Quercetin Suppresses the Migration and Invasion in Human Colon Cancer Caco-2 Cells Through Regulating Toll-like Receptor 4/Nuclear Factor-kappa B Pathway

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ABSTRACT

Objective: The migration and invasion features, which were associated with inflammatory response, acted as vital roles in the development of colon cancer. Quercetin, a bioflavonoid compound, was widely spread in vegetables and fruits. Although quercetin exerts antioxidant and anticancer activities, the molecular signaling pathways in human colon cancer cells remain unclear. Hence, the present study was conducted to investigate the suppression of quercetin on migratory and invasive activity of colon cancer and the underlying mechanism. Materials and Methods: The effect of quercetin on cell viability, migration, and invasion of Caco-2 cells was analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, wound-healing assay, and transwell chambers assay, respectively. The protein expressions of toll-like receptor 4 (TLR4), nuclear factor-kappa B (NFκB) p65, mitochondrial membrane potential-2 (MMP-2), and MMP-9 were detected by Western blot assay. The inflammatory factors, such as tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (Cox-2), and interleukin-6 (IL-6), in cell supernatant were detected by enzyme-linked immunosorbent assay. Results: The concentration of quercetin <20 μM was chosen for further experiments. Quercetin (5 μM) could remarkably suppress the migratory and invasive capacity of Caco-2 cells. The expressions of metastasis-related proteins of MMP-2, MMP-9 were decreased, whereas the expression of E-cadherin protein was increased by quercetin in a dose-dependent manner. Interestingly, the anti-TLR4 (2 μg) antibody or pyrrolidine dithiocarbamate (PDTC; 1 μM) could affect the inhibition of quercetin on cell migration and invasion, as well as the protein expressions of MMP-2, MMP-9, E-cadherin, TLR4, and NFκB p65. In addition, quercetin could reduce the inflammation factors production of TNF-α, Cox-2, and IL-6. Conclusion: The findings suggested for the 1st time that quercetin might exert its anticolon cancer activity via the TLR4- and/or NFκB-mediated signaling pathway.

Key words: Colon cancer, invasion, migration, quercetin, toll-like receptor 4

SUMMARY

• Quercetin could remarkably suppress the migratory and invasive capacity of Caco-2 cells
• The expressions of metastasis-related proteins of mitochondrial membrane potential-2 (MMP-2), MMP-9 were decreased, whereas the expression of E-cadherin protein was increased by quercetin in a dose-dependent manner.
• The anti-toll-like receptor 4 (TLR4) antibody or pyrrolidine dithiocarbamate affected the inhibition of quercetin on cell migration and invasion, as well as the protein expressions of MMP-2, MMP-9, E-cadherin, TLR4, and nuclear factor-kappa B p65.
• Quercetin could reduce the inflammation factors production of tumor necrosis factor-α, cyclooxygenase-2, and interleukin-6.

INTRODUCTION

Colon cancer, one of the most frequent malignant tumors worldwide, causes relatively high rates of morbidity and mortality.[1,2] Patients suffering from colon cancer often receive radiotherapy, hormonal therapy, immune therapy, chemotherapy, and symptomatic and supportive therapy. However, the current treatment was usually inefficient or caused a serious side effect. Therefore, it was urgent to explore the pathological mechanisms of colon cancer and find new strategies for the treatment of colon cancer.
The relationship between colitis and the occurrence of colon cancer has been well-recognized. It has been reported that the inflammatory bowel disease was widely acknowledged as the precancerous lesion associated with two-thirds of colitis-associated colon cancer. Toll-like receptor 4 (TLR4), one mechanism for chronic inflammation in colon cancer tumorigenesis and progression, has been reported to be responsible for carcinogenesis, invasion, metastasis, and cancer progression. TLR4 expression could be a useful prognostic marker in colon cancer. TLR4/nuclear factor-kappa B (NF-kB) signaling pathway, frequently overexpressed in human colon cancer, plays crucial roles in colon cancer development. Accumulating literatures demonstrated that the increased level of NF-kB phosphorylation in cancer tissues was associated with the clinical stage, migration, and invasion of colon cancer, indicating an important role of NF-kB activation in mediating the signaling event.

