Pre-diagnostic role of platelet miRNA in coronary heart disease of healthy overweight subjects via platelet leptin receptor activation

Yakubu Abdulrahman[1,2], **Azrina Azlan[3]**, **Loh Su Peng[3]**, **Sabariah Md Noor[1,*]**

ABSTRACT

Obesity and overweight have become a global problem that development of various coronary artery diseases (CAD), such as myocardial infarction, atherosclerosis and congestive heart failure. Effective diagnosis is needed for effective treatment and prevention, particularly in healthy overweight subject. Platelets is an important component of hemostatic balance, that maintains the coagulation physiology. Platelets are involved in different pathological events such as thrombosis and CAD in various inflammatory conditions. There are evidences that highlight an important role of miRNA in regulation of gene expression profiling in platelets. Current hypothesis has shown the likelihood of using miRNAs as diagnostic markers in the event of CAD. This review article describes the association between overweight/obesity and platelets activation in elucidating the gene expression profiling in platelet miRNAs in CAD patient. The application of platelet miRNAs as predictive markers in overweight/obese individuals may become a marginal milestone in the history of this diet-related disorder treatment.

Key words: Overweight, Obesity, Platelet activation marker, Platelet miRNA, Coronary Artery Disease.

[1]*Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400 Serdang, Selangor, Malaysia*

[2]*Department of Haematology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto, P.M.P 2346, Sokoto, Northern western Nigeria*

[3]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400 Serdang, Selangor, Malaysia*

Correspondence

Sabariah Md Noor, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400 Serdang, Selangor, Malaysia

Email: md_sabariah@upm.edu.my

Check for updates
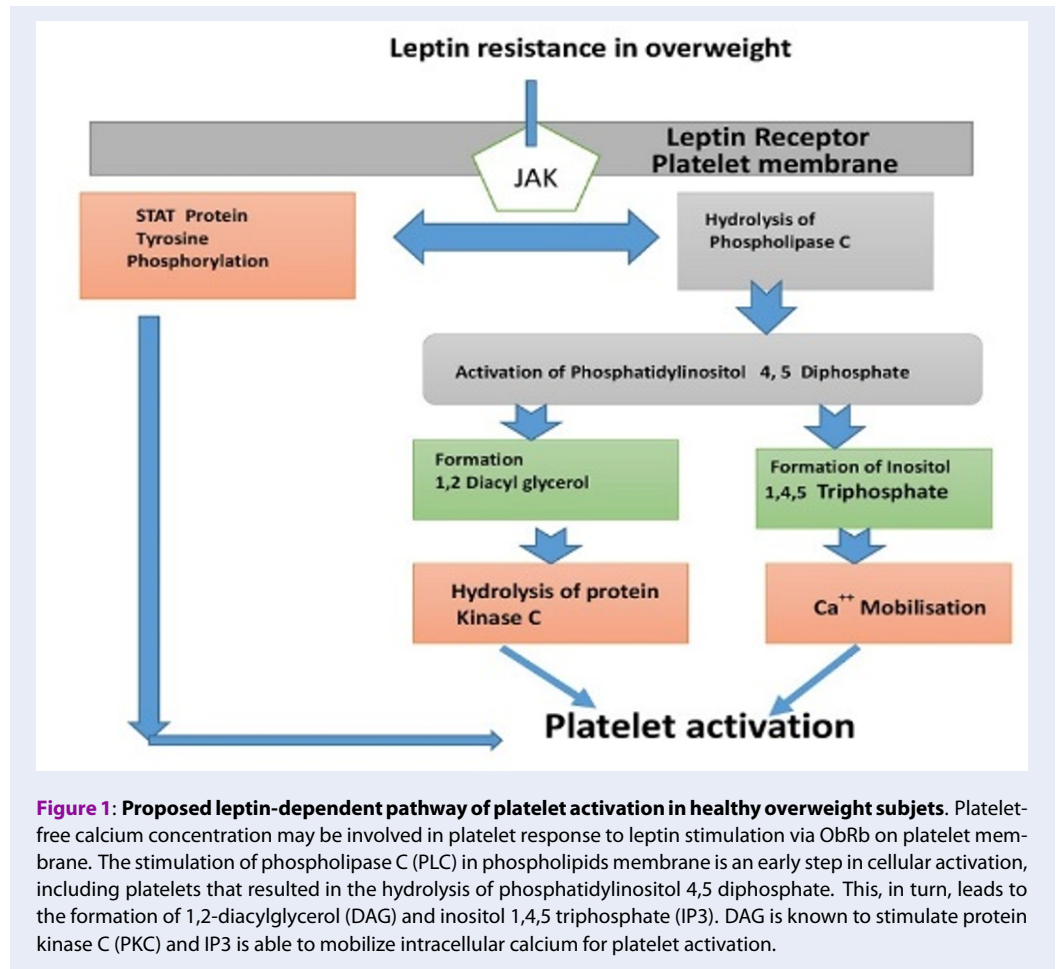
BioMedPress
The Open Access Publisher

INTRODUCTION

Overweight is a major public health issue, which affects conspicuous portion of the world population. It constitutes a risk factor for metabolic events that predispose an individual to diabetes, hypertensions and atherosclerosis. Overweight has been recently linked to low grade chronic inflammatory diseases, like type 2 diabetes, cardiovascular disease and cancer. However, acute inflammatory responses are important for defensive and homeostatic mechanisms of healing, repair and tissue regeneration[1]. Chronic inflammatory conditions include hypertension, diabetes, cardiovascular disorders (CVDs) and cancer[2]. A report by World Health Organization (WHO) in 2016 showed that over 1.9 billion adults above 18 were overweight worldwide, and 650 million adults were obese. These figures show, that 39 % of adults $\geq$ 18 years of age (representing 40 % women and 39 % men) were overweight, while 13 % of world adult population (representing 15 % women and 11 % men) were obese. Thus, from 1975 to 2016[3], the global prevalence of overweight and obesity cases has nearly tripled. Epidemiological evidence and clinical studies have clearly demonstrated, that overweight predisposed an individual to an increased incidence of thrombotic occlusion, or atherosclerosis that corre-

lated with epicardial fat thickness[4] and visceral obesity[5]. Endothelial dysfunction, monocytes recruitment, inflammation and platelet activation are associated with dyslipidemia. Studies showed that leptin resistance could be a possible candidate that links obesity with cardiovascular diseases[6–8]. Meta-analysis data by Coronary Heart Prevention of West Scotland Studies suggested, that leptin could induce CHD independently[9]. Although, leptin receptor (ObRb) on platelet membrane can signal angiogenesis, regulate bone formation, accelerate vascular endothelial injuries and further enhance platelet aggregation via platelet leptin receptor[10,11] (**Figure 1**).

