The effect of quercetin on microRNA expression: A critical review

Zdeněk Dostal\textsuperscript{a,b}, Martin Modriansky\textsuperscript{a,b}

Quercetin, a flavonoid with multiple proven health benefits to both man and animals, displays a plethora of biological activities, collectively referred to as pleiotropic. The most studied of these are antioxidant and anti-inflammatory but modulation of signalling pathways is important as well. One of the lesser-known and recently discovered roles of quercetin, is modulation of microRNA (miRNA) expression. miRNAs are important posttranscriptional modulators that play a critical role in health and disease and many of these non-coding oligonucleotides are recognized as oncogenic or tumor suppressor miRNAs. This review is an evaluation of the recent relevant literature on the subject, with focus on the ability of quercetin to modulate miRNA expression. It includes a summary of recent knowledge on miRNAs deregulated by quercetin, an overview of quercetin pharmacokinetics and miRNA biogenesis, for the interested reader.

**Key words:** polyphenols, microRNA, biogenesis, expression

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\textsuperscript{a}Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

\textsuperscript{b}Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

Corresponding author: Martin Modriansky, e-mail: martin.modriansky@upol.cz

**INTRODUCTION**

Quercetin, a biologically active compound, is a member of an extensive group of natural compounds called polyphenols. These are ubiquitous in the human diet and an average person can consume more than 1 g per day\textsuperscript{1,2}. The primary role of polyphenols in plants is defense against environmental stress such as UV-irradiation and predators. They also affect growth (development regulators), mediate pigmentation and attract pollinators\textsuperscript{3,4}. Polyphenols are classified as flavonoids, lignans, stilbenes, phenolic acids, coumarins, hydroxycinnamic acids and others\textsuperscript{3}. They display a number of biological activities such as radical scavenging, antioxidant and anti-inflammatory properties as mentioned\textsuperscript{5}. They can also modulate cell signalling cascades\textsuperscript{6,7}. Some cellular signals are transmitted and amplified via kinases, some of which can be inhibited by quercetin and other polyphenols\textsuperscript{7-9}.

Quercetin, whose chemical structure is shown in Fig. 1, is one of the most abundant flavonoids, belonging to the flavonol subgroup\textsuperscript{10}. Its protective effects are mediated by multifaceted, pleiotropic action.

![Chemical structure of quercetin.](image-url)

Quercetin is found abundantly in various foods such as fruits (apple and black currant), vegetables (onion and green beans), beverages (tea) and spices\textsuperscript{11,12}. It exists in two forms: an aglycone or a glycosylated form. The sugar moiety, glucose, other monosaccharides or disaccharides e.g. rhamnose or rutinose, is connected to different carbons of the structure via \(\text{O}-\)glycosidic linkage. The C-3 carbon is the most common position but the glycosylation may appear on carbons 4' and 7 (ref.\textsuperscript{13}). 4'-\(\text{O}\)-glucoside is considered the major representative of quercetin glycosides in onion\textsuperscript{14} but C-glycosides have also been described as well\textsuperscript{15}. From a dietary point of view, mostly quercetin glycosides are found in food with a negligible portion of the aglycone\textsuperscript{16,17}, although some authors have suggested that the aglycone form may be present in significant quantities in some red wines\textsuperscript{18}. Chemically, quercetin is a bright yellow crystallic substance with very good solubility either in DMSO (150 g/L, RT) (ref.\textsuperscript{19}), inferior in 50% ethanol (4.02 g/L, 37 °C) (ref.\textsuperscript{20}) and practically insoluble in water (4.7/10.28 mg/L, 37 °C, based on solubility in PBS) (ref.\textsuperscript{20,21}).

**Pharmacokinetics of quercetin**

The bioavailability of quercetin in a single oral dose is fairly low (≤ 1% of unchanged compound) (ref.\textsuperscript{22}). In the high doses used by Gugler et al.\textsuperscript{22} (4 g), quercetin solubility is probably the main limitation as more than 50% of unchanged quercetin was found in the feces. Absorption of quercetin takes place mainly in the intestine\textsuperscript{23} but also in the epithelium of the oral cavity\textsuperscript{17}. Older literature suggested that quercetin glucosides are not absorbed at all and absorption occurs with aglycone alone\textsuperscript{24}. It has since been suggested that hydrolysis of the glucosides is mediated by oral and gut microflora\textsuperscript{17,25}. On the other hand,
intestinal microflora and chemical reactions can cause quercetin degradation. In 2001, Walle et al. published data obtained with radiolabeled quercetin and showed that quercetin is metabolized in the human body with CO$_2$ generation as the end product (mean value 52.1/43.2% of administrated dose; oral/intravenous application) (ref.26). This study has the limitation of using only one radiolabeled carbon that restricts tracking of other products. Similar results were published with data obtained using a rat model where CO$_2$ generation was also observed. The degradation of flavonoids by intestinal microflora was found in both in vitro and in vivo and it is usually linked with ring-fission products.

Other documented possibilities of quercetin glucoside hydrolysis are enteroocyte or liver cytosolic β-glucosidase, whose activities depend on the sugar moiety and its position, and lactase phloridzin hydrolase (LPH) enzymes. LPH could be important in absorption of quercetin due to its localization: it resides on the luminal side of the brush border. The effect of cytosolic broad specificity β-glucosidase in enteroocytes and hepatocytes is questionable because the quercetin glucosides would be transported into the cell before the hydrolytic cleavage occurs in the intestine. On the other hand, some publications describe the uptake of a small amount of flavonol glucosides into the circulation. Hollman et al. showed that quercetin glucosides are better absorbed than the aglycone in ileostomy patients but these authors used only indirect calculation. In fact all samples were subjected to hydrolysis which means that there is lack of information on the aglycone:glucoside ratio in ileostomy fluid or other tested samples (see Walle et al.). Also, quercetin and its glycosides were evaluated for stability during a two/four/3.25 h long incubation period with gastric fluid/duodenal fluid/ileostomy effluent, respectively. The results indicated high stability of tested compounds but it appears that at least free quercetin tested during the study exceeded its expected solubility in aqueous solution. The study also showed only small hydrolysis of rutin and this corresponds to previously published results.

