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Original Article

The effect of quercetin on cervical cancer cells as determined by inducing tumor endoplasmic reticulum stress and apoptosis and its mechanism of action

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Abstract: Objective: This study aimed to explore the effect of quercetin on cervical cancer cells by inducing tumor endoplasmic reticulum stress (ERS) and apoptosis and its mechanism of action. Methods: HeLa cells were treated with different concentrations of quercetin, and the cell viability was measured using methyl thiazolyl tetrazolium (MTT) colorimetric assays. The apoptosis rate was measured using flow cytometry. The changes in the related protein X (Bax), B-cell lymphoma/leukemia-2 (Bcl-2), and G1/S-specific cyclin-D1 (Cyclin D1) levels after the HeLe apoptosis were determined using Western blot, and the changes in the human cystinase-3 (Caspase-3), glucoprotein 78 (GRP78), and enhancer-binding protein homologous protein (CHOP) levels, and the receptor-related protein levels in the ERS pathway/endoribonuclease inositol requiring enzyme 1 (IRE1), and the phosphorylated pancreatic endoplasmic reticulum stress kinase (p-Perk), and the activated transcription factor-6 (ATF6) levels were also quantified. Results: After treating the HeLa cells with different concentrations of quercetin, the cell viability was inhibited to varying degrees, showing a significant time and concentration dependence. The apoptosis rate in the quercetin group increased significantly in comparison with the blank control group, and the apoptosis rate also showed a tendency to increase progressively with an increasing concentration of the quercetin ($P<0.05$). The Bax and Bcl-2 levels in the quercetin intervention group showed a tendency to increase progressively in comparison with the blank control group, and Cyclin D1 showed a tendency to decrease progressively ($P<0.05$). The of Caspase-3, GRP78, and CHOP expression levels in the quercetin intervention group rose significantly in comparison with the blank control group ($P<0.05$). The IRE1, p-Perk, and c-ATF6 levels in the quercetin intervention group showed a tendency to rise gradually in comparison with the blank control group ($P<0.05$). Conclusion: Quercetin may promote the apoptosis of cervical cancer HeLe cells by inducing the tumor ERS pathway.

Keywords: Quercetin, tumor endoplasmic reticulum stress, apoptosis, cervical cancer cells

Introduction

Cervical cancer is the fourth most common cancer in the world and is associated with a large number of deaths annually [1]. Currently, surgery is the mainstay of the treatment, but due to the insidious onset of the disease, and limitations in the screening techniques, most patients are diagnosed at the advanced stage [2] after becoming metastatic at a distant site, and with a poor prognosis. The transcription factor enhancer-binding protein homologous protein (CHOP) is a member of the C/EBP transcription factor family, and its gene promoter contains the binding site of the UPR-induced proteins, including ATF4, ATF6 and XBP. PERK, ATF6, and IRE1 can induce the transcription of CHOP. Under non-stress conditions, its expression level is very low. In endoplasmic reticulum stress, the transcription factors ATF4, ATF6, and XBP1 enter the nucleus and activate its transcription level. Their expression levels increase greatly, and they eventually induce apoptosis by activating Caspase-3. Endoplasmic reticulum stress (ERS) is a stress-responsive mechanism produced by cells when they are stimulated externally, which is associated with the occurrence of cervical cancer and which can induce apoptosis in the presence of serious ERS [3, 4]. Therefore, the inhibition of

the tumor cell proliferation and the promotion of apoptosis are crucial in the treatment of cervical cancer. Quercetin is a flavonoid with diverse biological activities, and it has various antibacterial, antiviral, antioxidant, free radical scavenging and immune regulating effects [5]. According to published research on this topic [6], quercetin can potently inhibit and prevent the proliferation of several cancers, such as colon cancer, breast cancer, ovarian cancer, liver cancer, cervical cancer, and other malignant tumor cells, and it can regulate the cell cycle and induce apoptosis, which has been confirmed in in vitro and in vivo clinical studies of cancer strains. In recent years, the dual role of quercetin in the chemotherapy and radiotherapy of cervical cancer has attracted attention in medical circles, but its role and specific mechanism have not been fully clarified. Current studies indicate that Quercetin's mechanism of action is related to inhibiting cell growth and inducing tumor cell apoptosis, but few studies have been conducted to explore the specific apoptotic mechanisms. Therefore, the present trial is the first attempt to test the effect of quercetin on human cervical cancer cells by inducing ERS and apoptosis based on their actions on cervical cancer HeLe cells, and to explore the mechanisms of action, with the goal of providing information for the treatment of cervical cancer.

