Original Article

Quercetin Can Inhibit Angiogenesis via the Down Regulation of MALAT1 and MIAT LncRNAs in Human Umbilical Vein Endothelial Cells

Somayeh Esteghlal, Mohammad Javad Mokhtari*, Zahra Beyzaei[1]

Department of Biology, Zarghan Branch, Islamic Azad University, Zarghan, Iran, [1]Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Angiogenesis is an important step in cancer metastasis since it enables the growing tumor to receive nutrients and oxygen. Quercetin is a generic flavonoid and has been investigated for its ability to inhibit angiogenesis in different types of cancers. *MALAT1* and *MIAT lncRNAs* are associated with the angiogenesis process. *MALAT1* induces hypoxia-driven angiogenesis via the overexpression of angiogenic genes. Down regulation of *MIAT1* could inhibit the proliferation of endothelial cells, tube formation, and migration. In this study, we assessed the anti-angiogenic activity of quercetin on human umbilical vein endothelial cells (HUVEC) via the expression of *MALAT1* and *MIAT* genes. **Methods:** In the present study, HUVEC cells were incubated with various concentrations of quercetin for 24, 48, and 72 h. Cell proliferation was then evaluated by MTT assay. RNA was extracted by TRIzol and cDNA synthesis. The expression levels of *MALAT1* and *MIAT* genes relative to the *GAPDH* gene were quantified using the highly sensitive real-time PCR method. **Results:** Our results demonstrated that quercetin has an inhibitory impact on the cell viability of HUVEC cells. The IC_{50} values of quercetin after 24, 48, and 72 h were 282.05 µM, 228.25 µM, and 131.65 µM, respectively. The *MALAT1*/*GAPDH* ratio was computed as 0.21 for 24h, 0.18 for 48h, and 0.29 for 72 h. The *MIAT*/*GAPDH* ratio was computed as 0.82 for 24h, 0.84 for 48h, and 0.78 for 72 h. **Conclusions:** In conclusion, quercetin treatment had an anti-angiogenic effect on HUVEC cells, at least partially via the down regulation of *MALAT1* and *MIAT LncRNAs* gene expression.

Keywords: *Angiogenesis inhibitors, endothelial cells, long non-coding RNA, quercetin*

Introduction

Angiogenesis is a crucial step in the growth, progression, and metastasis of cancer cells, which help the growing tumor to receive nutrients and oxygen.[1,2] Angiogenesis is the physiological process that involves the activation, migration, and growth of endothelial cells toward angiogenic stimuli and chemotactic produced by the cancer cells.[3] When the angiogenesis is initiated the proliferation of was activated.[4] One of the main predictors of cancer's stages and prognostic indicator is tumor vasculature.[5]

One of the main integral parts of tumor control is chemo-preventive agents such as natural products. Many phytochemicals could have great potential as anti-angiogenic agents for inhibiting cancer metastasis.[6] Quercetin exists in many plant foods such as capers, parsley, apples, broccoli, tea, lovage, red onions, and red grapes.[7] The chemopreventive effects of quercetin have been related to different mechanisms, including its antioxidative activity, modify signal transduction cascades, and remove enzymes that activate carcinogens.[8] Quercetin may inhibit angiogenesis in different types of cancer cells such as; ovarian cancer, breast cancer, prostate cancer, rat, and human glioblastoma cells.[9]

Many angiogenic agents have been reported to change the morphogenesis, growth, and blood vessel formation.[10] Previous researches have showed the main roles of noncoding RNAs (ncRNAs) in vascular disease and angiogenesis.[11] These ncRNAs can be classified as circRNA, microRNAs, long ncRNAs (lncRNAs), and other small RNAs.[12] Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) are overexpressed in hypoxia condition that can result in different phenotypes such as; endothelial cell growth, metastasis, and angiogenesis, as well as cell cycle arrest and cell death.[13] It was also found to be up regulated in aqueous tumors.[14] Interestingly, *MALAT1*

How to cite this article: Esteghlal S, Mokhtari MJ, Beyzaei Z. Quercetin can inhibit angiogenesis via the down regulation of MALAT1 and MIAT LncRNAs in human umbilical vein endothelial cells. Int J Prev Med 2021;12:59.

