Vitamin C supplementation reduces expression of circulating miR-451a in poorly controlled type 2 diabetes mellitus

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Background: Vitamin C is an essential element required for the normal metabolic function. In this study, we investigated the effect of vitamin C supplementation on circulating miRNA (miR) expression in subjects with poorly controlled type 2 diabetes mellitus (T2DM) and correlated with clinical measures.

Methods: Pre- and post-vitamin C supplementation samples from five participants who had increased vitamin C levels, improved oxidative status and polymorphonuclear (PMN) function after receiving 1,000 mg vitamin C daily for six weeks were screened for miRNA expression using the NanoString miRNA assay. Differences in miRNA expression identified from the miRNA screen were validated by qRT-PCR.

Results: Four miRNAs showed significantly different expression post-vitamin C supplementation including the down-regulation of miR-451a (-1.72 fold change (FC), p=0.036), and up-regulation of miR-1253 (0.62 FC, p=0.027), miR-1290 (0.53 FC, p=0.036) and miR-644a (0.5 FC, p=0.042). Subsequent validation study showed only miR-451a expression was significantly different with supplementation. The miR-451a expression was negatively correlated with vitamin C levels (r=-0.497, p=0.049) but positively correlated with levels of malondialdehyde (MDA) (r=0.584, p=0.017), cholesterol (r=0.564, p=0.022) and low-density lipoproteins (LDL) (r=0.522, p=0.037). Bioinformatics analysis of the putative miR-451a target genes indicated gene functions related to signalling pathways involved in cellular processes such as the mammalian target of rapamycin (mTOR) signalling pathway.

Conclusions: Supplementation of vitamin C altered circulating miR-451a expression. This miRNA could be used as a biomarker to indicate the oxidative status in T2DM subjects with poor glycemic control and lead to a novel molecular strategy to reduce oxidative stress in T2DM.

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Abstract

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Methods: Pre- and post-vitamin C supplementation samples from five participants who had increased vitamin C levels, improved oxidative status and polymorphonuclear (PMN) function after receiving 1,000 mg vitamin C daily for six weeks were screened for miRNA expression using the NanoString miRNA assay. Differences in miRNA expression identified from the miRNA screen were validated by qRT-PCR.
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Introduction

Vitamin C, or ascorbic acid, is well known as an antioxidant required for the normal metabolic function of the body and associated with a wide spectrum of biological processes *(Mandl et al. 2009)*. It can diminish the effect of oxidative stress and protect cells or tissue injury from oxidative damages. Several studies reported a beneficial effect of vitamin C supplementation especially in type 2 diabetes mellitus (T2DM), chronic metabolic disease in conjunction with chronic hyperglycemia and excess free fatty acids (FFAs) *(Hawkins et al. 2003)*. Overproduction of reactive oxygen species from the disease can cause DNA and protein damage, lipid peroxidation, cellular and vascular dysfunction leading to diabetic complications, which have been associated with high levels of oxidative stress as indicated by MDA and F_2-Isoprostanes (F_2IsoPs) and low levels of vitamin C *(Bhatia et al. 2003; Johansen et al. 2005; Kaviarasan et al. 2009)*. There is evidence that supplementation with 1000 mg/day of vitamin C for six weeks can improve blood glucose, lipid profiles, Glycated Haemoglobin (HbA1C) and insulin in T2DM *(Afkhami-Ardekani & Shojaoddiny-Ardekani 2007)*. Furthermore, previous studies have shown the effect of vitamin C on gene expression through epigenetic regulation including microRNA (miRNA or miR) *(Kolhe et al. 2018)*. A study from Kim et al revealed dietary consumption of a high dose of vitamin C enhances anti-oxidation and anti-glycation as well as reduction of inflammatory miRNA in lipoproteins *(Kim et al. 2015)*. Treatment of human bone marrow stem cells with vitamin C can modulate miRNA expression and might have an impact on the biology of the cells *(Kolhe et al. 2018)*. These studies indicate that vitamin C could affect miRNA expression and may associate with the pathophysiology of T2DM.

