Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Mir-485-5p on the Morphology and Function of Cardiovascular System

Delai Zhang[#], Qian Nie[1#], Yanru Liu, Zaoying Li, Wen Xie[*], Li Jiang[*]

Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, 610072, China

#These authors contributed equally to this work as Co-first author

ARTICLE INFO

Original paper

Article history:
Received: October 25, 2021
Accepted: December 13, 2021
Published: January 30, 2022

Keywords:
mir-485-5p, cardiovascular system, morphology and function, target cells, cardiac fibrosis

ABSTRACT

Cardiovascular disease is a global problem that seriously endangers human health and life. At present, hypertension is considered to be a polygenic disease caused by the interaction of environmental factors and genetic factors. Various environmental factors have been proved to promote the occurrence and development of cardiovascular disease. MicroRNA (miR), as an essential gene regulatory factor in vivo, has been confirmed to participate in the regulation of many cell pathways, and its abnormal expression is closely related to a variety of human diseases. MiR-485-5p is located at 7q22.1, which has been proved to play an essential role in the tumor and cardiovascular system. Therefore, this paper discussed the mechanism of miR-485-5p on the morphology and function of the cardiovascular system. It is believed that miR-485-5p will impact the morphology and function of the cardiovascular system. Therefore, in the current study, 1655 unrelated patients with cardiovascular system diseases were simulated and analyzed based on the above background. A double luciferase reporter gene detection system verified the combination of miRNAs target recognition. The results showed that miR-485-5p significantly inhibited the luciferase activity of pGL-miR-wt but had no effect on pGL6-miR-mut. The lack of miR-485-5p can promote the activation of cardiac fibroblasts. The findings of this study can provide a new understanding and direction for the study of cardiovascular system morphology and function.

DOI: http://dx.doi.org/10.14715/cmb/2022.68.1.15

Introduction

The study of cardiovascular morphology provides a reliable basis for the clinical judgment of normal degeneration and pathological process (1). With the progress of clinical medicine, the requirement of medical quality assessment is higher and higher. Correct diagnosis is the basis of targeted and effective treatment. Traditional medicine is based on empirical medicine, while the modern medicine model is based on empirical medicine, according to the principle of evidence-based medicine, emphasizing the treatment of patients according to the basis of scientific research (2). Mir-485-5p plays an inhibitory role in many tumors (3). However, its biological characteristics and function in the morphology and function of the cardiovascular system are not clear (4).

MicroRNAs (Mirs) are endogenous, noncoding regulated RNAs that participate in the regulation of a large number of target genes (5, 6). Mir-485-5p can affect the cardiovascular system directly or indirectly, by protecting vascular endothelium from damage and inhibiting myocardial cell apoptosis, improving cardiac ischemia-reperfusion injury, preventing atherosclerosis, improving blood pressure, reducing blood lipids, and weight loss (1, 7). Jing and Mo (8) investigated the expression of mir-485-5p in gastric cancer and its prognostic value. The expression level of mir-485-5p was determined in 132 paired GC and adjacent non-tumor tissues by quantitative real-time PCR (QRT PCR). They assessed the correlation between mir-485-5p expression and clinic-pathological factors and examined overall survival using Kaplan Meier curves and Cox proportional risk regression models. Through multivariate analysis, it was confirmed that the low expression level of mir-485-5p was an independent predictor of poor prognosis in GC patients. They concluded that the expression level of mir-485-5p could be a new biomarker for the overall survival of gastric cancer patients. For the first time, Pan *et al.* (9) isolated CD44 + / CD133 + glioma stem cells (hugcs) from the glioma tissues of patients. The results of qPCR and Western blot showed that the expression of Tie1 in hugcs was significantly higher than that in CD44 - / CD133

