Quercetin induces growth arrest through activation of FOXO1 transcription factor in EGFR-overexpressing oral cancer cells

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Abstract

The squamous cell carcinomas of the head and neck (SCCHNs) with aberrant epidermal growth factor receptor (EGFR) signaling are often associated with poor prognosis and low survival. Therefore, efficient inhibition of the EGFR signaling could intervene with the development of malignancy. Quercetin appears to be antitumorigenesis, but the underlying mechanism remains unclear in oral cancer. Fork-head box O (FOXO) transcription factors, Akt downstream effectors, are important regulators of cell growth. Here, we hypothesized that FOXO1 might be crucial in quercetin-induced growth inhibition in EGFR-overexpressing oral cancer. Quercetin treatment suppressed cell growth by inducing G2 arrest and apoptosis in EGFR-overexpressing HSC-3 and TW206 oral cancer cells. Quercetin inhibited EGFR/Akt activation with a concomitant induction of FOXO1 activation. FOXO1 knockdown attenuated quercetin-induced p21 and FasL expression and subsequent G2 arrest and apoptosis, respectively. Likewise, quercetin suppressed tumor growth in HSC-3 xenograft mice. Taken together, our data indicate that quercetin is an effective anticancer agent and that FOXO1 is crucial in quercetin-induced growth suppression in EGFR-overexpressing oral cancer.

Keywords: EGFR; FOXO1; Oral cancer; Quercetin

1. Introduction

The invasive squamous cell carcinomas of the head and neck (SCCHNs) frequently overexpress epidermal growth factor receptor (EGFR) [1], leading to unfavorable clinical outcome—high recurrence and low survival rates [2,3]. Aberrant EGFR/Pi3K/Akt signaling pathway is often responsible for the malignant phenotype [4]. Although several EGFR-targeting drugs are clinically available [5–7], their wide application is limited by the resistance to the drugs and mutations in the EGFR downstream effectors in some patients [8]. Therefore, to improve therapeutic efficacy, new anticancer agent should be considered accordingly.

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the major dietary flavonoids found in a wide range of fruits, vegetables and beverages [9]. Among those, onion, apple and red wine are good sources of quercetin. The antioxidant, anti-inflammatory, as well as antiproliferative and apoptosis aspects of quercetin have been widely investigated [9]. A phase I clinical trial indicated that quercetin can be safely administered and its plasma levels are sufficient to inhibit lymphocyte tyrosine kinase activity [10]. Consumption of quercetin from onions and apples was inversely associated with lung cancer risk, supporting the chemopreventive effect of quercetin [11]. The chemotherapeutic efficacy of quercetin has also been demonstrated in many types of cancer [12–18]. Quercetin exerts its anticancer property mainly through induction of growth arrest in G1 or G2 [14,15,17,19], apoptosis [15,16,20] and inhibition of angiogenesis [13] as evidenced by down-regulating the expression of oncogenes (HER2 [21], H-ras, K-ras, c-myc [22] or COX-2 [13]) and mutant p53 [23] or up-regulating cell cycle control proteins (p21WAF1 and p27KIP1) [18,19,24]. In addition, quercetin inhibits several tyrosine and serine–threonine kinases, whose activities are linked to cell surface receptors-transduced survival pathways (P13K/Akt/PKB) [25–27]. Nonetheless, the effect of quercetin on oral cancers remains uncertain. Quercetin seems to induce apoptosis in some human oral cancer cell lines [28–30]; however, the underlying mechanisms are still elusive.

Fork-head box O (FOXO) transcription factors share a conserved “fork-head box” DNA-binding domain, which comprise four members in mammals: FOXO1, FOXO3a, FOXO4 and FOXO6 [31,32]. FOXO factors are involved in a wide range of biological processes, including cell cycle arrest, apoptosis, DNA repair, glucose metabolism, oxidative...
stress resistance and longevity [32]. The biological activity of FOXO factors is highly dependent on their intracellular trafficking, mediated by posttranslational modifications in phosphorylation, acetylation or ubiquitination [33]. In addition, FOXO factors share highly conserved sites within and nearby their DNA binding domains for phosphorylation by the survival PI3K/Akt signaling pathway. The growth-factors-mediated Akt phosphorylation on these conserved residues negatively regulates FOXO expression and activity [34]. In this regard, FOXO factors are implicated in pathogenesis including aging, diabetes, immune disease, infertility, neurodegeneration and cancer [35]. Among FOXO factors, increased p-FOXO1 or decreased FOXO1 dependent expression is often associated with tumorigenesis [36]. FOXO1 may exert its tumor suppression function through its transcription-dependent expression on growth arrest and apoptosis-related genes, including p15, p19, NOXA, FasL, TRAIL and Bim [37–39]. The promoters of these genes comprise the consensus sequence T/C-G/A- A-A-A-C-A-A recognized by FOXO1 [35]. Alternatively, FOXO1 can mediate cancer cell death through induction of autophagy which is independent of its transcriptional activity [40]. Similarly, FOXO1 inhibits Runx2-mediated cell migration and invasion independent of its transcriptional activity [41]. Together, FOXO1 appears to be a potential therapeutic target of anticancer reagents [42–46].