Address for correspondence:
Dr. Mohammad Javad Mokhtari, Assistant Professor, Department of Biology, Zarghan Branch, Islamic Azad University, Zarghan, Iran.
E-mail: mj.mokhtari@zariau.ac.ir

Access this article online

Website: www.ijpvmjournal.net/www.ijpm.ir

DOI: 10.4103/ijpvm.IJPVM_103_20

Quick Response Code:

is drastically overexpressed by transforming growth factor-β, which has inhibitory or stimulatory impacts on angiogenesis.[15] Also, it modulates cell-autonomous angiogenesis via the regulation of VEGFR2.[16] Myocardial infarction associated transcript (MIAT) regulates angiogenesis through interaction with miR-150-5p, which can target VEGF.[11] Also, VEGF stimulation generally promotes cell growth and the migration of HUVECs through regulating MIAT/miR-1246/ACE.[17]

In the ongoing battle against cancer, the development of novel therapeutic strategies, therefore, remains an essential goal. Therefore, the prevention of angiogenesis is presently perceived as one of the favorable strategies in the treatment of tumors. So, the aim of this study was to evaluate the quercetin effect on HUVEC cell viability, and to confirm the probability that quercetin exerted its role in an angiogenesis inhibition-dependent fashion, also the expressions of *MALAT1* and *MIAT* LncRNAs were evaluated.

Methods

Cell culture and quercetin treatment

This experimental study was carried out on HUVEC cell line. This cell line was purchased from the National Cell Bank at Pasteur Institute of Iran. The cells were cultured in DMEM medium containing 10% serum at 37°C in an incubator containing 95% O2 and 5% CO_2.

The cytotoxicity of quercetin was determined in the HUVEC cell line using MTT assay. Quercetin (Sigma, USA) was dissolved in DMSO (Sigma, USA). The amount of DMSO in culture media never exceeded 0.1% (v/v).[18] HUVEC cells were then seeded into 96-well plates (1×10^4 cells/well) in the complete medium. After overnight incubation, the medium was removed and 100 µL growth culture containing a series of different concentrations of quercetin (0, 6.25, 12.5, 25, 50, 100, and 200 µM)[19,20] at 24, 48, and 72 h was added. After a specified time, the culture medium from each well was discarded, and 100 µL of the DMEM containing 20 µL MTT solutions was added to each well. After 3-5 h of incubation, the formazan were solubilized with 150 µL DMSO. The determination of the amount of formazan was performed at 540 nm. The cell viability in different quercetin concentrations was assessed as a percentage relative to the untreated groups. Pharm statistical package (Springer Verlag, New York, NY) was used to evaluate the half maximal inhibitory concentration (IC_{50}) values.

Total RNA extraction and cDNA synthesis

Following pretreatment of HUVEC cells by quercetin at IC_{50} concentrations for 24, 48 and 72 h, total RNA was extracted from cell pellets using a trizol solution. RNA samples with the A260/A280 and A260/A230 ratios higher than 1.7 were chosen for the synthesis of cDNA. cDNA synthesis was performed by cDNA Synthesis Kit (Favorgen, Thailand) according to the manufacturer's instructions.[21]

Primer design

In the present study, *GAPDH* gene was chosen as the reference, and *MALAT1* and *MIAT* genes were chosen as target genes. Primers were designed with Gene Runner and Primer Express software. The sequence of forward and reverse primers for the PCR amplification of *MALAT1* and *MIAT* transcripts was 5′- TATAAATACGCCTCGCCCGAG -3′ and 5′- ATCTGCGGTTTCCTCAAGCTC -3′ (amplicon size, 98 bp) and 5′- CACAAAGAGCCCTCTGCACTAG -3′ and 5′- TGGCCACATGAACGTGTCTG -3′ (amplicon size, 94 bp), respectively.

The forward and reverse primers' sequence for the housekeeping gene *GAPDH* was 5′-CATGAGAAGTATGACAACAGCCT-3′and 5′-AGTCCTTCCACGATACCAAAGT-3′, respectively (amplicon size, 113 bp).[22]

Quantitative real-time PCR

The SYBR Green PCR Master Mix (Takara, Japan) was employed in carrying out quantitative RT-PCR on the Rotor-Gene 6000 (Corbett Research, Australia) with thermal-cycling settings of 10 min at 95°C (1 repeat) followed by 40 cycles for 15 s at 95°C and 1 min at 60°C. Every complete amplification phase was accompanied by a melting phase. Ct values were plotted against the log cDNA concentrations of five serial two-fold adynamic ranges of the target and reference genes to compute PCR efficiency. Response efficiency was calculated via the equation below: $E = [10^{(-1/slope)} - 1]$.[23]

Statistical analysis

The statistical data represent the mean ± SD and coefficient of determination (R^2). In this work, the statistical analyses were performed using Two-way ANOVA (for MTT assay) and one-way ANOVA (for qRT-PCR assay) followed by Tukey's multiple comparison post hoc tests. *P* value of <0.05 was regarded as statistically significant. All statistical analyses were performed with statistically available software (SPSS 24 for WINDOWS). Graphs preparation was done using Microsoft Office Excel software (version 2016).