*Corresponding author. E-mail: 1131649799@qq.com; 4712627@qq.com

glioma cells. Their study showed that Tie1 is an important factor to maintain the activity of glioma stem cells. SPIONs can induce over-expression of mir-485-5p in cells, inhibit the expression of endogenous Tie1, down-regulate the expression level of FGF2 / GDNF / GFAP / BDNF protein, and significantly reduce the survival ability of glioma *in vitro* and in *vivo* (9, 10). Kang *et al*. (11) found that mir-485-5p was down-regulated in the gastric cancer cell line. Mir-485-5p can inhibit the growth of gastric cancer cells in *vitro* and *in vivo*. They also found that mir-485-5p inhibited the metastasis and globular formation of gastric cancer cells. Flotillin-1 (flot1) is the direct target of mir-485-5p. Up-regulation of mir-485-5p can reduce the expression of flot1 in gastric cancer cells. Their study suggested that mir-485-5p may be a potential prognostic marker and play an anti-cancer role in gastric cancer by targeting flot1 after transcription. Also, they showed the expression of mir-485-5p is out of control, which is helpful for the differentiation between NSCLC patients and healthy controls, suggesting that mir-485-5p has potential for diagnosis. However, the significance and biological function of mir-485-5p in the morphology and function of heart blood system are not clear (12).

The cardiovascular system is also called the "circulatory system (2). It is composed of the heart, artery, capillary, and vein. It is a closed circulation pipe in which blood flows, supplying organs and tissues with oxygen, various nutrients, hormones, etc., and transporting wastes of tissue metabolism to excretory organs to maintain the homeostasis of the internal environment, metabolism, and everyday life activities (13). Cardiovascular disease is the leading cause of mortality worldwide. The research on the role of autophagy in heart and vascular tissue has opened up a new way for the treatment of cardiovascular diseases (14). The Kheloufi *et al*. study (15) indicated that autophagy activity needs to be maintained at an optimal level to maintain cardiovascular function. Achieving this goal is a challenge for effective treatment strategies in the future. Therefore, Kheloufi *et al*. (15) emphasized the recognition of autophagy in cardiovascular disease in recent years. Paneni *et al*. (16) provided some examples of major unresolved clinical problems encountered in the daily cardiovascular practice of caring for elderly patients. They summarized the current understanding of the mechanisms related to cardiovascular aging and the potential of new pathways related to endothelial dysfunction, mitochondrial oxidative stress, chromatin remodeling and genomic instability. Foster *et al*. (17) reviewed the properties, molecular composition, and pharmacological effects of KATP channels in various cardiovascular components (atrium, special conduction system, ventricle, smooth muscle, endothelium and mitochondria). They summarized the experiences from the existing mouse genetic model and discussed the known role of the KATP channel in cardiovascular disease, and how genetic variation of the KATP channel gene can cause human disease. Because of the importance of the cardiovascular system to the human body, cardiovascular disease has a huge impact on the human body. Therefore, based on the study of the morphology and function of the cardiovascular system, the effect of mir-485-5p on the cardiovascular system is considered (18, 19).

To explore the mechanism of the mir-485-5p influence on the morphology and function of the cardiovascular system, researchers used TargetScan, pictar, miRBase, mirwalk and other databases to query the miRNAs of Corin, and found that mir-485-5p and 3'UTR of Corin mRNA may regulate the expression of Corin (20, 21). In a study by Zhang *et al*. (21), mir-485-5p was transfected into acl6 cells to detect the expression of Corin at gene and protein levels by RT-PCR, ELISA and WB. According to the above experimental results, mir-485-5p can down-regulate the expression of endogenous Corin in acl6 cells and mir-485-5p can combine with the target recognition of Corin 3'utr and down-regulate the expression of exogenous Corin. Their study provided a new theoretical basis for further elucidating the molecular genetic mechanism of mir-485-5p on the morphology and function of the cardiovascular system).

This study aimed to evaluate the VEGF signal pathway through the regulation of VEGFR expression by mir-485-5p and explore the effect and mechanism of mir-485-5p on endothelial cell angiogenesis. Therefore, It aimed to provide a theoretical basis for designing drugs targeting mir-485-5p and then carry out targeted therapy of heart

diseases to bring new hope to patients with cardiovascular diseases.

Materials and methods

Research Object

From December 2019 to March 2020, 1655 unrelated patients with cardiovascular diseases were selected from the Affiliated Hospital of a Medical University. All patients were self-described as Han Chinese. According to the blood pressure measurement, they were divided into the EH group and control group. EH diagnosis and classification criteria are systolic blood pressure ≥ 140mmHg and diastolic blood pressure > 90mmHg without antihypertensive drugs, or antihypertensive drugs are currently taken, except for patients with obvious eh family history, secondary hypertension, chronic kidney disease, alcoholism, pulmonary hypertension, hyperthyroidism and tumor. People without major cardiovascular diseases such as eh, AF and HF were included in the control group of 786 cases, and finally, 869 cases were included in the EH group.