Aberrant EGFR expression and PI3K mutation are often observed in invasive SCCHN; however, the role of transcription factor FOXO1 as a downstream target of activated EGFR/PI3K/Akt signaling axis in oral cancer still remains unclear. Therefore, in the present study, we aimed to investigate the anticancer efficacy of quercetin in human oral cancer cell lines with elevated EGFR expression and to further identify the crucial role of FOXO1 factor in quercetin-mediated growth suppression.

2. Materials and methods

2.1. Reagents, antibodies and plasmids

All chemicals including quercetin were purchased from Sigma (St. Louis, MO, USA), and antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA), respectively, unless specified otherwise. Polyvinylidene difluoride (PVDF) membrane and ECL detection reagents were from Perkin Elmer Life Sciences, Inc. The annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit was purchased from BD Biosciences (San Jose, CA, USA). Antibody against cyclin D1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and anti-α-tubulin antibody was purchased from Abcam (Cambridge, MA, USA). FOXO1-specific siRNA and the Lipofectamine 2000 transfection reagent were purchased from Invitrogen (Grand Island, NY, USA). The pGL3-FHRE-luc plasmid was obtained from Addgene (Cambridge, MA, USA; Addgene database plasmid 1789), and the PolyJet In Vitro DNA Transfection Reagent was purchased from SignaGen Laboratories (Ijamsville, MD, USA). The NE-PER Nuclear and Cytoplasmic Extraction Reagents were purchased from Thermo Fisher Scientific (Rockford, IL, USA).

2.2. Cell culture and treatment

HSC-3 and TW206 human oral squamous carcinoma and HGF human gingival fibroblasts cells were kind gifts of Dr. Hsin-Ling Yang at China Medical University (Taiichung, Taiwan); HSC-3 and HGF cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM)-F12 and DMEM (Invitrogen), respectively, supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic ( Gibco). All cells were cultured with 5% CO2 at 37 °C.

2.3. Cell viability assay

Cell viability was measured by trypan blue method. Cells were allowed to grow in six-well culture plates and then treated with various concentrations of quercetin (0–50 μM) for indicated hours. Then, cells were trypsinized, and viable cells were stained with trypan blue for hemocytometric counting.

2.4. Western blotting

Cells were washed with cold phosphate-buffered saline (PBS) and lysed in radiomimunoprecipitation assay (RIPA) buffer containing 150 mM NaCl, 10 mM Tris (pH 7.2), 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 1% deoxycholate, 5 mM EDTA and protease/phosphatase inhibitors. Protein concentration was determined by bichinchoninic acid (BCA) protein assay, and denatured proteins were separated in 8% to 15% SDS polyacrylamide gel electrophoresis and transferred onto PVDF membranes. Nonspecific binding was blocked with 5% milk in TBST buffer (20 mM Tris base, 140 mM NaCl, pH 7.6, 0.1% Tween-20) for 1 h, followed by incubation with primary antibodies at 4 °C overnight and secondary antibodies at room temperature for 1 h. Blots were visualized by ECL detection reagents.

2.5. Cell cycle analysis

HSC-3 and TW206 cells were seeded onto six-well plates and serum-starved for 18 h followed by quercetin treatment in growth media for 24 h. Cells were then trypsinized, washed with PBS and fixed in cold 70% ethanol at –20 °C. The fixed cells were collected and stained in a solution containing 0.5 ml of 4 μg/ml of propidium iodide (PI), 0.5 mg/ml of RNase and 1% Triton X-100 for 30 min at 4 °C. DNA content of these cells was analyzed using a FACScan flow cytometer (Becton Dickinson).

