RESEARCH ARTICLE

Editorial Process: Submission:08/19/2022 Acceptance:01/17/2023

Quercetin Induces Cell Cycle Arrest and Apoptosis in YD10B and YD38 Oral Squamous Cell Carcinoma Cells

Hwa-Kyung Son[1], Dokyeong Kim[2]*

Abstract

Objective: Oral squamous cell carcinoma (OSCC) exhibits the highest lethality among head and neck cancers. Treatment for OSCC is limited due to diverse side effects. Quercetin is a natural flavonoid compound found in many kinds of plants and foods. Quercetin has been reported to be a modulator of proliferation and survival in various types of cancers due to its cytotoxic effects. We aimed to investigating chemopreventative roles of quercetin in YD10B and YD38 OSCC cells. **Methods:** For our study, two different types of OSCC cells were used. YD10B cells are tongue SCC cells with the p53 mutation and YD38 cells are lower gingiva SCC cells without the p53 mutation, respectively. The anticancer effects of quercetin were examined by cell viability, cell cycle, annexin-PI staining, and western blot. **Result:** Our results showed that quercetin decreased cell viability and induced G1 cell cycle arrest in YD10B and YD38 OSCC cells. Moreover, quercetin remarkably decreased the expression of cell cycle upregulating proteins and increased the expression of a CDK inhibitor. Quercetin also significantly increased the number of annexin-V-positive cells in a dose-dependent manner in both types of OSCC cells. This apoptotic potential of quercetin triggered cleavage of PARP followed by activation of p38 MAPK signaling pathway. **Conclusion:** In conclusion, this study demonstrates that quercetin shows different anti-cancer responses in OSCC with and without p53 mutation, respectively. Despite different p53 status in OSCC cells, quercetin led to apoptotic signals in both cells. Quercetin repressed cell proliferation with G1 cell cycle arrest and apoptosis by activating the p38 signaling pathway in two OSCC cells with different p53 status. These findings might provide new strategy for OSCC therapy by quercetin.

Keywords: Quercetin- oral squamous cell carcinoma (OSCC)- apoptosis- cell cycle arrest

Asian Pac J Cancer Prev, **24 (1)**, 283-289

Introduction

Head and neck cancers represent the sixth most common cancer worldwide, with >830,000 patients diagnosed and >430,000 patients dying yearly from this cancer (Bray et al., 2018). Oral squamous cell carcinoma (OSCC) has the highest incidence rates and mortality (>40%) among the patients with head and neck cancers, predominantly occurring in the oral cavity and lips (Markopoulos, 2012; Liu et al., 2019) Treatment for OSCC involves surgery, or a combination of surgery, radiation and chemotherapy. However, these therapies can cause a variety of side effects such as facial defects, malformation, dysfunction, drug resistance, and toxicity which lead to poor quality of life (Blatt et al., 2017; Liu et al., 2019). Thus, many studies have shifted their attention to natural compounds, which have little toxicity as new therapeutic agents for treating cancers (Demain and Vaishnav, 2011; Khalifa et al., 2019)

Natural products have been used as one of therapeutic strategies for many diseases, and have been the foundation of the development of anti-cancer drugs such as paclitaxel and rapamycin (Ramawat and Goyal, 2008; Demain and Vaishnav, 2011). Quercetin is a plant flavonol derived from flavonoid polyphenols commonly found in foods such as onions, apples, grapes, peppers and green tea (Anand David et al., 2016). Quercetin has pharmacological effects such as anti-oxidant, anti-diabetic, and anti-inflammatory activities (Boots et al., 2008; Eid and Haddad, 2017; Kim et al., 2019). Furthermore, the chemopreventative effects of quercetin have been demonstrated in a variety of cancers, including ovarian, colon, and lung cancer(Nguyen et al., 2004; van Erk et al., 2005; Shafabakhsh and Asemi, 2019). The anti-cancer efficacy of quercetin was shown by inhibiting the tumor-promoting pathway, NF-κB and transforming growth factor (TGF)-β in OSCC (Zhang et al., 2017; Li et al., 2019b; Kim et al., 2020). Although many studies have reported that quercetin played a role as an anti-cancer agent in various cancers, including OSCC, clinical applications remain challenging. Thus, the

*[1]Department of Dental Hygiene, Division of Health Science, Yeungnam University of College, Daegu, 42415, Republic of Korea. [2]Precision Medicine Research Center, Department of Biomedicine & Health Sciences, College of Medicine, The Catholic University of Korea, Seoul, 06591, Republic of Korea. *For Correspondence: dkkim2908@gmail.com*

potentials of quercetin for preventing and treating OSCC should be further explored.