Materials and methods

Materials and reagents

Human cervical cancer HeLa cells (Shanghai Cell Bank, Chinese Academy of Sciences), quercetin (Sigma, USA), Fetal bovine serum (Nanjing Biochannel Biotechnology Co., Ltd.), Trypsin (Invitrogen, USA), RPMI 1640 medium (Gibco, USA), MTT (Ameresco, USA), DMSO (Sigma, USA), Caspase-3 (Santa Cruz, USA), GRP78, CHOP antibodies (Cell Signaling Technology, USA), β-actin (Shanghai SBS Genetech Co., Ltd., China), anti-IRE1 antibodies, anti-p-PERK antibodies, and anti-c-ATF6 antibodies (Abcam, USA). This study was approved by our hospital's ethics committee.

Methods

Cell culture and treatment: HeLa cells were cultured in a RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 U/mL penicillin at 37°C and 5% CO_2. The cell media were changed every two days. When the growth density of the HeLa cells exceeded 80%, the cells were passaged at a ratio of 1:3; subsequent experiments were performed when the cell culture was in the logarithmic growth phase.

Cell viability by MTT assay

The HeLa cells were digested with trypsin and made into a single cell suspension. Later, they were cultured in 96-well plates at the concentration of 4×10^4 cells well, and 100 μL of RPMI 1640 culture medium was added to each well. The supernatant was discarded 24 hours after the cell adherence, and then the cells were cultured with different concentrations of quercetin (20, 40, and 80 μmol/L) media. After it was cultured for 24 h, 48 h, and 72 h, the culture solution was vacuumed and discarded to leave only the HeLa cells in each well. 100 μL of MTT was added to each well with a concentration of 5 g/L, and the supernatant was discarded after it was incubated for another 4 h. 150 μL of DMSO was the added to each well, and this was shaken and mixed well. Afterwards, the absorbance (A) at the wavelength of 490 nm was measured. Cell viability = (absorbance of treatment samples - absorbance of sample control well)/(absorbance of maximum enzyme activity - absorbance of sample control well) × 100%.

Measuring the endoplasmic reticulum stress-related proteins and apoptosis using Western blot

The HeLa cells were digested with trypsin, and then they were treated with quercetin at different concentrations (20, 40, and 80 μmol/L) for 24 h. Later, these cells were digested, centrifuged, and washed. The cells in each group were placed into 1.5 mL EP tubes, mixed and lysed with a lysis solution and a phosphate buffer solution (PBS). Afterwards, the protein concentrations were measured with bicinchoninic acid (BCA) assays. The proteins were separated using SDS-PAGE and then stored in a freezer at -80°C. After electrophoresis, the PVDF membranes were transferred to the tris-buffered saline tween (TBST), buffer with 5% bull serum albumin (BSA) and blocked for 1 h at room temperature. The membranes were washed three times for 10 minutes each time in TBST, and the primary antibody was incubated overnight at 4°C. The secondary antibody

Table 1. The effect of quercetin on the viability of cervical cancer HeLe cells ($\bar{x}$ ± s, n=9)

Group	24 h	48 h	72 h
Blank control group	95.46±2.35	95.49±2.33	95.53±2.29
20 μmol/L quercetin	83.27±3.10*	63.22±4.03*	50.13±4.26*
40 μmol/L quercetin	67.48±3.12*	48.78±5.38*	32.26±4.78*
80 μmol/L quercetin	56.78±2.40*	27.78±5.23*	22.17±3.30*
F	342.200	374.200	557.900
P	<0.001	<0.001	<0.001

Note: *indicates a comparison with the blank control group (P<0.05).