Quercetin is metabolized by different phase I and II enzymes after absorption. Products are predominantly quercetin glucuronides and sulfates that are generated within hepatocytes and enterocytes. Other products of quercetin metabolism include methylated quercetin. While in the intestine, bacterial cleavage of the hydrophilic moiety can produce aglycone, i.e. cause deconjugation, from quercetin metabolites, which could be re-absorbed. The products of quercetin metabolism are excreted into urine and feces as well as being exhaled. Quercetin is capable of inhibiting some cytochromes P450 in vitro, and this may affect the metabolism of quercetin itself or other drugs. Moreover, a decrease in CYP1A1 and CYP1B1 mRNA was observed in non-cancerous colon cells after treatment with flavonol-rich fraction from yaupon holly containing quercetin-3-rutinoside. However, contradictory information is available for isoquercitrin, a quercetin glucoside. Three selected members of the P450 family showed an enhancement of liver P450 activity after isoquercitrin gastric gavage in rats.

Increasing the bioavailability of quercetin is another aim of current research. From results published by Gugler et al., we can conclude that a higher single dose of quercetin does not improve absorption for several reasons, the most important being solubility in water. It is well-known that the solubility of quercetin in water is poor and hence the goal is to improve aqueous solubility (see below). It is also necessary to facilitate transport across membranes. An interesting approach is enclosing quercetin into special micelles or mixing it with various lipids to create quercetin containing liposomes. Another idea is to overcome low bioavailability by using multiple doses of quercetin repeatedly. The idea was tested on a human model and a plasma quercetin concentration of 1.5 µM was achieved in the test subject when 1 g per day was applied for 28 days. This study has a limitation due the pre-analytic sample preparation, which included a hydrolysis step that discarded all information about quercetin metabolites formed by second phase enzymes such as amount of glucuronides, sulfates etc. The conclusion is that the “long” elimination time is connected with bioaccumulation of quercetin.

miRNA

miRNAs are defined as short non-coding single strands of RNA with approximately 18–25 bases. The first mention of these molecules appeared in 1993. Scientists from Harvard College found a small RNA product of the lin-4 gene that is able to control the lin-14 protein level in C. elegans. These molecules provide a modality for posttranscriptional modulation of gene expression in this organism. miRNAs have several valid targets, sometimes dozens of targets, and some miRNAs can even share their targets. As of the writing of this article, 1917 human miRNA precursors have been described in miRBase 22 (http://www.mirbase.org/).

miRNA biogenesis

The first step in canonical miRNA biogenesis is RNA polymerase II catalyzed transcription of their genes from DNA (ref.43). This reaction generates long primary transcripts typically containing over a thousand nucleotides (pri-miRNA). The process continues by cropping the primary transcript to the size of up to hundred nucleotides long products called precursor miRNA (pre-miRNA). The editing is mediated by a microprocessor complex that contains two major components Drosha (belongs to the RNase III family) and Dicer (also called DiGeorge Syndrome Critical Region 8) (ref.44,45). The next step is the shuttling of pre-miRNAs to the cytosol. This occurs in the presence of exportin-5 and RanGTP (ref.46,47). Dicer (RNase III enzyme) accesses the pre-miRNAs and digests it to mature, 18 – 25 nucleotide long double stranded products. Unwinding of miRNA duplexes initiates the N-domain of argonaute 2 during RISC (RNA inducing silencing complex) assembly while attracting other important proteins such as Dicer, TRBP (ref.48). Within the complex formation, a guide strand is incorporated into the complex (strand with less stable pairing at the 5’end), whereas a passenger strand is degraded. The ac-
tive RIS complex both reduces stability and cleaves the target mRNA, which is the case of full complementarity of miRNA against target mRNA. Partial complementarity of miRNA blocks mRNA for ribosomal translation but it does not cleave a target mRNA immediately\(^5\). Moreover, mRNA/RISC complexes are probably stored and also degraded in p-bodies. For more detailed information about p-bodies see a review by Parker et al.\(^4\). The canonical miRNA biogenesis pathway is summarized in Fig. 2.

miRNAs are interesting and important molecules in cell processes because a single miRNA has the ability to modulate gene expression of multiple targets, thereby changing the cell phenotype. There are a number of deregulated miRNAs in different types of diseases. They are therefore being extensively studied especially in relation to cancer and their level may be associated with specific types of tumor. Ouzang et al.\(^5\) for example identified a group of up- and down-regulated miRNAs compared to normal tissue expression. Experiments based on restoration of normal non-pathologic expression of selected miRNA or even exceeding it showed elevated sensitivity of tested cancer cells to conventional treatment\(^4\). Modulation of miRNA expression could be used in the future against a number of diseases.