In this study, we aimed to examine the anti-cancer effects and the underlying mechanism of quercetin in YD10B and YD38 OSCC (Lee et al., 2005). YD10B cells are a polygonal-shaped cell line from the tongue and have a mutation in the p53 gene in exon 7. YD38 cells are an oval-shaped cell lines from the lower gingiva and have a wild-type p53 gene. Histopathologically, these two cell lines are classified as squamous cell carcinoma differentiated moderately. Treatment of quercetin induced cell cycle arrest in the G1 phase and apoptosis in YD10B and YD38 OSCC cells.

Materials and Methods

Cell cultures

YD10B and YD38 OSCC cells were grown in F medium (DMEM:F12 mixture, 10% FBS, and 1% P/S) supplemented with 1 $\times 10^{-10}$ M cholera toxin, 0.4 mg/mL hydrocortisone, 5 μg/mL insulin, 5 μg/mL transferrin, and 2 $\times$ 10^{-11} M triiodothyronine (T3). All of the cells were incubated at 37 °C, with an atmosphere containing 5% CO_2, and the culture medium was changed every 3 days.

Preparation of quercetin

Quercetin powder (#Q4591) was purchased from Sigma-Aldrich. The powder was dissolved in dimethyl sulfoxide (DMSO) and stabilized overnight. The supernatant was filtered through a 0.45μm filter.

Cell viability and cytotoxicity assay

Cells seeded in 96-well culture plates (8 x 103/well) were treated with the indicated concentrations (5 μM to 200 μM) of quercetin for 48 h, and MTT assays were performed.

Protein extraction and Western blotting

Cells (1 x 10^6 cells/100 mm culture dish) were seeded and then stabilized overnight. Next, the cells were lysed with cell lysis buffer (#9803, Cell Signaling Technology, Inc.) containing phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich) and placed on ice for 30 min. After vortexing, the cell lysates were swirled every 5 min. Lastly, the lysates were centrifuged, and the supernatant was stored at -20°C until use. The protein complexes were mixed with 5X sodium dodecyl sulfate (SDS) sample buffer and subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Afterwards, the proteins were transferred to polyvinylidene fluoride (PVDF) membrane and then blocked with 5% skim milk in phosphate-buffered saline (PBS) containing Tween 20. After blocking, the primary antibodies indicated below were applied.

Antibodies against cleaved PARP (#9541, Asp214, 1:1,000), phopho-p38 (#9215, Thr180/Tyr182, 1:1,000), p38 (#9212, 1:1,000), cyclin D1 (#2922, 1:1,000), Cdk4 (#2906, 1:1,000), p21 (Waf1/Cip1, #2947, 1:1,000), and the secondary antibodies (anti-rabbit, #7074 and anti-mouse-IgG, #7076; HRP-conjugated) were purchased from Cell Signaling Technology (Danvers, MA, USA). Cdk4 and Bcl-2 were purchased from Santa Cruz. β-actin (1:5,000) was used as the housekeeping control and was purchased from Bioworld Technology, Inc. (St. Louis, MO, USA).

Annexin V/PI staining with flow cytometry analysis

OSCC cells (5×10^5) were grown in 100-mm dishes and incubated at 37 °C overnight. The cells were treated with quercetin for the indicated times. After, the cells were stained using an annexin V-fluorescein isothiocyanate (FITC) kit (#556547, BD Bioscience, CA, USA) according to the manufacturer's protocol. Stained cells were analyzed by flow cytometry (Becton Dickinson, CA, USA). The fraction of early apoptotic cells was measured by detecting cells stained only with annexin V and is shown as bar graphs. An FC500 series CXP cytometer and CXP analysis (Beckman Coulter, USA) were used for the data analysis.

Cell cycle analysis using flow cytometry

OSCC cells (6×10^5) were grown in 100-mm dishes and incubated at 37°C overnight. The cells were treated with quercetin (25 to 100 nM) or DMSO(control) for 24 h. Next, the cells were fixed with 50% ethanol in PBS, then incubated with RNase A in PBS (#10109142001, Roche) for 30 min. The cells were stained with propidium (PI) and analyzed by flow cytometry (Becton Dickinson). An FC500 series CXP cytometer and CXP analysis (Beckman Coulter, USA) were used for the data analysis.

Statistical analysis

Statistical results were analyzed by SPSS version 20 (SPSS Inc., Chicago, IL, USA). Differences between the experimental and control groups were analyzed using Mann-Whitney U tests after test of normality. Each experiment was performed at least in triplicates and reported as the mean ± standard deviation (SD). A value of $p < 0.05$ was considered statistically significant.

