Original Article

The effects of Quercetin on miRNA-21 expression in MCF-7 cells

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Abstract

Background: Cancer prevention, by the use of natural dietary or secondary metabolites in plants is one of the strategies has been attracting some of the scientific interests. One of these natural agents is Quercetin, which has anti-metastatic and anti-cancer effects. MiR-21 among different miRNAs is one of the most important frequently up-regulated miRs in numerous cancers including breast and increase cell proliferation and decrease apoptosis and therefore leads to increases in cancer incidence. We assessed the effects of Quercetin on cell viability, MiR-21, Maspin and PTEN gene expression in the MCF-7 cell line. **Materials and Methods:** The human MCF-7 breast cancer cell line was cultured in RPMI1640 and treated with different concentrations of Quercetin (0.01-100 μM) for 24 hours. The cytotoxic effect of Quercetin on MCF-7 viability was determined using Methyl-Thiazolyl-Tetrazolium (MTT) assay by IC50 determination. The relative expression of MiR-21, Maspin, and PTEN gene expression were determined by real-time Polymerase Chain Reaction (PCR). **Results:** The maximum inhibitory effect of Quercetin on cell viability was observed at 100 μM after 24-hour incubation. The expression of MiR-21 in the treated cells compared to controls was significantly decreased after treatment with three different concentrations of Quercetin. In addition, expression of Maspin and PTEN in the treated cells compared to controls was significantly increased. **Conclusion:** Quercetin decreases cell viability and miR-21 gene expression in a dose-dependent manner. Also, Quercetin decreases mir-21 gene expression and increases Maspin and PTEN expression in MCF-7 breast cancer cell line. The growth inhibitory properties and therapeutic effect of Quercetin on the breast cancer may be mediated by reduction of miR-21 expression, and for verifying this hypothesis and the possible therapeutic implication of Quercetin in this direction further studies are necessary.

Keywords: Breast cancer, MiR-21, Maspin, PTEN, MCF-7 cell line, Quercetin

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Introduction

More than several hundred polyphenols have been characterized as secondary metabolites in plants. Based on the function of their structure, Polyphenols are classified into different groups including phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids group are divided into six different types including flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols[1]

Quercetin is a polyphenol belonging to the class of flavonoids molecule found in significant quantities ubiquitous in nature and in our diet with potential beneficial effects on health, including anti-cancer, anti-thrombotic, anti-inflammatory, and anti-neoplastic properties [2, 3]. Animal studies and in-vitro studies have been reported Quercetin inhibits tumor growth through Anti-cancer activities including

cell cycle regulation, interaction with type II estrogen binding sites, and tyrosine kinase inhibition[4]. Inducing cell death by Quercetin at high concentration has been reported in different human cells [5]. Nevertheless, the mechanism by which Quercetin leads to apoptosis is not well understood.

MicroRNAs (miRNAs) are small noncoding RNAs molecules with approximately 19–23 nucleotides in length, which modulate gene expression by suppressing translation and/or degradation by binding to 3́-UTR of their target mRNAs[6, 7]. miRNAs are transcribed from longer precursors (pri-miRNAs) with distinctive hairpin structures. Pri-miRNAs by the Drosha complex is cleaved in the nucleus into miRNA precursors (pre-miRNAs). These pre-miRNAs cleaved by Dicer ribonuclease in the cytoplasm and generate mature miRNAs. These mature miRNAs with Argonaute proteins are included in the RISC complex that binds to the 3-untranslated region of target mRNA [8-10]. miRNAs have a key role in regulating crucial cell processes such as proliferation, differentiation, and apoptosis. Thus, RNAs are involved in normal human development as well as in the initiation of various disease such as diabetes and cancers, where miRNAs have been found to be significant prognostic and predictive markers [11-14].

miR-21 is one of the pioneers identified 'oncomiRs' which abundantly expressed in mammalian cells and targets numerous tumor suppressor genes associated with proliferation, apoptosis, and invasion[15-17]. In breast cancer cell lines as well as in tissue of breast cancer samples, studies have been reported the expression of miR-21 was significantly increased and correlated with a key role in all phases of breast cancer pathogenesis and clinical stage, lymph node metastasis and poor prognosis[18, 19].