Results

Quercetin cytotoxicity and IC_{50} determination

The cytotoxicity of quercetin in the HUVEC cell line was evaluated by MTT assay. Cells were incubated with different doses of quercetin for 24, 48, and 72 h. The cytotoxic impacts of the quercetin concentrations are depicted in Figure 1, which demonstrates that quercetin reduced the viability of HUVEC cells in a time-dependent fashion.

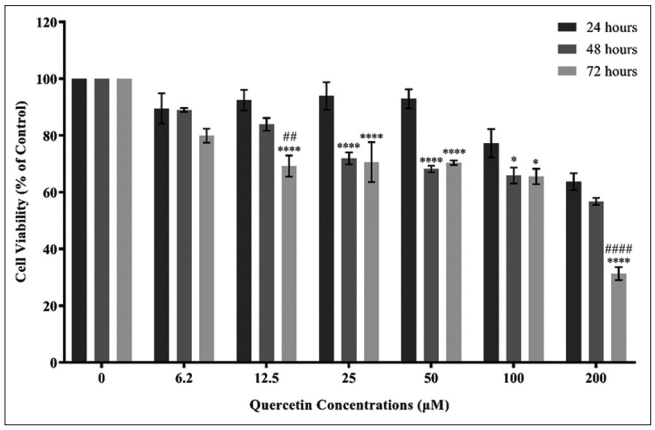

Figure 1: Quercetin effects on cell proliferation. The HUVEC cells were treated with different concentrations of quercetin for 24, 48, and 72 h and their viability was measured using MTT assay. Results are expressed as the percentage of viability compared with the control and are presented as mean ± SEM from at least three independent experiments. The statistical analyses were performed using Two-way ANOVA and Tukey multiple comparison tests. *$P < 0.05$ and ****$P < 0.0001$ represent significant differences with 24 h treatment and $^{\#\#}P < 0.01$ and $^{\#\#\#\#}P < 0.0001$ represent significant differences with 48 h treatment

Compared to the controls, different concentrations of quercetin (6.25, 12.5, 25, 50, 100, and 200 µM) decreased the cell viability of HUVEC cells by 10.47% ($P > 0.05$), 7.59% ($P > 0.05$), 6.07% ($P > 0.05$), 7.10% ($P > 0.05$), 22.74% ($P < 0.001$), and 36.30% ($P < 0.001$), respectively, after 24 h. Similarly, compared to the controls, various concentrations of quercetin (6.25, 12.5, 25, 50, 100, and 200 µM) decreased the cell viability of HUVEC cells by 11.02% ($P < 0.01$), 16.11% ($P < 0.01$), 28.11% ($P < 0.001$), 31.81% ($P < 0.001$), 31.14% ($P < 0.001$), and 43.27% ($P < 0.001$), respectively, after 48 h. After 72 h, compared to the controls, various concentrations of quercetin (6.25, 12.5, 25, 50, 100, and 200 µM) decreased cell viability of HUVEC cells by 20.10% ($P < 0.001$), 30.81% ($P < 0.001$), 29.38% ($P < 0.001$), 29.63% ($P < 0.001$), 34.45% ($P < 0.001$), and 68.71% ($P < 0.001$), respectively. In concentrations 12.5, 25, 50 and 100 µM of quercetin, significant differences were shown in 24h vs 48h and 24h vs 72h in cell viability ($P < 0.05$). In 200 µM concentration of quercetin significant differences were observed in 24 h vs 72 h and 48 h vs 72 h after treatment ($P < 0.05$).

The IC_{50} values of quercetin after 24, 48, and 72 h were 282.05 µM, 228.25 µM, and 131.65 µM, respectively.