Activated platelet releases cytokines, chemokines, hemostatic factor, adhesion protein, antigen receptor, mitogens and platelet miRNA[12]. Platelet miRNA gene expression pattern has been demonstrated to be more accurate and precise, compared to mRNA expression pattern in differentiation and characterizing various diseases (such as atherosclerosis, myocardial infarction and cancers[13]). Thus, this review article describes the relationship between overweight, leptin resistance, platelet activation and platelets miRNAs expression profiling, as well as to advocates the usefulness of miRNAs as predictive biomarkers of CAD in healthy overweight subjects.

Figure 1: **Proposed leptin-dependent pathway of platelet activation in healthy overweight subjects**. Platelet-free calcium concentration may be involved in platelet response to leptin stimulation via ObRb on platelet membrane. The stimulation of phospholipase C (PLC) in phospholipids membrane is an early step in cellular activation, including platelets that resulted in the hydrolysis of phosphatidylinositol 4,5 diphosphate. This, in turn, leads to the formation of 1,2-diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP3). DAG is known to stimulate protein kinase C (PKC) and IP3 is able to mobilize intracellular calcium for platelet activation.

THE ASSOCIATION BETWEEN PLATELET ACTIVATION AND ATHEROSCLEROSIS IN OVERWEIGHT

Overweight can be referred as a leptin resistance condition. Leptin is a product of adiponectin from *ob* gene[14], that signals the energy stores level via receptor in central nervous system. Leptin regulatesactivity of the hypothalamic nuclei, responsible for appetite and energy homeostasis. This hormone is anti-overweight, with its level decreasing during fasting and increasing after overfeeding in order to maintain energy status. Thus, leptin resistance in overweight subjects may signify the relationship between leptin concentration and overweight[15]. Multiple evidences have demonstrated that this hormone could engage in many pathophysiological pathways that could result in leptin resistance in different cells, arterial thrombosis formation[16], platelet activation and aggregation[17], arterial hypertension[18] and vascular response to in-

flammation[19,20]. Other studies have shown that leptin is a prognosticator of atherosclerosis, myocardial infarction, stroke and coronary artery episode which is independent of body fat[21].

The overall ability of this hormone to increase human platelet activation has been suggested in OB/OB mice and healthy control subjects[11,21]. However, Ozata *et al.* did not observe leptin on epinephrine, collagen and ADP platelet activation in both obese and overweight healthy subjects, and in leptin-deficient individuals[22]. Therefore, the notion of presence of leptin receptor on platelet membrane, signaling the prothrombotic episode of leptin resistance is still controversial. However, the association between platelet activation in overweight/obesity with dyslipidemia and endothelial dysfunction, triggers human platelet activation and aggregation, thus further enhancing the risk of atherosclerosis events[23]. Furthermore, studies have shown that obesity triggers platelet activation via release of an inflammatory mediator that activates multiple internal signaling networks, includ-

ing platelet miRNA [24]. The role of miRNA (miR) on leptin resistance has been demonstrated in several gene expression profiles. MiRNA plays a modulation role in different metabolites. Thus, the exact effect of platelet miRNA on leptin in hypothalamic center remains elusive. The up-regulation of miR-200b and miR-200a were observed in hypothalamus center of obese-deficient ob/ob mice, and down-regulation of miR-200b and miR-200a were reported after leptin treatment in the hypothalamus [25]. Reduced level of miR-200b and miR-200a in the hypothalamic center induced the expression of both insulin and leptin receptors that signal the reduction of fat deposit and further restored insulin function in the liver [26]. These miRNAs were present in activated platelets, suggesting that up regulation of miR-200a and miR-200b from activated platelets in overweight/obesity was altered (**Table 1**). Such alteration influenced leptin and insulin receptor signaling pathways in the hypothalamic nuclei [25] which could serve as a target for therapeutic intervention in obesity/overweight individuals.

PLATELET ACTIVATION AND PROTEIN SYNTHESIS

Platelets are made up of three secretory granules: the α-granules, lysosomes and dense granules [27]. The content of these granules is released into plasma after platelet activation, which may enhance and promote elevation levels of pro-atherogenic proteins. This includes growth factors (TGF-β, PDGF, bFGF and EGF), adhesion proteins (fibronectin, fibrinogen, P-selectin, thrombospondin, vWF, vitronectin, receptor complex glycoproteinIba-V-IX (GPIba-V-IX,), collagen receptor GP VI and GPIIb-IIIa), epithelial neutrophil activating protein 78 (ENA-78), chemokines CXC chemokine ligand 4 (CXCL4), RANTES (CCL5), platelet factor 4 (PF4), coagulation factors (factor V, XI, XIII), α2-antiplasmin, PAI-1 (plasminogen activator inhibitor), TFPI (tissue factor pathway inhibitor), protein S, antithrombin, plasminogen, cytokine-like factors (CD40L, β-thromboglobulin and IL-1β) and miRNA [28] (**Figure 2**).

REGULATORY AND BIOGENESIS OF PLATELET MIRNA

MiRNAs are a short class of non-coding endogenous RNAs, that post-regulate gene expression transcriptionally via either translational mRNA degradation, or repression. MiRNAs play crucial roles in controlling biochemical functions and mechanisms

of different types of cells, including cell differentiation, developmental timing, tumorigenesis, proliferation, apoptosis as well as thrombosis and platelet functions. Similarly, miRNA itself as a regulatory element, is coordinately modulated by multifarious effectors when carrying out basic functions, such as miRNA editing, single-nucleotide polymorphism, circadian clock and methylation. Platelet miRNAs act together in order to fine-tune and regulate a wide variety of this molecules in form of mRNA in different cellular functions. Those include aggregation, cell adhesion, proliferation, activation, chemotaxis, coagulation, proteolysis and cell survival. During these events, the mRNA is synthesized from DNA transcription and further translated into protein. Certain specific regions of these mRNA molecules are not normally translated into proteins. These regions include the 5′ untranslated region (5' UTR), 5′ cap, poly-A tail and 3′ untranslated region (3' UTR). 3′ UTR often contains regulatory site that influences and regulates the post-transcriptional gene expression profiling [29]. Britton and Davidson, (1969) [30] postulated that "activator" mRNA transcript may work to turn on and off genes as predicted by Watson-Crick base pairing to region, located within genes [31]. Three major forms of sRNAs regulation in plants and animals were identified, which include small interfering RNAs (siRNA), miRNAs (miRNA) and piwi-interacting RNAs (piRNA). Landry *et al.*, 2009 [32] found, that the platelet miRNA was not usually translated into protein, but it regulated mRNA by annealing to the recognition site on 3' UTR of mRNA, coding gene sequence of 2-8 nucleotide, complementary to miRNA seed region [33]. This pairing was based on Watson-Crick nucleotide base pairing, such as miRNA-mRNA. The hybridization of these complexes does not require full complementary homology [34] and complete homolog usually leads to degradation of a target mRNA, while partial pairing homology is a translational repression. Therefore, miRNA influences degradation to regulate mRNA by switching the functional gene transcript on and off, while translational repression of miRNA regulates the function of the protein expression [34,35]. This functional role of miRNA presumes a wide range of miRNA-mRNA interactions, following either convergent (many miRNA-Single targets), or divergent (single miRNA-many target mRNA) interaction. This results in robust pathways that regulate the synthesis of protein in a cell [35].