MiRNAs are a functional non-coding small RNAs typically 18-22 nucleotides (nt) in length that have been identified as an important regulator in fine-tuning of gene expression. These RNA molecules mainly act by repressing mRNA translation at the 3' untranslated region (3'UTR) of the target mRNA and can function in cell-cell communication *(Bartel 2004; Turchinovich et al. 2016)*. One of the properties of these small RNAs is that they are highly stable in various sample types such as serum or plasma and accordingly can be used as a biomarker or target therapeutics in several diseases including T2DM, in which miRNA deregulation has been associated with progression of the disease *(He et al. 2017; Jimenez-Lucena et al. 2018; Maqbool & Ul Hussain*

2014; Regazzi 2018). However, less is known about the effect of vitamin C supplementation on miRNAs in T2DM subjects, especially for those subjects with poor glycemic control. In such cases, the high oxidative stress and low levels of plasma vitamin C found in these subjects may be influenced by miRNAs and vice versa.

To determine the effect of vitamin C supplementation on miRNA expression in T2DM, we investigated circulating miRNAs after vitamin C supplementation using a miRNA screen. We hypothesized that miRNAs may be affected by vitamin C supplementation and correlate with the clinical characteristics involved in pathophysiological processes. We have chosen subjects who have been affected by vitamin C supplementation on oxidative stress and PMN function. Such associations may provide greater insights into the molecular mechanisms involved in these biological processes and may be used as biomarkers for responses to the dietary treatment of the disease leading to novel strategies in molecular targeting of relevant genes in T2DM.

Materials & Methods

Study design and participants

Archived plasma samples were a subset of the participants who have received vitamin C from the project of vitamin C supplementation in T2DM patients (*Chuangchot et al. 2020*). The study of leftover samples was approved by the Human Ethical Committee of Khon Kaen University in accordance with the 1964 Declaration of Helsinki (HE571264). Diagnostic measures of T2DM and clinical data of the subjects were received without personal identification from Srinagarind Hospital, Faculty of Medicine, Khon Kaen, Thailand. The criteria for the screening process included age between 30 and 60 years old, diagnosed as having T2DM for at least 12 months, poor glycemic control (HbA1c $\geq$8.5% or 69.4 mmol/mol), treated with oral diabetic drugs only (no insulin injection), sedentary and lack of any regular exercise program for at least 6 months as well as living in Khon Kaen Province, Thailand. Some subjects were treated with lipid-lowering drugs and/or anti-hypertensive drugs for the duration of the study to maintain blood pressure at $\leq$ 140/90 mmHg. The participants received 1,000 mg/day vitamin C for six weeks. Blood samples from the subjects who had increased plasma vitamin C levels, increased phagocytosis and oxidative burst, and reduced oxidative stress at the post-vitamin C supplementation timepoint compared to pre-supplementation were collected to investigate miRNA expression.

miRNA extraction

Total RNA was extracted from archived plasma using the miRNeasy Serum/Plasma kit (Qiagen, Germany) according to the manufacturer's instruction. In brief, 200 uL of the plasma was mixed with five volumes of Qiazol and a fold of chloroform. Spike-in controls, cel-miR-39 and cel-miR-254, were added as a normalizer and indicator of extraction efficiency. After absorption, 14 uL of RNase-free water was added to the spin column to elute total RNA and then miRNA concentration was measured by a Nanophotometer.

miRNA analysis

To explore miRNA profiling in response to vitamin C supplementation, a miRNA microarray screen was performed to discover mature-miRNA expression in plasma samples of five T2DM subjects individually by using the NanoString platform (NanoString Technologies, Seattle, WA, USA). This screen includes 800 human mature-miRNAs and can be profiled without an amplification step. Briefly, 3 uL of total RNA was ligated to a specific miR-Tag and hybridized to the color-coded probe. Counting of miRNA and digital analysis were performed using the nCounter human v3 miRNA expression platform. A conservative background threshold of 100 counts was set to eliminate all targets with low expression for further analysis. Six positive controls and cel-miR-254 were used for data normalization.

qRT-PCR

Qualitative real-time polymerase chain reaction (qRT-PCR) was performed to validate miRNA expression for a select set of miRNAs. The small RNAs were converted to cDNA using the miScript II RT kit (Qiagen, Germany) following the manufacturer's protocol. The miScript primer assays and miScript SYBR green PCR kits from the Qiagen were used to measure the specific miRNAs on a Bio-Rad CF96^{TM} real time PCR machine (Bio-Rad, CA, USA) according to the manufacturer protocols. The cel-miR-39 spike-in control was used as a reference gene. Ct values >35 were deemed background. Relative expression of individual miRNAs was calculated by the $2^{-\Delta Ct}$ method.

