miR-21 promotes non-small cell lung cancer cells growth by regulating fatty acid metabolism

Kewei Ni1†, Dimin Wang2†, Heyun Xu1, Fuyang Mei1, Changhao Wu1, Zhifang Liu1 and Bing Zhou1*

Abstract
Background: Lung cancer is one of the most common malignant tumors worldwide. CD36 is a receptor for fatty acids and plays an important role in regulating fatty acid metabolism, which is closely related to tumorigenesis and development. The regulation of miR-21 and its role in tumorigenesis have been extensively studied in recent years. However, the relationship between miR-21 and CD36 regulated fatty acid metabolism in human non-small cell lung cancer remains unknown.

Methods: In this study, lentivirus transfection, qRT-PCR, cell migration, immunofluorescence, and western blot were used to examine the relationship between miR-21 and CD36 regulated fatty acid metabolism and the regulation role of miR-21 in human non-small cell lung cancer.

Results: This study demonstrated that up-regulation of miR-21 promoted cell migration and cell growth in human non-small cell lung cancer cells. Moreover, the intracellular contents of lipids including cellular content of phospholipids, neutral lipids content, cellular content of triglycerides were significantly increased following miR-21 mimic treatment compared with control, and the levels of key lipid metabolic enzymes FASN, ACC1 and FABP5 were obviously enhanced in human non-small cell lung cancer cells. Furthermore, down-regulation of CD36 suppressed miR-21 regulated cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells, which suggested that miR-21 promoted cell growth and migration of human non-small cell lung cancer cells through CD36 mediated fatty acid metabolism. Inhibition of miR-21 was revealed to inhibit cell growth, migration, intracellular contents of lipids, and CD36 protein expression level in human non-small cell lung cancer cells. In addition, PPARC1B was a direct target of miR-21, and down-regulation of PPARC1B reversed the inhibition of CD36 expression induced by miR-21 inhibitor.

Conclusions: These results explored the mechanism of miR-21 promoted non-small cell lung cancer and might provide a novel therapeutic method in treating non-small cell lung cancer in clinic.

Keywords: miR-21, Non-small cell lung cancer cells, CD36, Fatty acid metabolism, Migration, Cell growth
expression of other genes [6]. miRNAs are closely related to the occurrence of various tumors, and can play an important role in tumor cell proliferation, apoptosis, differentiation, and metastasis by regulating target mRNA [7, 8]. miR-21 is one of the miRNAs that promotes cancer progression and targets multiple tumor suppressor genes involved in proliferation, apoptosis and invasion [9, 10]. The regulation of miR-21 and its role in tumorigenesis have been extensively studied in recent years [11, 12]. A number of studies have found the elevated expression of miR-21 in various cancer cells [13, 14]. Previous study has shown that miR-21 enhances drug-resistant lung adenocarcinoma cancer cell invasion and migration through targeting HBPI [15]. However, the detailed mechanisms of miR-21 regulated human non-small cell lung cancer remain to solve.

Lipids mainly include fatty acids (FA), triglycerides and cholesterol, which are important sources of energy metabolism in the body [16]. Metabolites of fatty acids are involved in the regulation of the expression of a variety of genes [17, 18]. Recent studies have shown that many fatty acid metabolic pathways have changed in various cancer cells [19, 20]. In the process of cancer development, changes in fatty acid metabolism pathways not only provide energy for the occurrence and development of cancer, but also play an important role in biofilm macromolecules and signaling molecules [21]. Previous study demonstrated that LNMICC accelerated metastasis by reprogramming fatty acid metabolism in cervical cancer [22]. CD36 is a receptor for lipids and fatty acids and plays an important role in metabolic syndrome and related cardiac activities, which involved in lipid metabolism, long-chain fatty acid adsorption, apoptotic residue clearance, and macrophage phagocytosis [23–25]. Studies have found that CD36-regulated fatty acid metabolism is closely related to tumorigenesis and development [26, 27]. Gloria Pascual et al. found the ability of fatty acid receptor CD36 and lipid metabolism genes, in initiating metastasis, and palmitic acid or a high-fat diet enhanced the metastatic potential of CD36+ metastasis-initiating cells in a CD36-dependent manner [28].