Real-time PCR validation

A melting curve analysis verified that we have only one product for each gene. The melting peaks were at 88.7°C for the *MALAT1* gene, 88.2°C for the *MIAT* gene, and 85.8°C for the *GAPDH* gene [Figure 2].

Referring to the initial data, it was demonstrated that in the treated samples the expression differences in the control and target genes would not be more than tenfold. So, we used two-fold serial dilution in the standard curves to confirm the test accuracy. This allowed us to increase the dynamic range resolution and make changes at the gene expressions.[24] The standard curves slopes were −3.36, −3.35, and −3.41 for *MALAT1*, *MIAT,* and *GAPDH*, respectively. PCR efficiency was computed as 98.15% for *MALAT1*, 98.60% for MIAT, and 96.33% for *GAPDH* [Figure 3].

Relative quantification of MALAT1 and MIAT expression

Between the two samples (treated and untreated), the relative gene expression may be defined by the difference in CT numbers of the exponential phase. The mCT value for *GAPDH* gene was 23.26 in various concentrations of quercetin. The *MALAT1*/*GAPDH* and *MIAT*/*GAPDH* gene expressions ratio in HUVEC cells that were treated with IC_{50} concentrations of quercetin at different times (24, 48, 72 h) was computed using the formula $2^{-\Delta\Delta CT}$. The *MALAT1*/*GAPDH* ratio was computed as 0.21 ($P < 0.001$) for 24h and 0.18 ($P < 0.001$) for 48h, and 0.29 ($P < 0.001$) for 72 h [Figure 4a]. The *MIAT*/GAPDH ratio was computed as 0.82 ($P > 0.05$) for 24 h and 0.84 ($P > 0.05$) for 48 h, and 0.78 ($P > 0.05$) for 72 h [Figure 4b].

Discussion

Angio-LncRs including *MALAT1* and *MIAT* have a function in the angiogenesis process and vascular disease. In the present study, we evaluated the expression of *MALAT1* and *MIAT LncRNAs* genes in HUVEC cells treated with quercetin. Quercetin treatment reduced the viability of HUVEC cells in a dose- and time-dependent fashion. We also observed the down regulation of *MALAT1* and *MIAT LncRNAs* in HUVEC cells treated by quercetin. Thus, the present study suggests that quercetin inhibits angiogenesis via the down regulation of *MALAT1* and *MIAT* LncRNAs genes. To the best of our knowledge, this is the first study to examine the effects of quercetin on the expression of *MALAT1* in HUVEC cells and *MIAT* LncRNAs genes.

Flavonoids are polyphenolic substances that possess anti-inflammatory, antimicrobial, antiviral, anti-thrombotic, anti-allergic, antineoplastic, antimutagenic, and cytoprotective effects on various cells.[25] Epidemiologic research has proposed that high flavonoid consumption might be correlated with a reduced risk of different types of tumors.[26] Quercetin is a member of a potent antioxidant flavonoid family that inhibits the oxidation of LDL.[27] It has previously been demonstrated to be a potent inhibitor against ovarian, colon, and prostate cancer.[28] The diameter of solid tumors cannot grow more than 3 mm because of the limits of diffusion for nutrients, metabolic wastes, and gases. The tumor growth needed new vasculature is recruited, facilitating gaseous and nutrients exchange.[29,30] One the effective strategies for cancer prevention is phytochemical angiopreventive agents

