, Department of Biochemistry, Near East University, Nicosia, Mersin 10 Turkey; [4]Faculty of Medicine, Department of Histology and Embryology, Manisa Celal Bayar University, Manisa, Turkey

Abstract. Resveratrol is a phytochemical that is regarded as a potential anticancer agent in liver cancer prevention. It had also been shown that resveratrol has role in the prevention of cancer and anti-cancer properties. In this study, we aimed to investigate the effects of resveratrol on cell viability, apoptosis, cellular proliferation, JAK/STAT pathway and epithelial-mesenchymal transition (EMT) in human hepatocellular carcinoma cell (*HepG2)* line. Cell growth and cytotoxicity were evaluated with MTT assay with different concentrations (5, 10, 25, 50, 100 μM) of resveratrol in HepG2 cells. The distribution of FasL, cyt-c, caspase-3, Ki-67, ACTA2, CD133, JAK2, N-cadherin, vimentin and STAT3 in HepG2 cells were analyzed using indirect immunoperoxidase technique. The effective dose and incubation time for inhibition of cell growth in HepG2 cells was determined as 100 μM for 48 hours. Decreased Ki-67 immunoreactivity following resveratrol application was significant in HepG2 cells. Increased cytc-c, STAT3, vimentin, N-cadherin and CD133 immunoreactivities were significant between resveratrol applicated HepG2 cells and control group. According to our results, resveratrol induced mitochondrial-dependent cell death and suppressed proliferation in HepG2 cells. On the other hand, our results showed that resveratrol stimulated cellular self-protection responses through activation of EMT and STAT3 protein expression in HepG2 cells.

Keywords: Cancer, resveratrol, EMT, apoptosis

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and is the third common cause of cancer mortality worldwide (1). Liver transplantation, chemotherapy, surgical resection and radiotherapy are current curative treatment options in HCC. However, recurrence and metastasis are very common in HCC. For this reason the length and quality of life are still poor in HCC (2). Extensive scientific researches over the past decade showed that critical mutations and epigenetic alterations in cellular signaling pathway molecules are involved in hepatocarcinogenesis (3). In this respect, development of new target therapeutics against critical cellular signaling pathways such as receptor tyrosine kinase, JAK/STAT, p53, Wnt/βcatenin, phosphatidylinositol-3 kinase (PI3K)/AKT, pRb, mitogen-activated protein kinase (MAPK) pathways are very important for halting the progression of HCC (4). However, poorly understood and complex molecular crosstalk in signaling pathways cause chemoresistance and/or decrease effectiveness of cancer therapy in HCC (5).

In HCC treatment, systemic chemotherapy is still lack. Although, FDA-approved sorafenib is used for HCC treatment, it causes many adverse effects. Thence, alternative therapeutic strategies are urgent to improve liver cancer treatment (6). Recent studies have shown that natural multifunctional polyphenolic compound such as resveratrol has protective impacts to

reduce the side effects that are induced by chemotherapeutic drugs. Resveratrol is one of the polyphenolic compounds which is found in various dietary sources such as red wine, white, peanut, blueberry, green grape, black grape, and raisin, etc (7). Moreover, resveratrol has been shown to modify molecular pathways involved in redox signaling and inflammation which are involved in HCC development and progression (7,8). Within the past decade, many research groups have evaluated the anticancer potential of resveratrol using in vitro studies. Several research demonstrated that resveratrol was shown to have cytotoxic and proapoptotic effects on HepG2 cells (9-18). Sun et al. reported that resveratrol inhibited the H22 hepatoma cell's growth in a dose and time-dependent manner (13). Additionally, Michels et al. observed that resveratrol stimulated the apoptosis of metabolically active H4IIE rat hepatoma cells by caspase activation (14).

In HCC, metastasis of cancer cells and recurrence after chemotherapy are reported problems. Poor prognosis of patients indicates that epithelial-mesenchymal transition (EMT) mediates tumor progression in HCC (15). EMT is a crucial key event in the early step of tumor metastasis. EMT is a molecular process which epithelial cells lose polarity and cell-cell contacts, and gain invasive mesenchymal stem cell like properties. Additionally, the expression of mesenchymal cell proteins such as E-cadherin is loss while N-cadherin and vimentin are expressed in cancer cells (16). HCC cells gain a more invasive, aggressive, chemoresistance and stemness properties like cancer stem cells through EMT (17-19). Recent studies reported that EMT markers including E-cadherin, N-cadherin and vimentin expression were correlated with a shorter survival, worse prognosis and extrahepatic recurrence in HCC (20-23).

Several studies demonstrated that resveratrol suppresses the tumor metastasis via inhibiting signaling pathways related with EMT in different types of cancer cells (24,25). Ji et al. reported that resveratrol inhibited EMT and invasion via reducing Smad2/3 expression in colorectal cancer (26). It has also shown that resveratrol inhibited EMT in A549 lung cancer cells through inhibiting the E-cadherin expression and attenuating the vimentin expression (27). Moreover, resveratrol has been shown to suppress the pancreatic cancer PANC-1 cell's metastatic potential via regulating EMT-associated factors such as vimentin and N-cadherin (28,29). To the current knowledge, there is no reported in vitro studies addressing the effects of resveratrol for EMT related proteins such as vimentin and N-cadherin in HepG2 cells. The specific effects of resveratrol to EMT in HepG2 cells remain undefined.