Polyphenols such as Quercetin and resveratrol have beneficial properties in almost all chronic diseases and recently have been shown to modulate the expression of miRNAs. In this regard, has been reported, Quercetin which is a flavonoid modulate miRNAs expression. Specifically, Quercetin upregulates miR-155 levels in macrophages activated by LPS[20]. In this study, we evaluated the mechanism of influence of Quercetin on apoptosis in MCF-7 breast cancer cell line. And also roles of miR-21 in the anticancer mechanism of flavonoid.

Methods

Material. Quercetin and second antibody were purchased from Sigma Aldrich. Primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). RPMI 1640, fetal bovine serum (FBS), Penicillin-Streptomycin (Pen-Strep) and trypsin EDTA were purchased from Gibco. All primers were produced by Pishgam company (Iran).

Cell culturing and cytotoxicity. MCF-7 cells were obtained from the Pasteur Institute of Iran and cultured in RPMI 1640 medium supplemented with 10% FBS, glutamine, HEPES, streptomycin/penicillin (100µg/ml, 100 units/ ml), in a 5% CO2 incubator at 37°C for 24 hours. It should be noted that for performing Real-time, PCR ٥×10٥ cells for in a six-well plate were cultured .The cytotoxic effect of Quercetin on the MCF-7 was examined by the MTT assay after 24 hours of treatment. Briefly, 15×103 cells/well were cultured in a 96-well plate then cells were treated in fourplicates with different concentrations of Quercetin (0.01 to 100 µM) for 24hours. Next, the medium was removed, and 20 µL of MTT (1 mg/mL dissolved in PBS) and 180 µL of RPMI1640 was added to each well and after 4.5 hours the absorbance of each well was read at 490 nm using a microplate reader (BioTek® ELx800, USA):

Real-time PCR. Total RNAs and miRNA were prepared from treated cells using GeneAll RibospinTM total RNA purification kit and GeneAll Hybrid-R RNA purification kit (GeneAll Biotechnology). A total of 1 µg RNA isolated from the treated cells was converted to cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Gene expression was evaluated by using quantitative real-time polymerase chain reaction (qRT-PCR) assay. qRT-PCR was performed in triplicates for two independent experiment sets with SYBR Green RealQ Plus 2x Master Mix Green (Ampliqon)on Corbett Rotor-Gene 6000 Light Cycler (Qiagen, Hilden, Germany) and its conditions were 15 min at 95 C, 15s at 95 C, and the 60s at 60 C. The specific primers were designed for human ACTB, PTEN, and Maspin . The following primers were used in qRT-PCR: hACTB F: CGCAAAGACCTGTACGCCAAC,

hACTB GAGCCGCCGATCCACACG, hPTEN F: TCCCAGACATGACAGCCATC, hPTEN R: TGCTTTGAATCCAAAAACCTTACT, h Maspin F: CTTGCCTGTTCCTTTTCCAC, h Maspin R: TGGAGAGAAGAGGACATTGC and miR-21, forward 5'- TAGCTTATCAGACTGATGTTGA-3.

Statistical analysis. Statistical analyses were performed using SPSS22.0. (SPSS, Chicago, IL, USA). Comparison of the experimental groups was performed by one -way ANOVA or independent sample t-test analysis. Values of $p<0.05$ were considered statistically significant. Results are expressed as the mean±SEM of at least two independent experiments.