Cell Culture

Cells were frozen in liquid nitrogen or refrigerated at - 80 ℃ were thawed and cultured again. Cell culture is usually heated at a very fast speed, and it will return to normal temperature within 1-2min, so as to prevent water from entering the cell, forming ice crystals and affecting cell survival during the process of thawing. Adjust the temperature of the water bath pot to 37 ℃, preheat the complete culture medium containing 10% FBS, wipe it with 75% ethanol for sterilization after rewarming, and transfer it into the sterile operation platform; Add 5ml of complete culture medium containing 10% FBS to 15ml sterile centrifuge tube, take out the cells stored in liquid nitrogen tank or - 80 ℃ refrigerator, put them into water bath quickly, shake them gently until the frozen solution is completely dissolved; transfer the melted cells to the above centrifuge tube, blow them gently and mix them evenly.

Wash and dry the blood cell counting plate and cover glass, and cover the cover glass on the counting plate grid for use; add a proper amount of culture medium to blow the cells evenly to make a single cell suspension; add culture medium in 1.5ml centrifuge tube and add a certain proportion of cell suspension to dilute the cell suspension multiple times; slowly add 10 μ l to the counting pool along the side of the cover glass For the cell suspension mixed left and right, note that there is no bubble under the cover glass; count the number of cells in the four corners of the blood cell counting plate under the microscope.

MiRNA-485-5p Testing

The main components of the Trizol reagent are guanidine isothiocyanate and phenol. Guanidine isothiocyanate can lyse cells, promote the dissociation of ribosomes, separate RNA from protein, and release RNA into solution. At the same time, Trizol can maintain the integrity of RNA due to its ribonuclease inhibitor. When chloroform is added, acid phenol can be extracted, and acid phenol can promote RNA to enter the water phase. After centrifugation, the water phase layer and organic layer can be formed, so RNA can be separated from protein and DNA still in the organic phase. The RNA can be reduced by isopropanol precipitation. The expression level of miRNA-485-5p in tissues and cells was detected by miDETECT A Track™ miRNA qRT-PCR Starter Kit, and U6 was used as endogenous control. For the role of Mir-485-5p in cardiovascular disease, we describe it in Figure 1.

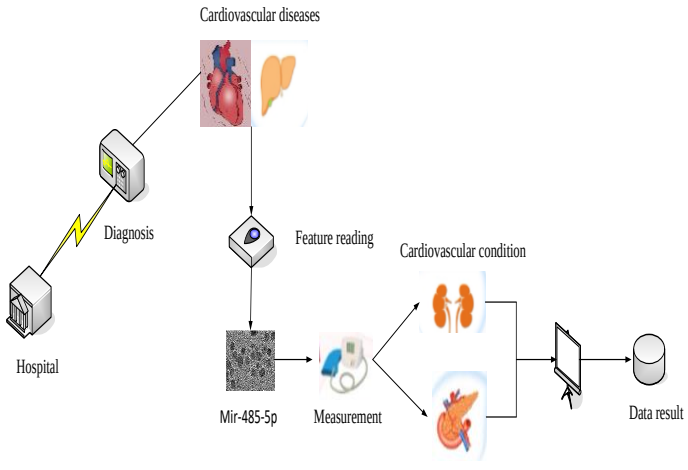

Figure 1. Mir-485-5p action process

Cell Transfection

Colorectal cancer cells were inoculated into 6-well plates and transfected when the cell density reached 30-50%. Dilute an appropriate amount of mir-485-5p

mic with 120 μ L 1 × ribofecttm CP buffer and mix gently; add 120 μ L 1 × ribofecttm CP reagent and mix gently, incubate at room temperature for 0-15min; add the mixture of ribofecttm CP to the cell culture medium and mix gently; place the culture plate in a carbon dioxide incubator of 37 °C.

Results and discussion

Detection of Corin Expression in Lysate of Transfected Cells by ELISA

Acl6 cells were cultured in vitro and divided into blank control group, miRNA-nc group and mir-485-5p group. The expression of endogenous Corin mRNA in acl6 cells was detected by relative quantitative RT-PCR. The results showed that the expression of Corin mRNA in the mir-485-5p group was significantly lower than that in the blank control group and mir-nc group ($P < 0.001$), indicating that mir-485-5p can down-regulate the expression of endogenous Corin mRNA in acl6 cells.