Currently, the nature and extent of the involvement of platelet miRNAs in a non-(protein)-coding ribonucleic acids (RNAs) in physiology and pathological
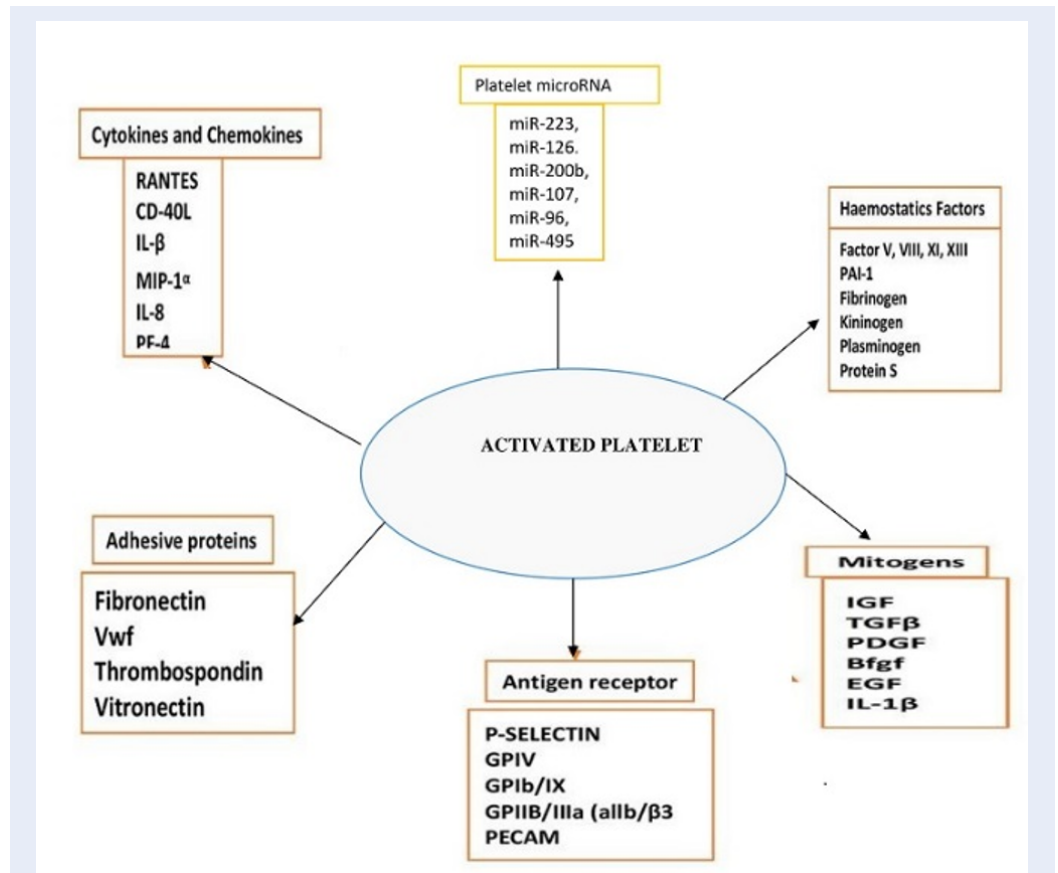
Figure 2: **Activated platelet releases both activation and inflammation makers, including the platelet miRNA**. Blood platelets are important for coagulation physiology and maintaining hemostatic balance, which is involved in various pathologies, such as thrombosis and atherosclerosis. Anucleated platelets are able to trigger protein synthesis via mRNA translation for blood platelets function and is regulated by miRNA molecules. Recent works postulated the possibility of using miRNAs as biomarkers for atherosclerosis and ischemic episodes.

processes (in both plant and animals) has become clearer [35]. More than 280 miRNAs have been recognized and characterized from human nucleated platelets [31,35]. The miRNAs biosynthesis in biological systems is strictly regulated.

The biogenesis of platelet miRNAs began with transcription of the gene in non-coding region of miRNA using RNAs poly-III from the genome of megakaryocytes, harboring miRNA gene in either intergenic or intronic at promoter site. The first primary miRNA as a non-coding transcript is known as pri-miRNA, which acts upon by RNA poly III (called "Drosha"), that bounds to the microprocessor subunits DGCR-8 (di-george syndrome critical region-8) to produce a small length hairpin called pre-miRNA stemloop with 80-110 nucleotides base in length. Exporting-5 assists the nuclear export of pre-miRNA into the cytoplasm [36]. The presence of pre-miRNAs in the cellular cytoplasm is identified by ribonuclease (RNase) or Dicer enzymes which is a part of transactivation response binding protein (TRBP) complex. The complex Dicer/TRBP cleaves the pre-miRNAs loop to release a short length of double stranded miRNA, such as miRNA/miRNA, * or duplex [37]. The duplex (such as miRNA/miRNA) are transported into the RNA Induced Silencing Complex (RISC) [38,39] and Helicase enzymes are used to unwind the double stranded miRNA into a single 22 nucleotides base, which is released as matured miRNA. The 2nd strand miRNA, known as passenger strand is thought to be digested by the RISC complex substrate. Argonauts (Ago) protein family will assemble in RISC and provide stability and protection to the mature miRNA strand against RNase enzymes activities and will further guide the matured miRNA to its target $3'$-untranslated region (UTR) of mRNA transcripts [40] (**Figure 3**). However, the inverse modulation of platelet miRNA profiling during adipose tissue development in overweight and

obesity is important for understanding miRNA dys-regulation in adipose tissue of both obese humans and mice. Such modulation enhances chronic inflammation observed in obesity subject with insulin resistance [41]. Thus, a single nucleotide base change of mature and pre-miRNA may drive the emerging of new miRNA by influencing the biological function of these cells [40].

PATHOLOGICAL ROLE OF PLATELET MIRNA IN CORONARY ARTERY DISEASE

The mutations or single-nucleotide polymorphism (SNP) that occur in the biogenesis of both mRNA and miRNA can be classified as follows:

(1) Mutations, that affect biogenesis enzymes of miRNA, or the promoter region of mRNA.

(2) Mutations, that affect the 3' UTR region of the mRNA.

(3) Mutations that affect the miRNA seed region. [42]

Mutation or SNP that affects miRNA biogenesis enzymes

SNP in the processing regions can be categorized as pri-miRNA, pre-miRNA, mature miRNA sequences and mRNA biogenesis machinery (promoter region). In fact, gene variations may influence either miRNA hairpin and biogenesis proteins, or enzymes in the processing accuracy [43].

Mutation in the miRNA seed region

SNP in the seed region of miRNA may reduce the binding strength between miRNA and mRNA target sites, which will influence hundreds of gene expressions [44].