2.6. Colony formation assay

After incubation with the indicated concentration of quercetin, cells were washed with PBS, fixed with 10% formalin (Mallinckrodt Chemicals) for 10 min and stained with 0.05% crystal violet (Panreac Quimica S.A.U.) for 30 min. Dishes were subjected to colony counting and optical density measurement at 540 nm.

Fig. 1. Quercetin inhibits cell growth in human oral cancer cell lines. (A) HGF (control) and two human oral cancer cell lines, TW206 and HSC-3 cells, were treated with quercetin (0–50 μM) dissolved in dimethyl sulfoxide (DMSO) for 24 h. The final DMSO concentration in the culture media was no more than 0.3%. Viable cell numbers were counted (P < .05 as compared with control, 0 μM). The cell number of each quercetin-un-treated group was set as 1. (B) Effect of quercetin on colony formation. HSC-3 cells were incubated with the indicated concentration of quercetin for 7 days. Cell colonies were stained with crystal violet and quantified. Bars with different letters indicate statistically significant difference from each other (P < .05).
2.7. Apoptosis analysis

Cells were seeded onto six-well plates in growth media and treated with quercetin (20 μM) for 24 h. Cells were then trypsinized, washed with PBS and resuspended in binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl and 2.5 mM CaCl₂) at a concentration of 1 × 10⁶ cells/ml. One hundred microliters of the lysis solution was transferred to a polystyrene tube, followed by 5 μl of annexin V-FITC and 5 μl of PI. The mixtures were sheltered from light and incubated for 15 min at room temperature. Finally, 400 μl of 1× binding buffer was added to each tube, and cells were analyzed using a FACScan flow cytometer (Becton Dickinson).

2.8. Reporter assay

The pGL3-FHRE-luc and β-galactosidase plasmids with or without FOXO1 siRNA were cotransfected into HSC-3 cells for 24 h, followed by another 24 h of quercetin treatment. Cell lysates were collected in lysis solution (Applied Biosystems, Carlsbad, A0 20 40 60 80 100 120 0 Q0 Q20 Q0 Q20 G2 S G1 HSC-3 TW206 * * *

Fig. 2. Quercetin induces cell cycle arrest and apoptosis in human oral cancer cells. (A) Effect of quercetin on cell cycle progression. Both HSC-3 and TW206 cells were treated with quercetin (20 μM) for 24 h and then underwent flow cytometric analysis for cell cycle distribution (*P < .05). (B) Effect of quercetin on cell-cycle-related genes. HSC-3 cells were treated with quercetin (0–20 μM) for 24 h, and the protein lysates were analyzed by Western blotting with the indicated antibodies where α-tubulin served as a loading control. (C–E) Effect of quercetin on apoptosis. Both HSC-3 and TW206 cells were subjected to annexin V-FITC and PI staining after 24-h treatment of quercetin (0–30 μM). Annexin-V-positive cells were analyzed and quantified using a FACScan flow cytometer (*P < .05 as compared with Q0, quercetin 0 μM). (C) HSC-3 cells after 24-h treatment of quercetin were stained with 4′,6-diamidino-2-phenylindole (DAPI) (D) or analyzed for apoptosis-related gene expression by Western blotting with the indicated antibodies where GAPDH served as a loading control (E).