Quercetin (3,3’,4’,5,7-pentahydroxyflavone, molecular weight = 302 g/mol), a bioactive flavonoid, is widely spread in various edible plants, and the primary sources are red onions, apples, and tea. It was reported that quercetin-induced apoptosis through increased intracellular ROS in colon cancer cells. In addition, the anti-invasion and anti-migration ability of quercetin has been explored in other cells, such as human oral squamous cell carcinoma cell line SAS. However, the underlying mechanism of quercetin suppressing the migration and invasion characteristics of colon cancer cells remains poorly understood. In the current study, we explored the regulation effect of quercetin on the migration and invasion of colon cancer Caco-2 cells and investigated its role on TLR4/NF-kB pathway. The effect of quercetin on metastasis-related proteins and inflammatory factors was also evaluated.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Quercetin (purity: >98%) was purchased from Sigma Chemical Co. (St. Louis, MO), and it was dissolved in dimethyl sulfoxide (DMSO) for the next experiments. Fetal bovine serum (FBS) and Dulbecco modified Eagle medium (DMEM) were obtained from Gibco BRL Co. (Gaithersburg, MD, USA). Rabbit anti-human TLR4 antibody was obtained from AMS Biotechnology (Abington, UK), whereas pyrrolidine dithiocarbamate (PDTC, an NF-kB inhibitor) was purchased from Beyotime Institute of Biotechnology (Nantong, China). The antibodies to TLR4, NF-kB p65, mitochondrial membrane potential-2 (MMP-2), and MMP-9 were obtained from Cell Signaling Technology (Beverly, MA, USA). The enzyme-linked immunosorbent assay (ELISA) kits for tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (Cox-2), and interleukin-6 (IL-6) were purchased from KeyGEN Biotech Co., LTD. (Nanjing, China). Growth factor-reduced Matrigel was purchased from Becton Dickinson (BD Biosciences, San Jose, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other materials were from commercial sources.

**Cell culture**

The Caco-2 colon cancer cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). Caco-2 cells were cultured in high glucose DMEM supplemented with 10% FBS, 80 units/ml of penicillin/streptomycin, 1% glutamine, and 1% nonessential amino acid. Cells were maintained on plastic cell culture flasks in a humidified atmosphere of 5% CO₂ at 37°C. Medium was changed every 2 days.

Cells were applied to the experiments when 80–90% confluent layer was generated.

**Cell viability assay by**

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

The effect of quercetin on cell viability was determined by MTT assay. Briefly, Caco-2 cells were seeded in 96 well-plate (5 × 10⁴ cells/well) and treated with quercetin (5, 10, and 20 μM) for 24 h. Then, the medium was removed and incubated with 10 μl of MTT (0.5 mg/ml) for 4 h. The formed crystals were dissolved by 100 μl of DMSO, and the optical density (OD) was measured at 490 nm on a Multiskan spectrum microplate reader (Thermo, Waltham, MA, USA). Cell viability was calculated by the following equation: Cell viability (%) = (OD_quercetin-OD_blank)/(OD_control-OD_blank) ×100%. The test was performed in triplicate.

**Wound-healing assay**

Caco-2 cells (1 × 10⁶ cells/well) were seeded in 24 well-plates and allowed to grow until they reached confluence. Subsequently, the cells were scratched by 100 μl pipette tips and then cell debris was removed by washing with phosphate-buffered saline (PBS), and the wounded cell monolayer was cultured in FBS-free medium with quercetin or other agents for 24 h. Photographs of the wound area were captured at the 0 and 24 h with C × 31-32FRL microscope (Olympus, Tokyo, Japan). The relative wound area was obtained by quantitatively analyzing the areas in the scratch not overlapped by Caco-2 cells using Image-Pro-plus (IPP) software (Media Cybernetics, Rockville, MD, USA).

**Invasion assay of Caco-2 cells**

Cell invasion assay was performed by Matrigel-coated transwell cell culture chambers (8 μm pore size). Caco-2 cells were cultured in FBS-free DMEM overnight, then were collected and resuspended. The upper side of the membranes in the transwell chamber was coated with Matrigel (30 mg/L) in 50 μL ice-cold serum-free medium and air dried at the room temperature. The cell suspension of 250 μL at a density of 1 ×10⁵ cell/well was added to the upper and 10% FBS DMEM medium of 500 μL was added to the lower chamber. Caco-2 cells were treated with quercetin, anti-TLR4 antibody or PDTC for 24 h. Subsequently, the transwell chamber was removed, and the medium in the upper chamber was washed twice with PBS. The nonpenetrated cells were removed by a cotton swab. Then, the transwell chamber was dried at room temperature. The invasive cells in the back of the membrane were stained with 0.1% crystal violet. The invasiveness of Caco-2 cells was defined according to the total number of cells in 3 randomly selected microscopic fields. Finally, the membrane was washed in 96 well plates with 100 μl acetic acid (30%). The OD value of washing solution was detected at 580 nm with a microplate reader.