Results

Quercetin decreases cell viability in YD10B and YD38 OSCC cells

To examine the anti-cancer effect of quercetin in YD10B and YD38 OSCC cells, we first observed cell viability using MTT assays after treating with 5 - 200 μM quercetin for 48 h. Treatment with quercetin remarkably

Table 1. Cell Cycle Analysis of YD10B and YD38 OSCC Cells by Flow Cytometry

Cells	Phase	Percentage of cells in cycle	
		Control	Quercetin
YD10B	SubG0/G1	0.18 ± 0.16	0.55 ± 0.74
	G1	67.75 ± 11.70	71.07 ± 11.34
	S	18.10 ± 6.11	19.18 ± 13.71
	G2/M	13.97 ± 7.88	9.19 ± 4.84
YD38	SubG0/G1	0.19 ± 0.42	1.08 ± 2.62
	G1	64.52 ± 8.46	72.75 ± 5.21
	S	17.85 ± 1.80	16.37 ± 4.45
	G2/M	17.44 ± 0.4	9.80 ± 0.2

The data are reported as mean ± SD

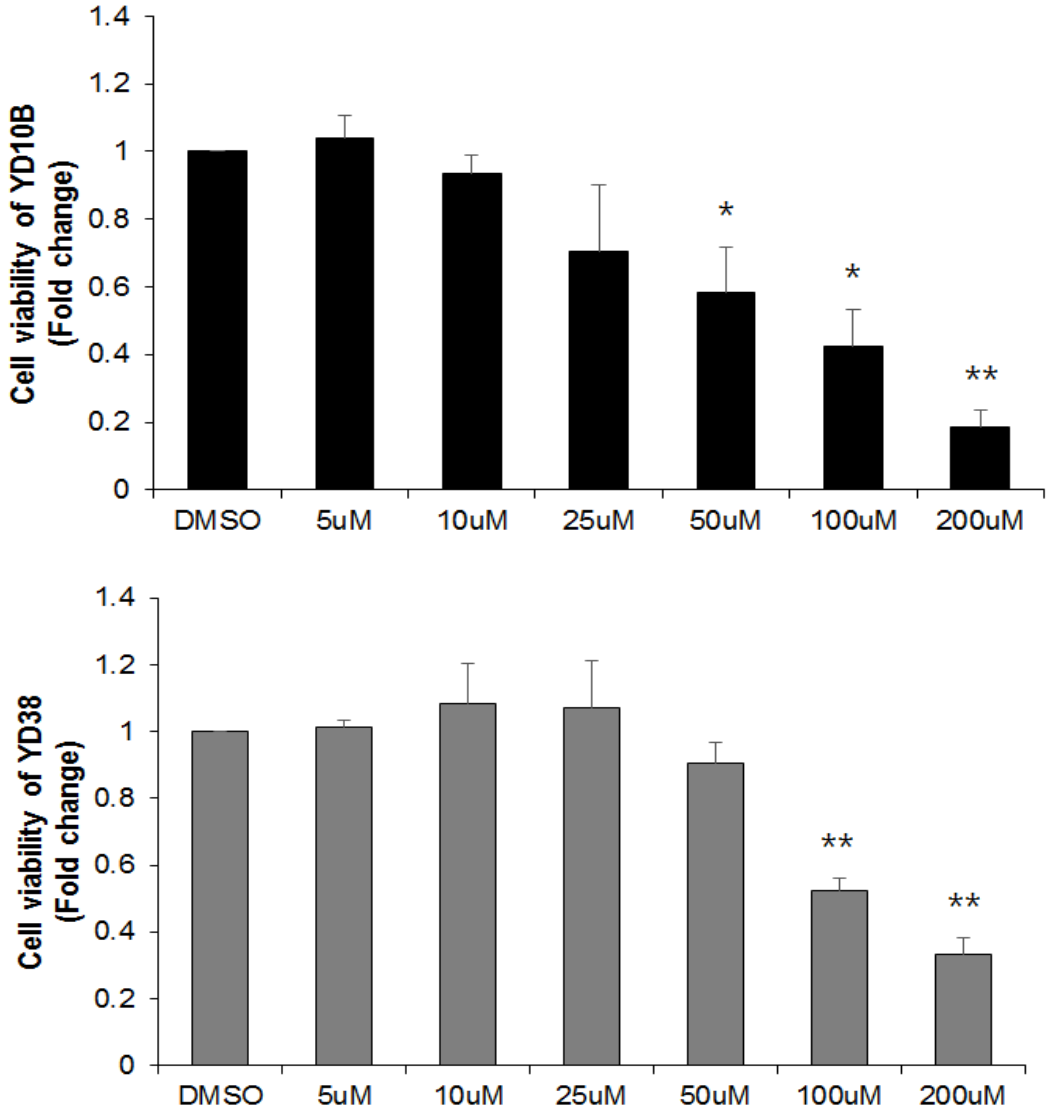

Figure 1. The OSCC Cell Viability by Treatment with Quercetin. OSCC cells (YD10B(upper) and YD38(lower)) were seeded in 96-well culture plates (2 x 103/well) and treated with the indicated concentrations (5 μM to 200 μM) of Quercetin for 48 h, and the MTT assays were performed. (*p < 0.05, **p < 0.01, Mann Whitney U test.)

decreased cell viability in a dose-dependent manner: YD10B and YD38 OSCC cells showed statistically significant reduction in cell viability from treatment with 25 μM and 50 μM quercetin, respectively. DMSO (control) treatment had little effect on OSCC cell viability (Figure 1A and 1B).