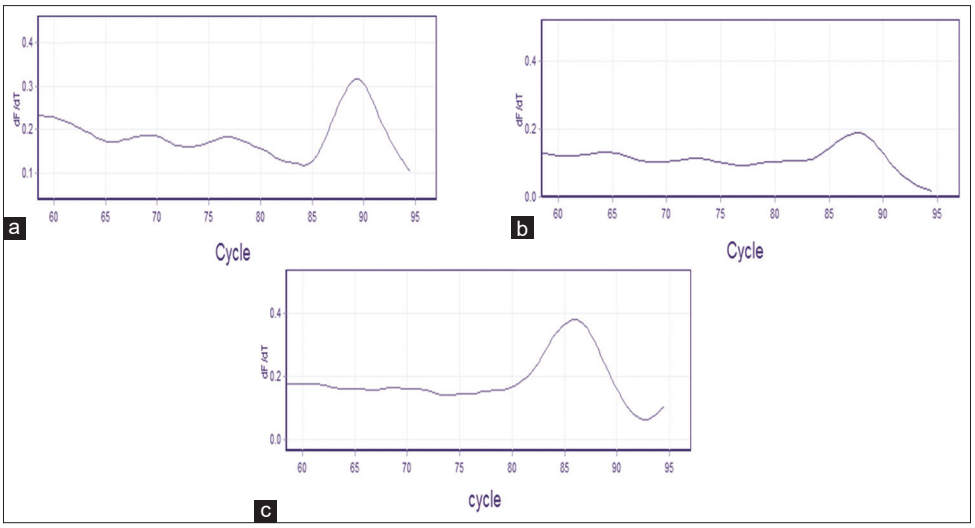

Figure 2: The melting analysis of the Real-time PCR assays. Melting peaks at (a) 88.7°C for *MALAT1* gene, (b) 88.2°C for *MIAT* gene and (c) 85.8°C for *GAPDH* gene indicate the formation of the tree specific products with different Tm temperatures

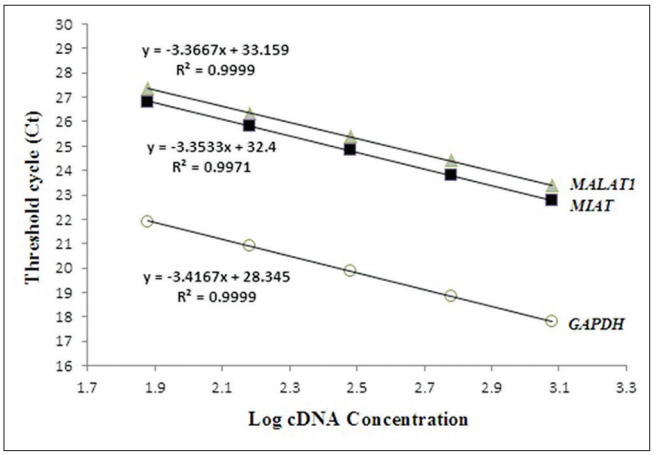

Figure 3: Standard curve for *MALAT1*, *MIAT* and *GAPDH*. Standard curve is generated by plotting CT values against the logarithm of the cDNA concentration. Pearson's correlation coefficients (square values – R^2) is shown. Standard curve of *MALAT1*, slope=−3.36, y-intercept = 33.15, R^2 = 0.999. Standard curve of *MIAT*, slope=−3.35, y-intercept = 32.4, R^2 = 0.997. Standard curve of *GAPDH*, slope=−3.41, y-intercept = 28.34, R^2 = 0.999

that can inhibit tumor proliferation and cancer metastatic in animal models.[6] However, we do not have enough knowledge about the relationship between phytochemicals and lncRNAs. Therefore were need more information about the role of phytochemicals and herbal derivatives in the regulation of lncRNA in cancer therapy.

We showed that the concentrations of 6.25-50 μM quercetin for 24 h does not significantly change the viability of HUVEC cells. Yang *et al*. showed that at concentrations of 30210 μM, quercetin induced the proliferation and viability of HUVEC cells.[28] HUVEC cells have been used in angiogenesis studies in many researches.[31,32] So, we used HUVEC cells because they are easier to culture and more readily available. In this study, quantitative real-time RT-PCR also showed that the expression of *MALAT1* was markedly lower in HUVEC cells treated by quercetin than under control conditions. The expression of *MALAT1* was shown to be upregulated in many types of cancers such as lung, liver, bladder, breast, cervix, colorectal, renal cell carcinoma, uterine endometrial, stromal sarcoma, and osteosarcoma. Also, *MALAT1* showed important effects on tumor cell proliferation, migration, and invasion.[33] Pan *et al*. showed that quercetin inhibits cell proliferation and promotes the apoptosis of fibroblast-like synoviocytes. Their results also showed that *MALAT1* expression was significantly overexpressed in quercetin-treated fibroblast-like synoviocytes.[27] *MALAT1* is significantly overexpressed by hyperglycemia and hypoxia in endothelial cells.[34] In hyperglycemic conditions, the knockdown of *MALAT1* shows reduction in cell proliferation and migration in endothelial cells.[34-36] Although, the main role of *MALAT1* in the angiogenesis mechanism is not clearly understood despite its extensive expression in the vasculature. In this study, we showed that the expression of *MIAT* was downregulated in HUVEC cells treated by quercetin, but there were no significant differences between untreated HUVEC cells and HUVEC cells treated by quercetin. It is possible that this result is due to the amount of quercetin concentration and if the higher concentration is used, it may lead to significant results. The upregulation of *MIAT lncRNA* in tumor tissues has been reported in different cancers such as breast cancer, clear cell renal cell carcinoma, papillary thyroid carcinoma. In colorectal cancer cells, *MIAT* bound to miR-132 and help the proliferation and migration of malignant tumor cells. Also, downregulation of *MIAT* represses migration in HeLa cells.[37] *MIAT* is related with angiogenesis following myocardial infarction, microvascular dysfunction, and upregulation of *VEGF*.[38] Several studies have demonstrated the impacts of quercetin and associated flavonols on cell signaling associated with carcinogenic processes, cell cycle distribution, apoptosis,