Target gene prediction and pathway analysis

To identify target genes of relevant miRNAs, four bioinformatics tools were selected to retrieve the predicted target genes namely Tarbase v. 8.0, miRTarBase v. 7.0, TargetScan v. 7.2 and miRDB v. 6.0. The biological functions and enrichment pathways of these target genes were analyzed using the Database for Annotation, Visualization and Integration Discovery (DAVID) v. 6.8. A false discovery rate (FDR) with an adjusted P-value <0.05 was set as the significance threshold.

Statistical analysis

Normal distribution was tested using the Shapiro-Wilk test. Log2 transformed data by nSolver Analysis Software v. 4.0 (NanoString Technologies) was applied to analyze the expression of each miRNA using the visual genomics analysis studio (VGAS) program (an in-house program for genomics analysis and visualization; http://www.iiid.com.au/software/vgas). Comparison of the pre- and post-vitamin C supplementation data was performed using a paired t-test. Laboratory measures and values from the validation study were compared between pre-and post-supplementation by paired t-test and Wilcoxon signed ranks test using SPSS statistics v. 19 (SPSS Inc., Chicago, IL, USA). A power of test (1-β) was checked by post-hoc analysis using G*Power v. 3.1.9.2 (Heinrich-Heine-Universität Düsseldorf, Germany). Spearman correlation was used to analyze the correlation between circulating miRNA expression and blood parameters

using GraphPad Prism v. 5.0 (GraphPad Software Inc., CA, USA). A p-value of <0.05 was set as the significance threshold. All data expressed as mean ± SD.

Results

Characteristics of T2DM subjects at pre- and post-vitamin C supplementation

Subjects had significantly increased levels of plasma vitamin C, raised PMN phagocytosis and oxidative burst, reduced products of lipid peroxidase (MDA and F_2IsoPs), as well as reduced levels of cholesterol after vitamin C supplementation for six weeks (Table 1).

MiRNA screen revealed differences in the expression level for a subset of miRNAs at pre- and post-vitamin C supplementation

A screen of circulating miRNAs in the plasma of five T2DM subjects at pre- and post-supplementation was performed using the NanoString platform. Of the 800 target miRNA in the screen, 26 miRNAs were expressed above background in all samples (Supplementary Fig. S1 and Table S1). Compared to the pre-supplementation, four miRNAs showed significantly different expression at post-supplementation. Of these, miR-451a was down-regulated post-supplementation (-1.72 fold change (FC), p=0.036), while miR-1253 (0.62 FC, p=0.027), miR-1290 (0.53 FC, p=0.036) and miR-644a (0.5 FC, p=0.042) were up-regulated post-supplementation (Fig. 1A). MiR-451a showed the highest reduced fold change of any of the other miRNAs tested. To validate these differences, samples from three additional subjects were included in the qPCR analysis (total n=8). Only miR-451a expression was found to be significantly different post-supplementation compared to the pre-supplementation timepoint (p=0.030) (Fig. 1B). The qPCR results for other targets from the screen were not examined further. The Ct values of miR-644a were greater than 35 (>background threshold; data not shown). For miR-1253 and miR-1290, there was no significant difference between pre- and post-supplementation (p=0.812 and p=0. 218, respectively; data not shown).

Correlation between circulating miRNA expression and clinical laboratory measures of T2DM

From the initial results, the expression of miR-451a was further correlated with clinical laboratory measures for the T2DM subjects and included both the pre-and post-vitamin C supplementation data to increase power of the statistic. Expression of miR-451a was negatively correlated with the plasma vitamin C levels (r=-0.497, p=0.049, Fig. 2A) but positively correlated with the MDA (r=0.584, p=0.017, Fig. 2B), cholesterol (r=0.564, p=0.022, Fig. 2C) and LDL (r=0.522, p=0.037, Fig. 2D) levels, suggesting that this miRNA might play a role in oxidative status and lipid metabolism.