**Western blot analysis**

To explore the effect of quercetin on colon cancer-related protein expression and investigate the underlying mechanism, Western blot analysis was conducted for the expressions of MMP-2, MMP-9, E-cadherin, TLR4, and NF-kB p65. After treatment with quercetin and other agents, Caco-2 cells were washed twice with PBS. Cell lysates were centrifuged at 13,000 × g at 4°C for 10 min to extract proteins. Proteins were separated by 10% SDS-PAGE gel and transferred onto a polyvinylidene difluoride membrane. In addition, membranes were blocked with 5% skimmed milk at room temperature for 1 h.
Subsequently, the membranes were probed with 1:1000 diluted primary antibodies including MMP-2, MMP-9, E-cadherin, TLR4, and NF-kB p65 at 37°C for another 2 h. Membranes were rinsed with TBST for 4 times and then incubated with the horseradish peroxidase bound secondary antibody (1:5000) in a shaker. Finally, membranes were washed with PBS for 3 times and then chemoluminescence reagents were added for the visualization of the protein bands. The quantification of proteins was analyzed by IPP software (Media Cybernetics, Rockville, MD, USA).

**Determination of tumor necrosis factor-α, cyclooxygenase-2, and interleukin-6 by enzyme-linked immunosorbent assay kits**

The levels of inflammatory cytokines, such as TNF-α, Cox-2, and IL-6, in cells culture supernatant, were determined by ELISA kits (KeyGEN, Nanjing, China). Finally, the absorbance of each sample was read at 450 nm with a microplate reader within 3 min. The content of TNF-α, Cox-2, and IL-6 were calculated according to the standard curve.

**Statistical analysis**

All values in this study were taken from three independent experiments and expressed as means ± standard deviation (SD). The statistical significance was analyzed using the one-way analysis of variance with the Statistical Package for the Social Sciences (SPSS, 13.0) software (Chicago, IL, USA). Differences with $P < 0.05$ were considered statistically significant.

**RESULTS**

**Quercetin suppressed the migratory ability of Caco-2 cells**

To investigate the ability of quercetin to possibly suppress cell migration, confluent monolayers of Caco-2 cells were scratched as described above. Subsequently, the cells were treated with quercetin and other agents for 24 h. The closure rate of Caco-2 cells in each group was evaluated by IPP software. As depicted in Figure 2, the Caco-2 cells in control blank group migrated and covered most of the wound area during the 24 h of incubation, and its wound closure was $75.2 ± 5.3\%$, which was significantly larger than the cells exposed to 5 μM quercetin, whose closure rate was $42.6 ± 4.4\%$ ($P < 0.01$). To verify whether quercetin affected the migratory capacity of Caco-2 cells via TLR4 or NF-κB-mediated signaling pathway, Caco-2 cells were pretreated with anti-TLR4 antibody (2 μg) or PDTC (1 μM) for 1 h and then incubated in the presence or absence of quercetin (5 μM) for 24 h. Both anti-TLR4 antibody and PDTC significantly diminished the closure rate of Caco-2 cells ($P < 0.05$, $P < 0.01$). Moreover, there was a statistical difference between the co-incubation group and the alone one. Interestingly, the incubation of quercetin in the presence of anti-TLR4 antibody or PDTC could also decrease the closure rate ($P < 0.05$). The above evidence suggested that the inhibition of quercetin on the migration of Caco-2 cells might be related to TLR4 and/or NF-κB-mediated signaling pathway.