Results

Cytotoxicity Effect of Quercetin on MCF-7 cell Viability. The cytotoxicity effect of Quercetin on cell viability of the MCF-7 cell line was investigated through the MTT assay at different concentrations at 24 hours. The growth of the MCF-7 cell line was inhibited by Quercetin in a dose-dependent manner. As shown in Figure 1, compared to the controls, treatment of the MCF-7 cell line with Quercetin at 1, 5, 25, 50, and 100 µM for 24 hours reduced the cell viability of MCF-7 cells by 110.1%, 13.8%, 19.9%, 34.6 and 65.6%, respectively. Data analysis showed that IC50 of quercetin on MCF-7 cell line was 7.06 µM during the 24-hour time by the MTT assay.

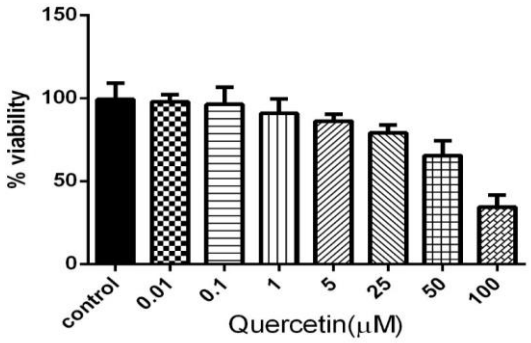

Figure 1. Effect of Quercetin on cell viability of MCF-7 Cell Line at 24 hours. MCF-7 cells were treated with Quercetin (at different concentrations of) for 24 hours and cell viability was assessed by the MTT assay. Results are presented as a percentage of cell viability as mean ± SEM, from three independent experiments.

Effect of Quercetin on miR-21 Gene Expression. The miR-21 gene expression in cancer cell line after exposure to Quercetin at 1, 5 and 10 µM after 24 hours of incubation was assessed by the real-time PCR method. The results of the real-time PCR showed a significant decrease in miR-21 gene expression in the treated cells compared to the controls (Figure 2). A significant decrease was detected for miR-21expression in the presence of 5 and 10 µM of Quercetin, at 24 hours ($P < 0.01$) and ($p <0.001$) respectively. While no significant difference was detected for miR-21 gene expression in the presence 1 µM of Quercetin (Figure 2)

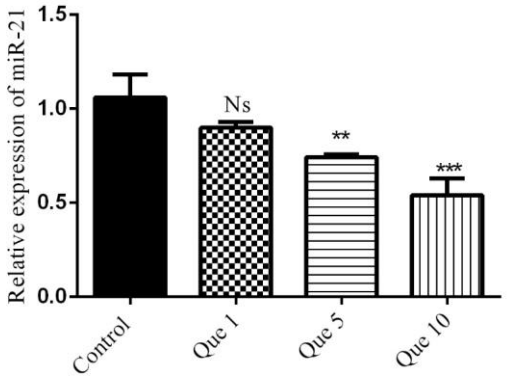

Figure 2. Effect of Quercetin on miR21 Expression in MCF-7 cell line. Quercetin in high concentration resulted in a significant decrease of miR21 expression. Values are the mean ±S.E. of at least three separate experiments. Ns= not significant, ** =P values of < 0.01 and *** =P values of < 0.001are considered significant.

Effect of quercetin on Maspin and PTEN Genes Expression. The expression of Maspin and PTEN mRNAs was determined using the real-time PCR method. Maspin and PTEN are known targets of mir-21 which are important in apoptosis pathway. The results of the real-time PCR showed that expression of Maspin and PTEN in MCF-7 cell line was increased by incubation at 5 to 10 µM concentration of Quercetin (Figure 3). five and 10 µM of Quercetin lead to significant increases in Maspin expression after 24 hours ($P <0.001$) and ($p <0.0001$) respectively. While 1 µM of Quercetin cannot lead to a significant difference in Maspin expression after 24 hours (Figure 3). Otherwise a significant increase was detected for PTEN expression in the presence 3 different Quercetin concentration at 24 hours ($P < 0.01$), ($p <0.0001$) and ($p <0.0001$) respectively.