Target gene prediction and pathway enrichment analysis

To identify target genes and pathway enrichments likely to be affected by miR-451a, bioinformatics analysis was performed using both experimentally validated and prediction tools. A total of 138 target genes of miR-451a were retrieved from four bioinformatics tools to identify relevant KEGG pathways using DAVID. The data indicated the most significant target gene enrichments for miR-451a were involved in the mTOR signalling pathway as well as other signalling pathways that overlap with the target genes such as AKT1 and MAPK1 as shown in Table 2 (Supplementary Table S2).

Discussion

Type 2 diabetes mellitus (T2DM) is a worldwide health problem and continues to increase in prevalence *(Cho et al. 2018)*. Long-standing T2DM can result in diabetic complications and increased susceptibility to infections resulting in increased morbidity and mortality, especially in uncontrolled diabetes *(Critchley et al. 2018)*. To our knowledge, the present study is the first report to investigate the effect of vitamin C supplementation on miRNA expression in T2DM subjects with poor glycemic control. We hypothesized that supplementation of vitamin C could modulate miRNA expression and associate with the blood parameters of the subjects for which we observed i.e. improved plasma vitamin C levels, reduced oxidative stress and increased PMN function following receiving 1,000 mg of vitamin C daily for six weeks. The result from the initial miRNA screen revealed that miR-451a was downregulated, with the highest negative fold change, after the supplementation and this was validated using qRT-PCR. However, the three significantly upregulated miRNAs from the microarray screen did not show differences in expression in the validation step with additional samples using qPCR. The discrepancy between the two platforms likely reflects the low template of these miRNAs and as such we focused only on miR-451a for further analysis.

MiR-451a (or miR-451, miRBase 22) is dicer independent and can be transcribed into a hairpin structure and then alternatively processed with the Argonaute 2 (Ago 2) protein *(Wang et al. 2019)*. This miRNA is stable in peripheral blood and can be found in erythrocytes, PMN, mononuclear cells and platelets *(Ghai et al. 2019; Masaki et al. 2007)*. MiR-451a has been reported in the blood circulation of the elderly T2DM subjects and is up-regulated in plasma samples of subjects with diabetic nephropathy as well as in the serum of subjects with acute diabetic Charcot foot, indicating the expressed miRNA may play a role in pathological processes of the disease *(Catanzaro et al. 2018; Pasquier et al. 2018; Sayilar et al. 2016)*. Furthermore, the expression of miR-451a was negatively correlated with the vitamin C levels but positively correlated with the MDA levels suggesting its role in oxidative status in the subjects. MiR-451a is also important in the erythroid lineage and plays a role in ROS production in erythrocytes by targeting 14-3-3zeta and via the inhibition of FoxO3 *(Yu et al. 2010)*. High oxidative stress may affect miR-451a production as a study from Ranjan et al found that deficient miR-451a expression was associated with defective ROS generation due to reduced Ago2 protein levels in macrophages *(Ranjan et al. 2015)*. Inhibition of miR-451a also reduced ROS, lipid peroxidation and DNA damage *(Zhu et al. 2018)*. These data suggest that miR-451a expression may positively

correlate with ROS production. In addition, miR-451a expression showed a positive correlation with cholesterol and LDL. These factors may exacerbate the progression of the disease accompanied with miR-451a expression. However, further work is needed to confirm the correlation and mechanism of miR-451a in oxidative stress found in the current study.