The aims of this study were: (i) to investigate the effect of resveratrol on cell viability in HepG2 cells; (ii) to determine the effect of resveratrol on expression of EMT markers, N-cadherin, vimentin and ACTA2 in HepG2 cells; (iii) to examine proapoptotic effects of resveratrol via FasL, cytochrome-c (cyt-c) and caspase-3 expression in HepG2 cells; (iv) to investigate effects of resveratrol on JAK/STAT pathway through JAK2 and STAT3 expression in HepG2 cells; and (v) to determine antiproliferative and stemness effects of resveratrol via Ki-67 and CD133 expression in HepG2 cells.

Materials and Methods

Cell Line and Cell Culture

HepG2 (human liver hepatocellular carcinoma cell line, ATCC: CRL-10741) was used in this study. RPMI 1640 (Gibco, Catalog no: 11875101) were used as supplements that contained 10% heat-inactivated fetal bovine serum (FBS) (Capricorn Scientific, Catalog no: FBS-HI-11B), 1% penicillin-streptomycin (Capricorn Scientific, Catalog no: PS-B) and 1% L-glutamine (Capricorn Scientific, Catalog no: GLN-B) for HepG2 cells culture. Cells were cultured in a humidified atmosphere at 37 °C and 5% CO_2. When the cultured cells reached confluency state, they were sub-cultured using a 0.25% trypsin-EDTA solution (Capricorn Scientific, Catalog no: TRY-1B).

Cell Viability and Growth Assay

Effect of resveratrol ((E)-5-(2-(4-hydroxyphenyl) ethenyl)-1,3-benzenediol; 99% pure) (Sigma, Catalog no: R5010) on cell proliferation was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Glentham Life Sciences, Catalog no: GC4568). Assay as described our previous report

with slight modifications (30). The stock resveratrol solution was prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Catalog no: D2650). At the time of analysis, resveratrol diluted in culture medium with five concentrations (5, 10, 25, 50, and 100 μM). The final concentration of DMSO in culture medium of the cells was less than % 0.05. HepG2 cells were cultured in 96-well plates for 24 h and 48h, cell density in each well was $5x10^3$ in 100 **μL** of culture medium. After the incubation, 10 μl MTT solution was added into each well and incubated for 4 h at 37 β Subsequently, 50 μl of DMSO was added to dissolve the formazan crystals. Absorbance of plate was measured at 540 nm with a UV-visible spectrophotometer (Versa Max, Molecular Device, Sunnyvale USA). All of the experiments were repeated minimum of three times independently.

Immunocytochemistry

The previously described indirect immunoperoxidase staining protocol was used for evaluating FasL, cyt-c, caspase-3, Ki-67, ACTA2, CD133, JAK2, N-cadherin, vimentin and STAT3 distributions in HepG2 cells (28). Briefly, $5x10^3$ HepG2 cells were plated in each well of 24-well plate and allowed to attach overnight and, then were applied with resveratrol, which was diluted with HepG2 culture medium, in resveratrol group. The control group cells were cultured with HepG2 culture medium as same culture time with study group cells. Both resveratrol and control HepG2 cells were fixed with 4% paraformaldehyde after incubation. After washing with phosphate buffer saline (PBS), they were incubated with blocking solution for 1 h, cells were then incubated with primary antibodies; FasL (Santa Cruz Biotechnology, Catalog no: sc-834), cyt-c (Santa Cruz Biotechnology, Catalog no: sc-13156), caspase-3 (Santa Cruz Biotechnology, Catalog no: sc-7272), Ki-67 (Thermofisher Scientific, Catalog no: B-081-A1), ACTA2 (Proteintech, Catalog no: 14395-1-AP), CD133 (Abcam, Catalog no: ab19898), JAK-2 (Abcam, Catalog no: ab32101), N-cadherin (Proteintech, Catalog no: 13769-1-AP), vimentin (Proteintech, Catalog no: 10366-1-AP) and STAT3 (Abcam, Catalog no: ab68153) for overnight at 4°C. Then washing with PBS, the cells were subsequently incubated with biotinylated secondary antibody (Thermo Fischer, Histo Stain-Plus, Catalog no: HRP 859043) and streptavidin-peroxidase complex, respectively. For immune labeling, they were incubated with diaminobenzidine (DAB) (Scytec, Catalog no: 36038) for 5 minutes. Then, HepG2 cells were counterstained with Mayer's hematoxylin solution (Bio Optica, Catalog no: 1213) for 5 minutes. They were mounted using the mounting medium (Merck Millipore, Catalog no: 107961, Germany) and examined under a light microscope (Olympus BX40, Tokyo, Japan).