Quercetin induces G1 cell cycle arrest in YD10B and YD38 OSCC cells

To investigate the role of quercetin in cell cycle progression, we analyzed cell cycle distribution after treating YD10B and YD38 OSCC cells with quercetin. Quercetin increased the proportion of cells in the G1 phase to 71.07% and 72.75% in YD10B and YD38 OSCC cell, respectively, simultaneously with decreases in the proportion of cells in S phase and G2/M phase compared to DMSO-treated control cells (67.75% and 64.52%, respectively). The number of apoptotic cells in the subG0/G1 phase was weakly increased by quercetin to 0.55% and 1.08%, respectively, after treatment with quercetin for 24 h compared to DMSO-treated control cells (0.18% and 0.19%, respectively) (Figure 2A and Table 1). Consistent with these results, we identified the expression of cell cycle-related proteins. Quercetin remarkably decreased the protein expression of cyclin D1 and CDK4, whereas p21, a CDK inhibitor, was increased by treatment with quercetin in both OSCC cell lines, except at a concentration of 50 μM in YD10B OSCC cells (Figure 2B). These results suggest that quercetin mediated G1 cell cycle arrest in both OSCC cell lines, indicating apoptosis.

Quercetin causes apoptotic cell death by p38 signaling in YD10B and YD38 OSCC cells

To verify the apoptotic cell death caused by quercetin, we performed an annexin-PI staining analysis. Treatment with quercetin significantly increased the number of annexin-V-positive cells in a dose-dependent manner in both OSCC cell lines. Compared to DMSO-treated control cells, treatment of YD10B cells with 25 μM and 50 μM