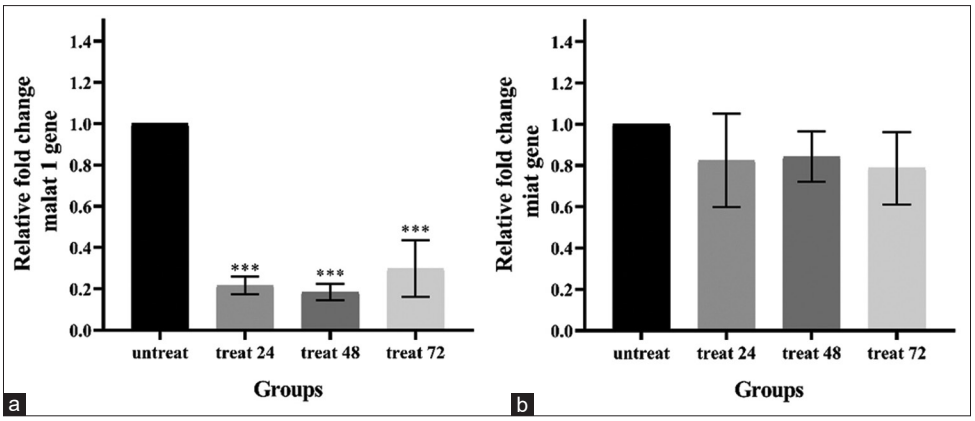

Figure 4: Quercetin effects on expression of target genes mRNA. All data of qRT-PCR were normalized against GAPDH. Bars represent fold differences of the mean of normalized expression values ± SDEM. (a) Quercetin effect on LncRNA MALAT1 mRNA expression. (b) Quercetin effect on LncRNA MIAT mRNA expression. The statistical analyses were performed using ordinary one-way ANOVA and Tukey multiple comparison tests. * *P* < 0.001 represents significant difference with untreated group**

and angiogenesis.[8] In addition, the effect of quercetin on cancer angiogenesis was validated.

Quercetin also prevents angiogenesis in HUVEC cells by suppressing the VEGF through the inhibition of kinases and through the induction of apoptosis.[39] By targeting VEGFR-2, quercetin could inhibit the proliferation of human prostate tumors in an angiogenesis inhibitiondependent fashion.[40] Microvessel density and serum VEGF levels are biomarkers of tumor angiogenesis, and they were both reduced in quercetin-treated animals.[41] In choroid-retinal endothelial cells, quercetin inhibited the VEGFR-2 regulated signaling pathways and the expression of COX-2 in breast cancer cells.[9] In a previous study it was demonstrated the efficiency in controlling murine T-cell lymphoma by reducing angiogenesis via the inhibition of AKT signaling pathway.[42] Quercetin showed an anti-invasive impact on glioblastoma cells via the down regulation of MMPs and VEGF protein levels.[43]

Conclusions

We showed the *in vitro* anti-proliferative and anti-angiogenic impacts of quercetin on HUVEC cells. This study demonstrated that quercetin inhibits angiogenesis, at least in part via the downregulation of *MALAT1* and *MIAT LncRNAs* genes. The present study therefore calls for the further study of quercetin as a new antiangiogenic factor in the treatment of solid tumors. In addition, other possible mechanisms of quercetin on tumor angiogenesis might be identified.