Khaidakov et al. demonstrated that Oxidized LDL enhanced upregulation of proliferative and pro-inflammatory signaling in human breast mammary epithelial cells, which involved in stimulation of miR-21 expression, as well as upregulation of CD36 expression [29]. These results suggested that miR-21 might positively correlated with CD36 in human breast mammary epithelial cells. A recent study has shown that lipids could promote the growth of breast cancer cells via regulation of CD36 expression [30]. On the other hand, Calo et al. Revealed that miR-21 deletion in hepatocytes increases modulated the expression of multiple key metabolic transcription factors involved in fatty acid uptake, de novo lipogenesis, gluconeogenesis and glucose output [31]. However, the relationship between miR-21 and CD36 regulated fatty acid metabolism in human non-small cell lung cancer remains unknown.

In this study, we focused the effect of miR-21 on lung cancer cell growth and migration and the tentative relation to CD36 regulated lipid metabolism. We found miR-21 enhanced cell growth, migration, intracellular contents of lipids and key lipid metabolic enzymes in human non-small cell lung cancer cells. Moreover, down-regulation of CD36 suppressed miR-21 regulated cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells. In addition, we also found inhibition of miR-21 suppressed cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells. These results explored the mechanism of miR-21 promoted non-small cell lung cancer and might provide a novel therapeutic method in treating non-small cell lung cancer in clinic.

**Materials and methods**

**Cell culture**

The A549 and H1703 human non-small cell lung cancer cells were obtained from the cell bank of Shanghai Institutes for Biological Sciences. Cells were cultured in RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 10% fetal bovine serum at 37 °C in an atmosphere of 5% CO2.

**miRNA**

The miR-21-3p mimic, inhibitor and the respective negative controls were obtained from GenePharma (Shanghai, China). The cell transfection was performed using Lipfectamine 2000 Reagent (Invitrogen) according to the manufacturer's instruction. The cell number was determined after different treatments.

**Plasmid, lentivirus construction, and transfection**

Oligonucleotides of shRNA for CD36 and PPARG1B were obtained from Sangon Biotech (Shanghai). After co-transfection, the virus media were harvested. Cells were infected for 72 h with lentivirus contained CD36 and control shRNA respectively. A549 cells were seeded in 6-well plate overnight, and then washed these cells with PBS for three times and these cells were finally both received lentivirus containing CD36 or control shRNA. The sequence of shRNA was as follows: CD36, TTGTACCTAATGAGAC; PPARG1B, GAGTGCCAGGTGC TGGCAAGAAATAGGA.
Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Trizol Reagent (Invitrogen) was used to extract RNA from A549 cell lines. An ABI PRISM 7500 real-time PCR System and SYBR Green Master Mix (Roche, Shanghai, China) were used to perform polymerase chain reactions. β-actin and U6 were used as the internal references for mRNA and miRNA respectively. PCR primers were as follows, miR-21 forward: 5′-GGACTAGCTTATCAGACTG-3′, reverse: 5′-CATCAGATGCCTTGCGTA-3′; CD36 forward: 5′-GAACCTGCTTTCAAAAACTGG-3′, reverse: 5′-GTCCTGAGTTATATTTTCTTGG-3′; PPARGC1B forward: 5′-GAACCTGACCTCTTCCCAGCT-3′, reverse: 5′-AGGGCCTCATCTCACTGTGG-3′; U6 forward: 5′-GCTTTCCTCAAGTTGCGTTGCTGTCAT-3′, reverse: 5′-CTCTGAGGTCTTACCGATGAGCTGCACATATCTAAGAAAT-3′, reverse: 5′-CGCTTCAAGTATTGCTGTGCT-3′; GAPDH forward: 5′-ATCTGGAGTTACCGCTTG-3′; reverse: 5′-ACTCGTGGCATGAGCTGCACATATCTAAGAAAT-3′.

Cell migration

Wound-healing assays were performed to evaluate cell migration ability. Cells were transfected with miR-21 mimic or control in culture medium containing 2% FBS. Wound closure was observed at 0 h, 24 h, and 48 h and photographed using a microscope (Olympus, Tokyo, Japan) after scratch at the bottom of the plates. The migration distance of wound closure was calculated between each group.