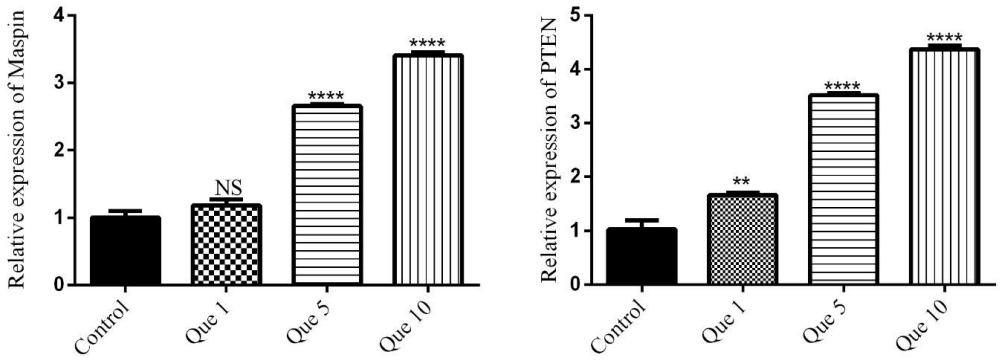

Figure 3. Effect of Quercetin on Maspin and PTEN expression in MCF-7 cell line. Quercetin leads to a significant increase of Maspin and PTEN expression. Values are the mean ±S.E. of at least three separate experiments. Ns= not significant, *** =P values of < 0.001 and **** =P values of < 0.0001.

Discussion

Cancer prevention, by the use of natural dietary or secondary metabolites in plants that can reverse, suppress or prevent carcinogenic progression, has become an appealing strategy to fight with increasing cases of cancers worldwide[21]. Studies have been shown this fact that consumption of a diet rich in foods and vegetables for long-term of time reduces the risk of different diseases, especially cancers [22]. Flavonoids are secondary metabolites of many groups of the plant including fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine[23]. Flavonoids are plant-based drugs represent high chemo-preventive properties and low toxicity for normal tissue . They have key role in a variety of functions involved in enzyme inhibitors and ligands of receptors in signal transduction. Studies have been shown; Flavonoids can also induce apoptosis and cell cycle arrest and regulate other signaling pathways involved in the development and progression of cancer in the breast and other cancers [24, 25]. One of these natural agents is Quercetin, which has anti-metastatic and anti-cancer effects, in several types of cancers[26].

In the other hand, MiR-21 among different miRNAs is one of the most important frequently up-regulated miRs in numerous cancers including breast, gastric, colon, lung, pancreatic and ovarian cancers[27, 28]. MiR-21 increase cell proliferation and decrease apoptosis and therefore leads to increases in cancer incidence [28]. In addition, in vitro, and in vivo studies reported MiR-21 stimulates multiple survival signaling pathways to mediate its roles in cancers. Therefore, targeting miR-21 has therapeutic implications[29].

Our data indicate that the Quercetin at a concentration more than 1µM inhibits the growth of human breast cancer cells. This finding is in agreement with previous studies showing that Quercetin has an anti-proliferative action against human cancer cells in vitro[30]

The Quercetin concentrations used in the current study for the assessment of cell viability were 0.01, 0.1, 1, 5, 25, 50 and 100 µM, respectively, which were selected based on a previous study[26]. No effect on cell viability was observed at low concentrations (<1 µM). However, at high concentrations (100 µg/mL), cell viability significantly decreased to approximately 34% compared to the controls. These results showed a potent reduction in MCF-7 cell viability in a dose-dependent manner, which is in agreement with the results of a previous study[26]. In our study, the IC50 value of Quercetin for this cell line during a 24-hour time interval was determined as 7.06 µM.