**Quercetin inhibited the invasive ability of Caco-2 cells**

In this study, transwell invasion assay was performed to evaluate the effect of quercetin on the invasive capability of Caco-2 cells. The invasive cells in the lower surface of the filter were stained and counted. As it can be seen from Figure 3, the concentration of quercetin at 5 μM could remarkably reduced the number of penetrated cells compared with the control blank group ($P < 0.05$). As expected, the anti-TLR4 antibody (2 μg) and NF-κB inhibitor PDTC (1 μM) could also significantly inhibit the number of penetrated cells. Furthermore, quercetin of 5 μM in the presence of anti-TLR4 antibody (2 μg) or PDTC (1 μM) evidently prevented the cells penetrating through the Matrigel compared with the control blank group ($P < 0.05$). Furthermore, there was a significant statistical difference of the number of penetrated cells between the co-incubation of quercetin (5 μM) and anti-TLR4 antibody group and anti-TLR4 antibody alone group ($P < 0.05$). Interestingly, the same result could be seen in the co-incubation of quercetin (5 μM) and PDTC group and PDTC alone group ($P < 0.01$). The above results suggested that quercetin could suppress the invasive capability of Caco-2 cells. This suppression might be associated with the TLR4 and/or NF-κB-mediated signaling pathway.

**Effect of quercetin on the protein expressions of mitochondrial membrane potential-2, mitochondrial membrane potential-9, E-cadherin, toll-like receptor 4, and nuclear factor-kappa B p65 in Caco-2 cells**

The degradation of extracellular matrix played an important role in the migration of tumor cells, which suggested that matrix-degrading proteinases were required. To investigate whether or not quercetin suppressed the migration of Caco-2 cells via the inhibition of related proteins, the expressions of E-cadherin, MMP-2, and MMP-9 protein were evaluated by Western blot analysis. As presented in Figure 4, the
Caco-2 cells after exposure to 5, 10, and 20 μM quercetin for 24 h, the expression of E-cadherin protein was 134.7 ± 8.1%, 171.4 ± 13.6%, and 186.3 ± 15.3% compared with the control blank group, respectively. The overexpression of MMP-2 and MMP-9 had been found to contribute to the migration of tumor cells. With the increment of quercetin dose from 5 μM to 20 μM, the expressions of MMP-2 and MMP-9 protein could be significantly decreased (P < 0.05, P < 0.01).

To further determine the specific effect of quercetin on TLR4/NF-κB pathway, Caco-2 cells were pretreated with anti-TLR4 antibody (2 μg) or PDTC (1 μM) for 1 h and then incubated in the presence or absence of quercetin (5 μM) for 24 h. As shown in Figure 5, the expressions of MMP-2, MMP-9, TLR4, and NF-κB p65 protein could be significantly diminished, and the expression of E-cadherin protein was evidently increased when the Caco-2 cells were incubated with anti-TLR4 antibody (2 μg) or PDTC (1 μM), compared with the control blank group (P < 0.05, P < 0.01). In addition, the co-treatment of quercetin and anti-TLR4 or quercetin and PDTC could markedly reduce the expressions of MMP-2, MMP-9, TLR4, and NF-κB p65 protein and increase the expression of E-cadherin protein, compared with the Caco-2 cells only been treated with quercetin (P < 0.05, P < 0.01). These findings indicated that the suppression of quercetin on migratory and invasive ability of Caco-2 cells through regulating the levels of MMP-2, MMP-9, and E-cadherin protein, which were affected by quercetin via TLR4- and/or NF-κB-mediated signaling pathway.

**Figure 2:** Effect of quercetin on migration of Caco-2 cells. (a) Cells were incubated in 24 well plates and treated with quercetin (5 μM) for 24 h in the presence or absence of anti-toll-like receptor 4 antibody (2 μg) or pyrrolidine dithiocarbamate (1 μM). a': Blank control; b': Quercetin of 5 μM; c': Anti-toll-like receptor 4 antibody (2 μg); d': Pyrrolidine dithiocarbamate (1 μM); e': Quercetin (5 μM) + anti-toll-like receptor 4 antibody (2 μg); f': Quercetin (5 μM) + pyrrolidine dithiocarbamate (1 μM). (b) The closure rate of cells was recorded, and the data were obtained from triplicate experiments and expressed as the means ± standard deviation (n = 3). *P < 0.05, **P < 0.01, versus blank control; #P < 0.05, quercetin (5 μM) + anti-toll-like receptor 4 versus anti-toll-like receptor 4 alone; &&P < 0.05, quercetin (5 μM) + pyrrolidine dithiocarbamate versus pyrrolidine dithiocarbamate alone.