We also investigated the target genes of miR-451a and their functional enrichment using both experimentally validated and predicted programs. This was done to include more candidate target genes with a high cut-off set to reduce false positives *(Assmann et al. 2018)*. The target genes were significantly involved in signalling pathways that play an important role in cellular functions of biological processes as shown in Fig. 3 and Table 2. To support our results, studies in T2DM cardiomyopathy-induced mice with high ROS production displayed up-regulation of miR-451a that directly targeted calcium-binding protein 39 (CAB39) and resulted in down-regulation of the LKB1/AMPK signaling pathway. The target genes were listed in Table 2. Furthermore, knockout of this miRNA showed the opposite effect *(Kuwabara et al. 2015)*. Thus, the miR-451a expression was induced by high oxidative stress leading to suppression of the AMPK pathway, a central energy-sensing of metabolic regulation, which was found to have reduced activity in insulin-resistant individuals *(Li et al. 2019; Xu et al. 2012)*. Reduced ROS production found in the T2DM subjects in this study after vitamin C supplementation and down-regulation of miR-451a might be involved through the CAB39/LKB1/AMPK signaling, as PI3K/AKT/mTOR signaling is the central pathway of glucose and lipid metabolism *(Huang et al. 2018; Tuo & Xiang 2018)*. Hyperglycemia and excess FFAs lead to insulin resistance and high levels of ROS resulting in impaired PI3K/AKT/mTOR signaling found in T2DM subjects *(Huang et al. 2018)*. Moreover, reduction of oxidative stress has been reported in the up-regulation of AKT and mTOR after pretreatment with vitamin C *(Lin et al. 2016)*. MiR-451a could target multiple genes involved in the PI3K/AKT/mTOR signalling pathway including AKT1, PIK3CA, PIK3R1, and MAPK1 (as indicated in Table 2). Supplementation with vitamin C in the subjects might help to promote these pathways through the regulation of miR-451a. Further studies are required to explore the mechanisms behind the effects of vitamin C supplementation on this circulating miRNA associated with systemic oxidative stress in the subjects.

The participants in this study showed enhanced phagocytosis and oxidative burst after supplementation of vitamin C, which is consistent with the previous study that showed the effect of vitamin C in enhancing PMN function such as motility, chemotaxis, phagocytosis and microbial killing *(Bozonet et al. 2015; Carr & Maggini 2017)*. However, knowledge of the molecular mechanisms of the effect of vitamin C on these processes, especially phagocytosis and oxidative burst, are limited. MiR-451a has been reported to impact neutrophil chemotaxis by suppressing p38 MAPK through targeting Rab5a and 14-3-3zeta *(Murata et al. 2014)*. Rab5a, a small GTPase, regulates intracellular membrane trafficking involved in phagolysosome fusion during bacteria phagocytosis of neutrophils *(Perskvist et al. 2002)*. Moreover, treatment of human bladder cancer cells by vitamin C could suppress p38 MAPK activity and ROS production *(Kim et al. 2008)*. As suggested from these data, vitamin C may affect miR-451a

expression and associate with neutrophil function through targeting Rab5a and the p38 MAPK pathway. However, investigation of intracellular miRNAs in responding to vitamin C supplementation may help us to better understand the molecular mechanism underlying PMN function that we clearly observed in these subjects. A proposed mechanism of actions for miR-451a in response to vitamin C supplementation was shown in Fig. 3.

Limitations of the current study include a small sample size. Increasing the number of the participants may better clarify the association of the candidate miRNA and the laboratory measures including improved oxidative status and PMN functions as well as reduced cholesterol after vitamin C supplementation. Nevertheless, the power of test based on difference between the two dependent groups using mean and S.D. of relative miR-451a data revealed that the power was approximated 80%. More research is also needed to confirm and validate the finding that vitamin C intake modulates circulating miR-451a expression and to determine the functional role of this miRNA in subjects with uncontrolled T2DM.

Conclusions

In conclusion, this study has identified a novel association between vitamin C supplementation for six weeks and reduced circulating miR-451a expression in poorly controlled T2DM. This candidate miRNA might be used as a biomarker to identify subjects that respond to vitamin C treatment or oxidative status in plasma.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare no competing interests.

Author contributions

Laongthip Ruknarong performed experiments, analysed the data, prepared figures and tables, prepared the first draft manuscript and the final draft.

Chongchira Boonthongkaew and Nisa Chuangchot enrolled participants, performed experiments and analysed the data
Amonrat Jumnainsong, Naruemon Leelayuwat and Apinya Jusakul reviewed and approved the final draft.
Silvana Gaudieri and Chanvit Leelayuwat analysed, criticised the data, presentations of the manuscript, reviewed and approved the final draft.
Chanvit Leelayuwat design of the study, oversaw and supervised the whole project.