Fig. 3. Effect of quercetin on EGFR/Akt signaling. (A) The endogenous levels of EGFR in HSC-3, TW206 and HGF cell lines were detected by Western blotting, where p38 served as a loading control. Quercetin suppresses phosphorylation of EGFR and its downstream effector, Akt, in time- (B) and dose-dependent (C) manners. HSC-3 cell lysates from each respective treatment were extracted and analyzed by Western blotting with the indicated antibodies. The experiments repeated three times with consistent results.
Fig. 4. Quercetin induces FOXO1 activation. Quercetin induces FOXO1 expression in time- (A) and dose-dependent (B) manners. HSC-3 cell lysates from each respective treatment were extracted and analyzed by Western blotting with antibodies against FOXO1, FOXO3a or α-tubulin. (C–D) Effect of quercetin on subcellular localization of FOXO1. (C) HSC-3 cells were treated with quercetin (0 or 10 µM) for 24 h and then underwent cytoplasmic and nuclear extraction. Protein lysates were subjected to Western blotting analysis of p-FOXO1 and α-tubulin. (C) HSC-3 cells were seeded onto a chamber slide, treated with quercetin for 24 h, and then fixed and stained with an antibody against FOXO1 (green). Nuclei were counterstained with DAPI (blue). (E) Quercetin induces FOXO1 transactivation. HSC-3 cells, in triplicate, were cotransfected with pGL3-FHRE-luciferase (0.25 µg) and β-galactosidase (0.05 µg) plasmids, in the presence or absence of FOXO1 siRNA, for 24 h and then treated with quercetin (0, 20 µM) for another 24 h. The luciferase activity was normalized to β-galactosidase for transfection efficiency and presented as fold activation. Each bar represents the mean ± S.D. (*P < 0.05). (F) LY294002 (LY), a PI3K inhibitor, mimics quercetin’s effect on FOXO1. HSC-3 cells were treated with LY at the indicated time points, and cell lysates were then analyzed by Western blotting for p-Akt and FOXO1 expression.

CA, USA) according to the manufacturer’s protocol. The Dual Light System (Applied Biosystems) was used to quantify luciferase and β-galactosidase activities.

2.9. Xenograft mouse

Seven-week-old male nude mice were purchased from National Laboratory Animal Center (Taipei, Taiwan). The animal study protocol, Institutional Animal Care and Use Committee (IACUC) 98-71-N, was reviewed and approved by the IACUC of the China Medical University. The animals were given free access to autoclaved food and water during the study. After 2 weeks of acclimation, animals were implanted subcutaneously with HSC-3 cells (3 × 10^6) suspended with liquid Matrigel to a final volume of 100 µl into the flank regions and randomly assigned into the control or quercetin group. Tumor size was recorded every other day for 40 days. Two weeks after the implantation, quercetin (3 mg/kg/day) or PBS was given via gavage feeding. Tumor volume = ½(length × width^2).

2.10. Statistical analysis

Data are expressed as mean ± S.D. from at least three independent experiments. Statistical significance was analyzed using Student’s t test or Duncan’s multiple range tests. Results were considered significantly different at P < 0.05.

3. Results

3.1. Quercetin inhibits cell growth in human oral cancer cells

The growth-inhibitory effect of quercetin has been shown in various types of cancer; however, its efficacy on oral squamous cancer cells remains elusive. To examine the effect of quercetin on oral cancer, we exposed two human tongue squamous carcinoma cell lines, HSC-3 and TW206 [47], and a control, human gingival fibroblast HGF cells, to various concentrations of quercetin. Quercetin potently inhibited HSC-3 cell growth as compared with TW206 and HGF cells (Fig. 1A). The IC50 of quercetin in HSC-3 cells was around 20 µM, much less than that of TW206 (45 µM). These results indicate that the metastatic HSC-3 cell line is more susceptible to quercetin than the other two cell lines tested. Indeed, quercetin supplementation for 7 days significantly suppressed HSC-3 colony formation in a dose-dependent manner with an IC50 value around 7 µM (Fig. 1B). These data support quercetin as a potential anticancer reagent in oral squamous carcinomas.

Cell cycle arrest and apoptosis are two main factors responsible for growth suppression. Quercetin has been shown to cause growth arrest in various cancer cells [14,15,17,19]. To investigate the effect of quercetin on cell proliferation in oral cancer, we performed flow cytometric analysis for cell cycle distribution upon quercetin addition. In both HSC-3 and TW206 cells, quercetin (20 µM) treatment for 24 h resulted in a significant increase of cell numbers in the G2/M phase (Fig. 2A), with concomitant reductions of the G2/M phase cyclins, cyclin A and cyclin B1 [48], whereas quercetin exhibited no apparent effect on cyclin D1, a G1/S phase cyclin (Fig. 2B). In addition, quercetin induced the expression of p21, but not p15, a G1/S inhibitor [49] (Fig. 2B). These results demonstrate that quercetin is a potent inducer of growth arrest in human oral squamous carcinoma cells.
Next, we evaluated the apoptotic efficacy of quercetin in oral cancer cells. Upon quercetin treatment (0–30 μM), both HSC-3 and TW206 cells displayed an accumulation of apoptotic cells in a dose-dependent manner (Fig. 2C). The apoptosis-inducing effect was further confirmed by the observations of the condensed chromatin staining (Fig. 2D) and the increased FasL and cleaved caspase 3, the active form of executioner caspase 3 (Fig. 2E), upon quercetin treatment in HSC-3 cells. However, quercetin had little effect on the expression of Bax, a proapoptotic member of the Bcl-2 family (Fig. 2E). These results suggest the potential involvement of the FasL-mediated extrinsic apoptotic pathway upon quercetin treatment in HSC-3 cells. Taken together, both growth arrest and apoptosis are responsible for the anticancer effect of quercetin in oral squamous carcinoma cells.