**Figure 3:** Effect of quercetin on invasion of Caco-2 cells. (a) The invasion of Caco-2 cells was evaluated by cell invasion assay, which was performed on 8 μm polycarbonate Matrigel-coated transwell cell culture chambers. Cells were incubated in 24 well plates and treated with quercetin (5 μM) for 24 h in the presence or absence of anti-toll-like receptor 4 antibody (2 μg) or pyrrolidine dithiocarbamate (1 μM). a': Blank control; b': Quercetin of 5 μM; c': Anti-toll-like receptor 4 antibody (2 μg); d': Pyrrolidine dithiocarbamate (1 μM); e': Quercetin (5 μM) + anti-toll-like receptor 4 antibody (2 μg); f': Quercetin (5 μM) + pyrrolidine dithiocarbamate (1 μM). (b) Optical density for the permeated cells. Data were obtained from triplicate experiments and expressed as the means ± standard deviation (n = 3). **P < 0.01, versus blank control; #P < 0.05, quercetin (5 μM) + anti-toll-like receptor 4 versus anti-toll-like receptor 4 alone; &&P < 0.05, quercetin (5 μM) + pyrrolidine dithiocarbamate versus pyrrolidine dithiocarbamate alone.

**Figure 5:** Relative invasion ability (% of control) of Caco-2 cells treated with quercetin, anti-TLR4, PDTC, or their combination. Data were obtained from triplicate experiments and expressed as the means ± standard deviation (n = 3). *P < 0.05, **P < 0.01, versus blank control; #P < 0.05, quercetin (5 μM) + anti-toll-like receptor 4 versus anti-toll-like receptor 4 alone; &&P < 0.05, quercetin (5 μM) + pyrrolidine dithiocarbamate versus pyrrolidine dithiocarbamate alone.

**Regulation of quercetin on the levels of inflammation factors in Caco-2 cells**

It has been reported that inflammation factors, such as TNF-α, Cox-2, and IL-6, played crucial roles in cancer cell migration. ELISA kits assay was carried out to determine the levels of the inflammation factors. As presented in Figure 6, quercetin of different concentrations...
Effect of quercetin on the expressions of metastasis-related protein with Western blot assay. (a) Western blot for the expressions of E-cadherin, MMP-2, and MMP-9 in Caco-2 cells. (b) E-cadherin protein level; (c) mitochondrial membrane potential-2 and mitochondrial membrane potential-9 in Caco-2 cells. (d) Mitochondrial membrane potential-2 and mitochondrial membrane potential-9 protein level. Data were expressed as the means ± standard deviation (n = 3). *P < 0.05, **P < 0.01 versus control blank group.

DISCUSSION

Recently, medical scientists paid more attention to natural chemical compounds, which were vital targets in anticancer field, because of the drug resistance and toxic side effects of current chemotherapy.[29] Growing evidence indicated that quercetin could fight against different types of cancer, including Dalton’s lymphoma, prostate cancer, hepatocellular carcinoma, breast cancer, glioma, and so on.[30-34] Quercetin has been reported to possess antiproliferation and antimigration capacity of cancer cells.[35] It has been reported to possess antiproliferation and antimigration capacity of cancer cells.[35] Quercetin has been shown to possess antimigratory and anti-invasive activity and the underlying mechanism of quercetin on Caco-2 cells remains unclear. The present study found that quercetin could suppress the migration and invasion of Caco-2 cells through inhibiting MMP-2 and MMP-9 enzyme activity in HepG2 cells.[36] In the present study, we also found that the expressions of E-cadherin, MMP-2, and MMP-9 could be suppressed by quercetin in a concentration-dependent manner as well as migration and invasion. The results suggested that the antimetastatic feature of quercetin on Caco-2 cells was associated with its suppression on E-cadherin, MMP-2, and MMP-9 expression.

Colonial inflammation could promote colon cancer development, especially for colitis-associated cancer.[8,39] TNF-α was a pro-inflammatory cytokine, which played an important role in tumor malignancy, including tumor cell motility, invasion, and metastasis.[40] High expression of TNF-α in the tumor microenvironment was a common characteristic of numerous malignant tumors. It has been reported that the increment of MMP-9 activity and expression was associated with the stimulation of TNF-α in cancer cells.[41] Furthermore, evidence suggested that the inflammatory cytokine of COX-2 could promote tumor angiogenesis and tumor invasion, and the downregulation of COX-2 inhibited cancer metastasis through preventing migration and invasion.[42,43] IL-6, linking the relationship between inflammation and cancer, was an indispensable inflammatory cytokines for cancer cell proliferation and invasion.[44,45] In the present study, we demonstrated that the levels of inflammation factors of TNF-α, COX-2, and IL-6 in cell supernatant were suppressed by quercetin in a dose-dependent manner. The result suggested that the attenuation of quercetin on the inflammatory responses could prevent the development of Caco-2 cells.