Staining of FasL, cyt-c, caspase-3, Ki-67, ACTA2, CD133, JAK2, N-cadherin, vimentin and STAT3 was graded semi-quantitatively using the H-SCORE. In H-SCORE=Σ(i+1) formulation, i is the intensity of dyeing with a value of 1, 2 or 3 (mild, moderate, or strong, respectively). βis the percentage of cells stained (between 0 and 100%) with each intensity.

Statistical Analysis

Mean ± SD (standard deviation) was used for data expression. Graph Pad Prism 7 software was used for analyzing and group differences were analyzed with Mann-Whitney U tests. A $p < 0.05$ was regarded as statistically significant.

Results

Cell Viability and Cytotoxicity

In this study, we determined the cytotoxicity of resveratrol on HepG2 cells using MTT assay. HepG2 cells were treated with five different resveratrol concentrations (5-100 μM) for 24 and 48 h. After MTT assay, resveratrol application at 100 μM concentration was more effective in suppressing HepG2 cell viability when compared with other dilutions for 48 h (Figure 1 A and B).

Cell Morphology

HepG2 cells are epithelial-like and adhesive cells (Figure 2B). After application with resveratrol, HepG2 cell number decreased and their shapes changed to oval (Figure 2A).

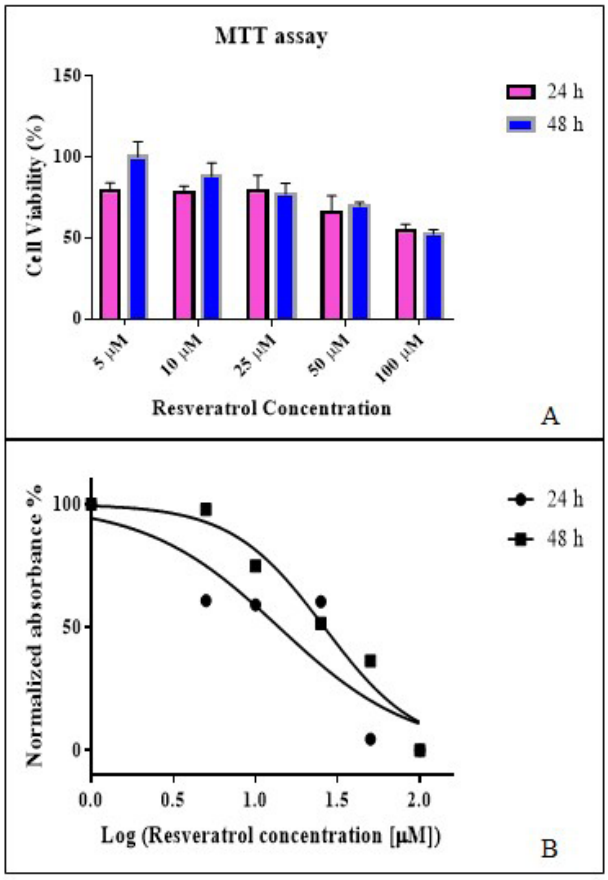

Figure 1. Cell viability and IC50 value. (A) MTT assay showing the viability of HepG2 cells after application increasing concentrations of resveratrol for 24 h and 48 h. (B) IC50 values of resveratrol for HepG2 cells. IC50 of the resveratrol was calculated as 111.6 µM.

Immunohistochemical evaluation

Regarding to the apoptotic markers, the immunostaining intensity of FasL and caspase-3 were moderate in HepG2 cells after resveratrol application (Figure 3A and 3E) and these intensities were higher than control HepG2 cells (Figure 3B and 3F), but they were not statistically significant ($p > 0.05$, Table 1).

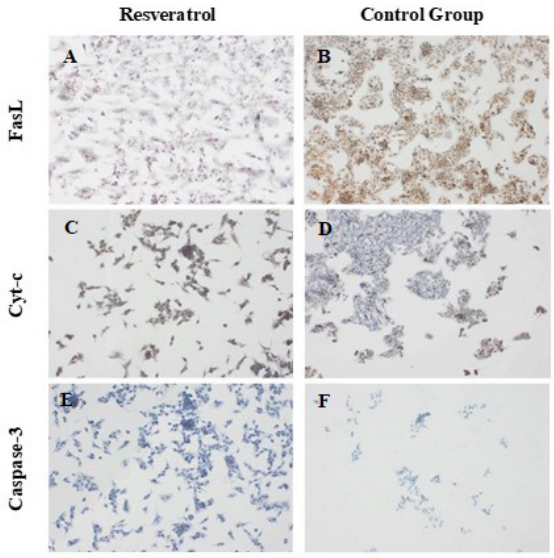

Figure 3. Immunoreactivity of FasL, cyt-c and caspase-3 in HepG2 cells in 48 h culture exposed with 100 µM resveratrol (A, C, E) or standard culture conditions (control group) (B, D, F). Scale bars: 500 µm.