Western blot

After transfection, cells were treated in a lysis buffer containing a complete protease inhibitor cocktail (Shanghai Roche Pharmaceuticals), followed by the extraction and quantification of proteins. Subsequently, the protein was transferred into nitrocellulose membranes, blocked, and incubated in specific primary antibody at 4 °C overnight. The membranes were washed and incubated with second antibody for 2 h at room temperature. Finally, the protein bands were visualized using chemiluminescence detection system. The following antibodies were used: anti- FASN (Abcam, UK), anti- ACC1 (Cell Signaling Technology, Danvers, MA, USA), anti-FABP5 (Cell Signaling Technology, Danvers, MA, USA); anti-CD36 (Santa CruzBiotechnology, Santa Cruz, CA, USA).

Quantification of neutral lipid

The lipophilic fluorescence dye BODIPY 493/503 (Invitrogen) was employed for monitoring the neutral lipid accumulation in A549 cells according to the manufacturer’s instructions. Briefly, cells were washed in Phosphate Buffered Saline (PBS), fixed with 4% paraformaldehyde and stained with BODIPY 493/503 (1 µg/mL) for 45 min at room temperature, and then nuclei were counterstained with Hoechst for 15 min. The results of immune staining were detected using a fluorescence microscope (Olympus, Tokyo, Japan).

Quantification of phospholipids and triglycerides

Intracellular phospholipids and triglycerides content were assayed by EnzyChrom™ phospholipids assay kit (BioAssay Systems, Hayward, CA, USA) and EnzyChrom™ triglycerides assay kit (BioAssay Systems, Hayward, CA, USA) respectively, according to the procedure provided in the kit.

Dual luciferase assays

The fragments contain the wild type (wt) and the mutant type of PPARGC1B gene were respectively cloned into the pGL3 luciferase miRNA target expression vector (Promega, Madison, Wisconsin). And then the recombinant reporter vector named pGL3-PPARGC1B-wt or pGL3-PPARGC1B-mut were synthesized. The reporter vector pGL3-PPARGC1B-wt/pGL3-PPARGC1B-mut and miR-21 mimic were co-transfected into A549 cells with Lipofectamine 200 (Invitrogen). The luciferase activity of the cells were detected using a Dual Luciferase Reporter Assay System (Promega).

Statistical analysis

The data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as the mean±SD of at least three independent experiments. The independent sample t test was used for comparing groups for statistical differences. Statistical significance was defined as P<0.05.

Results

miR-21 enhanced cell growth and migration in human non-small cell lung cancer cells

To investigate the effect of miR-21 on human non-small cell lung cancer cells, miR-21 mimic was constructed and treated to A549 cells. The transfection efficiency of miR-21 mimic in A549 cells were determined. The result revealed miR-21 mimic treatment up-regulated more than fourfolds of miR-21 expression (Fig. 1c). To further explore the effect of miR-21 on cell migration ability, A549 cells were transfected with or without miR-21 mimic for 24 h and 48 h. The results exhibited miR-21 mimic treatment significantly enhanced cell migration abilities compared with control (Fig. 1a, b). Moreover, the cell growth was significantly increased following miR-21 mimic treatment compared with control in A549 cells and H1703 cells (Fig. 1d, e). These results suggested that
miR-21 enhanced cell growth and migration in human non-small cell lung cancer cells.

miR-21 enhanced the intracellular contents of lipids and key lipid metabolic enzymes in human non-small cell lung cancer cells
To investigate the tentative mechanism of miR-21 regulated human non-small cell lung cancer cells, the intracellular contents of lipids and key lipid metabolic enzymes were determined following miR-21 mimic treatment. The result demonstrated that miR-21 mimic treatment increased nearly threefolds of cellular phospholipids compared with control in A549 cells or H1703 cells (Fig. 2a). Moreover, the neutral lipids content was detected by staining with BODIPY 493/503 dye and DAPI in human non-small cell lung cancer cells. The result showed that miR-21 mimic treatment obviously promoted the neutral lipids content in A549 and H1703 cells (Fig. 2b). Furthermore, cellular content of triglycerides was significantly increased following miR-21 mimic treatment compared with control in human non-small cell lung cancer cells (Fig. 2c). In addition, to further explore the effect of miR-21 on fatty acid metabolism, the protein expression levels of key lipid metabolic enzymes FASN, ACC1 and FABP5 were detected and the result revealed that miR-21 mimic treatment apparently promoted the expression levels of key lipid metabolic enzymes in A549 cells (Fig. 2d, e). These results suggested that miR-21 enhanced the intracellular contents of lipids and key lipid metabolic enzymes in human non-small cell lung cancer cells.

