ORIGINAL RESEARCH

Qing Yan Li Ge Tang Induces Apoptosis in Human OEC-M1 Oral Cancer Cells

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ABSTRACT

Background • Since most patients with oral cancer do not benefit from current treatments, new therapeutic strategies or drugs must be developed to improve patient prognosis. Qing Yan Li Ge Tang (QYLGT), a Chinese herbal medicine, is known for its anticancer activity. This study aimed to investigate whether QYLGT has anticancer effects on human OEC-M1 oral cancer cells.

Methods • To evaluate whether QYLGT affects viability, morphology, and colony formation ability of the OEC-M1 cells, the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, morphology study, and colony formation assay were performed, respectively. Each assay was carried out in triplicate, and the whole set of experiments was performed three times independently. To investigate whether QYLGT induces apoptotic effects in OEC-M1 cells, the enzyme-linked immunosorbent (ELISA) was carried out to quantify cytokeratin 18 fragment (an apoptosis marker). Each assay was carried out in triplicate, and the whole set of experiments was performed three times independently. The immunoblotting assay was performed to detect the protein expression after QYLGT treatment. The whole set of experiments was performed two times independently.

Results • The results from the MTT and colony formation assays indicate that QYLGT inhibited the cell viability and clonogenic growth capacity of OEC-M1 cells. The morphology study shows that QYLGT increased plasma membrane blebbing in OEC-M1 clles. The results of ELISA and an immunoblotting assay show that QYLGT increased cytokeratin 18 fragment release and poly ADP-ribose polymerase cleavage (another apoptosis marker) in OEC-M1 cells. In addition, the results from immunoblotting assay show that QYLGT also activated apoptotic executor proteins, including caspase-8, caspase-9, and caspase-3, and the results of ELISA indicate that treatment with the pan-caspase inhibitor, Z-VAD-FMK, inhibited QYLGT-induced cytokeratin 18 fragment release. These results indicate that QYLGT inhibited cell viability in OEC-M1 cells and induced OEC-M1 apoptosis through caspase activation. Additionally, QYLGT-activated c-Jun N-terminal kinase, extracellular signal-regulated kinase, p38 mitogen-activated protein kinase, and nuclear factor-kappa B (NF-κB), and the related inhibitors, including SP600125, PD184352, SB202190, and Bay11-7082, were used to confirm which signaling was involved in QYLGT-induced apoptosis. Moreover, only Bay11-7082, the NF-κB inhibitor, could suppress QYLGT-induced the release of cytokeratin 18 fragments from OEC-M1 cells.

Conclusions • QYLGT induced apoptosis in OEC-M1 cells via the NF-κB pathway. (*Altern Ther Health Med.* 2023;29(8):594-600).

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INTRODUCTION

Oral cancer is a type of head and neck cancer that develops in the mouth or throat tissue, and most cancers are squamous cell carcinoma.[1] Relevant risk factors for oral cancer include tobacco use, betel quid chewing, alcohol consumption, and chronic oral inflammation.[2,3] Oral cancer is a public health problem with a high incidence rate in India, Sri Lanka, Thailand, South China, and Taiwan.[4] Although

modern medicine has been developed for the treatment of oral cancer, the 5-year survival rate for patients with oral cancer is approximately 50%,[5] indicating that most patients do not benefit from current treatments. Therefore, discovering new therapeutic strategies and drugs to improve the survival rates of patients with oral cancer is crucial.

Chinese herbal medicines are useful in the prevention and treatment of cancers. For example, Guizhi Fuling Wan and Tien-Hsien liquid are Chinese herbal formulas that can promote cancer cell apoptosis.[6,7] Additionally, some natural products extracted from plants have anticancer properties. For example, *Elaeagnus angustifolia* plant extract can induce apoptosis in breast cancer cells.[8] Additionally, the *Hibiscus sabdariffa* leaf extract, also triggers apoptosis in melanoma cancer cells.[9]

Qing Yan Li Ge Tang (QYLGT), a Chinese herbal medicine, is widely used to treat acute upper respiratory infections, allergic rhinitis, cough, and chronic pharyngitis. Recently, we demonstrated that QYLGT could induce autophagic cell death in nasopharyngeal carcinoma (NPC) cells[10]; however, it remains unclear whether it also has anticancer activity against other types of cancer cells.