**miRNAs modulated by quercetin**

**miR-146a**

Tao et al.\(^5\) found that quercetin increased the expression of miR-146a in human breast cancer cells\(^5\). Generally, miR-146a is known as a posttranscriptional modulator of several important genes. For example, its validated targets are BRCA1, BRCA2 (ref.\(^5\)) involved in repairing double strand breaks in DNA or the EGFR receptor\(^6\), a transmembrane tyrosine kinase connected with pro-survival signalling. Overexpressed EGFR is often found in tumors and linked to aggressive behavior in cancer cells. This study found significant upregulation of miR-146a, approximately four- to five-fold of control for MCF-7/MDA-MB-231 cells, caused by the highest quercetin concentration tested during 48 h treatment. The result was a reduction in cell survival to below 40%. The observed effect was linked to negatively affected expression of EGFR, increase in Bax protein level and downstream activation of caspase-3 during 24 h treatment. In addition, the authors used miR-146a/anti-miR-146a transfection for validation of the observed effects. Moreover, a mouse xenograft model was used during the study. These experiments showed decrease in cancer volume and an increase in expression of miR-146a, almost two-fold higher than control, after quercetin treatment (10 mg/kg for 8 weeks). Unfortunately, the authors did not describe the method of application of quercetin\(^5\). The article contains discrepancies between text and images and authors used a non-standard unit for the concentrations of the tested compound (see Table 1). The literature suggests conflicting effects of miR-146a in cancer cell line MCF-7. For example, Gao et al.\(^6\) discusses the impact of the miR-146a-5p overexpression on enhanced proliferation in this cell line\(^6\). Overall, these results suggest another contribution of quercetin that cooperates with miR-146a up-regulation.

An article published by Tao et al.\(^5\) is not the only article showing that miR-146a is modulated by quercetin. Noratto et al.\(^6\) examined fractions from yaupon holly leaf extract. The flavonol-rich fraction turned out to be effective. Quercetin-3-rutinoside and kaempferol-3-rutinoside were determined as major flavonol compounds of this fraction by HPLC/MS (MS\(^2\), MS\(^3\)) and were characterized as gallic acid equivalents (GAE). The experiments were performed with CCD-18Co cells (normal colon cells), in which LPS treatment down-regulated miR-146a.
expression. Combined treatment of LPS with the highest concentration of 40 mg GAE/L resulted in the return of miR-146a expression to the level of control. Further experiments yielded interesting data because the relative expression of miR-146a exceeded the level in control (non-treated) cells after combination of the extract fraction (20 mg GAE/L) with miR-146a inhibitor as well as combination of the flavonol rich extract + LPS + miR-146a inhibitor. The authors suggested that miR-146a contributes to the anti-inflammatory properties of the tested fraction due to the regulation of its targets IRAK-1 and TRAF-6 (ref.37). IRAK-1 and TRAF-6. a part of the TLR pathway, were validated as miR-146a targets elsewhere41.

miR-27a

Another example of combined study is Del Follo Martinez et al.62 who used a quercetin:resveratrol mixture in a 1:1 ratio. The researchers used the HT-29 cell line as a model of colon cancer and tested the mixture for its potential anticanceer properties. A decrease in miR-27a was found. This miRNA is discussed as oncogenic because it is linked to regulation of ZBTB10, a zinc finger protein. The main effect of ZBTB10 is probably mediated via repression of Sp transcription factors. These are a part of the transcription factor family that regulates several housekeeping genes related to initiation of cancer and its progression. miR-27a was downregulated two-fold after the treatment that resulted in upregulation of ZBTB10. The impact was partially demonstrated using miR-27a mimics. On the other hand, modulation of miR-27a by the mixture did not respond in a dose dependent manner whereas ZBTB10 mRNA did. The data suggest another effect of this mixture42.

miR-27a was downregulated in the same way by another combination of polyphenols, namely quercetin and hyperoside (quercetin-3-O-galactoside), also in a 1:1 ratio. 786-O renal cancer cells were used as the model for these experiments. It is remarkable that almost all the figures, results, experimental design and even text bear a strong resemblance to those presented in Del Follo Martinez et al.62 with few exceptions. Many results were surprisingly similar, with IC50 differences smaller than 0.1 µg/mL between the articles43. It is possible to speculate from the similarities that quercetin is responsible for the effect and the other compound plays a support role.

miR-21

The same research group published another paper in 2015 describing the effect of the same combination quercetin:hyperoside (1:1 ratio) in a different cell model – prostate cancer cells PC3 cell line. The results show deregulation of miR-21, a well-known oncogenic miRNA, caused by quercetin/hyperoside combination. The miR-21 was downregulated compared to control cells by as much as 4.3-fold at the highest concentration, and the deregulation was accompanied by upregulation of PDCD4, a tumor suppressor. The influence of miR-21 was validated via pre-miR-21 transfection44. Because hyperoside is a quercetin glycoside, its combination with quercetin aglycon suggests that the synergistic effect may be linked to a simple increase in free quercetin via deglycosylation. The same could actually apply to miR-27 as discussed in the previous paragraph.

Wang and colleagues45 used on a quercetin combination as well, in this case a mix of quercetin and arctigenin evaluated in prostate cancer cell models (LAPC-4 and LNCaP cell lines). They compared miRNA expression of control cells with those treated with the arctigenin/quercetin combination and uncovered several miRNAs that were downregulated. Both cell lines showed a decrease of at least 20% relative to negative control in miR-21, miR-19b and miR-148a expression. However, LAPC cells were more sensitive to the treatment (Table 1). Moreover, the LAPC-4 cell line treated by arctigenin showed almost the same expression of miR-19b and miR-21 even displayed a decreasing trend compared to arctigenin/quercetin samples. However, quercetin monotherapy was usually weaker and, surprisingly, quercetin displayed an opposite behavior for miR-21 in both cell lines45. MiR-19b and miR-21 are usually designated as oncogenic46 and their downregulation is recognized as a positive effect. The last positive information in the paper is that no type of treatment had any effect on proliferation of normal prostate epithelial cells (PrEC) (ref.45).

Quercetin monotherapy modulates miR-21 as published by an Iranian group of scientists, who tested the effects of quercetin on breast cancer cell line MCF-7. The proliferation of the cell line was strongly affected only at very high concentrations (50 and 100 µM quercetin) after 24 h treatment. However, the paper presents two conflicting IC50 values: the data from Fig. 1 do not correspond with the 7.06 µM value presented in the text. Nevertheless, relative expression of miR-21 was significantly downregulated by quercetin, approximately two-fold at a concentration of 10 µM. The authors performed RT-PCR analysis of gene expression of PTEN and Maspin, targets of miR-21 and showed that both miRNA were upregulated47.