Down-regulation of CD36 suppressed miR-21 regulated cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells
To further investigate the effect of miR-21 on fatty acid metabolism, the key receptor CD36 in the upstream
of fatty acid metabolism was detected. The results demonstrated that miR-21 mimic increased CD36 expression level compared with control, and obviously promoted CD36 protein expression level (Fig. 3a, b). To investigate the effect of CD36 on miR-21 regulated cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells, CD36 was subsequently knockeddown by transfection with lentivirus shRNA. After transfection with CD36 shRNA (shCD36), the expression level of CD36 was significantly decreased compared with control (Fig. 3c). Moreover, cellular content of phospholipids and triglycerides were significantly decreased after transfection with shCD36 compared with miR-21 mimic treatment (Fig. 3d, e). In addition, the cell number and migration ability were also significantly down-regulated compared with miR-21 mimic treatment (Fig. 3f, g). These results suggested that miR-21 promoted cell growth, migration and fatty acid metabolism through regulating CD36 expression.

Inhibition of miR-21 suppressed cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells
To further examined the effect of miR-21 on cell growth, migration, and fatty acid metabolism, miR-21 inhibitor was constructed and treated to A549 cells. As expected, miR-21 inhibitor treatment significantly decreased miR-21 expression level compared with control (Fig. 4a). Moreover, the cell number and migration ability were significantly down-regulated compared with control (Fig. 4b, f). Furthermore, cellular content of phospholipids was slightly decreased, and cellular content of triglycerides was significantly decreased after transfection with miR-21 inhibitor compared with control (Fig. 4d, e). In addition, miR-21 inhibitor treatment also obviously decreased CD36 protein expression level compared with control in A549 cells (Fig. 4c). These results suggested that inhibition of miR-21 suppressed cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells.
PPARGC1B was involved in miR-21 regulated CD36 expression

To investigate the detailed mechanism of miR-21 regulated fatty acid metabolism, miR-21 was predicted to have the binding site with the 3’UTR of PPARGC1B (Fig. 5a). PPARGC1B was known to play an important role in multiple metabolic processes. To investigate the relationship between miR-21 and PPARGC1B, dual
luciferase assay was conducted in A549 cells. The luciferase activities were significantly decreased by treatment with pGL3-PPARGC1B-wt and miR-21 mimic. However, the luciferase activity in cells transfected with pGL3-PPARGC1B-mut revealed no significant difference following treatment of miR-21 mimic (Fig. 5b).

Next, to further examine the effect of PPARGC1B on miR-21 regulated CD36 expression, PPARGC1B was down-regulation by shRNA and the efficiency was determined in Fig. 5c. The relative expression of CD36 was decreased by the treatment of miR-21 inhibitor. However, after combined treatment with miR-21 inhibitor and shPPARGC1B, the relative expression of CD36 was significantly reversed compared with miR-21 inhibitor treatment group (Fig. 5d). These results suggested that PPARGC1B might involve in miR-21 regulated CD36 expression.

**Discussion**

Abnormal lipid metabolism and related pathologies are associated with cd36-mediated signal transduction dysfunction [32]. Several studies have found that fatty acid receptor CD36 is highly expressed in many types of tumors [28, 33]. Previous study has shown that a kind of cells expressing high level of the fatty acid receptor CD36
has been found in human oral cancer samples, and they have a high metastatic potential in mice [34]. miRNAs play an important regulatory role in post-transcriptional regulation of gene expression, and participate in a variety of biological processes including intercellular signal transmission, cell proliferation, growth, differentiation, apoptosis, and cell metabolism [35]. Recent studies have found that miRNA plays an important role in energy metabolism such as glucose metabolism, fat metabolism and amino acid metabolism [36, 37]. Specific miRNA regulates lipid metabolism by binding to the 3′ UTR of the related lipid metabolism regulating gene. Cruz-Gil et al. found miR-19b-1 suppressed invasion in colon cancer cells by targeting the lipid metabolic axis ACSL/SCD [38]. Cheng et al. demonstrated that down-regulation of miR-148a was revealed to be correlated with poor clinical outcomes in hepatocellular carcinoma (HCC) patients, and miR-148a deletion accelerated hepatocarcinogenesis through promoting lipid metabolic disorders in mice [39].