Apoptosis, a type of programmed cell death, is characterized by a series of biological features, such as cell shrinkage, plasma membrane blebbing, apoptotic body formation, chromatin condensation, cytokeratin 18 cleavage, and DNA fragmentation.[11-13] Apoptosis is mediated by the extrinsic pathway (death receptor pathway) or intrinsic pathway (mitochondrial pathway).[12,14] Caspases are a family of endoproteases that are divided into initiator and effector caspases.[12,14] It is known that the initiators, caspase-8, and caspase-9, are mediators of the death receptor and mitochondrial pathways, respectively.[12,14] Once activated, initiator caspases can cleave and activate downstream effector caspases, such as caspase-3, leading to poly ADP-ribose polymerase (PARP) cleavage [12,14] and finally contributing to apoptosis.

This study aimed to examine whether QYLGT can suppress the viability of OEC-M1 oral cancer cells and investigate the underlying mechanisms. QYLGT inhibited cell viability and induced apoptosis in OEC-M1 oral cancer cells. In addition, QYLGT induced apoptosis in OEC-M1 oral cancer cells by activating the nuclear factor-kappa B (NF-κB) pathway. Our findings suggest that QYLGT exhibits anticancer activity against OEC-M1 oral cancer cells, which may contribute to future clinical applications in oral cancer treatment.

MATERIALS AND METHODS

Cell Culture and Drug Treatment

Dr. Bu-Miin Huang (National Cheng Kung University, Taiwan, R.O.C) provided OEC-M1, a human oral cancer cell line derived from gingival epidermal carcinoma[15] that was cultured in the RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (Hyclone, UT, USA) at 37°C and 5% CO_2. To investigate whether caspases, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and NF-κB are involved in QYLGT-induced apoptosis, their specific inhibitors were used, respectively, based on the following conditions: to inhibit caspases, cells were treated with 10 μM Z-VAD-FMK (R&D Systems, MN, USA) for 48h; to inhibit JNK, cells were treated with 10 μM SP600125 (Sigma, MO, USA) for 48 h; to inhibit ERK, cells were treated with 5 μM PD184352 (Enzo Life Sciences, NY, USA) for 48 h; to inhibit p38 MAPK, cells were treated with 5 μM SB202190 (Sigma, MO, USA) for 48 h; and to inhibit NF-κB, cells were treated with 20 μM Bay 11-7082 (Sigma, MO, USA) for 48h.

Preparation of QYLGT

QYLGT is a commercial Chinese herbal medicine that consists of 10 herbs, including Radix scrophulariae, Rhizoma cimicifuga, Radix platycodi, Radix glycyrrhiza preparata, Poria, Rhizoma coptidis, Radix scutellariae, Fructus arctii, Radix saposhnikoviae, and Radix paeoniae alba. It was purchased from Ko Da Pharmaceutical Co., Ltd. (Taoyuan, Taiwan, R. O. C). We mixed 5 g of QYLGT with 50 ml of DMSO and shook it with a shaker (100 rpm) for 24 h at room temperature (RT). After 24 h, the mixture was centrifuged at 3000 rpm for 10 min at RT. Then, we collected the supernatant and dried it into powder by freeze-drying. To make a 100 mg/ml stock solution, we dissolved 5 g of the powder in 50 ml of PBS and performed an autoclave. Then, the stock solution was filtered by a 0.22 μm syringe filter and stored at 4°C.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

The cells (3×10^5/well) were seeded into a 6-well plate. Following incubation for 24 h at 37°C with 5% CO_2, cells were treated with QYLGT. At the indicated time points, the supernatants were removed, and MTT reagent (0.5 mg/ml in phosphate-buffered saline) was added to each well. After 4 h of incubation at 37°C with 5% CO_2, the supernatants were discarded, and 1 ml of dimethyl sulfoxide (DMSO) was added to each well to dissolve the crystals. Furthermore, 100 μl DMSO lysate from each well of 6-well plates was transferred into a 96-well plate. The optical density was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (BMG LABTECH, Ortenberg, Germany). Each experiment was performed in triplicate.

Morphology study

OEC-M1 cells (3×10^5/well) were seeded into a 6-well plate. After cells reached 80% confluence, cells were treated with or without different concentrations of QYLGT for 48 h. Cell morphology was then observed and recorded using light microscopy (Olympus, CK 40; Tokyo, Japan). The appearance of plasma membrane blebbing of the cells characterizes apoptosis.