Expression of miR-21 responds to the rate of oxidative stress, e.g. as result of environmental pollutant exposure. Cs2+ ions, inducres of ROS formation, are designated as carcinogenic and are connected with lung cancer. The study evaluated quercetin for its effects on acute Cs2+ response and alleviation of Cs2+ induced malignant transformation. During the study it was discovered that quercetin regulates the transformation through miR-21 and its target protein PDCD4. Three sets of experiments were performed (in vitro and in vivo). The first set was done on BEAS-2B cells, which are lung epithelial cells. miR-21 was upregulated four-fold by Cs2+ ions, compared to untreated control (3.4/6.0/18 for chronic exposure – 2/4/6 months, respectively). The upregulation was reduced by quercetin approximately three-fold compared to Cs2+ treated cells in acute Cs2+ exposure and 2.1/2.5/4.3 fold (roughly) in chronic Cs2+ exposure (2/4/6 months). The chronic Cs2+ treatment with quercetin resulted in modulation of colony number. It is promising that the chronic effect of Cs2+ was reversible by quercetin at a relatively low concentration. The second series of experiments included an athymic nude mouse xenograft model with injected chromium transformed cells. When the tumor reached
a given volume, the quercetin treatment began and took 30 days (10 mg/kg/day, intraperitoneally). miR-21 was downregulated in the tumor cells as well, confirming the positive effect of quercetin in vivo. The third approach consisted of a mouse xenograft model with application of pre-treated BEAS-2B cells. The pre-treatment of the cells was identical to that described in the chronic experiment. Tumor analysis was done 30 days later. Quercetin pre-treated BEAS-2B produced smaller tumors with lower expression of miR-21 (ref.68).

Finally, a Chinese research group form Daqingshi No. 4 Hospital published an article describing attenuation of fibrosis induced by transforming growth factor-β (TGF-β) in HK-2 cell line (renal tubular epithelial cells) as a result of quercetin treatment (15 mg/mL) in 2018. The effect is associated with suppression of miR-21-5p overexpression caused by TGF-β treatment. The effects of quercetin are partially reversed by miR-21 mimics. On the other hand, the doses of quercetin used were extremely high and unachievable in aqueous medium. It seems that the concentrations reported correspond to stock solutions, not the final concentrations in the medium. In addition, important information is missing in the paper, e.g. duration of incubation during selected experiments69.

miR-155

Quercetin causes changes in levels of miR-155 (ref.70) that are induced by the inflammation signalling pathway but react in the opposite way compared to the effect on miR-146a. The study assessed modulation of the miRNA by quercetin and its two important metabolites in murine RAW264.7 macrophages. Normally, miR-155 is increased 12-fold by LPS treatment, relative to negative control. However, the presence of quercetin or its metabolite isorhamnetin reduced the effect of LPS by approximately 1.8/1.5 fold. The assumed mechanism of miR-155 regulation by quercetin is via direct and indirect modulation through NFκB. Quercetin-3-glucuronide, that was also used in the study, had no impact70.

let-7 family

The influence of quercetin on let-7a in pancreatic ductal adenocarcinoma was shown by Appari et al.71. These authors demonstrated that quercetin treatment for 72 h increased the amount of let-7a 2.45/1.6/1.45 fold in MIA-PaCa2/BxPC-3/PacaDD-183 cells compared to control, 3.0/2.3/2.45 fold in MIA-PaCa2/BxPC-3/PacaDD-183 cells in combination treatment with sulforaphane and 3.2/3.1/3.1 fold in MIA-PaCa2/BxPC-3/PacaDD-183 cells with green tea catechins, respectively. let-7a enhancement was accompanied by K-Ras downregulation on both mRNA and protein levels with the exception of quercetin only treated cells, in which no changes in K-Ras protein levels were observed71. The effect of let-7a against Ras protein correlates with the findings of Johnson et al.72. Non-malignant pancreatic ductal cells showed minimal changes71.

Similar data for let-7 family, 7c isoform in particular, in pancreatic ductal adenocarcinoma were published by Nwaeburu et al.73. The miRNA showed approximately 1.8/1.3/1.9 fold higher expression after 50 µM quercetin treatment in AsPC-1/AsanPACA/PANC-1 cells. A result of let-7c modulation in AsPC-1 was positive regulation of Numb protein, inhibitor of Notch, accompanied by a decrease in Notch protein level. The authors also confirmed an additional five miRNAs with response to quercetin via RT-PCR (miR-200a/200b/103/125b/1202) and published a heatmap of 24 miRNAs with the highest deregulation after quercetin treatment73.

miR-200b-3p

A follow-up study by Nwaeburu et al.74 focused on miR-200b-3p that was significantly modulated (upregulated more than 2.5 times) by quercetin (50 µM) in pancreatic ductal adenocarcinoma (AsPC-1). Activity of miR-200b-3p against Notch 3' UTR region was demonstrated. The article describes an unusual combination of effects. miR-200b caused attenuation of luciferase activity of reporter gene containing 3' UTR of Notch1, but there was no effect on Notch1 mRNA expression. This suggests that miR-200b-3p only blocks translation and does not cause cleavage of mRNA. The inhibition of Notch protein is associated with cell fate decision therefore the quercetin treated cells prefer an asymmetric cell division. On the other hand, the results of miR-200b-3p transfection showed activation of Numb transcription75 similar to let-7c transfection in Nwaeburu et al.76 published in 2016. It seems that miR-200b-3p associated activation of Numb is more important than 3' UTR anti-notch activity. If we consider both articles published by Nwaeburu et al.73,74, we can recognize a synergy in the effect of the two miRNAs, miR-200b-3p and let-7c, on quercetin treated PDA cells. Both miR-200b-3p, and let-7c, upregulate Numb protein, the Notch1 inhibitor.