Recently, the role of miRNAs in the regulation of various tumor lipids is gradually elucidated [40]. However, whether miRNAs regulate fatty acid metabolism in non-small cell lung cancer, and its role in the progression of non-small cell lung cancer remain unclear. Here, in this study, we found miR-21 mimic treatment significantly enhanced cell migration abilities and cell growth compared with control in human non-small cell lung cancer cells. Moreover, to investigate the effect of miR-21 on fatty acid metabolism in human non-small cell lung cancer cells, the intracellular contents of lipids and key lipid metabolic enzymes were determined and the results revealed cellular content of phospholipids, neutral lipids content, cellular content of triglycerides, and the levels of key lipid metabolic enzymes FASN, ACC1 and FABP5
were significantly increased following miR-21 mimic treatment compared with control in A549 cells. Furthermore, CD36 was subsequently knockdown by transfection with lentivirus shRNA to explore the mechanism of miR-21 regulated fatty acid metabolism. The results demonstrated that down-regulation of CD36 suppressed miR-21 regulated cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells, which suggested that miR-21 promoted cell growth, migration and fatty acid metabolism through up-regulated CD36 expression. In addition, we also found inhibition of miR-21 suppressed cell growth, migration, intracellular contents of lipids, and CD36 protein expression level in human non-small cell lung cancer cells. In order to investigate the detailed mechanism of miR-21 regulated CD36 expression, PPARGC1B was revealed to be a direct target of miR-21, and down-regulation of PPARGC1B reversed the inhibition of CD36 expression induced by miR-21 inhibitor. Although PPARGC1B was revealed to involve in miR-21 regulated CD36 expression, the mechanism of PPARGC1B in miR-21 regulated fatty acid metabolism needed further explore.

Conclusion
This study demonstrated that up-regulation of miR-21 promoted cell migration and cell growth in human non-small cell lung cancer cells. Moreover, the intracellular contents of lipids including cellular content of phospholipids, neutral lipids content, cellular content of triglycerides were significantly increased following miR-21 mimic treatment compared with control, and the levels of key lipid metabolic enzymes FASN, ACC1 and FABP5 were obviously enhanced in human non-small cell lung cancer cells. Furthermore, down-regulation of CD36 suppressed miR-21 regulated cell growth, migration and intracellular contents of lipids in human non-small cell lung cancer cells, which suggested that miR-21 promoted cell growth and migration of human non-small cell lung cancer cells through CD36 mediated fatty acid metabolism. In addition, inhibition of miR-21 was revealed to inhibit cell growth, migration, intracellular contents of lipids, and CD36 protein expression level in human non-small cell lung cancer cells. The results also demonstrated that PPARGC1B was a direct target of miR-21, and down-regulation of PPARGC1B reversed the inhibition of CD36 expression induced by miR-21 inhibitor. These results explored the mechanism of miR-21 promoted non-small cell lung cancer and might provide a novel therapeutic method in treating non-small cell lung cancer in clinic.

Acknowledgements
Not applicable.

Authors’ contributions
BZ and ZL designed this study. KN, DW and HX carried out the study. FM and CW performed the statistical analysis. DW wrote the manuscript and BZ revised the manuscript. All authors read and approved the final manuscript.

Funding
This project is funded by Zhejiang Provincial Lung Cancer Diagnosis and Treatment Technology Research Center.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
Not applicable.

Consent of publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Cardiothoracic Surgery, Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College, Hangzhou 310014, Zhejiang, People’s Republic of China. 2 College of Basic Medical Sciences, Second Military Medical University, Shanghai, China.