Mutation in the mRNA 3' UTR-region

Approximately 180,000 mutations or SNP found within the 3' UTR region mRNA were demonstrated with its corresponding mature miRNA 2,600 sequences. These were found in database sequence [45]. Mutations in the seed region of miRNA and 3' UTR region of mRNA are almost identical in terms of variations and regulation of miRNA-mRNA* complex, affecting functions and disease vulnerability [42]. However, further molecular mechanism underlying disease-associated with 3' UTR SNPs in mRNA still require further investigation [46]. For example, SNP meta-analysis of 8,120 patients and 8,364 controls identified four different SNPs in the following miR-NAs (miRNA-149, miRNA-196a2, miRNA-499 and

miRNA-146a), which increased patients CAD vulnerability [47]. Other articles have demonstrated that miR-146a was upregulated in CAD [47,48] due to the change of G to C in pre-miRNA. That affected the expression of mature miRNA-146a in CAD patients [49]. That finding was confirmed by Wang et al. and showed, that C allele was greater than G allele in gene that predisposed individuals to CAD [50,51]. However, in type 2 diabetes patients with ischemic stroke, the miR-146a was downregulated (**Table 1**) [52]. Other abundant miRNAs found in platelets miRNA were miR-223 and miR-126. In addition, miR-223, miR-107, miR- 495, miR-96 and miR-200b were actively involved in various platelet functions, such as platelet release, adhesion and aggregation, [53] as summarized in **Table 2**.

MIRNA AS BIOMARKERS

The fact of discovery of miRNA as a stable molecule in plasma and serum is surprising despite the level of RNase enzymes activities [54]. The expression of miRNA in tissue, or organ-specific, and its release into the plasma in response to tissue injury is considered to be a potential biomarker for different diseases [55]. The hypothesis that miRNA in blood cells could be used as diagnostic parameter in different diseases was first postulated by Mitchell *et al.*. He showed, that miRNA remained highly stable in both plasma and serum even after prolonged storage at room temperature, or repeated cycles of freezing and thawing. For example, tumor-derived miRNA could easily be identified in plasma sample from cancer patient with standard laboratory procedure [54,56,57]. The diagnostic role of circulating miRNA has been evaluated not only in cancer, but also in other clinical disorders, such as hepatic disease [58], heart failure [59] and diabetes [60]. Few studies have claimed platelets in obesity and overweight healthy individuals as the culprit factor, responsible for coronary artery disease [61]. The application of specific platelet miRNAs as markers for platelet activation will be a marginal milestone in the history of this diet-related disorder.

PLATELET- MIRNA IN ENDOTHELIAL AND VASCULAR SMOOTH MUSCLE (VSCM) CELLS IN CORONARY ARTERY DISEASE (CAD)

Large scale studies, presented by Nagalla *et al.* and Landry *et al.* found, that thirty different platelet miRNAs modulate platelet and endothelial angiogenesis (angio-miRs) [32,35]. These miRNAs, (miR-21, miR-200, miR-210 and miR-126), are well known
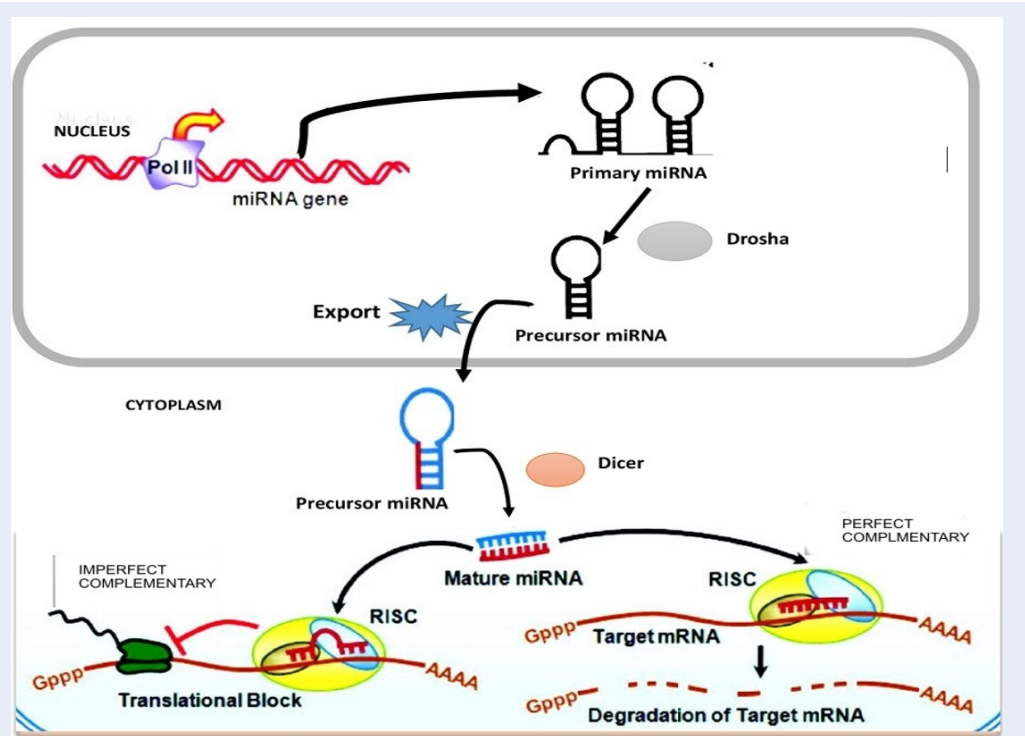
Figure 3: **Mechanism of platelet miRNA to induce repression of mRNA**. Production pri-miRNA* from miRNA genes is processed by RNase-II/III and the pri-miRNA form is cleaved by Drosha-DGCR8 complex to produce pre-miRNA* in the nucleus. Pre-miRNA* is exported by exportin-5 from the nucleolus to the cytoplasm. The pre-miRNA* in the cytoplasm is further digested by another enzme called RNase-Dicer complex with TRBP that catalyzed the pre-miRNA* hairpin to mature miRNA duplex. Matured miRNA strand is transported into RISC in assemble of argonaute-2 (Ago2) proteins to guide the silencing of target mRNA to fully complementary matched for degradation and partially complementary matched for repression. *This figure is adopted and modified DOI: 10.5772/ intechopen.81847.*

to play critical roles in the vessel and capillary formation. The regulation of angiogenesis by miRNA in the endothelial cell was confirmed, using Dicer-knockdown mice experiment, demonstrating the essential role of Dicer in miRNA biogenesis [62,63]. Furthermore, Dicer gene deletion was shown to cause early death of mice during the embryonic stage, due to impaired angiogenesis [64]. In addition, Dicer knockdown of vascular smooth muscle cells VSMC specific caused the late embryonic death due to internal bleeding [65], suggesting that platelet miRNA was an integral part of vascular development. Therefore, clusters of miR-221 and miR-222 are the most abundant and well distributed miRNAs across these three cells(platelet, endothelial and VSMC) [66,67]. Both miR-221 and miR-222 function as pro-inflammatory inhibitor of angiotensin II and reversed leucocytes adhesion (*in vivo* and *in vitro)* [68], suggesting a possible role of these miRNA clusters in CAD dysregulation. The endothelial-enriched miR-92a has been proposed as a possible therapeutic target after treatment with anti-miR-92a oligonucleotide.Such treatment improved the formation of blood vessel and recovery of cardiovascular disorder in acute myocardial infarction in mice [67]. MiR-143/145 is the most abundant miRNA found in VSCM and is well characterized as part of the same bi-cistronic cluster. These miRNAs target multiple mRNAs to influence VSCM differentiation and simultaneously reduce proliferation [68]. The delivery of miR-145 by lentiviral in endothelial and VSCM inhibits monocyte/macrophages recruitment and infiltration, thus reducing inflammation and limiting plaque formation. These results suggest a new therapeutic target in order to decrease atherosclerotic progression and increase plaque atrial stability [69].