Immunoblotting Assay

Total protein preparation and immunoblotting assay were performed as described previously.[16] Briefly, cells were

lysed using lysis buffer (3% sodium dodecyl sulfate (SDS), 1.6 M urea, 4% β-mercaptoethanol). Proteins were separated in an SDS-10% polyacrylamide gel and then electrotransferred onto Hybond-C extra membranes (Cytiva, MA, USA). The membranes were blocked with TBST (50 mM Tris-HCl [pH 7.4], 0.15 M NaCl, 0.05% Tween 20) containing 5% non-fat milk for 1 h at RT and then incubated with the primary antibodies at 4°C overnight. After primary antibodies incubation, the membranes were washed with TBST for 30 min and incubated with secondary antibodies for 1 h at RT. Then, the membranes were washed with TBST for 30 min. Protein bands were visualized using an ultra-sensitive enhanced chemiluminescent kit (Thermo Fisher Scientific, Inc., MA, USA). Quantity One (PDI, Huntington Station, NY, USA), a computer-assisted image analysis system, was used to quantify the optical density of protein bands. β-actin, a housekeeping gene, was used as an internal control in immunoblotting assay. Primary antibodies detecting cleaved PARP (cat. no. 9532; 1:1000), cleaved caspase-8 (cat. no. 9496; 1:1000), cleaved caspase-9 (cat. no. 7237; 1:1000), cleaved caspase-3 (cat. no. 9662; 1:1000), JNK phosphorylated at Thr183/Tyr185 (cat. no. 9251; 1:1000), total JNK (cat. no. 9252; 1:1000), ERK phosphorylated at Thr202/Tyr204 (cat. no. 9101; 1:1000), total ERK (cat. no. 9102; 1:1000), p38 MAPK phosphorylated at Thr180/Tyr182 (cat. no. 9215; 1:1000), total p38 MAPK (cat. no. 9212; 1:1000), phosphorylated NF-κB p65 at ser536 (cat. no. 3031; 1:1000), and total NF-κB p65 (cat. no. 6956; 1:1000) were purchased from Cell Signaling Technology (Cell Signaling Technology, MA, USA). The anti-β-actin antibody (cat. no. A5316; 1:5000) was purchased from Sigma-Aldrich (Sigma, MO, USA). Two secondary antibodies, including horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (cat. no.111-035-144; 1: 5000) and HRP-conjugated goat anti-mouse IgG (cat. no. 111-035-146; 1:5000), were purchased from Jackson ImmunoResearch, Inc. (PA, USA). Representative results from at least two independent experiments are shown.

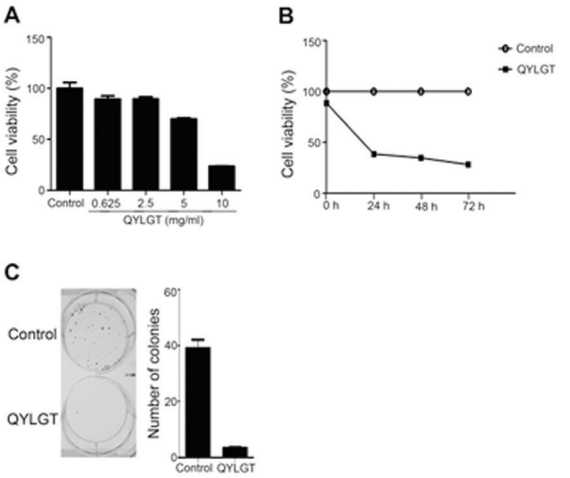
ELISA

OEC-M1 cells (3×10^5/well) were seeded into a 6-well plate. After cells reached 80% confluence, cells were treated with or without QYLGT for 24 and 48 h, respectively. After the indicated times, the cell supernatants were collected and subjected to quantify cytokeratin 18 fragments using the SimpleStep ELISA kit (cat. no. ab254515, Abcam, Cambridge, UK) according to the manufacturer's instructions.

Colony Formation Assay

OEC-M1 cells were treated with 6.7 mg/ml QYLGT for 24 h, trypsinized, and seeded into 6-well plates (200 cells/well). The cells were cultured in the RPMI-1640 medium supplemented with 5% FBS at 37°C with 5% CO_2 for 12 days. Furthermore, the cell colonies were fixed with ice-cold methanol for 10 min and stained with 0.1% crystal violet for 5min. Visible colonies were counted manually. Each assay was performed in triplicate, and the entire set of experiments was independently performed three times.