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Figure legends

Figure 1 Patterns of miRNA expression. (A) Analysis of circulating miRNA expression combined with clinical measures and qPCR data in response to vitamin C supplementation in T2DM patients with poor glycemic control (n=5). For the miRNA, blue and red bubbles

represent pre-and post-supplementation, respectively. The size of the bubbles reflects the count of the specific miRNA relative to other miRNAs. The laboratory measures and miR-451a qRT-PCR data are indicated as a heat map from minimum value to maximum value of each parameter independently of other parameters; green to red represents the low to high range. Pre; pre-vitamin C, Post; post-vitamin C supplementation. Numbers represent subjects 1-5. (B) Plot represents relative expression of miR-451a as validated by qRT-PCR (n=8).

Figure 2 Correlation between miR-451a expression and laboratory variables. (A) Vitamin C levels, (B) MDA, (C) Cholesterol and (D) LDL. Blue and red represent pre-and post-supplementation samples, respectively.

Figure 3 Proposed mechanism of the effect of miR-451a in response to vitamin C supplementation. Red lines represent the results of this study and blue lines indicate potential mode of interaction for miR-451a. Poor glycemic controlled T2DM subjects receiving vitamin C 1,000 mg dairy for six weeks showed significant down-regulation of circulating miR-451a accompanied with increased levels of vitamin C, reduced oxidative stress (MDA and F_2IsoPs) and increased PMN function (phagocytosis and oxidative burst). Changes in oxidative status may modulate miR-451a expression by altering Ago2 protein and/or increased involvement of signalling pathways such as AMPK signalling by repressing its target genes. MiR-451a may play a role in neutrophil chemotaxis by targeting RAB5A and 14-3-3zeta resulting in the activation of p38 MAPK signalling. However, the role of this miRNA in phagocytosis and oxidative burst of neutrophils is still to be explored (dotted line) and may be reflected in intracellular miRNA changes rather than circulating changes as identified in this study.

Table 1(on next page)

Table 1 Characteristics of the participants at the pre-and post-vitamin C supplementation timepoints.

Age, gender, BMI and blood chemistry data of participants pre- and post- vitamin C supplementation.

Table 1:

Characteristics of the participants at the pre-and post-vitamin C supplementation timepoints.

Parameters	Pre-supplementation (n=8)	Post-supplementation (n=8)	P-value
Gender (female/male)	7/1	7/1	-
Age (yr)	58.8±5.9	58.8±5.9	-
BMI (Kg/m^2)	26.2±4.1	26.2±3.9	0.861
HbA1c (mmol/mol)	95.0±15.0	95.0±14.8	0.909
FBS (mmol/L)	13.0±4.3	10.0±2.0	0.059
Plasma vitamin C levels (μmol/L)	57.8±11.0	90.5±55.5	0.017
Plasma MDA levels (μmol/mL)	17.0±8.6	10.6±3.8	0.031
Plasma F_2IsoPs (pg/mL)	16.9±4.8	12.0±4.3	0.012
Phagocytosis (%)	20.4±8.6	30.7±11.4	0.012
Oxidative burst (%)	5.9±3.5	11.1±4.5	0.006
Cholesterol (mg/dL)	239.3±60.1	187.9±45.9	0.050
Triglyceride (mg/dL)	168.8±63.8	195.9±100.1	0.217
HDL (mg/dL)	44.9±10.6	45.9±15.0	0.735
LDL (mg/dL)	164.9±53.7	126.5±32.3	0.154
Insulin (IU/mL)	14.1±4.6	11.5±3.6	0.057

Data represented as mean ± S.D. P-value was calculated using paired t-test.

Table 2(on next page)

Table 2 KEGG signaling pathways of miR-451a.

KEGG signaling pathways, Target gene, p-value and FDR prediction of miR-451a.

Table 2:
KEGG signaling pathways of miR-451a.