ROLE OF PLATELET MIRNA AS A MARKER IN CORONARY ARTERY DISEASE (CAD)

Previous reports showed that more than 80 different diseases were associated with dysregulation, or mutation in miRNAs[70]. The mechanisms of platelet activation in various thrombotic diseases were well established[71]. The accurate etiology of miRNA in activated platelet in overweight and obesity is not clear, but obesity is always a risk factor for atherothrombotic episodes[71]. Landry *et al.* 2009 demonstrated that the platelets and megakaryocyte miRNAs had 219 different types of miRNA in platelet expression patterns and profiles[72]. The authors observed three most abundant miRNAs in human platelets; miR-19a, let-7c, and miR-223. Binding of miRNA-223 to 3' UTR region of P2Y12 mRNA receptor in HEK293 cell line repressed P2Y12 gene and decreased the activities of both platelet and megakaryocyte. However, dysregulation of miRNA-233 was observed in hyperreactive platelet, leading to overexpression of miRNA-223[73]. Thus, miR-223, miR-197 and miR-126 were involved in platelet hyperreactive and endovascular inflammation, stretching the application of these miRNAs as predictive biomarkers for diagnosis of CAD (**Table 1**)[73].

CAD, CORONARY ARTERY DISEASE; QRT-PCR, REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION PCR

In 2013, Osman and Fälker discovered 281 transcripts, of which 228 were mature miRNAs and 53 were pre-miRNAs. Six miRNAs: miR-339-3 p, miR-15 a, miR-365, miR-495, miR-361-3 p and miR-98, were upregulated, or downregulated in hyperreactive platelets[28]. The level of expression pattern, or characteristic of miRNAs in platelets were associated with a procoagulant (such as thrombin stimulation). Nagalla *et al.,* 2011 demonstrated that there were 284 miRNA transcripts in human platelets, by which 74 were differentially expressed, based on the platelet reactivity[35]. Seven miRNA expression profiles (miR-320b, miR-190, miR-320d, miR-19b, miR-34b, miR-320c and miR-320a) were shown to have a strong relationship with the degree of platelet response to adrenaline[35]. In fact, the most abundant expressed platelets miRNA is miR-223, followed by miR-126[57]. Others were identified as miR-200b, miR-107, miR-96 and miR- 495[53]. All of these miRNAs were involved in platelet hyperreactivity (such as platelet activation, adhesion and aggregation, as summarized in **Table 2**).

Table 1: Changes in human platelets miRNA level in coronary artery diseases (CAD)

Platelet miRNA	Disease	Group	Methods	Reference
↓mir-223	Diabetes mellitus type 2 patients without ischemic stroke	Human/7 diabetes, 8 control	qRT-PCR	52
↓miR942, ↓20a ↑miR483-5p ↑miR146a-5p*	Acute coronary syndrome (NSTEMI)	Human/13 CAD, 13 non-	qRT- PCR	74
↑miR127-3p ↑miR221-3p ↓miR186-5p* ↓miR185-5p*	Acute coronary syndrome (STEMI)	Human/44 CAD, 22 non-CAD	qRT-PCR	75
↑miR454*↑miR545:9.1 ↑miR615-5p ↑miR340* ↑miR451*↑miR624 ↓ miR-12801*	Premature coronary artery disease	Human/12 premature CAD, 12 controls	Microarray confirmed by qRT-PCR	53
↑miR-144, ↓miR-146a*	Diabetes mellitus type 2 patients with ischemic stroke	HUMAN/6 ischemic stroke ,8 controls	Microarray qRT-PCR	52
↑miR340*↑miR624*	Mature coronary artery disease	Human/40 CAD, 40 controls	Microarray Confirmed by qRT-PCR	53

NSTEM, Non Segment Elevation Myocardial Infarction; STEM, Segment Elevation Myocardial Infarction. * Shows the platelet miRNA marker of atherosclerosis

Table 2: **Pathophysiology of platelet miRNA in platelet activation**

	Platelet miRNA	Target protein (mRNA)	Function	Pathophysiology	Implication	Ref
1.	miRNA-223	P2Y12 receptor for ADP	Regulate platelet Hyperreactive	miRNA-233: Dysregulation of miRNA-233 will results in hyper-reactive platelet, leading to upregulation miRNA-223↑	miR-223 and miR-197 are platelet activation miRNA involved in vascular inflammation and have been shown as markers in the diagnosis of CAD.	[73,76]
2.	miRNA-200b	cAMP-dependent PKA	Keep platelet in hyporeactive state	miRNA-200b: Dysregulation in obesity/overweight subjects lead to release of plate αlpha- and dense granules that harbor inflammatory molecules in platelet. miRNA-200b inhibits endothelial angiogenesis upregulates↑	Overexpression of miR-200b in platelet activation may be used as a diagnostic maker of CAD	[77,78]
3	miR-107	CLOCK/Bmal1	vWF gene is regulated by CLOCK/Bmal1 complex in normal physiological state	miRNA-107: Dysregulation increased the plasma level of vWF↑	Association with a prothrombotic state that promote CAD	[79]
4.	Member of miR-17-92 cluster (miR-17)	Fibronectin	Fibronectin aids in the formation of stable arterial thrombi at the site of endothelial injury	Inhibition of fibronectin by miR-17 leads to impeded platelet coagulation and wound healing and is downregulated ↓	miR-17-92 and its family were dysregulated in CAD	[80,81]
5	miR29a*/miR-409-3p* and fibrinogen	FGA, FGB, and FGG)	Fibrinogen functions as hemostatic plug formation,	miR-29 reduce the level of mRNA genes such as FGG, FGB and FGA. Dysregulation of these miRNAs has been linked with cardiac fibrosis ↓	miR-29 family are grouped with MI-regulated member	[82,83]