3.2. Quercetin modulates the EGFR/Akt signaling axis

Aberrant EGFR activation is the key cause of oral cancer malignancy. Therefore, we studied the effect of quercetin on EGFR signaling. In EGFR-overexpressing HSC-3 cells (Fig. 3A), the protein levels of p-Y1086-EGFR and its downstream effector, p-Akt, were decreased upon quercetin treatment in both time- (Fig. 3B) and dose- (Fig. 3C) dependent patterns, whereas the p-Y1045-EGFR levels were gradually increased (Fig. 3B). It is noteworthy that the phosphorylation at tyrosine 1045 is responsible for c-Cbl-mediated ubiquitination and degradation of EGFR protein [50]. These results suggest quercetin as an important modulator of the EGFR/Akt pathway.

3.3. Quercetin regulates FOXO1 activation

FOXO transcription factors play a role in the regulation of cell growth and apoptosis. The activity of FOXO factors can be regulated by Akt-mediated nucleus exclusion and subsequent degradation [33]. Therefore, we examined the effect of quercetin on FOXOs. While HSC-3 cells displayed increased FOXO1 protein levels in both dose- and time-dependent manners (Fig. 4A–B) upon quercetin treatment, no change of FOXO3a expression was observed (Fig. 4B). The finding of the increased FOXO1 expression upon quercetin treatment (Fig. 4A–B) prompted us to further differentiate the nuclear/cytoplasmic translocation of FOXO1; we then measured p-FOXO1 and total FOXO1 levels in the cytoplasm and nucleus, respectively. Quercetin reduced the phosphorylation of cytoplasmic FOXO1 at serine 256 (Fig. 4C) and induced the translocation of FOXO1 from cytoplasm to nucleus as shown by Western blotting (Fig. 4C) and immunofluorescence (Fig. 4D) in HSC-3 cells. These results suggest that quercetin induces the nuclear translocation of FOXO1 and thereby transactivates FOXO1.
Indeed, quercetin activated a luciferase reporter gene driven by three fork-head responsive elements (FHREs) in HSC-3 cells, whereas such activation was abolished upon FOXO1 knockdown (Fig. 4E). Since these FHREs were originally identified in FasL promoter [51], our data indicate that quercetin activates FOXO1 and induces FasL expression. Furthermore, as in the treatment of quercetin (Fig. 3A), the administration of LY294002, a PI3K inhibitor, increased FOXO1 activation was abolished upon FOXO1 knockdown (Fig. 4E). Since these results indicate that quercetin activates FOXO1 and induces FasL expression.

3.4. Quercetin mediates growth inhibition through FOXO1 activation

To verify if FOXO1 plays a key role in quercetin-mediated growth inhibition in oral cancer cells, we suppressed FOXO1 expression by FOXO1-specific siRNA in the presence of quercetin. FOXO1 knockdown relieved the suppression effect of quercetin on cell growth (Fig. 5A) and colony formation (Fig. 5B). In particular, FOXO1 suppression abolished quercetin-mediated G2/M arrest in both HSC-3 and TW206 cells (Fig. 5C). FOXO1 knockdown reduced quercetin-induced p21 expression (Fig. 5D); ectopic expression of FOXO1 resulted in increased p21 expression (Fig. 5E). These results indicate that quercetin mediates FOXO1-dependent p21 expression where the subsequent p21 expression might be responsible for the G2 arrest.

We next examined the role of FOXO1 in quercetin-mediated apoptosis. FOXO1 knockdown alleviated quercetin-induced apoptosis (Fig. 6A) and reduced the expression levels of FasL and cleaved caspase 3 (Fig. 6B) in HSC-3 cells. These results further suggest that quercetin-induced cell death might, in part, be through FOXO1-mediated FasL expression.