The expression of Corin in the lysate of each group was detected by the ELISA kit. The results showed that the expression of Corin in the mir-485-5p group was significantly lower than that in the blank control group and the mir-nc group ($P < 0.001$). The determination of Corin in the lysate of acl6 cells in each transfection group is shown in Table 1. The endogenous Corin protein of acl6 cells in each group was detected by ELISA as shown in Figure 2.

Table1. Determination of corin in the lysate of acl6 cells in each transfection group

Transfection group	Corin level (M±SD, ng/ml)	P value (Multiple group comparison))	P value (Inter group comparison)
Blank control group	0.83±0.075	P<0.001	MiR-NC group: 0.123 MiR-485-5p group: <0.001
MiR-NC group	0.77±0.033	P<0.001	Blank control group: 0.123 MiR-485-5p group: <0.001
MiR-485-5p group	0.62±0.074	P<0.001	Blank control group: <0.001MiR-NC group: <0.001

Inhibitory Effect of miR-485-5p on Cell Proliferation and Invasion

In order to determine the biological role of mir-485-5p, we carried out overexpression and knockout experiments in A549 and NCI-H1299 cells. Transfection of mir-485-5p can significantly reduce the proliferation and invasion ability of A549 and NCI-H1299 cells. There was a significant difference between the two groups ($0 P < 0.05$). After 72 hours of culture, mir-485-5p could significantly inhibit the proliferation of A549 and NCI-H1299 cells. The overexpression of mir-485-5p promoted the G0 / G1 phase arrest of A549 cells and blocked tgf-p-induced epithelial-mesenchymal transformation. In addition, knockdown of mir-485-5p expression with an anti-mir-485-5p inhibitor can promote cell proliferation and invasion. The inhibitory effect of mir-485-5p on cell proliferation and invasion is shown in Figure 3.

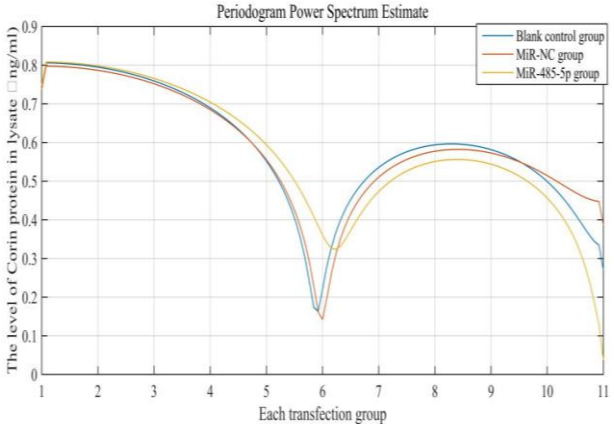

Figure 2. Detection of endogenous Corin protein in acl6 cells of each transfection group by ELISA

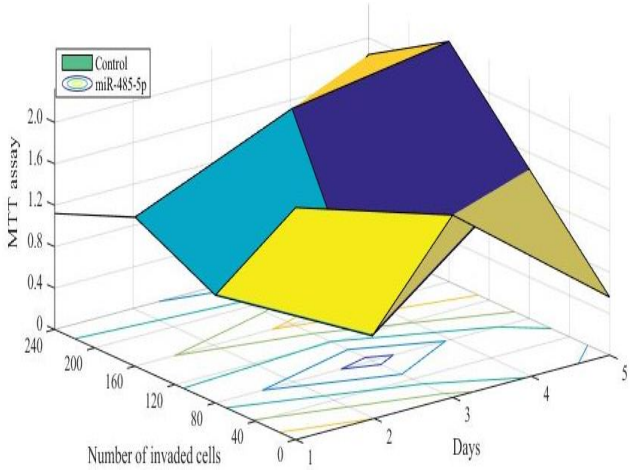

Figure 3. Inhibitory effect of mir-485-5p on cell proliferation and invasion

We performed statistics on cardiovascular heart rate, systolic blood pressure and diastolic blood

pressure in the control group and mir-485-5p group. The results are shown in Table 2:

Table 2. Cardiovascular information of control group and mir-485-5p

Grouping	Heart rate (beats/min)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Control	61.17±10.29	112.02±5.25	54.51±4.02
mir-485-5p	67.16±11.65	117.24±6.23	52.58±3.576
P	0.037	0.026	0.375

Mir-485-5p-dependent Regulation Mode of ago-2 inhibits NLRC5 Expression

MiRNA mainly recognizes the complete or incomplete complementary sites on the target gene by combining with the MRes on the target gene mRNA, that is, the seed sequence of miRNA, and most of them play the role of transcriptional inhibition, mRNA cutting or degradation. Therefore, in this paper, we use multiple miRNA gene libraries such as targets can and miRBase to predict miRNA targets. We find that nlrc5 may be the target gene of mir-485-5p. We use the double Luciferase Report to prove that the sequence of mir-485-5p and nlrc5cds are completely complimentary. In this paper, wild-type Luciferase Report vector and mutant luciferase report vector were constructed. Luciferase activity analysis showed that mir-485-5p significantly inhibited the luciferase activity of pgl-mir-wt, but had no effect on pgl6-mir-mut. In order to determine whether nlrc5mrna and mir-485-5p are directly combined in RISC, RNA immunoprecipitation experiment was carried out in this paper: the reaction between the antibody of ago-2 or IgG and cardiac fibroblast protein products was carried out. The results showed that nlrc5mrna and mir-485-5p were enriched in the ago-2 antibody group. The mir-485-5p dependent regulation mode of ago-2 inhibits the expression of nlrc5 as shown in Figure 4.

We made statistics on the changes before and after the patients in different groups, and the results are shown in Figure 5:

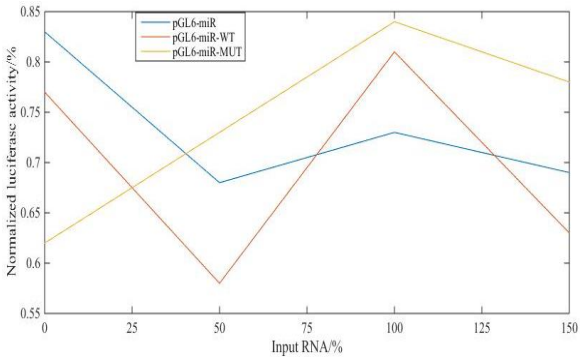

Figure 4. Mir-485-5p-dependent regulation mode of ago-2 inhibits nlrc5 expression.

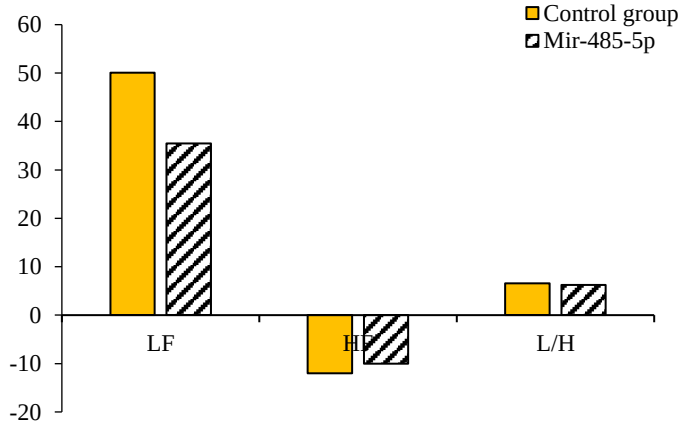

Figure 5. Changes in different groups of patients

Mir-485-5p Inhibits the Activation of Cardiac Fibroblasts

In this paper, the proliferation ability of cardiac fibroblasts and the transformation ability of fibroblasts to myofibroblasts were used to evaluate the activation level of cardiac fibroblasts. The results of CCK8 showed that the cell activity of the mir-485-5p mimic group was lower than that of the mimicnc group, indicating that mir-485-5p mimic inhibited the proliferation of cardiac fibroblasts, while mir-485-5p inhibitor promoted the proliferation of cardiac fibroblasts; edu proliferation experiment showed that the cell proliferation of mir-485-5p mimic group was lower than that of mimicnc group, indicating that mir-485-5p mimic inhibited the proliferation of cardiac fibroblasts, But mir-485-5p inhibitor promoted the proliferation of cardiac fibroblasts; WB results showed that compared with MIC NC group, the expression of col α 1 and α - SMA in mir-485-5p mimic group decreased, indicating that mir-485-5p

mimic inhibited the ability of fibroblasts to myofibroblasts, while mir-485-5p inhibitor promoted the ability of fibroblasts to myofibroblasts. The results showed that mir-485-5p could inhibit the activation of cardiac fibroblasts, and the lack of mir-485-5p could promote the activation of cardiac fibroblasts.The inhibitory effect of mir-485-5p on the activation of cardiac fibroblasts is shown in Figure 6.