Continued on next page

Table 2 continued

	Platelet miRNA	Target protein (mRNA)	Function	Pathophysiology	Implication	Ref
6.	miR-96 and miR-15	VAMP8	miR-96 and miR-15 regulate VAMP8. VAMP8 aids in platelet activation, secretion of α-granules and platelet function.	SNPs in the 3' untranslated region (UTR) of VAMP8 mRNA resulted in VMP8 mRNA dysregulation and is upregulated ↑	The consequence of reduced VAMP8 mRNA control is due to an excess of VAMP8 in platelets and higher platelet reactivity, leading to increased risk of myocardial infarction	84
7.	miR-495	Kelch protein	Platelet activation uses Kelch protein for actin and filament organization during platelet function	KLHL5 mRNA is required for platelet activation and miR-495 regulates the Kelch-like protein at 3' UTR region. Repression of this protein in megakroyocytes leads to platelet hyporeaction	?	85
8	miR-126 present in both platelet and endothelial cell	VCAM-1	The target of miRNA-126 is VCAM mRNA, and the suppression of VCAM leads to decreasing infiltration of leucocytes into the vascular endothelial cell	Mutation of miR-126 resulted in the expression of TNF-α that stimulate VCAM-1 expression and increase leukocyte adherence to endothelial ↓	Promotes CAD	86,87
9	miR-210.	ephrina3 receptor (efna3) and Tyrosine protein and Phosphatase(ptp1b)	The repression of the two molecules (ptp1b and efna3) by miRNA-210 enhances angiogenic property and endothelial angiogenesis	Mutation lead to downregulation of miR-210↓	Angiogenesis after MI	88
10	miR-21	Rho kinase, SPROYTY2, RhoB, BMPRII, SOD2 and PTEN	All possess anti-angiogenic functions. .	Increased expression of miR-21 inhibits angiogenesis of endothelial cells by downregulating several pro-angiogenic miR-21↑	Atherosclerosis, CAD, apoptosis and neoangiogenes	89–91

miRNA (miR), mRNA (messenger RNA), ↑: upregulation, ↓: down-regulation, CAD: Coronary ArteryDisease

Plé and colleagues in 2012 detected more than 492 different mature miRNA transcripts in active platelets[74,75,92]. A total of 15 novel miRNAs were identified from human platelet: miR-103, miR-140, miR-24, miR-185, miR-223, miR-23, miR-320, miR-25, miR-21, miR-26, miR-191, miR-423, miR-101, miR-199 and let-7[86]. This finding suggested a possible relationship between the platelet activation and the miRNA modification, which may an induce agonist-specific platelet function.

Jeanine et al., 2014 also reported the expression profile of platelet miRNA in patients with acute coronary syndrome after a series of diagnostic investigations. In blood sample of STEMI patients, miR185-5p and miR186-5p were downregulated, while miR221-3p and miR127-3p were upregulated in platelets. On the other hand, in blood samples of NSTEMI patients, miR942 and miR20a-5p were downregulated, whereas miR146a-5p and miR483-5p were upregulated in platelets (**Table 2**)[74]. Thus, in body circulation, the pathophysiology of platelet miRNA can exhibit clear characteristic differences of expression profiling in various platelet disorders. This condition however is dangerous if urgent measure is not taken, since the risk of heart failure (HF) in such case is eminent. Patients with systolic heart failure were found to have different platelet miRNA expression profiles, as compared to control subjects[56]. Platelets miRNA-150 expression level decreased more than three-fold in blood platelets of patients with heart failure, secondary to atrial fibrillation (**Table 2**)[75].

In addition, the study, conducted by Duan *et al.,* 2014 showed, that the expression of platelet miRNA-223 and miRNA-146a in patients with ischemic stroke and diabetes mellitus was significantly higher, compared to healthy donors. The expression level of these two platelet miRNAs was suggested to correlate with platelet activation (**Table 2**)[52].

CONCLUSION

The gene expression and bioinformatic analysis procedures have become the state-of-the-art methods for diagnosing a number of human diseases. Several studies have reported different miRNA expression profiles in platelets. The application of these miRNAs for diagnostic purpose in overweight healthy subjects will be a milestone in the history of treatment of this diet-related disorder. Six notable candidates of platelet miRNAs have been discovered and proposed for diagnostic accuracy in CAD upregulation: ↑ miR483-5p* ↑ miR146a-5p* ↑miR340* ↑miR624*, ↑miR451* and ↑miR454*. Current modern diagnostic laboratory method for identification of coronary obstruction is still in its early stage. Nevertheless, current molecular medicine and diagnostic methods are capable to identify if an individual is predisposed to the illness. Platelet miRNA expression profile detection is associated with platelet hyperactivity and may serve as an important marker for prevention of coronary artery disease (CAD) in human overweight/obesity.

ABBREVIATIONS

ADP: Adenosine diphosphate

BFGF: Basic Fibroblast Growth Factor

CAD: coronary artery disease

cAMP: cyclic adenosine monophosphate

CCL5: RANTES

CD40L: CD40 Ligand

CHD: Coronary heart disease

CVDs: cardiovascular disorders

CXC: Chemokines

CXCL4: Chemokine ligand 4

DAG: 1,2-diacylglycerol

DGCR 8: di-george syndrome critical region-8

DNA: Deoxyribonucleic Acid

EGF: Epidermal Growth Factor

ENA: 78- epithelial neutrophil activating protein 78

IL-1β: interleukin -1β

IP3: inositol 1,4,5 triphosphate.

miRNA: microRNA

mRNA: messenger RNA

NSTEM: Non Segment Elevation Myocardial Infarction;

ObRb: Leptin receptor

PAI-1: Plasminogen Activator Inhibitor,

PDGF: Platelet-derived growth factor

PF4: platelet factor 4

piRNA: piwi-interacting RNAs

PKC: protein kinase C

PLC: phospholipase C

RISC: RNA In-duced Silencing Complex

siRNA: small interfering RNAs

SNP: single-nucleotide polymorphism

STEM: Segment Elevation Myocardial Infarction

TFPI: tissue factor pathway inhibitor,

TGF-β: Transforming growth factor beta

TRBP: Transactivation Response Binding Protein

UTR: untranslated region

VAMP8: Vesicle-associated Membrane Protein 8

VCAM-1: Vascular Cell Adhesion Molecules-1

VSMC: Vascular Smooth Muscle Cells

vWF: von willbrand factor

WHO: World Health Organization

COMPETING INTERESTS

The author declares no conflict of interest regarding the article for publication.

AUTHORS' CONTRIBUTIONS

Sabariah Md Noor: initiate the conception and technicality; Azrina Azlan, and Loh Su Peng: guide the article publication along with flow of idea and amending the figure respectively; Yakubu Abdulrahman: do the written and revision of the paper.

ACKNOWLEDGMENTS

REFERENCES

1. Purkayastha S, Cai D. Neuroinflammatory basis of metabolic syndrome. Mol Metab. 2013;2(4):356–63. PMID: 24327952. Available from: 10.1016/j.molmet.2013.09.005.

2. Heap GA, van Heel DA. The genetics of chronic inflammatory diseases. Hum Mol Genet. 2009;18:101–6. PMID: 19297396. Available from: 10.1093/hmg/ddp001.