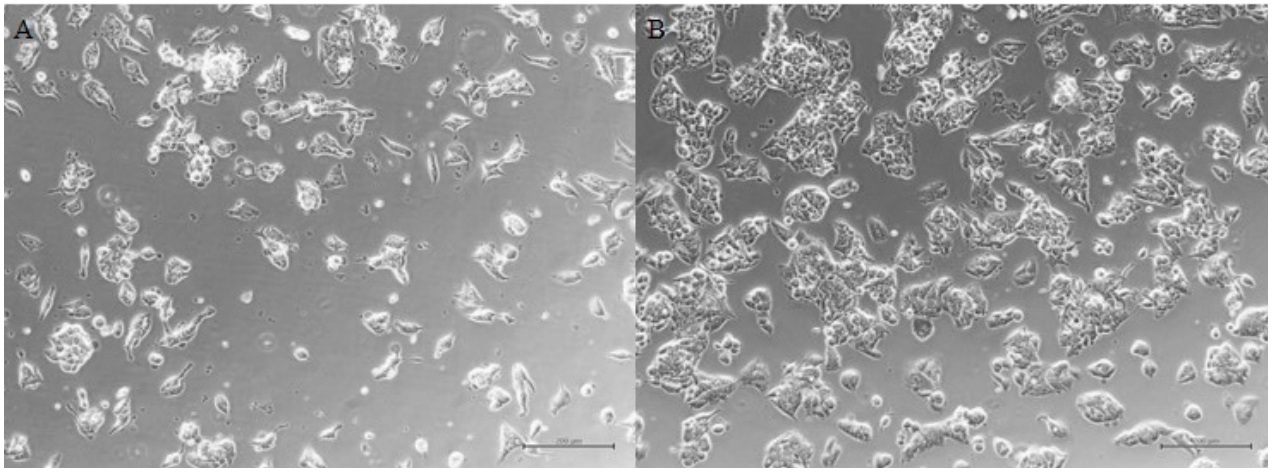

Figure 2. HepG2 cells imaged under the inverted microscope: (A) Resveratrol and (B) control groups of HepG2 cells. Scale bars: 200 µm.

On the other hand, strong immunostaining of cyt-c was detected in resveratrol group HepG2 cells (Figure 3C), this increased intensity was significant when compared to control group HepG2 cells (Figure 3D) ($p < 0.05$, Table 1).

The immunoreactivity of Ki-67 was weak in resveratrol group HepG2 cells (Figure 4A) and strong in control group HepG2 cells (Figure 4B). Also, the staining intensity of Ki-67 in resveratrol group HepG2 cells group was statistically significant ($p<0.05$, Table 1).

Immunostaining of CD133, N-cadherin, STAT3 and vimentin were very strong in resveratrol group HepG2 cells (Figure 4G, 4I, 4K and 4M). Additionally, increase staining intensity of CD133, N-cadherin, STAT3 and vimentin were detected in resveratrol group HepG2 cells in comparison with control group, and the differences were statistically significant ($p< 0.05$, Table 1). While the intensity of ACTA2 and JAK2 were very moderate in HepG2 cells after resveratrol application (Figure 4C and 4E) and these intensities were higher than control HepG2 cells (Figure 4D and 4F), but they were not statistically significant ($p> 0.05$, Table 1).

Discussion

HCC is one of the common cancers types in the world. HCC is an extremely challenging disease to treat, due to different disease molecular mechanisms. The goal of utilization a molecular target-specific drug is to suppress a target central to a main disease mechanism of interest in HCC. Whereas, single molecular target drugs have very little therapeutic impacts in complex disease as HCC. The multi-target treat-

Table 1. The H-SCORE of FasL, cyt-c, caspase-3, Ki-67, ACTA2, JAK2, STAT3 CD133, N-cadherin and vimentin immunolabelling in both resveratrol and control groups of HepG2 cells for 48 h.

	Resveratrol Group	Control Group
FasL	282.9 ± 47.4	238.6 ± 22.08
Cyt-c	310.3 ± 31.67*	244.5 ± 20.64
Caspase-3	120.5 ± 5.4	106.6 ± 8.36
Ki-67	120.4 ± 17.44*	319.5 ± 27.18
ACTA-2	274.9 ± 45.48	263.7 ± 43.82
JAK2	177.2 ± 45.37	161 ± 32.47
STAT3	228.9 ± 39.44*	116.5 ± 15.34
N-cadherin	309.5 ± 47.58*	122.7 ± 8.87
Vimentin	178.2 ± 44.69*	119.1 ± 3.39
CD133	330.1 ± 25.77*	193.6 ± 52.73

Data are expressed as means ± SD and were compared by Mann-Whitney U test.
* The data was significant when compared with control group ($p<0.05$).