Term	Gene targets	P-value	FDR
mTOR signaling pathway	AKT1, MAPK1, TSC1, STK11, PIK3CA, PRKAA1, CAB39, IKBKB, PIK3R1	1.29E-08	0.00002
Hepatitis B	AKT1, MAPK1, YWHAZ, IL6, MAP3K1, BCL2, MMP9, PIK3CA, IKBKB, MYC, PIK3R1, ATF2	1.75E-08	0.00002
PI3K-Akt signaling pathway	AKT1, MAPK1, IL6, YWHAZ, TSC1, STK11, BCL2, PIK3CA, PRKAA1, LPAR1, IL6R, IKBKB, MYC, PIK3R1, ATF2	2.5E-07	0.00031
FoxO signaling pathway	AKT1, MAPK1, IL6, CDKN2B, STK11, CDKN2D, PIK3CA, PRKAA1, IKBKB, PIK3R1	6.56E-07	0.00080
Estrogen signaling pathway	AKT1, MAPK1, GNAQ, MMP9, PIK3CA, MMP2, PIK3R1, ATF2	8.51E-05	0.01033
TNF signaling pathway	AKT1, MAPK1, IL6, MMP9, PIK3CA, IKBKB, PIK3R1, ATF2	1.87E-05	0.02266
Non-alcoholic fatty liver disease (NAFLD)	AKT1, IL6, PIK3CA, PRKAA1, IL6R, IKBKB, UQCRQ, EIF2AK3, PIK3R1	2.95E-05	0.03578
AMPK signaling pathway	AKT1, TSC1, STK11, RAB14, PIK3CA, PRKAA1, CAB39, PIK3R1	3.94E-05	0.04776

Figure 1

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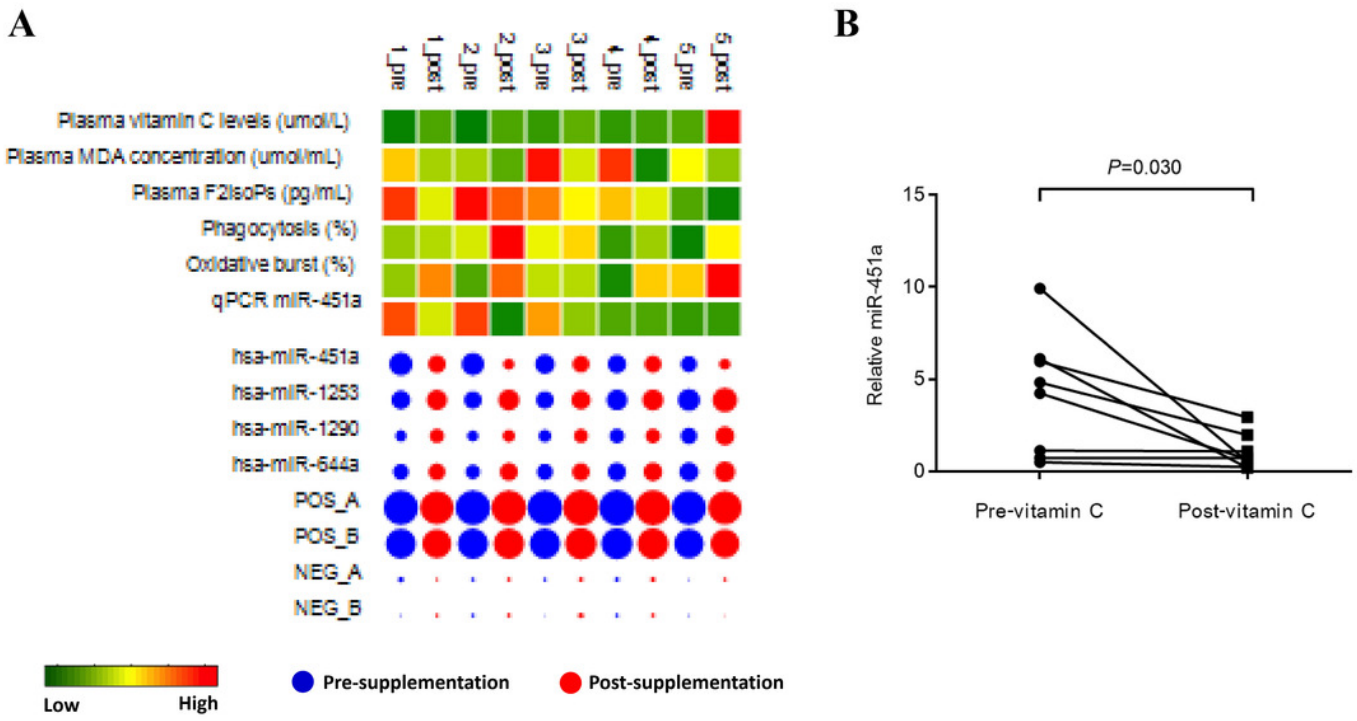

Figure 2

Figure 2 Correlation between miR-451a expression and laboratory variables.