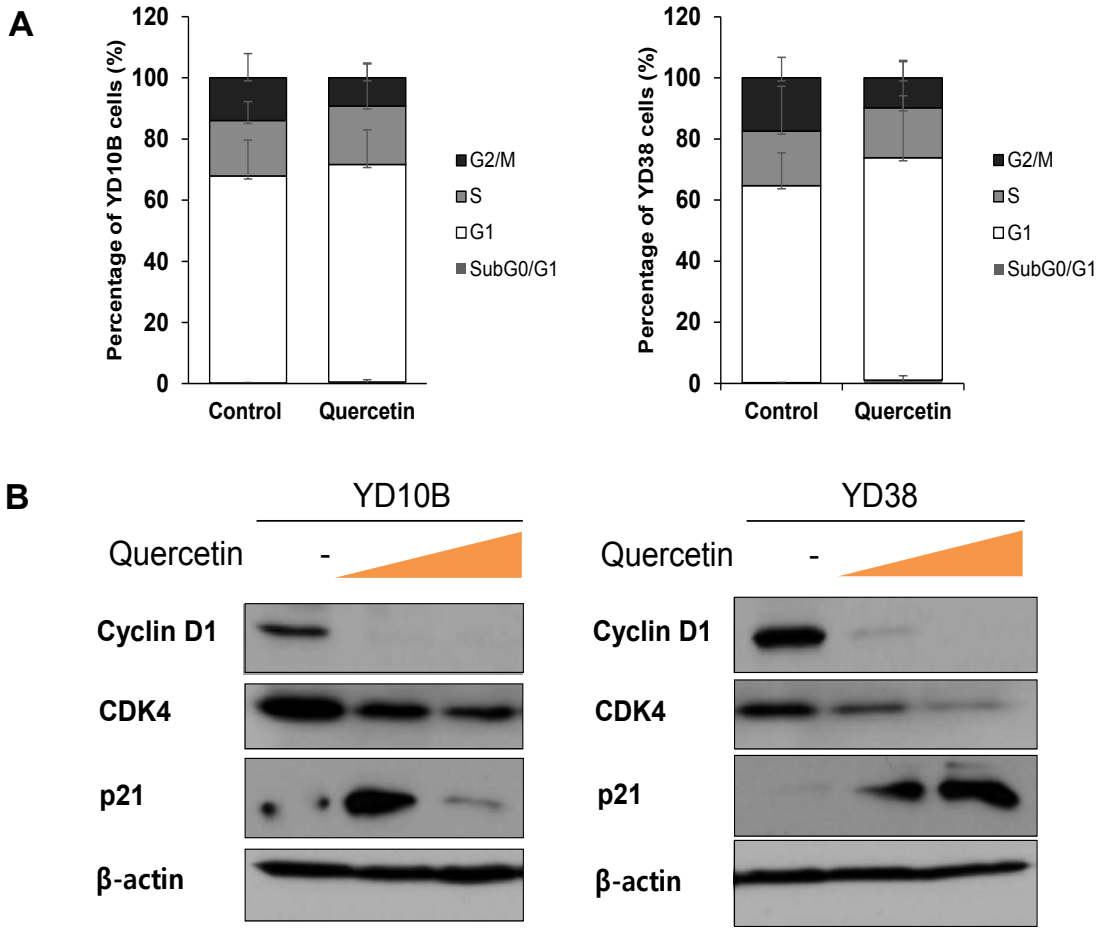

Figure 2. Quercetin Mediates Cell Cycle Arrest in G1 Phase. (A) OSCC cells were plated to 1 x 106 cells/100 mm culture dish. Each cell was maintained with presence or absence of Quercetin (50 μM in YD10B cells and 100 μM in YD38 cells) for 24 h. After incubation, Propidium iodide (PI) staining for cell cycle was performed and analyzed by flow cytometry. The representative histograms are shown, respectively. (*p < 0.05, Mann Whitney U test.) (B) OSCC cells were seeded into 1 x 106 cells/100 mm culture dish. YD10B cells were grown with DMSO (as a control), 25 μM and 50 μM of quercetin in P medium with 0.5% FBS, whereas YD38 cells were grown with DMSO (as a control), 50 μM and 100 μM of Quercetin in P medium with 0.5% FBS. After incubation for 48 h, cells were collected and lysated.

quercetin increased the number of annexin-V-positive cells 1.7- fold and 2.45-fold, respectively. Treatment of YD38 cells with 25 μM and 50 μM quercetin resulted I a 6.79-fold increase and an 8.37 fold-increase in annexin-V-positive cells, respectively (Figure 3A and 3B). To verify whether quercetin could induce apoptosis, we identified the expression of apoptosis-related proteins. Quercetin notably reduced the expression of the anti-apoptotic protein Bcl-2, whereas it triggered the cleavage of PARP. We further investigated whether the Mitogen activated protein kinase (MAPK) signaling pathway could induce apoptosis on YD10B and YD38 OSCC cells treated with quercetin. After treatment with quercetin, phosphorylated p38 was increased in both OSCC cell lines. However, phosphorylated Erk and JNK were not increased (data not shown) (Figure 3C). Collectively, these results suggest that quercetin-mediated apoptosis might be caused by PARP cleavage followed by p38 activation.

Discussion

Numerous studies have reported that quercetin played an important role in preventing the progression of various cancers (Ozsoy et al., 2020; Khan et al., 2021). In oral cancer, quercetin suppressed cell proliferation, migration, and invasion by inducing cell cycle arrest and apoptosis (Ma et al., 2018; Li et al., 2019a; Zhao et al., 2019). We intended to investigate the anticancer effects of quercetin in two different types of OSCC cells with and without p53 mutation. The p53 tumor suppressor protein, encoded by the TP53 gene, lies at the center of circuit regulating various cellular function, including induction of cell cycle arrest and apoptosis (Ozaki and Nakagawara, 2011). The therapeutic consequence by chemotherapy can be relied on p53 status (Goldstein et al., 2011; Li and Zhang, 2015). Mutant p53 can regulate the tumor immune microenvironment and immune checkpoint inhibitor responsiveness in OSCC (Shi et al., 2022). Hence, we selected two OSCC cell lines YD10B (with p53 mutation) and YD38 (without p53 mutation) to carry out experiments in vitro. Our finding showed that quercetin inhibited cell viability in both YD10B and YD38 OSCC cells, as in other OSCC cells (SAS and HN22), indicating its cytotoxic effects (Kim et al., 2020). The concentration of quercetin used in our study has selective