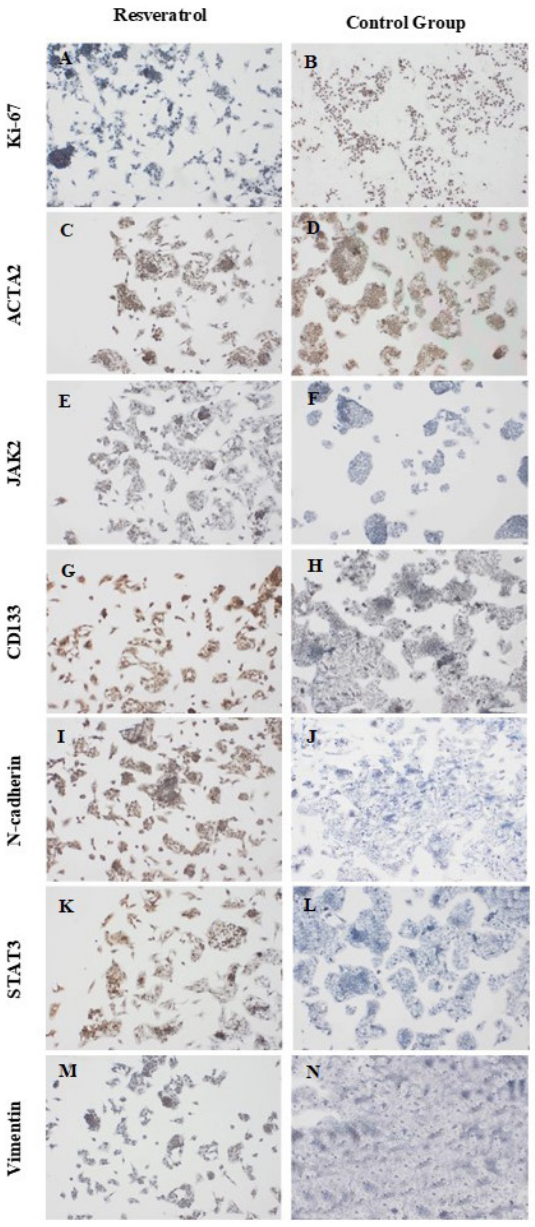

Figure 4. Immunoreactivity of Ki-67, ACTA2, JAK2, CD133, N-cadherin, STAT3 and vimentin in HepG2 cells in 48 h culture exposed to 100 μM resveratrol (A, C, E, G, I, K, M). or standard culture conditions (control group) (B, D, F, H, J, L, N). Scale bars: 500 μm

ment approach can be especially beneficial in HCC (1). In the last decade, natural compounds combined treatment strategies have been examined to suppress multi-molecular signaling cascades in HCC. However, resveratrol-related investigations for HCC treatment are still limited (31). In the present study, we demonstrated that resveratrol had pro-apoptotic and antiproliferative effects on HepG2 cells. Interestingly, resveratrol application stimulated EMT and expression of STAT3 protein and thereby activation cellular self-protection response in HepG2 cells.

Resveratrol can trigger mitochondrial-dependent cell death (intrinsic apoptotic pathway) to stimulate release of pro-apoptotic factors from mitochondria, and this capability has been used as a potential strategy in cancer treatment (32). Ou et al. found that resveratrol induces apoptosis through intrinsic pathway in HepG2 cells (33). Ma et al. showed that resveratrol stimulated cell death in HepG2 cells through intrinsic apoptotic pathway including mitochondrial membrane potential loss and releasing of cytochrome c (cyt-c). Also, authors demonstrated that resveratrol increased intracellular Ca^{2+} ion concentration in mitochondria that may compromise mitochondrial membrane potential. Progressive Ca^{2+} loading ultimately caused the mitochondrial permeability transition pore to open and released apoptotic signaling molecules such as cyt-c into the cytoplasm in HepG2 cells. Then, the cyt-c activated caspase-9 which cleaved and activated calpain enzymes, executioner caspase-3 and caspase-6 resulting in HepG2 cell apoptosis (34). In our results showed that cyt-c intensity was found to be increased after resveratrol application in HepG2 cell after 48 h culture time. Moreover, resveratrol application increased in both FasL and caspase-3 intensities in HepG2 cells while results did not statistically significant. The results indicated that resveratrol may be effective in triggering intrinsic apoptotic pathway in HepG2 cells, but, 48 h culture time may not be effective to be significant stimulation of the caspase-3 secretion.