Figure 1. QYLGT inhibits the cell viability of OEC-M1 cells. (A) OEC-M1 cells were treated without (control) or with various concentrations of QYLGT for 48 h, and the MTT assay was used to measure cell viability. (B) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT for 0, 24, 48, and 72 h, respectively, and the MTT assay was used to measure cell viability. (C) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT for 24 h, and the colony formation assay was used to measure clone formation ability.



RESULTS

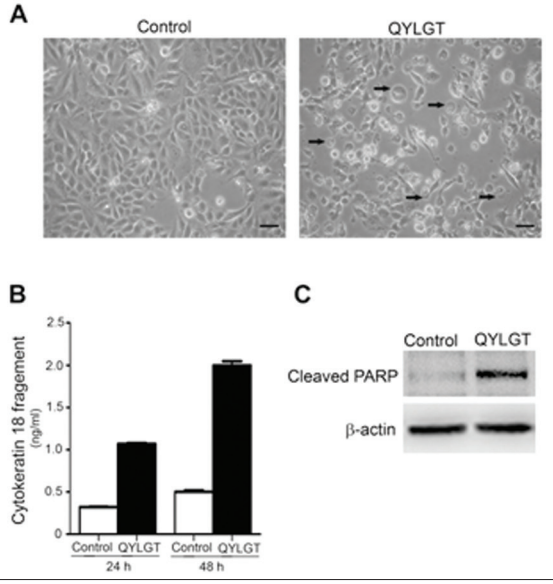
QYLGT inhibits the viability of OEC-M1 cells

We tested whether QYLGT affected OEC-M1 cell viability. OEC-M1 cells were treated with different doses of QYLGT (0.625-10 mg/ml), and cell viability was measured using the MTT assay. Figure 1A shows that 5 and 10 mg/ml QYLGT significantly inhibited OEC-M1 cell viability (Figure 1A). The IC50 value of QYLGT in the OEC-M1 cell was 6.7 mg/ml. Next, we used the IC50 value of QYLGT as the standard concentration to study the time-course effect of QYLGT on OEC-M1 cells. QYLGT significantly inhibited OEC-M1 cell viability at 24, 48, and 72 h than in the control (Figure 1B). We used a colony formation assay to confirm the inhibitory effect of QYLGT on OEC-M1 cell viability. Figure 1C shows that QYLGT suppressed the clonogenic growth of OEC-M1 cells.

QYLGT induces apoptosis in OEC-M1 cells

Next, we examined the morphology of the control and QYLGT-treated OEC-M1 cells. Figure 2A shows that QYLGT-treated cells exhibited more significant membrane blebbing than control, suggesting that apoptosis was induced by QYLGT. Caspase-cleaved cytokeratin 18 is a biomarker of apoptosis that is released from epithelial cells during apoptosis.[13] The release of cytokeratin 18 fragments from OEC-M1 cells was remarkably increased after 24 and 48 h of

Figure 2. QYLGT induces apoptosis in OEC-M1 cells. (A) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT. After 48 h, the morphological changes of cells were observed using light microscopy (scale bar: 100 µm; arrowheads: membrane-blebbed cells). (B) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT for 24 and 48 h. Cytokeratin 18 fragment concentration in the cell culture supernatants was measured by ELISA. (C) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT for 36 h. Protein expression of cleaved PARP and β-actin was examined using an immunoblotting assay. The relative expression levels of individual proteins were quantified by normalization with their corresponding β-actin, and the expression level in the control was set as 1.0.

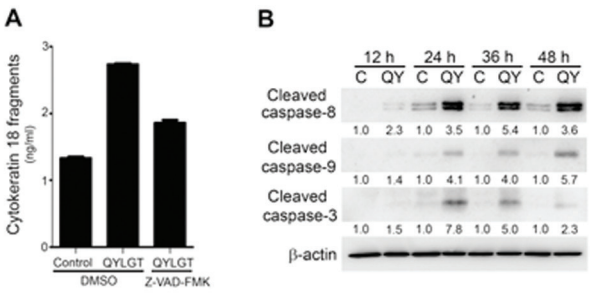


QYLGT treatment than in the control (Figure 2B). In addition, PARP cleavage is an important step in apoptosis, which is considered a hallmark of apoptosis.[12,14] The immunoblotting data revealed that QYLGT treatment induced PARP cleavage in OEC-M1 cells (Figure 2C). These results indicate that QYLGT induces OEC-M1 cell death through apoptosis.