Table 2. The effect of quercetin on the HeLe apoptosis ($\bar{x}$ ± s, n=9)

Group	Apoptosis rate (%)
Blank control group	4.30±0.78
20 μmol/L quercetin	10.03±0.60*
40 μmol/L quercetin	15.26±0.91*
80 μmol/L quercetin	23.19±1.75*
F	515.200
P	<0.001

Note: *indicates a comparison with the blank control group (P<0.05).

was incubated for 1 hour at room temperature. A luminescent solution was added to the PVDF membrane for color development and photographed in a dark room. The grayscale values of each protein band were analyzed using Image J software, and their relative expressions were calculated.

Apoptosis using flow cytometry

The HeLa cells treated with quercetin at different concentrations (20, 40, 80 μmol/L) were collected and centrifuged. 0.2% FBS-containing PBS was administered to wash the HeLa cells. Then 70% ethanol solution was added to the fix. The cell suspension density of quercetin at different concentrations (20, 40, 80 μmol/L) was adjusted to about 1×10^6/ml. Subsequently, Anexin V and PI (BD, USA) were used for double staining. The specific operation of the Annexin V apoptosis detection kit was performed as per the specifications. The apoptosis was measured using flow cytometry (BD, model FACSCalibur).

Statistical analysis

The data analysis was carried out using GraphPad Prism 7.0 statistical software. The measurement data were represented as ($\bar{x}$ ± s). T-tests, one-way analyses of variance, and multivariate analyses of variance were carried out to assess whether there was a significant difference between the intergroup or among the multi-group comparisons The significance level was set at P<0.05.

Results

The effect of quercetin on the viability of the cervical cancer HeLe cells

To investigate the effect of quercetin on the viability of the cervical cancer HeLe cells, we conducted MTT, and the findings revealed that the viability of the cervical cancer HeLe cells was inhibited markedly in the quercetin intervention group (20, 40, and 80 μmol/L) at 24 h, 48 h, and 72 h. Meanwhile, the viability of the HeLe cells showed a tendency to decrease progressively with an increase in the quercetin concentration. We found that the viability of the cervical cancer HeLe cells was inhibited by quercetin in a dose-dependent manner. The cell viability decreased progressively in the quercetin group (20, 40, and 80 μmol/L) versus the blank control group, and a statistically significant difference was noted (P<0.05) (**Table 1**).

The effect of quercetin on the HeLe apoptosis

Then, to test the effect of quercetin on the HeLe apoptosis, we carried out flow cytometry. We found that the apoptosis rate was remarkably increased in the quercetin group (20, 40, and 80 μmol/L) versus the blank control group, and the apoptosis rate also showed a tendency to increase progressively with an increase in the quercetin concentration, and a statistically significant difference was found (P<0.05) (**Table 2** and **Figure 1**).

Changes in the HeLe apoptosis-related proteins after the quercetin treatment

Next, western blot was performed to measure the changes in the HeLe apoptotic-related proteins after the quercetin treatment. We observed that the Bax and Bcl-2 levels rose gradually, but the Cyclin D1 level decreased progressively in the quercetin intervention group (20, 40, and 80 μmol/L) versus the blank