Abnormal activation of intracellular signaling pathways contained in cell proliferation, differentiation, survival and apoptosis have been found to contribute to development and progression of HCC. JAK/STAT signaling pathway is one of the signaling pathway which is involved in HCC development (35). The JAK/STAT signaling pathway has crucial roles in numerous cellular functions such as cell proliferation, differentiation, stem cell maintenance and immune/inflammatory response modulation. This pathway can be activated by cytokines and growth factors, including EGF family members, interferons and interleukins, which bind to their transmembrane receptors. The cytoplasmic tails of these transmembrane receptors are associated with JAKs that are activated onto ligand-induced and phosphorylate tyrosine residues on the receptors cytoplasmic tail (36). Then, JAKs phosphorylate STATs (signal transducers and activators of transcription) such as STAT3 which are form dimers and translocate to the nucleus of cell. Also, STAT3 signal transduction cascade is activated by distinct receptors stimuli such as growth factors, oxidative stress and cytokines (37). Studies into HCC have demonstrated that STAT3 is key role in various tumorigenesis processes including proliferation, invasion, metastasis, survival, inflammation and angiogenesis (38,39). Due to the oncogenic role of STAT3 dysregulation, many molecule inhibitors that target STAT3 have been developed and tested for against HCC (40,41). Moreover, Li et al. reported that resveratrol inhibited STAT3 signaling pathway via SIRT1 signaling in high glucose-exposed HepG2 cells (42). Trung et al. showed that resveratrol inhibited constitutively active STAT3 and JAK2 phosphorylation however did not effect on other STAT3 activation upstream mediators, such as JAK1, PTEN and TYK2 in natural killer (NK) (43). In contrast to previous studies, resveratrol increased STAT3 intensity in HepG2 cell in our study. Also, resveratrol application elevated JAK2 intensity but results were not statistically significant. We speculate that antitumor effects of resveratrol may be through different signaling pathways from JAK/STAT pathway in HepG2 cells.

Hepatocellular EMT is an important event in cancer cell invasion and metastasis in HCC progression. During the tumor cells invasion process, cancer cells lose cell-cell contacts and lose polarity due to loss of E-cadherin expression while mesenchymal proteins such as N-cadherin and vimentin expression are increased. Additionally, molecular signaling pathways which indicate partial recurrence of embryonic

programs such as TGF-βand Wnt/βcatenin signaling pathways are activated and play key role in the EMT of HCC cells (15). Especially, TGF-ß activated transcription ACTA2 (actin alpha 2) which is an EMT-associated marker and known to contribute to cell movement. Elevation of ACTA2 levels are related with cancer cells invasion and metastasis (44). When EMT is activated in HCC, cancer cells gain properties of stem cells that contribute to self-renew abilities and can be marked by CD133, CD44 and CD90 proteins (45). In HCC, the expression of CD133, CD90 and CD44 has been correlated to EMT and can predict metastasis. Many studies reported that resveratrol inhibited the development of tumor cell invasion and metastasis via suppressing signaling pathways related with EMT in different cancer types (26,27). In our previous study, we showed that resveratrol increased the N-cadherin and vimentin expression in CD133- and CD133+ pancreatic cancer cells when compared with the control groups but the differences were not significant (28). In the current study, the intensity of N-cadherin, vimentin and CD133 were significantly increased after resveratrol application in HepG2 cells. Also, resveratrol application increased in ACTA2 intensity in HepG2 cells while results did not statistically significant. Our results indicated that resveratrol application in different cancer cell types may be lead to diverse responses through signaling pathway activation or inhibition. EMT related signaling pathways may be activated by resveratrol in HepG2 cells.

Antiproliferative potential of resveratrol was revealed in cell growth experiments. The results showed that resveratrol inhibited proliferation and delayed or prevented the HepG2 cells from entering mitoisis. The tumor cell proliferation and growth are associated with Ki-67, nuclear protein expression (10,46,47). Clinically, Ki-67 protein is associated with the intrinsic cell populations proliferative activity in malignant tumors and it is used as a prognostic marker for cancer diagnosis and treatment (48). In our study, the intensity of Ki-67 was significantly decreased after resveratrol application in HepG2 cells. Our results exhibited that resveratrol inhibited cell proliferation in HepG2 cells.

The *in vitro* studies that investigate effects of the resveratrol on EMT, apoptosis, stemness properties, proliferation and JAK/STAT3 pathway in HCC are still limited (31). In conclusion, we showed that resveratrol induced mitochondrial-dependent cell death and suppressed proliferation in HepG2 cells. On the other hand, our results showed that resveratrol stimulated cellular self-protection responses through activation of EMT and STAT3 protein expression in HepG2 cells. Therefore, resveratrol could be able to trigger apoptotic cell death rather than metastatic properties in HepG2 cells. Our results presented new concerns on the resveratrol side-effects of administration which may break the balance of the pro and anti-growth signal in HCC. In order to verify exact anti-cancer activities of resveratrol on HepG2 cells, further assessment with different cellular signaling pathway molecules that include all possible cancer promotion, and metastasis mechanisms, is necessary.

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

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Chen C, Wang G. Mechanisms of hepatocellular carcinoma and challenges and opportunities for molecular targeted therapy. World journal of hepatology 2015; 7:1964.
2. Sia D, Villanueva A, Friedman SL, Llovet JM. Liver cancer cell of origin, molecular class, and effects on patient prognosis. Gastroenterology 2017; 152:745-61.
3. Alqahtani A, Khan Z, Alloghbi A, S Said Ahmed T, Ashraf M, M Hammouda D. Hepatocellular carcinoma: molecular mechanisms and targeted therapies. Medicina 2019; 55:526.
4. Cha C, DeMatteo RP. Molecular mechanisms in hepatocellular carcinoma development. Best practice & research Clinical gastroenterology 2005; 19:25-37.
5. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology 2008; 134:1752-63.
6. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. New England journal of medicine 2008; 359:378-90.
7. Ramos S. Cancer chemoprevention and chemotherapy: dietary polyphenols and signaling pathways. Molecular nutrition & food research 2008; 52:507-526.
8. Bishayee A, Politis T, Darvesh AS. Resveratrol in the chemoprevention and treatment of hepatocellular carcinoma. Cancer treatment reviews 2010; 36:43-53.