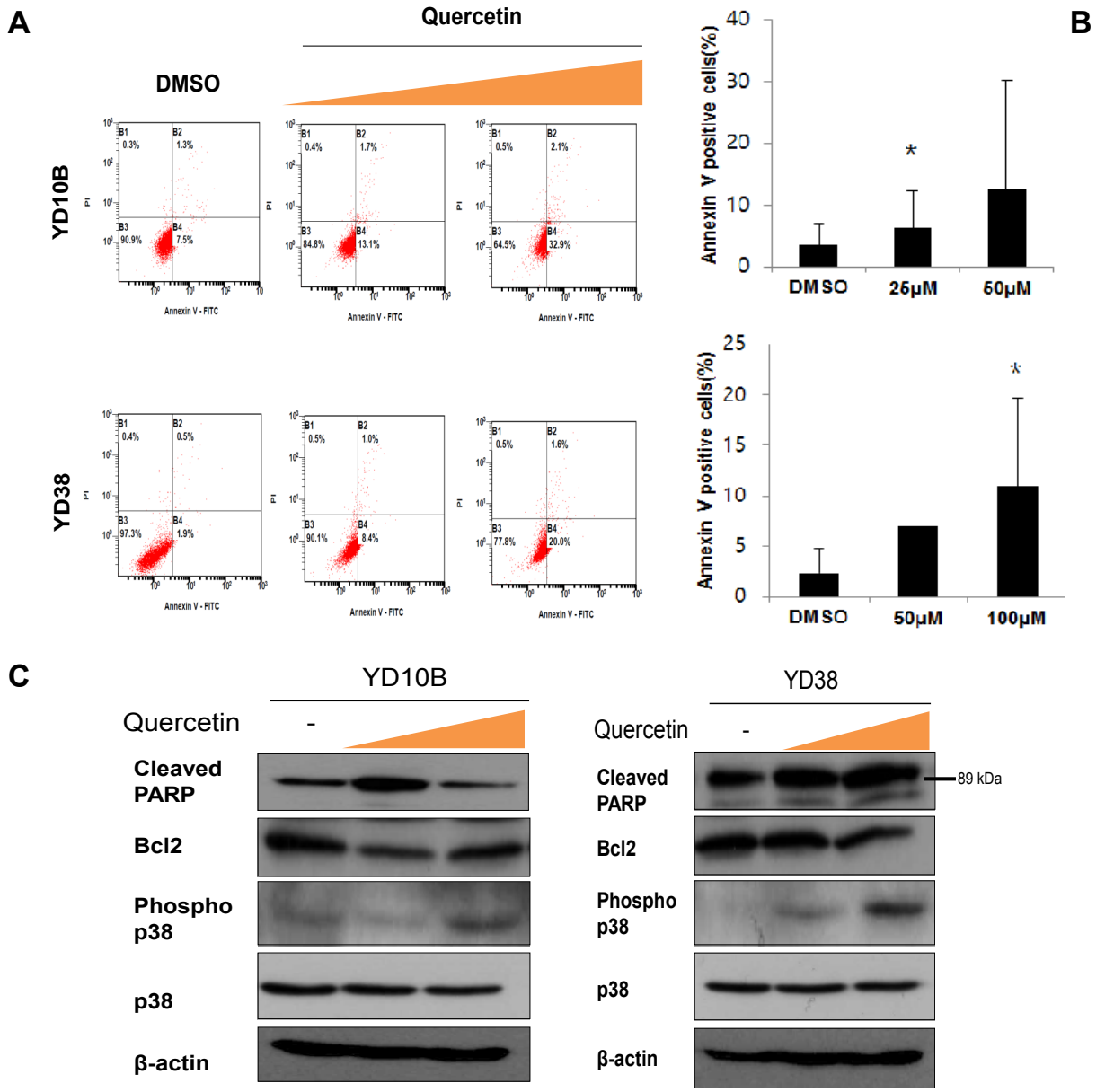

Figure 3. Quercetin Induces Apoptosis in OSCC Cells. (A) OSCC cells were plated to 1 x 106 cells/100 mm culture dishes, and then treated with indicated concentrations of quercetin for 24 h. After incubation, Annexin/PI staining was performed and analyzed by flow cytometry. The representative dot graphs are shown. (B) The bar graphs were shown by counting early-apoptotic cells (annexin-V positive cells relative to control) (*$p < 0.05$, Mann Whitney U test). (C) OSCC cells were seeded into 1 x 106 cells/100 mm culture dish. YD10B cells were grown with DMSO (as a control), 25 μM and 50 μM of quercetin in P medium with 0.5% FBS, whereas YD38 cells were grown with DMSO (as a control), 50 μM and 100 μM of Quercetin in P medium with 0.5% FBS. After incubation for 48 h, cells were collected and lysated.

cytotoxicity in only both OSCC cells, because quercetin has cytotoxicity more than 200 μM of quercetin in normal keratinocyte (Wang et al., 2020). Our results showed that quercetin suppressed the cell proliferation of OSCC cells by G1 cell cycle arrest, consistent with previous study that quercetin-mediated anti-cancer effects were shown by the inhibition of cell proliferation through cell cycle arrest (Huang et al., 2013). However, various cancer cells, including oral cancer cells, showed G2/M arrest after treatment with quercetin (Vidya Priyadarsini et al., 2010; Chen et al., 2013; Zhao et al., 2019). It was reported that type of quercetin-induced cell cycle arrest is dependent upon the concentration of anti-cancer drugs used or cell type (Nagy and Tora, 2007). Furthermore, we identified the expression of cell cycle-related proteins, such as cyclin D1 and CDK4, and the cyclin-dependent kinase inhibitor p21, following quercetin treatment. The expression of cyclin D1 and CDK4 was decreased dose-dependently in both OSCC cell lines. However, there were differences on the expression of some proteins between YD10B and YD38 cells. The expression of most protein in YD38 cells increased dose-dependently as the concentration of quercetin increases, whereas YD10B cells did not show dose-dependent response not only p21, but apoptosis related proteins such as PARP and p38. With regard to these results, we inferred that p38/PARP-mediated