Received: 1 May 2019   Accepted: 18 August 2019
Published online: 23 August 2019

References
1. Remon J, Ahn MJ, Girard N, Johnson M, Kim DW, Lopes G, Pillai RN, Solomon B, Villacampa G, Zhou Q. Advanced stage non-small cell lung cancer: advances in thoracic oncology 2018. Journal Thorac Oncol. 2019;14(7):1134–55.
2. Blandin Knight S, Crosbie PA, Balata H, Chudzik J, Russell T, Dive C. Progress and prospects of early detection in lung cancer. Open Biol. 2017;7(9):170070.
3. Jakobsen E, Rasmussen TR, Green A. Mortality and survival of lung cancer in Denmark: results from the Danish Lung Cancer Group 2000–2012. Acta Oncol. 2016;55(Suppl 2):2–9.
4. Janssen-Heijnen ML, van Erning FN, De Ruyscher DK, Coebergh JW, Green HJ. Variation in causes of death in patients with non-small cell lung cancer according to stage and time since diagnosis. Ann Oncol. 2015;26(5):902–7.
5. Griffin R, Ramirez RA. Molecular targets in non-small cell lung cancer. Ochsner J. 2017;17(4):388–92.
6. Tian K, Shi R, Gu A, Pennella M, Gu LQ. Polycationic probe-guided nanopore single-molecule counter for selective miRNA detection. Methods Mol Biol. 2017;1632:255–68.
7. Sandford OA, Moore CA, Du J, Boulad M, Gergues M, Eltouky H, Rameshwar P. Human aging and cancer: role of miRNA in tumor microenvironment. Adv Exp Med Biol. 2018;1056:137–52.
8. Qin X, Xu H, Gong W, Deng W. The tumor cytosol miRNAs, fluid miRNAs, and exosome miRNAs in lung cancer. Front Oncol. 2014;4:357.
9. Hermansen SK, Nielsen BS, Aaberg-Jessen C, Kristensen BW. miR-21 is linked to glioma angiogenesis: a co-localization study. J Histochem Cytochem. 2016;64(2):138–48.
10. Xu Z, Liu X, Wang H, Li J, Dai L, Li J, Dong C. Lung adenocarcinoma cell-derived exosomal miR-21 facilitates osteoclastogenesis. Gene. 2018;666:116–22.
11. Wang W, Songlin P, Sun Y, Zhang B, Jinhui W. miR-21 inhibitor sensitizes human OSCC cells to cisplatin. Mol Biol Rep. 2012;39(5):5481–5.
12. Frankel LB, Christofferson NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem. 2008;283(2):1026–33.
13. Liu L, Chen Q, Lai R, Wu X, Wu X, Liu F, Xu G, Ji Y. Elevated expression of mature miR-21 and miR-155 in cancerous gastric tissues from Chinese patients with gastric cancer. J Biomed Res. 2010;24(3):187–97.

14. Huang CS, Yu W, Cui H, Wang YJ, Zhang L, Han F, Huang T. Increased expression of miR-21 predicts poor prognosis in patients with hepatocellular carcinoma. Int J Clin Exp Pathol. 2015;8(6):7234–8.

15. Su C, Cheng X, Li Y, Han Y, Song X, Yu D, Cao X, Liu Z. MiR-21 improves invasion and migration of drug-resistant lung adenocarcinoma cell and transformation of EMT through targeting HBP1. Cancer Med. 2018;7(6):2485–503.

16. Bermudez-Cardona J, Velasquez-Rodriguez C. Profile of free fatty acids and fractions of phospholipids, cholesterol esters and triglycerides in serum of obese youth with and without metabolic syndrome. Nutrients. 2016;8(2):54.

17. Huang YL, Paulson KE, Lichtenstein AH, Lamon-Fava S. Regulation of the expression of key genes involved in HDL metabolism by unsaturated fatty acids. Br J Nutr. 2012;108(8):1351–9.

18. Huang T, Hu X, Khan N, Yang J, Li D. Effect of polyunsaturated fatty acids on homocysteine metabolism through regulating the gene expressions involved in methionine metabolism. Scientific World J. 2013;2013:931626.