9. Kocsis Z, Marcsek ZL, Jakab MG, Szende B, Tompa A. Chemopreventive properties of trans-resveratrol against the cytotoxicity of chloroacetanilide herbicides in vitro. International journal of hygiene and environmental health 2005; 208:211-218.
10. Stervbo U, Vang O, Bonnesen C. Time‐and concentration‐dependent effects of resveratrol in HL‐60 and HepG2 cells. Cell Proliferation 2006; 39:479-493.
11. Notas G, Nifli AP, Kampa M, Vercauteren J, Kouroumalis E, Castanas E. Resveratrol exerts its antiproliferative effect on HepG2 hepatocellular carcinoma cells, by inducing cell cycle arrest, and NOS activation. Biochimica et Biophysica Acta (BBA)-General Subjects 2006; 1760:1657-1666.
12. Yu H, Pan C, Zhao S, Wang Z, Zhang H, Wu W. Resveratrol inhibits tumor necrosis factor-α-mediated matrix metalloproteinase-9 expression and invasion of human hepatocellular carcinoma cells. Biomedicine & pharmacotherapy 2008; 62:366-372.
13. Sun ZJ, Pan CE, Liu HS, Wang GJ. Anti-hepatoma activity of resveratrol in vitro. World journal of gastroenterology 2002; 8:79.
14. Michels G, Wätjen W, Weber N, et al. Resveratrol induces apoptotic cell death in rat H4IIE hepatoma cells but necrosis in C6 glioma cells. Toxicology 2006; 225:173-82.
15. Giannelli G, Koudelkova P, Dituri F, Mikulits W. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. Journal of hepatology 2016; 65:798-808.
16. Gurzu S, Kobori L, Fodor D, Jung I. Epithelial mesenchymal and endothelial mesenchymal transitions in hepatocellular carcinoma: a review. BioMed research international 2019.
17. Seo DD, Lee HC, Kim HJ, et al. Neural cadherin overexpression is a predictive marker for early postoperative recurrence in hepatocellular carcinoma patients. Journal of gastroenterology and hepatology 2008; 23:1112-1118.
18. Yamanaka C, Wada H, Eguchi H, et al. Clinical significance of CD13 and epithelial mesenchymal transition (EMT) markers in hepatocellular carcinoma. Japanese journal of clinical oncology 2018; 48:52-60.
19. Yang MH, Chen CL, Chau GY, et al. Comprehensive analysis of the independent effect of twist and snail in promoting metastasis of hepatocellular carcinoma. Hepatology 2009; 50:1464-1474.
20. Zhai X, Zhu H, Wang W, Zhang S, Zhang Y, Mao G. Abnormal expression of EMT-related proteins, S100A4, vimentin and E-cadherin, is correlated with clinicopathological features and prognosis in HCC. Medical oncology 2014; 31:1-9.
21. Kim J, Hong SJ, Park JY, et al. Epithelial–mesenchymal transition gene signature to predict clinical outcome of hepatocellular carcinoma. Cancer science 2010; 101:1521-1528.
22. Mima K, Hayashi H, Kuroki H, et al. Epithelial-mesenchymal transition expression profiles as a prognostic factor for disease-free survival in hepatocellular carcinoma: Clinical significance of transforming growth factor-β signaling. Oncology letters 2013; 5:149-154.
23. Li YM, Xu SC, Li J, et al. Epithelial–mesenchymal transition markers expressed in circulating tumor cells in hepatocellular carcinoma patients with different stages of disease. Cell death & disease 2013; 4:e831.
24. Huber MA, Beug H, Wirth T. Epithelial-mesenchymal transition: NF-κB takes center stage. Cell cycle 2004; 3:1477-1480.
25. Maier HJ, Schmidt-Straßburger U, Huber MA, Wiedemann EM, Beug H, Wirth T. NF-κB promotes epithelial–mesenchymal transition, migration and invasion of pancreatic carcinoma cells. Cancer letters 2010; 295:214-228.
26. Ji Q, Liu X, Han Z, et al. Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-β1/Smads signaling pathway mediated Snail/E-cadherin expression. BMC cancer 2015; 15:1-2.
27. Wang H, Zhang H, Tang L, et al. Resveratrol inhibits TGF-β1-induced epithelial-to-mesenchymal transition and suppresses lung cancer invasion and metastasis. Toxicology 2013; 303:139-146.
28. Hoca M, Becer E, Kabadayı H, Yücecan S, Vatansever HS. The effect of resveratrol and quercetin on epithelial-mesenchymal transition in pancreatic cancer stem cell. Nutrition and cancer 2020; 72:1231-1242.
29. Li W, Ma J, Ma Q, et al. Resveratrol inhibits the epithelial-mesenchymal transition of pancreatic cancer cells via suppression of the PI-3K/Akt/NF-κB pathway. Current medicinal chemistry 2013; 20:4185-4194.
30. Özsoy S, Becer E, Kabadayı H, Vatansever HS, Yücecan S. Quercetin-Mediated apoptosis and cellular senescence in human colon cancer. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 2020; 20:1387-1396.
31. Ko JH, Sethi G, Um JY, et al. The role of resveratrol in cancer therapy. International journal of molecular sciences 2017; 18:2589.
32. De Oliveira MR, Nabavi SF, Manayi A, Daglia M, Hajheydari Z, Nabavi SM. Resveratrol and the mitochondria: From triggering the intrinsic apoptotic pathway to inducing mitochondrial biogenesis, a mechanistic view. Biochimica et Biophysica Acta (BBA)-General Subjects 2016; 1860:727-745.
33. Ou X, Chen Y, Cheng X, Zhang X, He Q. Potentiation of resveratrol-induced apoptosis by matrine in human hepatoma HepG2 cells. Oncology reports 2014; 32:2803-2809.
34. Ma X, Tian X, Huang X, Yan F, Qiao D. Resveratrol-induced mitochondrial dysfunction and apoptosis are associated with Ca 2+ and mCICR-mediated MPT activation in HepG2 cells. Molecular and cellular biochemistry 2007; 302:99-109.
35. Tang JJ, Thng DK, Lim JJ, Toh TB. JAK/STAT signaling in hepatocellular carcinoma. Hepatic oncology 2020; 7.
36. Murray PJ. The JAK-STAT signaling pathway: input and output integration. The Journal of Immunology 2007; 178:2623-2629.
37. Siveen KS, Sikka S, Surana R, et al. Targeting the STAT3 signaling pathway in cancer: role of synthetic and natural inhibitors. Biochimica et Biophysica Acta (BBA)-reviews on