miR-17-3p

Quercetin also affects expression of ferroportin, a membrane exporter of ferrous iron in the intestine, via miR-17-3p. The export is located in enterocytes, more precisely on the basolateral membrane76. The main role of the protein is to transport iron from intracellular to extracellular space. A specific block of enterocyte ferroportin expression should have an influence on intestinal iron absorption77. The research group used Caco-2 TC7 cell line as their cell model for miRNAs experiments. During miRNA array analysis, some miRNAs were identified with an increase in expression of over 1.5 fold after 10 µM quercetin treatment in AsPC-1/AsanPACA/PANC-1 cells. A result of let-7c modulation in AsPC-1 was positive regulation of Numb protein, inhibitor of Notch, accompanied by a decrease in Notch protein level. The authors also confirmed an additional five miRNAs with response to quercetin via RT-PCR (miR-200a/200b/103/125b/1202) and published a heatmap of 24 miRNAs with the highest deregulation after quercetin treatment73.

miR-16

Sonoki and colleagues focused on the impact of quercetin treatment in lung adenocarcinoma A549 cells. The
A549 cells were exposed to 50 µM quercetin for 24 h and observed induction of miR-16 expression, approximately 1.4 fold compared to untreated control. The result was a decrease in Claudin-2 mRNA and protein level, with the effect being partially reversed by miR-16 inhibitor77.

miR-217
Zhang et al.78 tested the effect of quercetin on cisplatin treatment in an osteosarcoma model. They used the 143B cell line and recognized that miR-217 is partially responsible for quercetin-mediated sensitivity of cells to cisplatin. The induced expression of miR-217 caused a decrease of K-Ras protein and mRNA expression as both quercetin and cisplatin upregulate miR-217 expression with a synergic effect if used together. The importance of miR-217 was shown via miR-217 mimics/anti-miR-217 that partially enhance/reverse cisplatin and/or quercetin outcome. The paper also includes the information that K-Ras regulates the PI3K/AKT pathway8. K-Ras is not the only player in PI3K/AKT regulation in the treatment, since quercetin is also a known regulator of the pathway.

miR-142-3p
miR-142-3p is another miRNA modulated by quercetin. MacKenzie et al.79 discovered that quercetin at 100 µM (as well as triptolide at 100 nM) upregulated the miRNA in three different types of pancreatic ductal adenocarcinoma cells: over three-fold in Mia PaCa-2 cells, almost eight-fold in Capan-1 cells and more than three-fold in S2-O13 cells. Most of the experiments however were performed only with triptolide89.

miR-145
Dose dependent induction of miR-145 was observed in ovarian cancer cells (SKOV-3 and A2780) as a result of quercetin treatment (0 – 100 µM/mL). miR-145 was increased approximately 3/3.5 fold for SKOV-3/A2780 at the highest concentration after 24 h treatment. Quercetin (50 µM/mL) was indicated as IC₅₀ for 48 h incubation and this concentration was used in further experiments. The miRNA upregulation is linked to growth inhibition and enhancement of caspase-3 cleavage that can be reversed by miRNA-inhibitor. However, the article does not reveal the molecular mechanism in detail such as which target protein is modulated by upregulated miR-145. We assume that the observed caspase effect is a consequence of miR-145 protein target regulation80. The article provides concentrations in “µM/mL”, however it is not clear what this non-standard unit represents (see also in Tao et al.23).

In vivo experiments (several miRNAs tested)

In vitro experiments aside, some in vivo studies into quercetin’s effect on miRNA were performed. An example is the study by Wien et al.81, in which male Wistar rats were fed a diet containing approximately 10 mg/kg per day quercetin for 7 weeks. PCR array analysis of 352 miRNA showed 23 deregulated hepatic miRNAs. Nineteen of them had more than three-fold lower expression than control group, although the published table in the article contains only 13 miRNAs. The remaining miRNAs (four species) showed increased expression by the same value (see Table 2). The group demonstrated that the most deregulated rno-miR-125b-3p (nine fold) presumably induces γ-glutamyl hydrolase expression (measured at mRNA level by RT-PCR) due to its downregulation82. The authors used a sample pooled from eight animals for each group tested, to evaluate miRNA expression via PCR array. γ-glutamyl hydrolase represents an important enzyme associated with cancer and its high levels are discussed as a poor prognosis marker during invasive breast cancer83. Rats are not the only model used, as there are also at least five articles using mice as an in vivo model (see Table 2).

Quercetin and 8 other polyphenols were investigated in apoE deficient mice, a model of deregulated lipid metabolism. The study used microarrays for miRNA (567 species) and mRNA (35,852 probes) expression patterns (signatures) as a powerful tool of comprehensive analysis. The experiment was designed as follows - two week’s exposure with 0.006% (w/w) of quercetin in the diet. The dose corresponds to 30 mg per day for humans. The results showed 47 miRNAs with different expression for quercetin and control group. 22 miRNAs had reduced expression and 25 miRNAs had induced expression. Moreover, five miRNAs displayed expression similarities across tested polyphenols. Three species exhibited lower expression (mmu-miR-30c-1*, mmu-miR-374* and mmu-miR-467b*) and two remaining miRNAs exhibited higher expression (mmu-miR-291b-5p and mmu-miR-296-5p) compared to control. Moreover, the authors discovered an interesting phenomenon. The ApoE miRNA signature was partially reversed by polyphenols, including quercetin toward the wild type signature87.