apoptotic pathways induced by quercetin were partially interrupted by mutation of the p53 gene in YD10B OSCC cells, unlike that of wild-type p53 in YD38 OSCC cells. These results provide that treatment for OSCC patient with p53 mutation should be combined with complex therapeutic strategies, rather than to conduct exclusive treatment with quercetin.

Many studies have reported that anti-cancer agents can induce apoptosis through various signaling pathways. The MAPK pathway is a crucial pathway that regulates cellular proliferation, differentiation, and apoptosis (Kim and Choi, 2010). In several studies related to oral cancer, some cancer preventive agents facilitate cell cycle arrest and apoptosis by activating p38 signaling (Chen et al., 2021; Su et al., 2021). Moreover, few studies have reported that the p38 MAPK pathway mediated apoptosis induced by quercetin in oral cancer (Kim et al., 2014; Wang et al., 2021). Similarity, our finding showed that quercetin induced apoptosis in OSCC cells by the cleavage of PARP, which may have promoted by the phosphorylation of p38.

In conclusion, this study demonstrates that quercetin induces different therapeutic sensitivity in OSCC with and without p53 mutation, respectively. Despite different p53 status in OSCC cells, quercetin induced apoptotic signals in both cells. Quercetin repressed cell proliferation with G1 cell cycle arrest and apoptosis by activating the p38 signaling pathway in two OSCC cells with different p53 status. These findings suggest the therapeutic potential of quercetin in OSCC.

Author Contribution Statement

All authors read and approved the final version of the paper. DK and HS designed the study. DK performed the experiments and analyzed the results with inputs from HS. DK and HS wrote and revised the manuscript.

Acknowledgements

Funding Statement

This study was supported by the Yeungnam University College Research Grants in 2020.

Conflict of Interest

The authors disclose no potential conflict of interest.

References

Anand David AV, Arulmoli R, Parasuraman S (2016). Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacogn Rev*, **10**, 84-9.

Blatt S, Krüger M, Ziebart T, et al (2017). Biomarkers in diagnosis and therapy of oral squamous cell carcinoma: A review of the literature. *J Craniomaxillofac Surg*, **45**, 722-30.

Boots AW, Haenen GR, Bast A (2008). Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol*, **585**, 325-37.

Bray F, Ferlay J, Soerjomataram I, et al (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **68**, 394-424.

Chen SF, Nien S, Wu CH, et al (2013). Reappraisal of the anticancer efficacy of quercetin in oral cancer cells. *J Chin Med Assoc*, **76**, 146-52.

Chen YT, Lin CW, Su CW, et al (2021). Magnolol Triggers Caspase-Mediated Apoptotic Cell Death in Human Oral Cancer Cells through JNK1/2 and p38 Pathways. *Biomedicines*, **9**.

Demain AL, Vaishnav P (2011). Natural products for cancer chemotherapy. *Microb Biotechnol*, **4**, 687-99.

Eid HM, Haddad PS (2017). The Antidiabetic Potential of Quercetin: Underlying Mechanisms. *Curr Med Chem*, **24**, 355-64.

Goldstein I, Marcel V, Olivier M, et al (2011). Understanding wild-type and mutant p53 activities in human cancer: new landmarks on the way to targeted therapies. *Cancer Gene Ther*, **18**, 2-11.

Huang CY, Chan CY, Chou IT, et al (2013). Quercetin induces growth arrest through activation of FOXO1 transcription factor in EGFR-overexpressing oral cancer cells. *J Nutr Biochem*, **24**, 1596-603.

Khalifa SAM, Elias N, Farag MA, et al (2019). Marine Natural Products: A Source of Novel Anticancer Drugs. *Mar Drugs*, **17**.

Khan K, Javed Z, Sadia H, et al (2021). Quercetin and MicroRNA Interplay in Apoptosis Regulation in Ovarian Cancer. *Curr Pharm Des*, **27**, 2328-36.

Kim EK, Choi EJ (2010). Pathological roles of MAPK signaling pathways in human diseases. *Biochim Biophys Acta*, **1802**, 396-405.

Kim GT, Lee SH, Kim JI, et al (2014). Quercetin regulates the sestrin 2-AMPK-p38 MAPK signaling pathway and induces apoptosis by increasing the generation of intracellular ROS in a p53-independent manner. *Int J Mol Med*, **33**, 863-9.