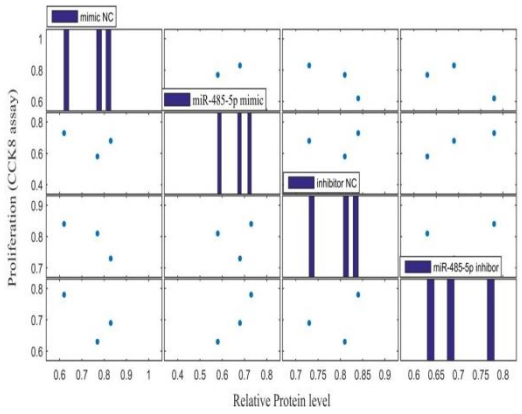

Figure 6. Mir-485-5p inhibits the activation of cardiac fibroblasts

MiRNA is a kind of small non-coding RNA, its expression level is lower than protein-coding mRNA, but it affects the occurrence and development of various diseases (22, 23). At present, the research on the effect of mir-485-5p on the morphology and function of the cardiovascular system shows contradictory conclusions, which may be caused by the selection of different myocardial fibrosis models (24). Mir-485-5p promoted cardiac fibrosis in the isoproterenol-induced model, while mir-485-5p showed cardioprotection in the acute myocardial infarction model (25). Therefore, the role of mir-485-5p in cardiac fibrosis deserves further study. In this paper, we used the TAC-induced stress overload model of cardiac fibrosis to confirm the protective effect of mir-485-5p on cardiac fibrosis. A large number of studies have shown that a variety of miRNAs are highly or specifically expressed in the heart. MiRNAs can regulate the differentiation, proliferation, hypertrophy and apoptosis of cardiac cells. The expression of miRNAs increased within hours after acute myocardial infarction, which may become a new biochemical marker to evaluate the severity of acute myocardial infarction; single nucleotide polymorphism in the process of miRNAs expression regulation, SNPs of miRNAs seed sequence may affect the specificity of silencing target mRNA. At present, the most classical experiment to verify miRNAs target recognition binding gene is double luciferase reporter gene detection system. If the luciferin expression of firefly is inhibited after the correction of luciferase, it can be concluded that miRNAs can regulate the target gene 3'UTR.

In this study, we found a new mir-485-5p / nlrc5 regulatory axis and its role in cardiac fibrosis and found that the lack of mir-485-5p in cardiac fibroblasts will aggravate the process of cardiac fibrosis. The results of this study provide strong evidence for mir-485-5p to protect the heart and reduce fibrosis. The discovery of the mir-485-5p / nlik5 regulatory axis can provide a new understanding and direction for the study of cardiovascular system morphology and function

Acknowledgments

None.

Conflict interest

The authors declare no conflict of interest.

References

1. Sartori EM, Magro-Filho O, Silveira Mendonca DB, Li X, Fu J, Mendonça G. Modulation of Micro RNA Expression and Osteoblast Differentiation by Nanotopography. nt J Oral Maxillofac Implants 2018; 33(2).
2. Shimokawa H, Sunamura S, Satoh K. RhoA/Rho-kinase in the cardiovascular system. Circ Res 2016; 118(2): 352-366.
3. Jiang P, Xu C, Chen L et al. Epigallocatechin-3-gallate inhibited cancer stem cell–like properties by targeting hsa-mir-485-5p/RXRα in lung cancer. J Cell Biochem 2018; 119(10): 8623-8635.
4. Reiss AB, Grossfeld D, Kasselman LJ et al. Adenosine and the cardiovascular system. Am J Cardiovasc Drugs 2019; 19(5): 449-464.
5. Petkevicius V, Salteniene V, Juzenas S et al. Polymorphisms of microRNA target genes IL12B, INSR, CCND1 and IL10 in gastric cancer. World J Gastroenterol 2017; 23(19): 3480.