3. Update OEO. Obesity Update 2017. Diabetologe (Heidelb). 2017;13(5):331–41. Available from: 10.1007/s11428-017-0241-7.

4. Mazzoccoli G, Copetti M, Dagostino MP, Grilli M, Fontana A, Pellegrini F, et al. Epicardial adipose tissue and idiopathic deep venous thrombosis: an association study. Atherosclerosis. 2012;223(2):378–83. PMID: 22748278. Available from: 10.1016/j.atherosclerosis.2012.05.033.

5. Vayá A, Martínez-Triguero ML, España F, Todolí JA, Bonet E, Corella D. The metabolic syndrome and its individual components: its association with venous thromboembolism in a Mediterranean population. Metab Syndr Relat Disord. 2011;9(3):197–201. PMID: 21352080. Available from: 10.1089/met.2010.0117.

6. Werner N, Nickenig G. From fat fighter to risk factor: the zigzag trek of leptin. Arterioscler Thromb Vasc Biol. 2004;24(1):7–9. PMID: 14707035. Available from: 10.1161/01.ATV.0000110908.43721.ad.

7. Sader S, Nian M, Liu P. Leptin: a novel link between obesity, diabetes, cardiovascular risk, and ventricular hypertrophy. Circulation. 2003;108(6):644–6. PMID: 12912793. Available from: 10.1161/01.CIR.0000081427.01306.7D.

8. Yang R, Barouch LA. Leptin signaling and obesity: cardiovascular consequences. Circ Res. 2007;101(6):545–59. PMID: 17872473. Available from: 10.1161/CIRCRESAHA.107.156596.

9. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation. 2001;104(25):3052–6. PMID: 11748099. Available from: 10.1161/hc5001.101061.

10. Nakata M, Yada T, Soejima N, Maruyama I. Leptin promotes aggregation of human platelets via the long form of its receptor. Diabetes. 1999;48(2):426–9. PMID: 10334326. Available from: 10.2337/diabetes.48.2.426.

11. Konstantinides S, Schäfer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. J Clin Invest. 2001;108(10):1533–40. PMID: 11714745. Available from: 10.1172/JCI13143.

12. Mitchell AJ, Gray WD, Hayek SS, Ko YA, Thomas S, Rooney K, et al. Platelets confound the measurement of extracel-lular miRNA in archived plasma. Sci Rep. 2016;6(September):32651. PMID: 27623086. Available from: 10.1038/srep32651.

13. Edelstein LC, Nagalla S, Bray PF. MicroRNAs in Platelet Production and Activation. MicroRNAs Med. 2013;2013:101–116. Available from: 10.1002/9781118300312.ch7.

14. Stern JH, Rutkowski JM, Scherer PE. Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. Cell Metab. 2016;23(5):770–84. PMID: 27166942. Available from: 10.1016/j.cmet.2016.04.011.

15. Jr MG, Leibel RL, Seeley RJ, Schwartz MW. Obesity and Leptin Resistance: Distinguishing Cause from Effect. Trends Endocrinol Metab. 2011;21:643–51. Available from: 10.1016/j.tem.2010.08.002.Obesity.

16. Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, et al. Leptin in human physiology and pathophysiology. Am J Physiol Endocrinol Metab. 2011;301(4):567–84. PMID: 21791620. Available from: 10.1152/ajpendo.00315.2011.

17. Elbatarny HS, Maurice DH. Leptin-mediated activation of human platelets: involvement of a leptin receptor and phosphodiesterase 3A-containing cellular signaling complex. Am J Physiol Endocrinol Metab. 2005;289(4):695–702. PMID: 15886225. Available from: 10.1152/ajpendo.00125.2005.

18. Bełtowski J. Role of leptin in blood pressure regulation and arterial hypertension. J Hypertens. 2006;24(5):789–801. PMID: 16612235. Available from: 10.1097/01.hjh.0000222743.06584.66.

19. Paz-Filho G, Mastronardi C, Franco CB, Wang KB, Wong ML, Licinio J. Leptin: molecular mechanisms, systemic pro-inflammatory effects, and clinical implications. Arq Bras Endocrinol Metabol. 2012;56(9):597–607. PMID: 23329181. Available from: 10.1590/S0004-27302012000900001.

20. Allman M, Wallace M, Gaskin L, Rivera CA. Leptin induces an inflammatory phenotype in lean Wistar rats. Mediators Inflamm. 2009;2009:738620. PMID: 20150963. Available from: 10.1155/2009/738620.

21. Chen TH, Lee CJ, Wang JH, Hsu BG. High Serum Leptin Level is Associated with Peripheral Artery Disease in Geriatric Individuals. Int J Gerontol. 2018;12(3):191–5. Available from: 10.1016/j.ijge.2018.02.001.

22. Ozata M, Avcu F, Durmus O, Yilmaz I, Ozdemir IC, Yalcin A. Leptin does not play a major role in platelet aggregation in obesity and leptin deficiency. Obes Res. 2001;9(10):627–30. PMID: 11595779. Available from: 10.1038/oby.2001.82.

23. Mao X, Ait-Aissa K, Lagrange J, Youcef G, Louis H. Hypertension, hypercoagulability and the metabolic syndrome: a cluster of risk factors for cardiovascular disease. Biomed Mater Eng. 2012;22(1-3):35–48. PMID: 22766701. Available from: 10.3233/BME-2012-0688.

24. Xia L, Zeng Z, Tang WH. The Role of Platelet Microparticle Associated microRNAs in Cellular Crosstalk. Front Cardiovasc Med. 2018;5:29. Available from: 10.3389/fcvm.2018.00029.

25. Dou L, Zhao T, Wang L, Huang X, Jiao J, Gao D, et al. miR-200s contribute to interleukin-6 (IL-6)-induced insulin resistance in hepatocytes. J Biol Chem. 2013;288(31):22596–606. PMID: 23798681. Available from: 10.1074/jbc.M112.423145.

26. Benoit C, Ould-Hamouda H, Crepin D, Gertler A, Amar L, Taouis M. Early leptin blockade predisposes fat-fed rats to overweight and modifies hypothalamic microRNAs. J Endocrinol. 2013;218(1):35–47. PMID: 23576026. Available from: 10.1530/JOE-12-0561.

27. Sharda A, Flaumenhaft R. The life cycle of platelet granules. F1000 Res. 2018;7(0):236. PMID: 29560259. Available from: 10.12688/f1000research.13283.1.

28. Bijak M, Saluk J, Ponczek MB, Nowak P, Wachowicz B. The synthesis of proteins in unnucleated blood platelets. Postepy Hig Med Dosw. 2013;506:672–679. PMID: 24018431. Available from: http://www.phmd.pl/fulltxt.php?ICID=1059587.

29. Patil VS, Zhou R, Rana TM. Gene regulation by non-coding RNAs. Crit Rev Biochem Mol Biol. 2014;49(1):16–32. PMID:

24164576. Available from: 10.3109/10409238.2013.844092.

30. Britten RJ, Davidson EH, Dike S. Gene Regulation for Higher Cells: A Theory. Science. 1969;165(3891):349–357. Available from: 10.1126/science.165.3891.349.