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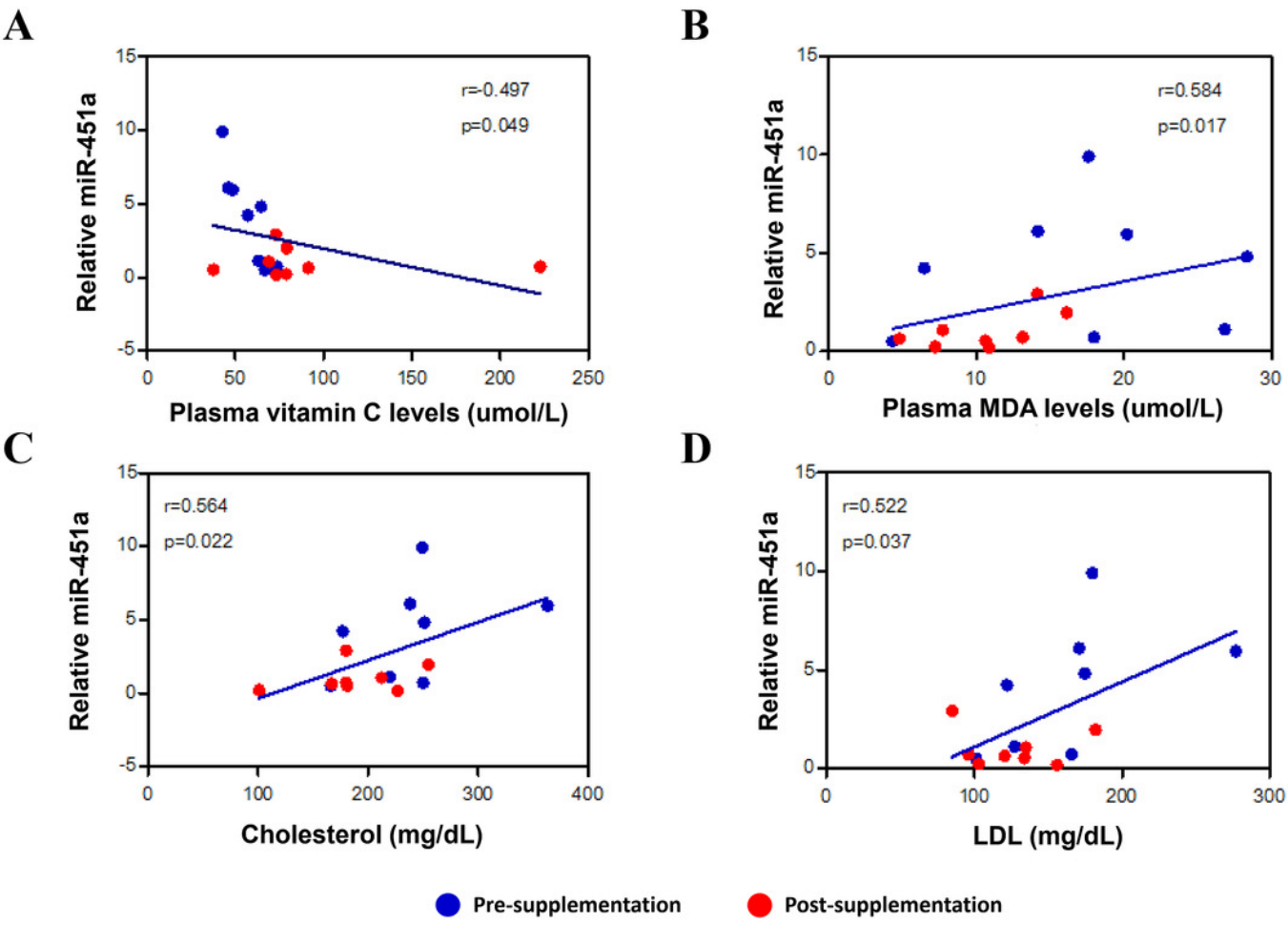

Figure 3

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