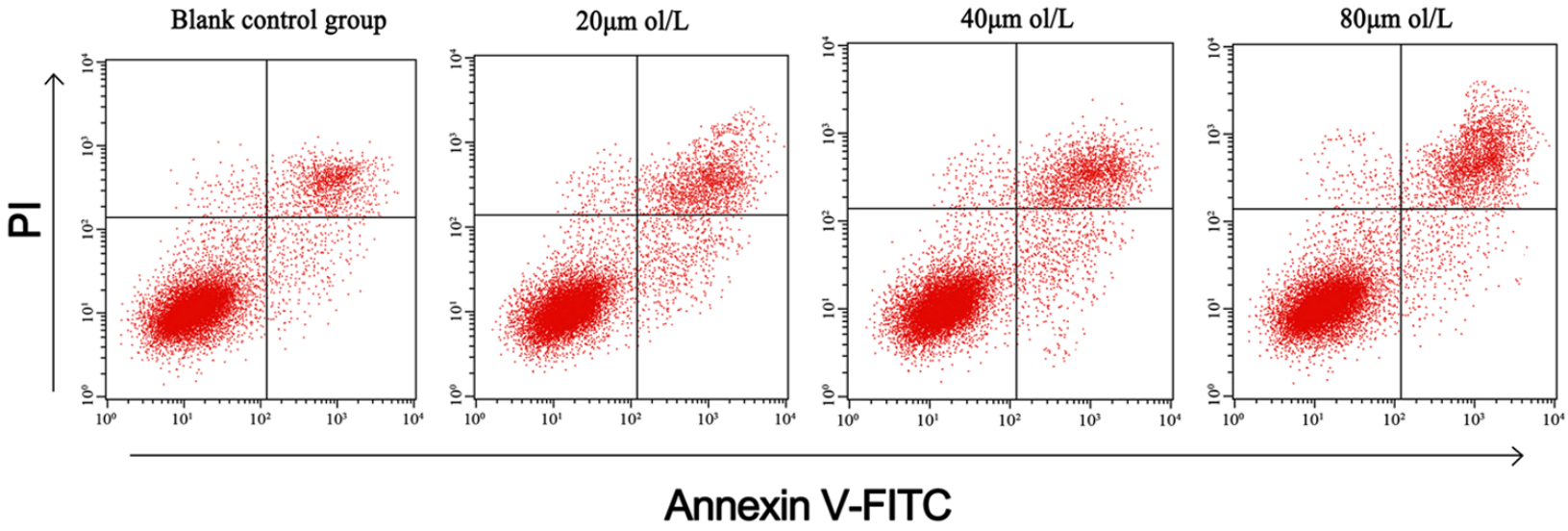

Figure 1. Flow cytometry to measure the cell apoptosis.

Table 3. Changes in the HeLe apoptosis-related proteins after the quercetin treatment

Group	Bax	Bcl-2	Cyclin D1
Blank control group	0.99±0.07	1.03±0.09	1.63±0.34
20 μmol/L quercetin	1.28±0.06*	1.26±0.07*	1.46±0.27*
40 μmol/L quercetin	1.43±0.08*	1.48±0.07*	1.22±0.25*
80 μmol/L quercetin	1.68±0.06*	1.81±0.08*	1.03±0.11*
F	151.300	163.400	9.675
P	<0.001	<0.001	<0.001

Note: *indicates a comparison with the blank control group (P<0.05).

Table 4. The effect of quercetin on the tumor endoplasmic reticulum stress pathway-related proteins in the HeLe cells ($\bar{x} \pm s$, n=9)

Group	Caspase-3	GRP78	CHOP
Blank control group	0.99±0.07	0.22±0.01	0.68±0.04
20 μmol/L quercetin	1.28±0.06*	0.36±0.02*	0.95±0.07*
40 μmol/L quercetin	1.46±0.08*	0.62±0.03*	1.15±0.05*
80 μmol/L quercetin	1.70±0.06*	0.81±0.05*	1.35±0.07*
F	174.400	592.200	212.100
P	<0.001	<0.001	<0.001

Note: *indicates a comparison with the blank control group (P<0.05).

control group, and a statistically significant difference was observed (P<0.05) (**Table 3**).

Effect of quercetin on the tumor endoplasmic reticulum stress pathway-related proteins in the HeLe cells

Further, the western blot results showed that the Caspase-3, GRP78, and CHOP expression levels rose notably in the quercetin intervention group (20, 40, and 80 μmol/L) versus the blank control group (P<0.05) (**Table 4** and **Figure 2A-D**).