This flavonoid may regulate cell growth through regulating of miRNAs. This hypothesis is supported by the following experiments. Real-time PCR was used to evaluate the miRNA-21 expression in MCF-7 cells treated with Quercetin. Quercetin

dose-dependently leads to down-regulation of miRNA-21 gene expression. While no significant difference was detected for a miRNA-21 level between controlled cells and treated and at the 1µM concentration of Quercetin, however, a significant decrease was seen for at 5 and 10 µM. This result showed that exposure to Quercetin at 5 and 10 µM resulted in a reduction of miRNA-21 expression.

While Yi Yang et al. identified PTEN and Maspin as candidate miR-21 targets using a genetic screen/selection system and observed that inhibition of miR-21 significantly
upregulated protein expression of PTEN and Maspin in the metastatic breast cancer cell
line MDA-MB-231[31]. We then investigated the effect of Quercetin on PTEN and Maspin gene expression. Since we didn't observe significant changes for miRNA-21 at 1µM concentration of Quercetin so according to the results no significant difference was observed for Maspin between controlled cells and treated at the 1µM concentration of Quercetin, however, a significant increase was seen for at 5 and 10 µM. Also, our finding showed that exposure to Quercetin at 1, 5 and 10 µM resulted in an increasing of PTEN expression.

According to the results, Quercetin -induced reduction of expression of miR-21 in MCF-7 breast cancer cell line seemed to result in increasing of PTEN and Maspin expression. This result is important because it has been seen in the most of breast carcinomas miR-21 are highly expressed than normal tissues [32-34]. In addition, the anti-proliferation-stimulatory effect of Quercetin is mediated through the activation of miR-21.

Conclusion

The results of our study indicated that treatment of MCF-7 human breast cancer cell line with Quercetin decreases cell viability and miR-21 gene expression in a dose-dependent manner. To the best of our knowledge, this is the first report that shows the effect of Quercetin on mir-21, Maspin and PTEN expression in MCF-7 breast cancer cell line. The growth inhibitory properties AND therapeutic effect of Quercetin on the breast cancer may be mediated by reduction of miR-21 expression, and further studies in this direction are necessary for identifying therapeutic implications of Quercetin.

Conflict of Interest

There is no conflict of interest

Acknowledgment

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References

1. Crozier, A., I.B. Jaganath, and M.N. Clifford, Dietary phenolics: chemistry, bioavailability and effects on health. Natural product reports, 2009. 26(8): p. 1001-1043.
2. D'Andrea, G., Quercetin: A flavonol with multifaceted therapeutic applications? Fitoterapia, 2015. 106: p. 256-71.
3. Scambia, G., et al., Inhibitory effect of quercetin on OVCA 433 cells and presence of type II oestrogen binding sites in primary ovarian tumours and cultured cells. British Journal of Cancer, 1990. 62(6): p. 942-946.
4. Lamson, D.W. and M.S. Brignall, Antioxidants and cancer, part 3: quercetin. Alternative medicine review: a journal of clinical therapeutic, 2000. 5(3): p. 196-208.
5. Matsuo, M., et al., Cytotoxicity of flavonoids toward cultured normal human cells. Biol Pharm Bull, 2005. 28(2): p. 253-9.
6. Lim, L.P., et al., Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature, 2005. 433(7027): p. 769.
7. Esquela-Kerscher, A. and F.J. Slack, Oncomirs—microRNAs with a role in cancer. Nature Reviews Cancer, 2006. 6(4): p. 259.
8. Bladé, C., et al., miRNAs, polyphenols, and chronic disease. Molecular nutrition & food research, 2013. 57(1): p. 58-70.
9. Brodersen, P. and O. Voinnet, Revisiting the principles of microRNA target recognition and mode of action. Nature reviews Molecular cell biology, 2009. 10(2): p. 141.
10. Carthew, R.W. and E.J. Sontheimer, Origins and mechanisms of miRNAs and siRNAs. Cell, 2009. 136(4): p. 642-655.
11. Bartel, D.P., MicroRNAs: target recognition and regulatory functions. cell, 2009. 136(2): p. 215-233.
12. Ghorbani, S., et al., Decreased serum microRNA-21 level is associated with obesity in healthy and type 2 diabetic subjects. Arch Physiol Biochem, 2017: p. 1-6.
13. Mahdavi, R., et al., Decreased Serum Level of miR-155 is Associated with Obesity and its Related Metabolic Traits. Clin Lab, 2018. 64(1): p. 77-84.
14. Alipoor, B., et al., Association of miR-146a rs2910164 and miR-149 rs2292832 Variants with Susceptibility to Type 2 Diabetes. Clin Lab, 2016. 62(8): p. 1553-1561.
15. Feng, Y.-H. and C.-J. Tsao, Emerging role of microRNA-21 in cancer. Biomedical Reports, 2016. 5(4): p. 395-402.
16. Fulci, V., et al., Quantitative technologies establish a novel microRNA profile of chronic lymphocytic leukemia. Blood, 2007. 109(11): p. 4944-51.