Kim HR, Kim BM, Won JY, et al (2019). Quercetin, a Plant Polyphenol, Has Potential for the Prevention of Bone Destruction in Rheumatoid Arthritis. *J Med Food*, **22**, 152-61.

Kim SR, Lee EY, Kim DJ, et al (2020). Quercetin Inhibits Cell Survival and Metastatic Ability via the EMT-mediated Pathway in Oral Squamous Cell Carcinoma. *Molecules*, **25**.

Lee EJ, Kim J, Lee SA, et al (2005). Characterization of newly established oral cancer cell lines derived from six squamous cell carcinoma and two mucoepidermoid carcinoma cells. *Exp Mol Med*, **37**, 379-90.

Li X, Guo S, Xiong XK, et al (2019a). Combination of quercetin and cisplatin enhances apoptosis in OSCC cells by downregulating xIAP through the NF-kappaB pathway. *J Cancer*, **10**, 4509-21.

Li X, Guo S, Xiong XK, et al (2019b). Combination of quercetin and cisplatin enhances apoptosis in OSCC cells by downregulating xIAP through the NF-κB pathway. *J Cancer*, **10**, 4509-21.

Li Y, Zhang J (2015). Expression of mutant p53 in oral squamous cell carcinoma is correlated with the effectiveness of intra-arterial chemotherapy. *Oncol Lett*, **10**, 2883-7.

Liu L, Chen J, Cai X, et al (2019). Progress in targeted therapeutic drugs for oral squamous cell carcinoma. *Surg Oncol*, **31**, 90-7.

Ma YS, Yao CN, Liu HC, et al (2018). Quercetin induced apoptosis of human oral cancer SAS cells through mitochondria and endoplasmic reticulum mediated signaling pathways. *Oncol Lett*, **15**, 9663-72.

Markopoulos AK (2012). Current aspects on oral squamous cell carcinoma. *Open Dent J*, **6**, 126-30.

Nagy Z, Tora L (2007). Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. *Oncogene*, **26**, 5341-57.

Nguyen TT, Tran E, Nguyen TH, et al (2004). The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. *Carcinogenesis*, **25**, 647-59.

Ozaki T, Nakagawara A (2011). Role of p53 in Cell Death and Human Cancers. *Cancers (Basel)*, **3**, 994-1013.

Ozsoy S, Becer E, Kabadayi H, et al (2020). Quercetin-Mediated Apoptosis and Cellular Senescence in Human Colon Cancer. *Anticancer Agents Med Chem*, **20**, 1387-96.

Ramawat K, Goyal S (2008). Natural Products in Cancer Chemoprevention and Chemotherapy. In Eds pp 153-71.

Shafabakhsh R, Asemi Z (2019). Quercetin: a natural compound for ovarian cancer treatment. *J Ovarian Res*, **12**, 55.

Shi Y, Xie T, Wang B, et al (2022). Mutant p53 drives an immune cold tumor immune microenvironment in oral squamous cell carcinoma. *Commun Biol*, **5**, 757.

Su CW, Chuang CY, Chen YT, et al (2021). FLLL32 Triggers Caspase-Mediated Apoptotic Cell Death in Human Oral Cancer Cells by Regulating the p38 Pathway. *Int J Mol Sci*, **22**.

van Erk MJ, Roepman P, van der Lende TR, et al (2005). Integrated assessment by multiple gene expression analysis of quercetin bioactivity on anticancer-related mechanisms in colon cancer cells in vitro. *Eur J Nutr*, **44**, 143-56.

Vidya Priyadarsini R, Senthil Murugan R, Maitreyi S, et al (2010). The flavonoid quercetin induces cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells through p53 induction and NF-kappaB inhibition. *Eur J Pharmacol*, **649**, 84-91.

Wang F, Ke Y, Yang L, et al (2020). Quercetin protects human oral keratinocytes from lipopolysaccharide-induced injury by downregulating microRNA-22. *Hum Exp Toxicol*, **39**, 1310-7.

Wang XP, Xie WP, Bi YF, et al (2021). Quercetin suppresses apoptosis of chondrocytes induced by IL-1beta via inactivation of p38 MAPK signaling pathway. *Exp Ther Med*, **21**, 468.

Zhang W, Yin G, Dai J, et al (2017). Chemoprevention by Quercetin of Oral Squamous Cell Carcinoma by Suppression of the NF-κB Signaling Pathway in DMBA-treated Hamsters. *Anticancer Res*, **37**, 4041-9.

Zhao J, Fang Z, Zha Z, et al (2019). Quercetin inhibits cell viability, migration and invasion by regulating miR-16/HOXA10 axis in oral cancer. *Eur J Pharmacol*, **847**, 11-8.