The effect of quercetin on the receptor protein levels in the tumor endoplasmic reticulum stress pathways in the HeLe cells

In the ERS pathway, the relevant receptor protein levels, such as IRE1, p-Perk, and c-ATF6, were measured, except for the relevant proteins. The IRE1, p-Perk, and c-ATF6 levels increased proportionally with the concentration of quercetin. The IRE1, p-Perk, and c-ATF6 levels in the quercetin intervention group (20, 40, and 80 μmol/L) rose gradually in comparison with the blank control group (P<0.05) (**Table 5**).

Discussion

Cervical cancer is a disease of public health importance affecting a significant number of women globally. Surgery and chemoradiotherapy have emerged over the years as the chief cervical cancer treatments. But chemoradiotherapy has many side effects, such as its high cost and its unsatisfactory prognosis [7]. It has been shown [8] that there is a key signaling pathway in the processes of cell proliferation, differentiation, and apoptosis, and changes in any component of the signaling pathway can lead to uncontrolled cell proliferation, resulting in carcinogenesis. ERS is an essential part of the tumor development and progression process in the signaling pathway. For example, when the body is invaded by viruses or when inflammation occurs, the endoplasmic reticulum is imbalanced and ERS responses occur to various degrees, but the expression levels of the ERS-related proteins can reflect different degrees of ERS conditions, and the processes such as cell proliferation and apoptosis are also destroyed after the occurrence of the ERS responses [8, 9]. Apoptosis is a programmed cell death process, and it maintains a balance of cell survival and death by regulating the intracellular genes and their products. Apoptotic signaling protects the integrity of the maintained genome, while both tumor development and progression are bound up with the apoptotic defects in cells [10, 11]. Thus, focusing on apoptosis is a hot topic in cancer therapy. Studies have confirmed [12] that quercetin shows anticancer activity in several malignant tumors, such as lung cancer, liver cancer, breast cancer, and leukemia. A growing body of studies provides evidence [13] that quercetin also has a biological potential for the treatment of cervical cancer. The prior findings revealed [14] that cancer cells are characterized by unlimited proliferation, and quercetin can inhibit the activity of cervical cancer HeLa cells in a time- and concentration-dependent manner. Tumor cells may inhibit apoptosis and acquire resistance to the apoptotic factors through multiple molecular mechanisms.

A study conducted by GU et al. [15] demonstrated that quercetin can selectively inhibit the