QYLGT activates caspase cascades to induce apoptosis in OEC-M1 cells

It is well known that caspase cascades play important roles in mediating the apoptotic processes.[12,14] Since QYLGT induces apoptosis in OEC-M1 cells, we examined whether caspases are involved in QYLGT-induced apoptosis. We found that treatment with Z-VAD-FMK, a pan-caspase inhibitor that blocks caspase activation, inhibited the release of cytokeratin 18 fragments from OEC-M1 cells (Figure 3A), indicating that activation of caspase cascades is required for QYLGT-induced apoptosis in OEC-M1 cells. In addition, we investigated whether QYLGT treatment activated the initiator caspases, caspase-8 and caspase-9, and their downstream effector caspase, caspase-3. OEC-M1 cells were treated with QYLGT for 12, 24, 36, and 48 h, and the activation of

Figure 3. QYLGT activates caspases, which are required for QYLGT-induced apoptosis in OEC-M1 cells. (A) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT and concurrently treated with the solvent control DMSO or a pan-caspase inhibitor, Z-VAD-FMK. Cytokeratin 18 fragment concentration in the cell culture supernatants was quantified by ELISA. (B) OEC-M1 cells were treated without (C = control) or with 6.7 mg/ml QYLGT (QY) for 12, 24, 36 and 48 h. Protein expression of cleaved caspase-8, cleaved caspase-9, cleaved caspase-3, and β-actin was examined using an immunoblotting assay. The relative expression levels of individual proteins were quantified by normalization with their corresponding β-actin, and the expression level in the control was set as 1.0.



caspase-8, caspase-9, and caspase-3 was detected by immunoblotting assay. Data showed that QYLGT activated caspase-8 (at 12, 24, 36, and 48 h posttreatment), caspase-9 (at 24, 36, and 48 h posttreatment), and caspase-3 (at 24, 36, and 48 h posttreatment), respectively (Figure 3B).

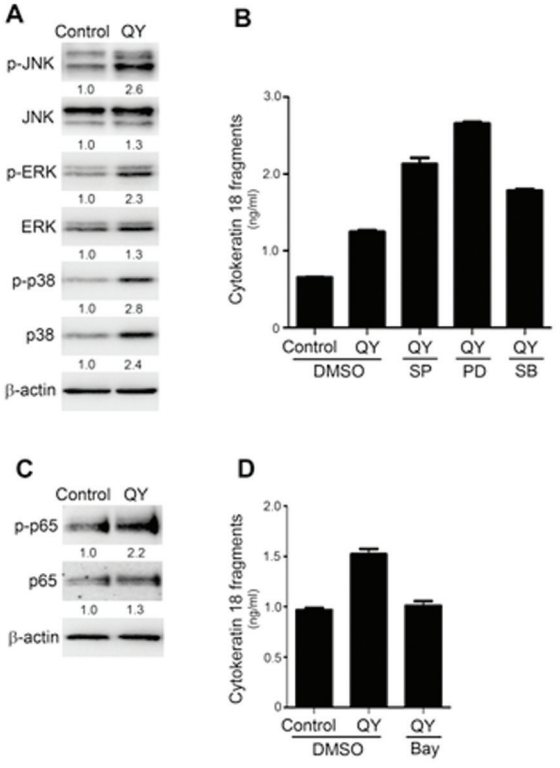
MAPKs are not involved in QYLGT-induced apoptosis in OEC-M1 cells

MAPKs consist of ERK, JNK, and p38 MAPK, which are known to be involved in regulating apoptosis.[17-19] We examined whether MAPKs are involved in QYLGT-induced apoptosis in OEC-M1 cells. Immunoblotting data showed that QYLGT activated JNK, ERK, and p38 MAPK (Figure 4A). However, treatment with JNK (SP600125), ERK (PD184352), and p38MAPK inhibitors (SB202190) did not suppress the QYLGT-induced release of cytokeratin 18 fragments from OEC-M1 cells (Figure 4B), suggesting that MAPKs are not involved in QYLGT-induced apoptosis.

NF-*κB* pathway is involved in QYLGT-induced apoptosis in OEC-M1 cells

NF-κB, a heterodimeric transcription factor, is commonly composed of p50 and p65 subunits.[20] Since NF-κB is involved in apoptosis,[21] we examined whether it is also involved in QYLGT-induced apoptosis in OEC-M1 cells. Figure 4C shows that QYLGT can activate NF-κB (p65 phosphorylated), and treatment with Bay11-7082, an NF-κB activation inhibitor, could remarkably reduce QYLGT-induced cytokeratin 18 fragments release from OEC-M1 cells (Figure 4D), indicating that NF-κB pathway is involved in QYLGT-induced apoptosis in OEC-M1 cells.