TLRs mediated signaling was thought to be closely relevant with cell migration and invasion during the development and progression of cancer.[46] TLR4, an important member of TLRs family, was known to recognize lipopolysaccharide on the surface of bacteria. TLR4 signaling pathway on colon cancer cell facilitated evasion of immune surveillance and mediated tumor progression, which was considered as one of the underlying mechanisms for chronic inflammation in colon cancer tumorigenesis and progression.[47] It has been reported that TLR4 signaling pathway was one of the most important pathways to regulate the proteinase activation of E-cadherin, MMP-2, and MMP-9.[48,49] The research of...
Byun et al. indicated that quercetin could negatively regulate TLR4 signaling in inflammatory disease. Although the model was different, the above literature evidenced our results that quercetin could decrease the protein expression of TLR4. In the present study, the treatment of anti-TLR4 could obviously affect the migration and invasion of Caco-2 cells, and the expressions of E-cadherin, MMP-2, and MMP-9 protein. The results demonstrated that quercetin might play its anti-migratory and anti-invasive effect via TLR4-mediated signaling pathway.

NF-κB was the key downstream signal transducer of TLR4 signaling pathway. Transcriptional factor NF-κB plays an important role in human colon cancer cells, and its constitutive activity has been found in cancer cell migration and invasion. A growing body of evidence indicated that quercetin could efficiently down-regulate the phosphorylation of NF-κB to inhibit cancer migration and invasion through the decrement of MMP-2/MMP-9 protein expression in cancer cells. Bhaskar et al. demonstrated that quercetin inhibited TLR4/NF-κB signaling pathway, thereby modulated the cytokine production and downregulated the activity of inflammatory enzymes, thus had protective effect against the ox-LDL-induced inflammation in PBMCs. Wang et al. indicated that isoquercetin, a flavonoid similar to quercetin, protected cortical neurons from oxygen-glucose deprivation-reperfusion induced injury via suppression of TLR4-NF-κB signal pathway. A study by Kaneko et al. showed that quercetin suppressed the TLR4/NF-κB signaling pathway to decrease the accumulation of lipid rafts. The similar results were present in our observations. Quercetin could inhibit the activation of TLR4 and NF-κB p65. Our results indicated that the suppression of quercetin on migration and invasion of Caco-2 cells might be relevant to TLR4-and/or NF-κB-mediated signaling pathway.

CONCLUSION

Our findings indicated that quercetin could inhibit the migration and invasion of Caco-2 cells. The treatment with quercetin in the presence or absence of anti-TLR4 antibody and NF-κB inhibitor (PDTC) could decrease the expressions of MMP-2, MMP-9, TLR4, and NF-κB p65.
protein, and increase the expression of E-cadherin protein. The results demonstrated that quercetin could inhibit the migration and invasion of Caco-2 cells by inhibiting TLR4/NF-κB signaling pathway. These results might provide new insight into the underlying molecular mechanisms of quercetin on treating colon cancer. Consequently, we advise that quercetin could be a promising candidate for the treatment of colon cancer.

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Conflicts of interest
There are no conflicts of interest.

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AIM: Quercetin suppresses the migration and invasion of colon cancer Caco-2 cells through the EGFR/PI3K/Akt signaling pathway.

METHODS: Caco-2 cells were treated with quercetin (0.1-10 μM) for 24 h. The wound healing, transwell migration, and invasion assays were performed. Western blot, RT-qPCR, and luciferase assays were conducted to determine the expression of EGFR, PI3K, Akt, TLR4, and NF-κB. The Caco-2 cell lysates were analyzed for the expression of IL-8 and COX-2 via ELISA.

RESULTS: Quercetin significantly suppressed the migration and invasion of Caco-2 cells in a dose-dependent manner. Quercetin treatment downregulated the expression of EGFR, PI3K, Akt, TLR4, and NF-κB, and upregulated the expression of IL-8 and COX-2. Quercetin also inhibited the phosphorylation of Akt, TLR4, and NF-κB.

CONCLUSION: Quercetin suppresses the migration and invasion of colon cancer Caco-2 cells through the EGFR/PI3K/Akt signaling pathway.

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