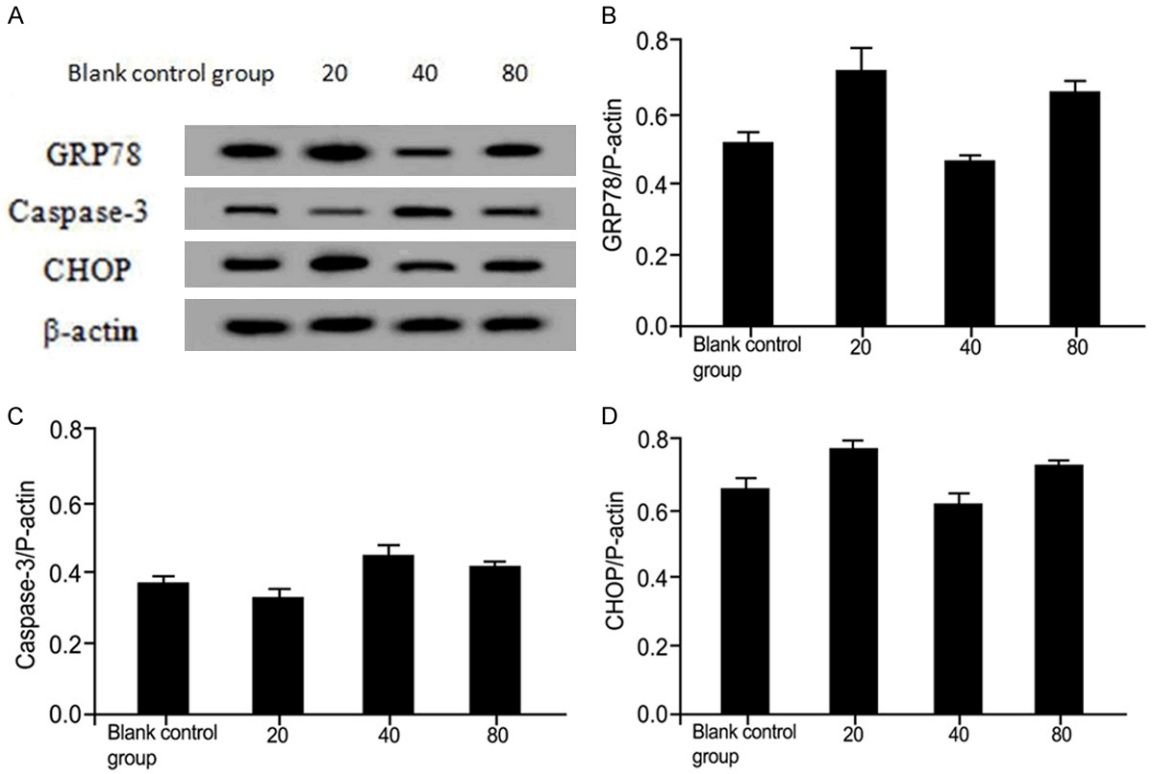

Figure 2. A Western blot Grey-analysis on the Caspase-3, GRP78, and CHOP expression levels, and the effect of quercetin on the HeLe cell tumor endoplasmic reticulum stress pathway related proteins.

Table 5. The effect of quercetin on the receptor proteins levels in the tumor endoplasmic reticulum stress pathways in the HeLe cells ($\bar{x} \pm s$, n=9)

Group	IRE1	p-Perk	c-ATF6
Blank control group	0.43±0.03	0.45±0.04	0.04±0.005
20 μmol/L quercetin	0.63±0.07*	0.69±0.06*	0.07±0.01*
40 μmol/L quercetin	0.79±0.07*	0.98±0.08*	0.13±0.01*
80 μmol/L quercetin	0.92±0.08*	1.25±0.10*	0.35±0.04*
F	94.090	201.300	259.300
P	<0.001	<0.001	<0.001

Note: *indicates a comparison with the blank control group (P<0.05).

growth of HeLa cells in a dose- and time-dependent manner. After the quercetin treatment of HeLa cells for 72 hours, nuclear condensation and edge phenomena can also be seen in the staining, and the characteristic apoptotic bands can be seen in DNA electrophoresis. In this study, MTT assays were used to determine the effects of the different concentrations of quercetin on the viability of human cervical cancer HeLa cells over different time periods. It was indicated that the viability of the cervical cancer HeLe cells was inhibited markedly in the quercetin intervention group (20, 40, and 80 μmol/L) at 24 h, 48 h, and 72 h. Meanwhile, the viability of the HeLe cells showed a tendency to decrease progressively with an increase in the concentration of quercetin. We observed that the viability of the HeLe cells was inhibited notably by quercetin in a dose- and concentration-dependent manner. The cell viability in the quercetin group (20, 40, and 80 μmol/L) was decreased progressively relative to the blank control group. It was proven that quercetin inhibits the viability of cervical cancer HeLe cells in a concentration-dependent manner. In this study, HeLa cells were treated with different concentrations of quercetin (20, 40, and 80 μmol/L) using flow cytometry, and the results showed that the apoptosis rates of the quercetin group (20, 40, and 80 μmol/L) increased remarkably relative to the blank control group, and the apoptosis rate also tended to increase progressively with an increasing concentration of quercetin (P< 0.05). We was demonstrated that the apoptosis rate rose proportionally with the concentra-

tion of quercetin, and our findings are supported by the results of Neeraja [16]. Additionally, it was shown by the Western Blot that the Bax and Bcl-2 levels rose gradually, but the Cyclin D1 level decreased progressively in the quercetin intervention group (20, 40, and 80 μmol/L) versus the blank control group (<0.05). We confirmed that quercetin underwent apoptosis by inducing the cervical cancer HeLe cells.