6. Chen R-C, Wang J, Kuang X-Y et al. Integrated analysis of microRNA and mRNA expression profiles in HBx-expressing hepatic cells. World J Gastroenterol 2017; 23(10): 1787.
7. Lu H, Lei X, Liu J, Klaassen C. Regulation of hepatic microRNA expression by hepatocyte nuclear factor 4 alpha. World J Hepatol 2017; 9(4): 191.
8. Jing L, Mo X. Reduced miR-485-5p expression predicts poor prognosis in patients with gastric cancer. Eur Rev Med Pharmacol Sci 2016; 20(8): 1516-1520.
9. Pan Z, Huang Y, Qian H, Du X, Qin W, Liu T. Superparamagnetic iron oxide nanoparticles drive miR-485-5p inhibition in glioma stem cells by silencing Tie1 expression. Int J Biol Sci 2020; 16(7): 1274.
10. Querfeld C. The emerging role of micro RNA s in the molecular diagnosis of mycosis fungoides. Br J Dermatol 2019; 180(5): 984-985.
11. Kang M, Ren M-P, Zhao L, Li C-P, Deng M-M. miR-485-5p acts as a negative regulator in gastric cancer progression by targeting flotillin-1. Am J Transl Res 2015; 7(11): 2212.
12. Gao F, Wu H, Wang R et al. MicroRNA-485-5p suppresses the proliferation, migration and invasion of small cell lung cancer cells by targeting flotillin-2. Bioengineered 2019; 10(1): 1-12.
13. Oesterle A, Laufs U, Liao JK. Pleiotropic effects of statins on the cardiovascular system. Circ Res 2017; 120(1): 229-243.
14. Lilleness BM, Frishman WH. Ghrelin and the cardiovascular system. Cardiol Rev 2016; 24(6): 288-297.
15. Kheloufi M, Rautou P-E, Boulanger CM. Autophagy in the cardiovascular system. Med Sci 2017; 33(3): 283-289.
16. Paneni F, Diaz Cañestro C, Libby P, Lüscher TF, Camici GG. The aging cardiovascular system: understanding it at the cellular and clinical levels. J Am Coll Cardiol 2017; 69(15): 1952-1967.
17. Foster MN, Coetzee WA. KATP channels in the cardiovascular system. Physiol Rev 2016; 96(1): 177-252.
18. Sack MN, Fyhrquist FY, Saijonmaa OJ, Fuster V, Kovacic JC. Basic biology of oxidative stress and the cardiovascular system: part 1 of a 3-part series. J Am Coll Cardiol 2017; 70(2): 196-211.
19. Duke JM, Randall SM, Fear MW, Boyd JH, Rea S, Wood FM. Understanding the long-term impacts of burn on the cardiovascular system. Burns 2016; 42(2): 366-374.
20. Liu H, Hu G, Wang Z et al. circPTCH1 promotes invasion and metastasis in renal cell carcinoma via regulating miR-485-5p/MMP14 axis. Theranostics 2020; 10(23): 10791.
21. Zhang F, Li Q, Zhu K et al. LncRNA LINC00265/miR-485-5p/IRF2-mediated autophagy suppresses apoptosis in acute myeloid leukemia cells. Am J Transl Res 2020; 12(6): 2451.
22. Abramowicz A, Story MD. The long and short of it: The emerging roles of non-coding RNA in small extracellular vesicles. Cancers 2020; 12(6): 1445.
23. Guo X, Yin B, Wang C, Huo H, Aziziaram Z. Risk assessment of gastric cancer in the presence of Helicobacter pylori cagA and hopQII genes. Cell Mol Biol 2021; 67(4): 299-305.
24. Song R, Hu X-Q, Zhang L. Mitochondrial MiRNA in cardiovascular function and disease. Cells 2019; 8(12): 1475.
25. Chen Y, Chen X, Li H et al. Serum extracellular vesicles containing MIAT induces atrial fibrosis, inflammation and oxidative stress to promote atrial remodeling and atrial fibrillation via blockade of miR-485-5p-mediated CXCL10 inhibition. Ciln Trans Med 2021; 11(8): e482.