17. Feng, Y.H., et al., Deregulated expression of sprouty2 and microRNA-21 in human colon cancer: Correlation with the clinical stage of the disease. Cancer Biol Ther, 2011. 11(1): p. 111-21.
18. Yan, L.X., et al., MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. Rna, 2008. 14(11): p. 2348-60.
19. Zhang, Z.J. and S.L. Ma, miRNAs in breast cancer tumorigenesis (Review). Oncol Rep, 2012. 27(4): p. 903-10.
20. Boesch-Saadatmandi, C., et al., Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. The Journal of nutritional biochemistry, 2011. 22(3): p. 293-299.
21. Batra, P. and A.K. Sharma, Anti-cancer potential of flavonoids: recent trends and future perspectives. 3 Biotech, 2013. 3(6): p. 439-459.
22. Wallstrom, P., et al., Fruit and vegetable consumption in relation to risk factors for cancer: a report from the Malmo Diet and Cancer Study. Public Health Nutr, 2000. 3(3): p. 263-71.
23. Nijveldt, R.J., et al., Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr, 2001. 74(4): p. 418-25.
24. Casagrande, F. and J.-M. Darbon, Effects of structurally related flavonoids on cell cycle progression of human melanoma cells: regulation of cyclin-dependent kinases CDK2 and CDK11. Biochemical pharmacology, 2001. 61(10): p. 1205-1215.
25. Martinez-Perez, C., et al., Novel flavonoids as anti-cancer agents: mechanisms of action and promise for their potential application in breast cancer. Biochem Soc Trans, 2014. 42(4): p. 1017-23.
26. Hashemzaei, M., et al., Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. Oncology Reports, 2017. 38(2): p. 819-828.
27. Si, M., et al., miR-21-mediated tumor growth. Oncogene, 2007. 26(19): p. 2799.
28. Krichevsky, A.M. and G. Gabriely, miR-21: a small multi-faceted RNA. Journal of cellular and molecular medicine, 2009. 13(1): p. 39-53.
29. Chen, J., T. Xu, and C. Chen, The critical roles of miR-21 in anti-cancer effects of curcumin. Annals of Translational Medicine, 2015. 3(21): p. 330.
30. Singh, B., et al., Dietary quercetin exacerbates the development of estrogen-induced breast tumors in female ACI rats. Toxicology and applied pharmacology, 2010. 247(2): p. 83-90.
31. Yang, Y., et al., Identification of miR-21 targets in breast cancer cells using a quantitative proteomic approach. Proteomics, 2009. 9(5): p. 1374-1384.
32. Frankel, L.B., et al., Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. Journal of Biological Chemistry, 2008. 283(2): p. 1026-1033.
33. Yan, L.-X., et al., MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. Rna, 2008. 14(11): p. 2348-2360.
34. Qian, B., et al., High miR-21 expression in breast cancer associated with poor disease-free survival in early stage disease and high TGF-β1. Breast cancer research and treatment, 2009. 117(1): p. 131-140.