Another interesting finding is the effect of quercetin, exercise or their combination on miRNA expression and their interaction with an atherogenic diet. Authors used C57BL/6j LDL−/− mice. miRNAs expression was assessed in aorta and liver tissues. Experiments suggested upregulation of miR-21 in the aorta after exercise and quercetin/exercise group. Aorta miR-125b was also upregulated in the quercetin/exercise group. However, miR-451 showed non-significant changes in the same tissue. In the liver tissue, expression of miR-21 displayed the same expression pattern as for the aorta. Moreover, quercetin slightly potentiated the effect of exercise on liver miR-21 expression, but quercetin monotherapy was not effective. On the other hand, exercise decreased expression of miR-451 in the liver and combination with quercetin reduced the effect of exercise. Quercetin alone caused non-significant downregulation. miR-125b in the liver samples displayed almost no effect of quercetin but the same compound in combination with exercise mildly attenuated (non-significant) the exercise induced upregulation of miR-125b. Quite a few of these results were trends84.

Boesch-Saadatemandi and colleagues published a paper in 2012 describing two other miRNAs regulated by quercetin achieved with in vivo experiment. They used a female mouse model C57BL/6J with chronic subacute inflammation induced by a high fat diet. Liver miR-122 and miR-125b were upregulated dose dependently after six weeks of consumption of the quercetin containing diet.
Table 1. Summary of quercetin mediated miRNAs with modulated targets and treatment characteristics – *in vitro*. The values for miRNA expression were usually estimated from article graphs.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Modulation of miRNA [folds of control]</th>
<th>Model</th>
<th>Quercetin treatment (highest concentration)</th>
<th>Length of treatment (h)</th>
<th>Involved proteins</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>miR-146a</td>
<td>↑ 4.0</td>
<td>MCF-7</td>
<td>100 µM/l</td>
<td>48</td>
<td>Bax ↑, Caspase-3 ↑, EGFR ↓</td>
<td>Tao et al., 2015 (ref.17)</td>
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<tr>
<td></td>
<td>↓ 4.5</td>
<td>MDA-MB-231</td>
<td></td>
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<tr>
<td>miR-146a</td>
<td>↑ restore to control level or exceed it</td>
<td>CCD-10Co</td>
<td>10-20 mg GAE/L (flavonol-rich fraction)</td>
<td>48</td>
<td>Pretreatment 30 min before stimulation with LPS or LPS + anti-miR</td>
<td>Proposal not confirmed directly in the article. For example via anti-miR experiment</td>
</tr>
<tr>
<td>miR-27a</td>
<td>↓ 2.0</td>
<td>HT-29</td>
<td>20 µg/ml</td>
<td>24</td>
<td>ZBTB10 (miRNA level) ↑</td>
<td>Del福洛-Martinez et al., 2013 (ref.25)</td>
</tr>
<tr>
<td>miR-27a</td>
<td>↓ 2.0</td>
<td>786-O</td>
<td>20 µg/ml</td>
<td>24</td>
<td>ZBTB10 (miRNA level) ↑</td>
<td>Li et al., 2014 (ref.25)</td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 4.3 (77 %)</td>
<td>PCI-3</td>
<td>20 µg/ml</td>
<td>24</td>
<td>PDCD4 ↑</td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 3.4</td>
<td>LAPC-4</td>
<td>1 µM - Arctigenin</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 1.7</td>
<td>BEAS-2B</td>
<td>10 µM (+ 5 µM Cr(VI)+)</td>
<td>24 (acute)</td>
<td>PDCD4 ↑</td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 1.2</td>
<td>LNCaP</td>
<td>1 µM - Arctigenin</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 1.4</td>
<td>LAPC-4</td>
<td>1 µM - Quercetin</td>
<td>48</td>
<td>no direct confirmation</td>
<td>Wang et al., 2015 (ref.16)</td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 1.5</td>
<td>LNCaP</td>
<td>10 µM - Quercetin</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 1.7</td>
<td>MCF-7</td>
<td>10 µM</td>
<td>24</td>
<td>PTEN ↑ and Maspin ↑ (not directly confirmed)</td>
<td>Tofigh et al., 2017 (ref.26)</td>
</tr>
<tr>
<td>miR-155</td>
<td>↓ ~ 1.8*</td>
<td>RAW264.7</td>
<td>10 µM</td>
<td>6</td>
<td>propsoal TNF-α (not directly confirmed)</td>
<td>Boesch-Saadatmandi et al., 2011 (ref.72)</td>
</tr>
<tr>
<td>les-7a</td>
<td>↓ 2.45</td>
<td>MIA-PaCa2</td>
<td>200 µM - Quercetin</td>
<td></td>
<td>K-Ras ↓ (miRNA level)</td>
<td>Appari et al., 2014 (ref.19)</td>
</tr>
<tr>
<td></td>
<td>↑ 1.45</td>
<td>Panc01D-185</td>
<td></td>
<td></td>
<td>(protein level showed exceptions after sulforaphane, quercetin treatment)</td>
<td></td>
</tr>
<tr>
<td>miR-200b</td>
<td>↓ 2.6</td>
<td>ASANPaCa1</td>
<td></td>
<td></td>
<td>Ferroptosis ↓ (not directly confirmed)</td>
<td>Nwaeburu et al., 2016 (ref.53)</td>
</tr>
<tr>
<td>miR-200a</td>
<td>↓ 2.1</td>
<td>Panc01C-1</td>
<td></td>
<td></td>
<td>Notch ↓</td>
<td>Nwaeburu et al., 2017 (ref.54)</td>
</tr>
<tr>
<td>miR-125b</td>
<td>↓ 1.4</td>
<td>Panc01C-1</td>
<td></td>
<td></td>
<td>No direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-120c</td>
<td>↓ 3.3</td>
<td>A2780</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-17-3p</td>
<td>↓ over 1.5</td>
<td>Caco-2-TC7</td>
<td>10 µM</td>
<td>18</td>
<td>Ferroptosis ↓ (not directly confirmed)</td>
<td>Lesjak et al., 2014 (ref.27)</td>
</tr>
<tr>
<td>miR-16</td>
<td>↑ 1.4</td>
<td>A549</td>
<td>50 µM</td>
<td>24</td>
<td>Claudin-2 ↑</td>
<td>Sonoki et al., 2015 (ref.18)</td>
</tr>
<tr>
<td>miR-217</td>
<td>↑ 1.45</td>
<td>143B</td>
<td>5 µM - Quercetin</td>
<td>48</td>
<td>K-Ras ↓</td>
<td>Zhang et al., 2015 (ref.28)</td>
</tr>
<tr>
<td>miR-142-3p</td>
<td>↓ 3.1</td>
<td>MIA-PaCa2</td>
<td>100 µM</td>
<td>24</td>
<td>no direct confirmation with quercetin (HSP70 ↓)</td>
<td>MacKenzie et al., 2013 (ref.29)</td>
</tr>
<tr>
<td>miR-345</td>
<td>↓ 3.0</td>
<td>SKOV-3</td>
<td>100 µM/ml</td>
<td>24</td>
<td>Caspase-3 ↑ (growth inhibition)</td>
<td>Zhou et al., 2015 (ref.30)</td>
</tr>
<tr>
<td></td>
<td>↓ 3.0</td>
<td>A2780</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Against positive LPS treatment
† Authors determined amount of polyphenols in the fraction via total reduction capacity and gallic acid was used as standard (20 mg GAE/L = mg gallic acid equivalents/L)