Financial support and sponsorship

Nil

Conflict of interest

There are no conflicts of interest

Received: 05 Mar 20 **Accepted:** 03 Jul 20
Published: 18 Jun 21

References

1. Rajabi M, Mousa SA. The role of angiogenesis in cancer treatment. Biomedicines 2017;5:34.
2. Bielenberg DR, Zetter BR. The contribution of angiogenesis to the process of metastasis. Cancer J 2015;21:267.
3. McMahon G. VEGF receptor signaling in tumor angiogenesis. Oncologist 2000;5:3-10.
4. Naumov GN, Folkman J, Straume O. Tumor dormancy due to failure of angiogenesis: Role of the microenvironment. Clin Exp Metastasis 2009;26:51-60.
5. Halvorsen OJ, Haukaas S, Høisaeter PA, Akslen LA. Independent prognostic importance of microvessel density in clinically localized prostate cancer. Anticancer Res 2000;20:3791-9.
6. Bhat TA, Singh RP. Tumor angiogenesis–a potential target in cancer chemoprevention. Food Chem Toxicol 2008;46:1334-45.
7. Bischoff SC. Quercetin: Potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care 2008;11:733-40.
8. Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. Cancer Lett 2008;269:315-25.
9. Rajasekar J, Perumal MK, Vallikannan B. A critical review on anti-angiogenic property of phytochemicals. J Nutr Biochem 2019;71:1-15.
10. Ahmed Z, Bicknell R. Angiogenic signalling pathways. Methods Mol Biol 2009;467:3-24.
11. Yu B, Wang S. Angio-LncRs: LncRNAs that regulate angiogenesis and vascular disease. Theranostics 2018;8:3654-75.
12. Yang S, Sun Z, Zhou Q, Wang W, Wang G, Song J, *et al*. MicroRNAs, long noncoding RNAs, and circular RNAs: Potential tumor biomarkers and targets for colorectal cancer. Cancer Management Res 2018;10:2249-57.
13. Voellenkle C, Garcia-Manteiga JM, Pedrotti S, Perfetti A, De Toma I, Da Silva D, *et al*. Implication of Long noncoding RNAs in the endothelial cell response to hypoxia revealed by RNA-sequencing. Sci Rep 2016;6:24141.
14. Yan B, Tao Z-F, Li X-M, Zhang H, Yao J, Jiang Q. Aberrant expression of long noncoding RNAs in early diabetic retinopathy. Invest Ophthalmol Vis Sci 2014;55:941-51.
15. Singh KK, Matkar PN, Quan A, Mantella LE, Teoh H, Al-Omran M, *et al*. Investigation of TGFβ1-induced long noncoding RNAs in endothelial cells. Int J Vasc Med 2016;2016:2459687.
16. Zhang X, Tang X, Hamblin MH, Yin KJ. Long non-coding RNA