19. Matthews GM, Howarth GS, Butler RN. Short-chain fatty acids induce apoptosis in colon cancer cells associated with changes to intracellular redox state and glucose metabolism. Chemotherapy. 2012;58(2):102–9.

20. Balaban S, Lee LS, Varney B, Aishah A, Gao Q, Shearer RF, Saunders DN, Grevel T, Hoy AJ. Heterogeneity of fatty acid metabolism in breast cancer cells underlies differential sensitivity to palmitate-induced apoptosis. Mol Oncol. 2018;12(9):1623–38.

21. Marques CN, Davies DG, Sauer K. Control of biofilms with the fatty acid signaling molecule cis-2-decenonic acid. Pharmaceuticals. 2018;10(8):234.

22. Chang C, Wang W, Liao Y, Chen Y, Liu T, Du Q, Huang J, Liang Y, Liu J, Zhao Y, et al. LNMMCC promotes nodal metastasis of cervical cancer by reprogramming fatty acid metabolism. Can Res. 2018;78(4):877–90.

23. Chen YP, Kuo WW, Baskaran R, Day CH, Chen RJ, Wen SY, Ho TJ, Padma VV, Kuo CH, Huang CY. Acute hypoxic preconditioning prevents palmitic acid-induced cardiomyocyte apoptosis via switching metabolic GLUT4-glucose pathway back to CD36-fatty acid dependent. J Cell Biochem. 2018;119(4):3363–72.

24. Zhao L, Varghese Z, Wang X, Cing H, Song G, Yu X, Zhu Y, Wang X, Zhang X, Di Y. Exogenous lipids promote the growth of breast cancer cells via CD36. Oncol Rep. 2017;38(4):2105–15.

25. Calo N, Ramadori P, Sobolevski C, Romero Y, Maeder C, Fournier M, Rantakari P, Zhang FP, Poutanen M, Dufour JF, et al. Stress-activated miR-21/miR-21* in hepatocytes promotes lipid and glucose metabolic disorders associated with high-fat diet consumption. Gut. 2016;65(11):1871–81.

26. Landberg N, von Paffly S, Askmyr M, Lilljebrond H, Sanden C, Rissler M, Mustjoki S, Hjorth-Hansen H, Richter J, Agerstam H, et al. CD36 defines primitive chronic myeloid leukemia cells less responsive to imatinib but vulnerable to antibody-based therapeutic targeting. Haematologica. 2018;103(3):447–55.

27. Pepino MY, Kuda O, Samovski D, Abumrad NA. Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. Annu Rev Nutr. 2014;34:281–303.

28. Chevrot M, Martin C, Pasilisky-Degrace P, Bernard P. Role of CD36 in oral and postural sensing of lipids. Handb Exp Pharmacol. 2012;209:295–307.

29. Lian J, Guo J, Huang X, Yang X, Huang G, Mao H, Sun HH, Ba Y, Zhou J. miRNAs regulate hERG. J Cardiovasc Electrophysiol. 2016;27(12):1472–82.

30. Baldan A, Fernández-Hernando C. Truths and controversies concerning the role of miRNAs in atherosclerosis and lipid metabolism. Curr Opin Lipidol. 2012;27(6):623–9.

31. Zhang HF, Wang YC, Han YD. MicroRNA34a inhibits liver cancer cell growth by reprogramming glucose metabolism. Mol Med Rep. 2018;17(3):4483–9.

32. Cruz-Gil S, Sanchez-Martinez R, Gomez de Cedron M, Martin-Hernandez I, Okamura DM. The macrophage phagocytic receptor CD36 promotes fibrogenic pathways on removal of apoptotic cells during chronic kidney injury. Am J Pathol. 2015;185(8):2232–45.

33. Sp N, Kang DY, Kim DH, Park JH, Lee HG, Kim HJ, Darvin P, Park YM, Yang YM. Nobiletin inhibits CD36-dependent angiogenesis in inflammation, migration, invasion, and sphere formation through the Cd36/Stat3/NF-Kb pathway. Nutrients. 2018;10(6):772.

34. Nath A, Chan C. Genetic alterations in fatty acid transport and metabolism genes are associated with metastatic progression and poor prognosis of human cancers. Scientific Rep. 2016;6:18669.