Proposal = not confirmed directly in the article. For example via anti-miR experiment

# = not statistically significant

x = compared to Cr(VI) treated cells

y = compared to TGF-β treated cells

101
2 mg quercetin/g diet caused 1.6 to 1.5-fold upregulation, 0.2 mg quercetin/g diet only resulted in non-significant enhancement.\textsuperscript{35}

**Patient samples**

The last set of findings is based on an epidemiologic study of Lung cancer tissues. The question was whether a quercetin-rich diet can modulate miRNA expression. Data presented in this study showed that there is a group of miRNAs which was significantly upregulated (2 miRNAs/4 miRNAs) or downregulated (2 miRNAs/8 miRNAs) in adenocarcinoma/squamous cell carcinoma between groups consuming high and low quantities of quercetin-rich food (see Table 3). The authors applied an advanced complex sorting based on former/current smoking + histology or sorting according to miRNA families in combined with smoking status and histology.\textsuperscript{36}

**Quercetin glycosides and its derivatives**

Scientists are interested in derivatives of quercetin such as rhamnetin that modulates miR-34a in different cell lines.\textsuperscript{37-39} Finally, we found an article, which reversed the usual logic. The authors used miR-143 as a molecule that increases chemosensitivity to quercetin in gastric can-

### Table 2. Summary of quercetin mediated miRNAs with modulated targets and treatment dose of the compound characteristics \textit{– in vivo}. The values for miRNA expression were usually estimated from article graphs.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Modulation of miRNA [folds of control]</th>
<th>Model</th>
<th>Quercetin treatment [highest concentration]</th>
<th>Length of treatment</th>
<th>Involved processes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-146a</td>
<td>↑ over 1.75</td>
<td>female BALB/c athymic nude mouse (xenograft)</td>
<td>10 mg/kg per day</td>
<td>8 weeks</td>
<td>authors only compare size of tumor and miR-146a expression</td>
<td>Tao et al., 2015 (ref.\textsuperscript{35})</td>
</tr>
<tr>
<td>miR-21</td>
<td>↓ 1.5</td>
<td>female NU/NU Athymic nude mouse</td>
<td>pretreated BEAS-2B cells injected into flank 2 µM (+ 0.5 µM Cr\textsuperscript{VI+})</td>
<td>30 days</td>
<td>PDCD4 ↑</td>
<td>Pratheeshkumar et al., 2017 (ref.\textsuperscript{36})</td>
</tr>
<tr>
<td>miR-125b-3p</td>
<td>↓ 9</td>
<td>male Wistar rat</td>
<td>10 mg/kg per day (100-ppm quercetin)</td>
<td>7 weeks</td>
<td>γ-glutamylhydrokse (not directly confirmed)</td>
<td></td>
</tr>
<tr>
<td>miR-133b</td>
<td>↓ 7</td>
<td>wild-type and apoE knock-out mouse (male, C57BL/6J)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-505</td>
<td>↓ 7</td>
<td>male C57BL/6J mouse (liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-1</td>
<td>↓ 6</td>
<td>male C57BL/6J mouse (liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-298</td>
<td>↓ 4</td>
<td>aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-503</td>
<td>↓ 4</td>
<td>aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-539</td>
<td>↓ 4</td>
<td>aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-33</td>
<td>↓ 4</td>
<td>liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-216a</td>
<td>↓ 4</td>
<td>liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-201a</td>
<td>↓ 3</td>
<td>liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-22</td>
<td>↓ 3</td>
<td>liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-205</td>
<td>↓ 3</td>
<td>liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-125a-3p</td>
<td>↓ 5</td>
<td>male C57BL/6J LDL\textsuperscript{−/−} mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-411</td>
<td>↓ 5</td>
<td>male C57BL/6J LDL\textsuperscript{−/−} mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-484</td>
<td>↓ 7</td>
<td>male C57BL/6J LDL\textsuperscript{−/−} mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 miRNAs</td>
<td>↓ over 1.5</td>
<td>wild-type and apoE knock-out mouse (male, C57BL/6J)</td>
<td>0.006 % (w/w)</td>
<td>2 weeks</td>
<td>several pathway discussed (not directly confirmed)</td>
<td>Milenkovic et al., 2012 (ref.\textsuperscript{35})</td>
</tr>
<tr>
<td>22 miRNAs</td>
<td>↓ over 1.5</td>
<td>wild-type and apoE knock-out mouse (male, C57BL/6J)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↑ 1.2</td>
<td>aorta</td>
<td>100 µg/day</td>
<td></td>
<td>no direct confirmation</td>
<td>Garelahti et al., 2014 (ref.\textsuperscript{35})</td>
</tr>
<tr>
<td>miR-21</td>
<td>↑ 1.12</td>
<td>liver</td>
<td>100 µg/day</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-125b</td>
<td>↑ 1.1</td>
<td>aorta</td>
<td>100 µg/day</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-125b</td>
<td>↑ 1.26</td>
<td>liver</td>
<td>100 µg/day</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↑ 1.2</td>
<td>aorta</td>
<td>100 µg/day</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-451</td>
<td>↑ 1.1</td>
<td>aorta</td>
<td>100 µg/day</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-451</td>
<td>↑ 1.4</td>
<td>liver</td>
<td>100 µg/day</td>
<td></td>
<td>no direct confirmation</td>
<td></td>
</tr>
<tr>
<td>miR-122</td>
<td>↑ 1.6</td>
<td>female C57BL/6J mouse (liver)</td>
<td>0.2 and 2 mg/g diet</td>
<td>6 weeks</td>
<td>acylcoxyacylhydrokse (not directly confirmed)</td>
<td>Boesch-Saadatmandi et al., 2012 (ref.\textsuperscript{35})</td>
</tr>
</tbody>
</table>