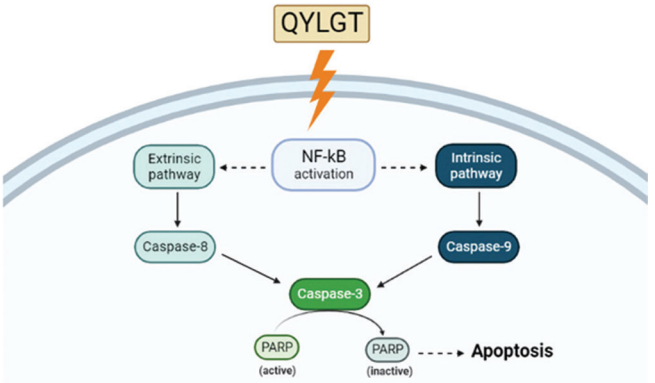
Figure 4. QYLGT-induced apoptosis is mediated by NF-κB rather than MAPKs. (A) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT (QY) for 36 h. Protein expression of phosphorylated JNK, total JNK, phosphorylated ERK, total ERK, phosphorylated p38 MAPK, total p38 MAPK, and β-actin was examined using an immunoblotting assay. The relative expression levels of individual proteins were quantified by normalization with their corresponding β-actin, and the expression level in the control was set as 1.0. (B) OEC-M1 cells were treated without (control) or with QYLGT (QY) and concurrently treated with solvent control DMSO, SP600125 (SP), PD184352 (PD), or SB202190 (SB), respectively. The concentration of cytokeratin 18 fragments in cell culture supernatants was quantified using ELISA. (C) OEC-M1 cells were treated without (control) or with 6.7 mg/ml QYLGT (QY) for 36 h. Protein expression of phosphorylated p65, total p65, and β-actin was examined using an immunoblotting assay. The relative expression levels of individual proteins were quantified by normalization with their corresponding β-actin, and the expression level in the control was set as 1.0. (D) OEC-M1 cells were treated without (control) or with QYLGT (QY) and concurrently treated with the solvent control DMSO or Bay 11-7082 (Bay). The concentration of cytokeratin 18 fragments in cell culture supernatants was quantified using ELISA.



Summary chart of the potential pathways implicated with QYLGT-induced apoptosis in OEC-M1 cells

Based on the findings of this study, we propose a hypothetical signaling network as a model of the molecular mechanism underlying QYLGT-induced apoptosis in OEC-M1 cells (Figure 5). QYLGT-activated NF-κB starts the signaling cascade of the network, which may contribute to the activation of caspase-8 and caspase-9 by regulating the extrinsic and intrinsic pathways. Activation of both caspase-8 and caspase-9 leads to activation of caspase-3, which ultimately promotes PARP cleavage to trigger apoptosis in OEC-M1 cells.

Figure 5. The potential pathways implicated with QYLGT-induced apoptosis in OEC-M1 cells. The QYLGT-induced NF-κB activation plays a crucial role in regulating the apoptotic signaling pathways, which results in apoptosis in OEC-M1 cells.



DISCUSSION

Chinese herbal medicine plays a vital role in cancer prevention.[22] Our previous study revealed that QYLGT has anticancer properties in nasopharyngeal carcinoma cells.[10] In this study, we further evaluated whether QYLGT also has anticancer properties in the human OEC-M1 oral cancer cells. We found that QYLGT effectively reduced viability in OEC-M1 cells (Figures 1A and 1B). Unlimited proliferation is one feature of cancer cells that enables a single cancer cell to grow into a colony.[23] The results from the colony formation assay showed that the clonogenic growth capacity of OEC-M1 cells was inhibited by QYLGT treatment (Figure 1C), indicating that a single OEC-M1 cell loses its ability to grow into a clonal population after QYLGT treatment. In addition, QYLGT also induced apoptosis in OEC-M1 cells (Figure 2). These results suggest that QYLGT has anticancer effects on OEC-M1 cells.