GRP78 is a marker protein for the detection of ERS. It was shown to promote protein assembly, folding and trafficking, maintaining calcium homeostasis, and regulating ERS signaling [17]. In the body's normal state, GRP78 expression is very low, but in the event of an ERS response, GRP78 is abundantly expressed. When quercetin treats Hela, the Caspase protein exhibits its maximum activity at different times. Studies by GU et al. [15] showed that Caspase-3 activity reaches its highest level at 48 h, while the Caspase-8 activity reaches its highest level at 24 h. Quercetin can specifically induce the apoptosis of human HeLa cells, and its mechanism of inducing apoptosis may be related to the activations of Caspase-3 and Caspase-8. Caspase-3 is an execution protein of apoptosis, an effector molecule in the apoptotic cascade signaling pathway, and ERS can induce apoptosis through the activation of the caspase-dependent apoptotic pathway [18]. The CHOP mechanism in the ERS response has not been fully clarified in current studies. However, it has been shown [19] that CHOP can promote apoptosis in some tumors by increasing TRB3 expressions and that clearance of the TRB3 expression also attenuates the ERS-induced apoptosis. The results of this study showed that the Caspase-3, GRP78, and CHOP expression levels rose notably in the quercetin intervention group (20, 40, and 80 μmol/L) versus the blank control group (P< 0.05), which suggests that quercetin can induce ERS in HeLa cells. The development of ERS is related to the unfolded protein response, and when a mild ERS response occurs, unfolded proteins can initiate the autophagic clearance of misfolded proteins to maintain endoplasmic reticulum homeostasis, thereby mitigating the cellular damage from the external stimuli and improving survival, but when a severe ERS occurs, unfolded proteins can initiate autophagic cell death and apoptosis [20]. The common signaling pathways that mediate the occurrence of ERS include IRE1, p-Perk, and c-ATF6. p-Perk has been shown to mediate eIF2α phosphorylation and inhibit the synthesis of intracellular related proteins, reducing the intensity of ERS and avoiding the induction of apoptosis [21]. The results show that the IRE1, p-Perk and c-ATF6 levels tended to increase progressively with an increasing quercetin concentration (P<0.05). It was further confirmed that quercetin can induce ERS to initiate HeLe cell apoptosis.

In summary, quercetin may promote the apoptosis of cervical cancer HeLe cells by inducing the tumor ERS pathway. However, the specific signal transduction mechanism of the quercetin-induced apoptosis of HeLa cells remains to be further studied.

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Disclosure of conflict of interest

None.

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References

[1] Jiang B, Sun R, Fang S, Qin C, Pan X, Peng L, Li Y and Li G. Lnc-CC3 increases metastasis in cervical cancer by increasing Slug expression. Oncotarget 2016; 7: 41650-41661.

[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.

[3] Li C, Chen J, Zhu J, Gong G, Tao C, Li Z, Lu J and Yin Y. Plan quality comparison for cervical carcinoma treated with halcyon and trilogy intensity-modulated radiotherapy. J Cancer 2019; 10: 6135-6141.

[4] Zhu G and Lee AS. Role of the unfolded protein response, GRP78 and GRP94 in organ homeostasis. J Cell Physiol 2015; 230: 1413-20.

[5] Said M, Brouard I, Quintana J and Estévez F. Antiproliferative activity and apoptosis induction by 3',4'-dibenzyloxyflavonol on human leukemia cells. Chem Biol Interact 2017; 268: 13-23.

[6] Souza RP, Bonfim-Mendonça PS, Damke GMZF, de-Assis Carvalho ARB, Ratti BA, Dembogurski DSO, da-Silva VRS, Silva SO, Da-Silva

DB, Bruschi ML, Maria-Engler SS and Consolaro MEL. Artepillin C induces selective oxidative stress and inhibits migration and invasion in a comprehensive panel of human cervical cancer cell lines. Anticancer Agents Med Chem 2018; 18: 1750-1760.

[7] Silva GÁF, Nunes RAL, Morale MG, Boccardo E, Aguayo F and Termini L. Oxidative stress: therapeutic approaches for cervical cancer treatment. Clinics (Sao Paulo) 2018; 73 Suppl 1: e548s.