ip = intraperitoneal

x = compared to Cr\textsuperscript{VI+} treated cells

CrT = chromium transformed cells
cer cells via autophagy inhibition. The autophagy inhibition was mediated by GABARAPL1, a miR-143 target.

CONCLUSION

We found a total of ninety-five different species of miRNA affected by quercetin in our literature research. Of these, 18 miRNAs were revealed as deregulated in in vitro experiments, 66 through in vivo experiments and finally, 15 were discovered in human tissue samples. Several miRNA molecules were expressed with a common pattern among in vitro and in vivo experiments as a result of quercetin treatment. miR-21 in particular was deregulated in two studies that reported quercetin mediated upregulation of miR-21 in the majority of samples across different cell lines and it seems that these miRNAs cooperate. The data suggest synergy in potential anti-cancer effects against pancreatic ductal cell adenocarcinoma. Further, the patient samples described in Lam et al. study, displayed ambiguous expression of miR-502 that was upregulated in lung adenocarcinoma tissues but lung squamous cell carcinoma tissues showed downregulation of the expression when tissues from highest/lowest quercetin-rich food consumers were compared.

Some miRNA molecules can serve as predictive markers of cancers, especially miR-21 and let-7 family that are modulated by quercetin. MiR-21 is commonly recognized as oncogenic and as an unfavorable prognostic factor. As expected, its expression was evaluated in many studies and quercetin or combination treatment usually downregulate miR-21 expression regardless of the tested model (in vitro/in vivo). The let-7 family, on the other hand, is widely viewed as a tumor suppressor miRNAs.

Different members of the miRNA family are often upregulated by quercetin treatment. Shin et al. discuss miR-27a as a diagnostic marker of gastric cancer and miR-146a as gastric cancer-associated miRNA. Both miRNAs are modulated by quercetin. These results suggest that quercetin could function prophylactically in cancer prevention (e.g. in nutraceutics) with minor exceptions, e.g. Wang et al. - quercetin only treatment enhanced expression of miR-21.

Many of the studies published used high concentrations, usually several tens of µM that are not physiologically achievable per os from a regular diet. The data could be viewed as an interesting option for adjuvant therapies.

ABBREVIATIONS

- DMSO, Dimethyl sulfoxide; PBS, Phosphate-buffered saline; ROS, Reactive oxygen species; LPH, Lactase phlorizin hydrolase; RISC, RNA-induced silencing complex; DGCR8, DiGeorge syndrome critical region 8; LPS, Lipopolysaccharide; AhR, Aryl hydrocarbon receptor; miRNA, micro ribonucleic acid; CYP, Cytochrome P450; RNA, messenger ribonucleic acid; GAE/L, Gallic acid equivalents per liter; TGF-β, Transforming growth factor β; TCDD, 2,3,7,8-Tetrachlorodibenzo-dioxin; TRBP, Trans-Activation Responsive RNA-Binding Protein; BRCA1/2, Breast cancer type 1/2 susceptibility protein; EGFR, Epidermal growth factor receptor; IRAK-1, Interleukin-1 receptor associated kinase 1; TRAF-6, Tumor necrosis factor receptor associated factor 6; TLR, Toll-like receptor pathway; ZBTB10, Zinc Finger And BTB Domain-Containing Protein 10; Sp transcription factors, Specificity protein transcription factors; PDCD4, Programmed cell death protein 4; PTEN, Phosphatase and tensin homolog; Masp1, Mammary serine protease inhibitor; NF-kB, Nuclear Factor Kappa B; PCR, Polymerase chain reaction; RT, Room temperature.
Search strategy and selection criteria

Our article focuses on the miRNA modulation as impact of quercetin treatment or combination of compounds containing quercetin. Minor emphasis was placed on pharmacokinetics of quercetin and miRNA biogenesis. The scientific articles from 1972 to 2019 were searched using the PubMed and Google Scholar. All the documents were searched through keywords such as “Quercetin, miRNA, modulation, microRNA, regulation and polyphenols”. Only English language articles were reviewed.

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