cancer 2014; 1845:136-154.
38. Li WC, Ye SL, Sun RX, et al. Inhibition of growth and metastasis of human hepatocellular carcinoma by antisense oligonucleotide targeting signal transducer and activator of transcription 3. Clinical Cancer Research 2006; 12:7140-7148.
39. Zhang CH, Guo FL, Xu GL, Jia WD, Ge YS. STAT3 activation mediates epithelial-to-mesenchymal transition in human hepatocellular carcinoma cells. Hepato-gastroenterology 2014; 61:1082-1089.
40. Okusaka T, Ueno H, Ikeda M, et al. Phase 1 and pharmacological trial of OPBβ31121, a signal transducer and activator of transcriptionβ3 inhibitor, in patients with advanced hepatocellular carcinoma. Hepatology Research 2015; 45:1283-1291.
41. Yoo C, Kang J, Lim HY, et al. Phase I dose-finding study of OPB-111077, a novel STAT3 inhibitor, in patients with advanced hepatocellular carcinoma. Cancer research and treatment: official journal of Korean Cancer Association 2019; 51:510.
42. Li Y, Zhu W, Li J, Liu M, Wei M. Resveratrol suppresses the STAT3 signaling pathway and inhibits proliferation of high glucose-exposed HepG2 cells partly through SIRT1. Oncology reports 2013; 30:2820-2828.
43. Trung LQ, Espinoza JL, Takami A, Nakao S. Resveratrol induces cell cycle arrest and apoptosis in malignant NK cells via JAK2/STAT3 pathway inhibition. PloS one 2013; 8:e55183.
44. Tomaskovic-Crook E, Thompson EW, Thiery JP. Epithelial to mesenchymal transition and breast cancer. Breast Cancer Research 2009; 11:1-0.
45. Yang HL, Chen WQ, Cao X, et al. Caveolin-1 enhances resveratrol-mediated cytotoxicity and transport in a hepatocellular carcinoma model. Journal of Translational Medicine 2009; 7:1-3.
46. Yu Z, Pestell TG, Lisanti MP, Pestell RG. Cancer stem cells. The international journal of biochemistry & cell biology 2012; 44:2144-2151.
47. Colin D, Lancon A, Delmas D, et al. Antiproliferative activities of resveratrol and related compounds in human hepatocyte derived HepG2 cells are associated with biochemical cell disturbance revealed by fluorescence analyses. Biochimie 2008; 90:1674-1684.
48. Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer. Molecular medicine reports 2015; 11:1566-1572.

Correspondence
Eda Becer, Near East University, Faculty of Pharmacy, Department of Biochemistry, Near East Boulevard, Nicosia, ZIP. 99138, Mersin 10 Turkey.
Phone: + 90 392 680 2000/5134 Fax: + 90 392 680 20 38
E-mail: edabecer@yahoo.com