Our results indicated that QYLGT induced OEC-M1 apoptosis by activating caspases (Figure 3A), and that both initiator caspase-8 and caspase-9 were activated by QYLGT (Figure 3B). Since caspase-8 and caspase-9 are mediators of the death receptor and the mitochondrial pathways, respectively, we speculated that treatment of QYLGT may induce the activation of both pathways. On the other hand, the activation of caspase-8 could influence the activation of the mitochondrial pathway.[12] We found that the activation of caspase-8 was earlier than that of caspase-9 following QYLGT treatment (Figure 3B). Therefore, we also speculated that caspase-8 activated by QYLGT may be one of the upstream

molecules linking the death receptor and mitochondrial pathways.

MAPKs are involved in the regulation of apoptosis,[17-19] and our findings also revealed that MAPKs, including JNK, ERK, and p38 MAPK, were activated by QYLGT (Figure 4A). However, treatment with inhibitors of JNK, ERK, and p38MAPK, respectively, did not inhibit QYLGT-induced apoptosis (Figure 4B), suggesting that these protein kinases were not involved in QYLGT-induced apoptosis. On the other hand, in addition to regulating apoptosis, some studies indicated that MAPKs also play an important role in regulating cell survival.[24-26] Treatment with inhibitors of JNK, ERK, and p38MAPK, respectively, promoted QYLGT-induced apoptosis (Figure 4B), suggesting that MAPKs may serve as survival pathways during QYLGT treatment. Previous studies have shown that inhibition of JNK, ERK, or p38 MAPK can enhance apoptosis induced by anticancer drugs.[27-29] We suggest that inhibition of MAPKs is a strategy for strengthening apoptosis induced by QYLGT.

NF-kB is involved in promoting apoptosis.[21] We found that treatment with QYLGT could activate NF-kB (Figure 4C), and inhibition of NF-kB reduced QYLGT-induced apoptosis (Figure 4D), indicating that NF-kB is involved in QYLGT-induced apoptosis. How NF-kB contributes to QYLGT-induced apoptosis is still unknown. Fas, a death receptor, binds to its ligand FasL. The binding of Fas to FasL activates the Fas/FasL death receptor pathway, leading to the activation of caspase-8.[12,14] A previous study showed that NF-kB can regulate Fas expression.[30] In addition, NF-κB can also promote Bax expression,[31] a pro-apoptotic Bcl protein that can trigger mitochondrial pathway activation, which leads to the activation of caspase-9.[12] According to these references, we speculated that NF-kB may activate the death receptor and mitochondrial pathways by promoting the expression of Fas and Bax, respectively, which contribute to QYLGT-induced apoptosis.

The mechanism by which QYLGT activates NF-kB in OEC-M1 cells remains unclear. Poria is a component of QYLGT, and its isolated product has been reported to activate NF-kB.[32] In addition, Coptischinensis is another component of QYLGT; its active constituent, berberine, can induce reactive oxygen species (ROS) production,[33] and ROS is known to promote the activation of NF-kB.[34,35] We speculated that QYLGT may activate NF-kB through the mechanisms described above.

In addition, our previous study found that QYLGT promotes cell death of NPC cells by inducing autophagy.[10] However, it causes oral cancer cell death by inducing apoptosis in the present study. Some previous studies also found that treating different cancer cells with the same drug can trigger different cell death mechanisms.[36,37] For instance, resveratrol is a natural substance found in red wine.[38] Previous studies indicate that it induces autophagic cell death in oral cancer cells[37] but triggers apoptosis in NPC cells.[36] We speculate that different cellular backgrounds may affect drug-induced cancer cell death through different mechanisms.

CONCLUSION

This study demonstrated that QYLGT can induce apoptosis in OEC-M1 cells through the NF-kB pathway. Our findings provide evidence supporting the clinical application of QYLGT in patients with oral cancer.

ABBREVIATIONS

DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; HRP, horseradish peroxidase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB, nuclear factor-kappa B; NPC, Nasopharyngeal carcinoma; PARP, poly ADP-ribose polymerase; QYLGT, Qing Yan Li Ge Tang; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate.

ACKNOWLEDGEMENT

BioRender.com was used to create Figure 5.

DECLARATION OF COMPETING OF INTEREST

The authors declare no conflicts of interest.

FOUNDING SOURCE

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AUTHOR'S CONTRIBUTIONS

CHY, BMH, and YYL designed the present study. YTW and KLT performed all the experiments. CHY, BMH, and YYL analyzed the data and interpreted the results. CHY and YYL wrote the initial manuscript. BMH and YYL revised the manuscript

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