[8] Zhang GQ, Tao YK, Bai YP, Yan ST and Zhao SP. Inhibitory effects of simvastatin on oxidized low-density lipoprotein-induced endoplasmic reticulum stress and apoptosis in vascular endothelial cells. Chin Med J (Engl) 2018; 131: 950-955.

[9] Siwecka N, Rozpędek W, Pytel D, Wawrzynkiewicz A, Dziki A, Dziki L, Diehl JA and Majsterek I. Dual role of endoplasmic reticulum stress-mediated unfolded protein response signaling pathway in carcinogenesis. Int J Mol Sci 2019; 20: 4354.

[10] Xu L, Liu JH, Zhang J, Zhang N and Wang ZH. Blockade of autophagy aggravates endoplasmic reticulum stress and improves paclitaxel cytotoxicity in human cervical cancer cells. Cancer Res Treat 2015; 47: 313-21.

[11] Jemu M, van Wijk L, Parker M and Jones G. Tumour and treatment factors influencing the outcome of chemo-radiation in stage IIB cervical cancer: a single institution experience. South Afr J Gynaecol Oncol 2018: 1-6.

[12] Anand David AV, Arulmoli R and Parasuraman S. Overviews of biological importance of quercetin: a bioactive flavonoid. Pharmacogn Rev 2016; 10: 84-89.

[13] Kanimozhi S, Subramanian P, Shanmugapriya S and Sathishkumar S. Role of bioflavonoid quercetin on expression of urea cycle enzymes, astrocytic and inflammatory markers in hyperammonemic rats. Indian J Clin Biochem 2017; 32: 68-73.

[14] Cai X, Bao L, Ding Y, Dai X, Zhang Z and Li Y. Quercetin alleviates cell apoptosis and inflammation via the ER stress pathway in vascular endothelial cells cultured in high concentrations of glucosamine. Mol Med Rep 2017; 15: 825-832.

[15] Gu C, Xu SL, Tang WW, Zhu FJ, Xu Q and Gao F. HeLa cell apoptosis induced by quercetin and effects of caspase-3, caspase-8 activation on apoptosis. Chin Pham J 2011; 46: 595-599.

[16] Furman RR, Eradat HA, DiRienzo CG, Hofmeister CC, Hayman SR, Leonard JP, Coleman M, Advani R, Chanan-Khan A, Switzky J, Liao QM, Shah D, Jewell RC, Lisby S and Lin TS. Once-weekly ofatumumab in untreated or relapsed Waldenström's macroglobulinaemia: an open-label, single-arm, phase 2 study. Lancet Haematol 2017; 4: e24-e34.

[17] Wang Y, Wang JH, Zhang XL, Wang XL and Yang L. Endoplasmic reticulum chaperone glucose-regulated protein 78 in gastric cancer: an emerging biomarker. Oncol Lett 2018; 15: 6087-6093.

[18] Su Q, Peng M, Zhang Y, Xu W, Darko KO, Tao T, Huang Y, Tao X and Yang X. Quercetin induces bladder cancer cells apoptosis by activation of AMPK signaling pathway. Am J Cancer Res 2016; 6: 498-508.

[19] Lee KJ, Jang YO, Cha SK, Kim MY, Park KS, Eom YW and Baik SK. Expression of fibroblast growth factor 21 and β-Klotho regulates hepatic fibrosis through the nuclear factor-κB and c-Jun N-terminal kinase pathways. Gut Liver 2018; 12: 449-456.

[20] Maeng HJ, Song JH, Kim GT, Song YJ, Lee K, Kim JY and Park TS. Celecoxib-mediated activation of endoplasmic reticulum stress induces de novo ceramide biosynthesis and apoptosis in hepatoma HepG2 cells mobilization. BMB Rep 2017; 50: 144-149.

[21] Ocaña GJ, Pérez L, Guindon L, Deffit SN, Evans-Molina C, Thurmond DC and Blum JS. Inflammatory stress of pancreatic beta cells drives release of extracellular heat-shock protein 90α. Immunology 2017; 151: 198-210.