3.5. Quercetin inhibits tumor growth with concomitant FOXO1 induction in vivo

The antigrowth effect of quercetin on HSC-3 cells was further tested with xenograft mice. Both tumor size and weight of HSC-3-engineered xenografts were significantly suppressed upon quercetin administration as compared with the control (Fig. 7A–B). In addition, quercetin-fed xenograft mice also exhibited higher FOXO1 expressions than those of control animals (Fig. 7C). Therefore, consistent with the in vitro results, the in vivo data indicate that quercetin inhibits tumor growth and induces FOXO1 expression.

4. Discussion

Aberrant activation of the EGFR/PI3K/Akt signaling pathway is often associated with cancer malignancy [52]. Accordingly, this deregulated signaling pathway becomes a conceivable therapeutic target in anticancer strategy. HSC-3 cell line, a cervical lymph node metastasis originating from a tongue primary tumor, displays activated EGFR/PI3K/Akt signaling by expressing augmented EGFR levels and a constitutively active PI3K mutant [52]. Quercetin has been reported as a potent PI3K inhibitor [25], and the efficacy of quercetin inhibition on tyrosine kinase may continue for 16 h [10]. In the current study, we observed down-regulation of EGFR and Akt phosphorylation upon quercetin treatment for 12 h in HSC-3 cells. It is possible that quercetin might compete with adenosine triphosphate for the binding to the catalytic site of the PI3K [25]. Our data indicate that quercetin activates FOXO1 as an alternative regimen for oral cancer patients carrying an aberrant EGFR/PI3K/Akt signaling axis.

Oral squamous cancer cells with EGFR overexpression appear susceptible to the antiproliferation effect of quercetin. The concentration of quercetin to inhibit SCC-1483 cell viability by half is about 40 μM [29], in contrast with 20 μM for EGFR-overexpressing HSC-3 oral cancer cell line. In the current study, quercetin-induced G2 arrest is associated with the induction of FOXO1-dependent p21 expression in HSC-3 human oral cancer cells. Similar induction effect on p21 has
Fig. 8. Role of FOXO1 in quercetin-mediated growth suppression of oral squamous carcinomas. Quercetin efficiently inhibits cell growth by targeting EGFR/Akt signaling in EGFR-overexpressing oral cancer. The resulting FOXO1 activation leads to elevated expression of p21 and Fasl, and, subsequently, growth arrest in G2 phase and apoptosis.

also been reported in quercetin-treated breast and prostate cancer cells [17,18]. However, the concentration of quercetin to induce p21 expression in PC-3 cells was much higher (50–100 μM) than that of HSC-3 cells.

The involvement of p21 in G2 arrest could result from either DNA damage or mitogen starvation [49]. Seoane et al. proposed that, in response to transforming growth factor-β, FOXO1 forms a complex with Smad 3/4, which further induces p21 expression through the binding of the fork-head binding element within the p21 promoter [53]. The link between FOXO1 and p21 was further supported by the finding that FOXO1-mediated p21 expression is crucial in the antineoplastic effect of calorie restriction [54].

Quercetin may exert apoptotic function in a FOXO1-dependent fashion. Here, we demonstrated that quercetin treatment caused apoptosis in both HSC-3 and TW206 cells (Fig. 2C). This phenomenon was supported by increased levels of cleaved caspase 3 and Fasl upon quercetin addition (Fig. 2E). In particular, FOXO1 suppression alleviated quercetin-mediated cell death and reduced the induction of Fasl and cleaved caspase 3 (Fig. 6). Mutant p53 could block apoptosis, whereas quercetin can down-regulate mutant p53 protein levels [23]. Since HSC-3 carries a truncated p53 mutant [55], this could be an alternative mechanism how quercetin facilitates cell death in HSC-3 cells.

In summary, our data support quercetin as an efficient anticancer agent in EGFR-overexpressing oral cancers; the schematic mechanism is illustrated in Fig. 8. By suppressing EGFR/Akt activation, quercetin stimulates FOXO1 activation and subsequently induces p21 and Fasl, which link to G2 arrest and apoptosis, respectively. The current study provides several lines of evidence both in vivo and in vitro to support quercetin as a potential therapeutic agent for a subset of oral squamous carcinomas. Specifically, the mechanism involving FOXO1 in the anticancer action of quercetin may be translated to the future application as a clinical stratagem in the cure of oral cancer.

References