Malat1 regulates angiogenesis in hindlimb ischemia. Int J Mol Sci 2018;19:1723.
17. Bai Y, Wang W, Zhang Y, Zhang F, Zhang H. lncRNA MIAT suppression alleviates corneal angiogenesis through regulating miR-1246/ACE. Cell Cycle 2019;18:661-9.
18. Mokhtari MJ, Motamed N, Shokrgozar MA. Evaluation of silibinin on the viability, migration and adhesion of the human prostate adenocarcinoma (PC-3) cell line. Cell Biol Int 2008;32:888-92.
19. Kittl M, Beyreis M, Tumurkhuu M, Fuerst J, Helm K, Pitschmann A, *et al*. Quercetin stimulates insulin secretion and reduces the viability of rat INS-1 beta-cells. Cell Physiol Biochem 2016;39:278-93.
20. Pereira DF, Cazarolli LH, Lavado C, Mengatto V, Figueiredo MSRB, Guedes A, *et al*. Effects of flavonoids on α-glucosidase activity: Potential targets for glucose homeostasis. Nutrition 2011;27:1161-7.
21. Rahimmi A, Peluso I, Rajabi A, Hassanzadeh K. miR-185 and SEPT5 genes may contribute to Parkinson's disease pathophysiology. Oxid Med Cell Longev 2019;2019:5019815.
22. Asgari R, Bakhtiari M, Rezazadeh D, Vaisi-Raygani A, Mansouri K. Autophagy related genes expression status in patients diagnosed with azoospermia: A cross-sectional study. J Gene Med 2020:22:e3161.
23. Mokhtari MJ, Koohpeima F, Mohammadi H. A comparison inhibitory effects of cisplatin and MNPs-PEG-cisplatin on the adhesion capacity of bone metastatic breast cancer. Chem Biol Drug Des 2017;90:618-28.
24. Mokhtari MJ, Akbarzadeh A, Hashemi M, Javadi G, Mahdian R, Ghasemi S, *et al*. Cisplatin induces up-regulation of KAI1, a metastasis suppressor gene, in MCF-7 breast cancer cell line. Trop J Pharm Res 2012;11:523-9.
25. García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA. Flavonoids as anti-inflammatory agents: Implications in cancer and cardiovascular disease. Inflamm Res 2009;58:537-52.
26. Yao H, Xu W, Shi X, Zhang Z. Dietary flavonoids as cancer prevention agents. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2011;29:1-31.
27. Pan F, Zhu L, Lv H, Pei C. Quercetin promotes the apoptosis of fibroblast-like synoviocytes in rheumatoid arthritis by upregulating lncRNA MALAT1. Int J Mol Med 2016;38:1507-14.
28. Hashemzaei M, Delarami Far A, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, *et al*. Anticancer and apoptosis-inducing effects of quercetin *in vitro* and *in vivo*. Oncology Reports 2017;38:819-28.
29. Naumov GN, Akslen LA, Folkman J. Role of angiogenesis in human tumor dormancy: Animal models of the angiogenic switch. Cell Cycle 2006;5:1779-87.
30. Bhat TA, Nambiar D, Tailor D, Pal A, Agarwal R, Singh RP. Acacetin inhibits *in vitro* and *in vivo* angiogenesis and downregulates Stat signaling and VEGF expression. Cancer Prev Res 2013;6:1128-39.
31. Gu N, Wang J, Di Z, Liu Z, Jia X, Yan Ye, *et al*. The effects of intelectin-1 on antioxidant and angiogenesis in HUVECs exposed to oxygen glucose deprivation. Front Neurol 2019;10:383.
32. Shi B, Andrukhov O, Berner S, Schedle A, Rausch-Fan X. The angiogenic behaviors of human umbilical vein endothelial cells (HUVEC) in co-culture with osteoblast-like cells (MG-63) on different titanium surfaces. Dent Mater 2014;30:839-47.
33. Gutschner T, Hämmerle M, Diederichs S. MALAT1—a paradigm for long noncoding RNA function in cancer. J Mol Med 2013;91:791-801.
34. Liu J, Yao J, Li X, Song Y, Wang X, Li Y, *et al*. Pathogenic role of lncRNA-MALAT1 in endothelial cell dysfunction in diabetes mellitus. Cell Death Dis 2014;5:e1506.
35. Tripathi V, Shen Z, Chakraborty A, Giri S, Freier SM, Wu X, *et al*. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. PLoS Genet 2013;9:e1003368.
36. Kumar M, Goyal R. LncRNA as a therapeutic target for angiogenesis. Curr Top Med Chem 2017;17:1750-7.
37. Liu W, Wang Z, Wang C, Ai Z. Long non-coding RNA MIAT promotes papillary thyroid cancer progression through upregulating LASP1. Cancer Cell Int 2019;19:194.
38. Ishii N, Ozaki K, Sato H, Mizuno H, Saito S, Takahashi A, *et al*. Identification of a novel non-coding RNA, MIAT, that confers risk of myocardial infarction. J Hum Genet 2006;51:1087-99.
39. Matsubara K, Ishihara K, Mizushina Y, Mori M, Nakajima N. Anti-angiogenic activity of quercetin and its derivatives. Lett Drug Des Discov 2004;1:329-33.
40. Pratheeshkumar P, Budhraja A, Son Y-O, Wang X, Zhang Z, Ding S, *et al*. Quercetin inhibits angiogenesis mediated human prostate tumor growth by targeting VEGFR-2 regulated AKT/mTOR/P70S6K signaling pathways. PloS One 2012;7:e47516.
41. Zhao X, Wang Q, Yang S, Chen C, Li X, Liu J, *et al*. Quercetin inhibits angiogenesis by targeting calcineurin in the xenograft model of human breast cancer. Eur J Pharmacol 2016;781:60-8.
42. Maurya AK, Vinayak M. Quercetin attenuates cell survival, inflammation, and angiogenesis via modulation of AKT signaling in murine T-cell lymphoma. Nutr Cancer 2017;69:470-80.
43. Liu Y, Tang Z-G, Yang J-Q, Zhou Y, Meng L-H, Wang H, *et al*. Low concentration of quercetin antagonizes the invasion and angiogenesis of human glioblastoma U251 cells. Onco Targets Ther 2017;10:4023-8.