31. Ui-Tei K, Naito Y, Nishi K, Juni A, Saigo K. Thermodynamic stability and Watson-Crick base pairing in the seed duplex are major determinants of the efficiency of the siRNA-based off-target effect. Nucleic Acids Res. 2008;36(22):7100–9. PMID: 18988625. Available from: 10.1093/nar/gkn902.

32. Landry P, Plante I, Ouellet DL, Perron MP, Rousseau G, Provost P. Existence of a microRNA pathway in anucleate platelets. Nat Struct Mol Biol. 2009;16(9):961–6. PMID: 19668211. Available from: 10.1038/nsmb.1651.

33. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature. 2011;469(7330):336–42. PMID: 21248840. Available from: 10.1038/nature09783.

34. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215–33. PMID: 19167326. Available from: 10.1016/j.cell.2009.01.002.

35. Nagalla S, Shaw C, Kong X, Kondkar AA, Edelstein LC, Ma L, et al. Platelet microRNA-mRNA coexpression profiles correlate with platelet reactivity. Blood. 2011;117(19):5189–97. PMID: 21415270. Available from: 10.1182/blood-2010-09-299719.

36. Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004;303(5654):95–98.

37. Zamore PD, Tuschl T, Sharp PA, Bartel DP, Phillip D. Double-Stranded RNA Directs the ATP-Dependent Cleavage of mRNA at 21 to 23 Nucleotide Intervals. Zamore Thomas Tuschl. 2000;101:25–33. Available from: 10.1016/S0092-8674(00)80620-0.

38. Preall JB, He Z, Gorra JM, Sontheimer EJ. Short interfering RNA strand selection is independent of dsRNA processing polarity during RNAi in Drosophila. Curr Biol. 2006;16(5):530–5. PMID: 16527750. Available from: 10.1016/j.cub.2006.01.061.

39. Siomi H, Siomi MC. On the road to reading the RNA-interference code. Nature. 2009;457(7228):396–404. PMID: 19158785. Available from: 10.1038/nature07754.

40. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005;123(4):631–40. PMID: 16271387. Available from: 10.1016/j.cell.2005.10.022.

41. Xie H, Sun L, Lodish HF. Targeting microRNAs in obesity. Expert Opin Ther Targets. 2009;13(10):1227–38. PMID: 19650761. Available from: 10.1517/14728220903190707.

42. Moszyńska A, Gebert M, Collawn JF, Bartoszewski R. SNPs in microRNA target sites and their potential role in human disease. Open Biol. 2017;7(4):170019. PMID: 28381629. Available from: 10.1098/rsob.170019.

43. Zhang W, Wang L, Zhou P, Song G, Shen M, Gan S, et al. Identification and analysis of genetic variations in pri-miRNAs expressed specifically or at a high level in sheep skeletal muscle. PLoS One. 2015;10(2):e0117327. PMID: 25699993. Available from: 10.1371/journal.pone.0117327.

44. Króliczewski J, Sobolewska A, Lejnowski D, Collawn JF, Bartoszewski R. microRNA single polynucleotide polymorphism influences on microRNA biogenesis and mRNA target specificity. Gene. 2018;640:66–72. PMID: 29032146. Available from: 10.1016/j.gene.2017.10.021.

45. Schwerk J, Savan R. Translating the Untranslated Region. J Immunol. 2015;195(7):2963–71. PMID: 26386038. Available from: 10.4049/jimmunol.1500756.

46. Vaishnavi V, Manikandan M, Munirajan AK. Mining the 3of autism-implicated genes for SNPs perturbing microRNA regulation. Genomics Proteomics Bioinformatics. 2014;12(2):92–104. PMID: 24747189. Available from: 10.1016/j.gpb.2014.01.003.

47. Xiong XD, Cho M, Cai XP, Cheng J, Jing X, Cen JM, et al. A common variant in pre-miR-146 is associated with coronary artery disease risk and its mature miRNA expression. Mutat Res - Fundam Mol Mech Mutagen . 2014;761:15–20. Available from: 10.1016/j.mrfmmm.2014.01.001.

48. Wang Y, Wang X, Li Z, Chen L, Zhou L, Li C, et al. Two single nucleotide polymorphisms (rs2431697 and rs2910164) of miR-146a are associated with risk of coronary artery disease. Int J Environ Res Public Health. 2017;14(5):4–10. PMID: 28489066. Available from: 10.3390/ijerph14050514.

49. Liu X, You L, Zhou R, Zhang J. Significant association between functional microRNA polymorphisms and coronary heart disease susceptibility UNKNOWN UNICODE CHARACTER 0202F (NARROW NO-BREAK SPACE) : a comprehensive meta-analysis involving 16484 subjects. Oncotarget. 2016;•••:1–11. PMID: 28035059. Available from: 10.18632/oncotarget.14249.

50. Xie X, Shi X, Xun X, Rao L. Association between microRNA polymorphisms and coronary heart disease : A meta-analysis. Herz. 2017;42(6):593–603. PMID: 27832287. Available from: 10.1007/s00059-016-4495-4.

51. Bao MH, Xiao Y, Zhang QS, Luo HQ, Luo J, Zhao J, et al. Meta-analysis of miR-146a polymorphisms association with coronary artery diseases and ischemic stroke. Int J Mol Sci. 2015;16(7):14305–17. PMID: 26114385. Available from: 10.3390/ijms160714305.

52. Duan X, Zhan Q, Song B, Zeng S, Zhou J, Long Y, et al. Detection of platelet microRNA expression in patients with diabetes mellitus with or without ischemic stroke. J Diabetes Complications. 2014;28(5):705–10. PMID: 24908639. Available from: 10.1016/j.jdiacomp.2014.04.012.

53. Gatsiou A, Boeckel JN, Randriamboavonjy V, Stellos K. MicroRNAs in platelet biogenesis and function: implications in vascular homeostasis and inflammation. Curr Vasc Pharmacol. 2012;10(5):524–31. PMID: 22338566. Available from: 10.2174/157016112801784611.

54. Mitchell PS, Parkin RK, Kroh EM. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences. 2008;105(30):10513–10518. Available from: 10.1073/pnas.0804549105.

55. Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, Muniappa N, Tanaka WK, et al. Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem. 2009;55(11):1977–83. PMID: 19745058. Available from: 10.1373/clinchem.2009.131797.

56. Schulte C, Zeller T. microRNA-based diagnostics and therapy in cardiovascular disease-Summing up the facts. Cardiovasc Diagn Ther. 2015;5(1):17–36. PMID: 25774345. Available from: 10.3978/j.issn.2223-3652.2014.12.03.

57. Pordzik J, Pisarz K, Rosa SD, Jones AD, Eyileten C, Indolfi C, et al. The potential role of platelet-related microRNAs in the development of cardiovascular events in high-risk populations, including diabetic patients: A review. Front Endocrinol (Lausanne). 2018;9(MAR):74. PMID: 29615970. Available from: 10.3389/fendo.2018.00074.

58. Ding X, Ding J, Ning J, Yi F, Chen J, Zhao D, et al. Circulating microRNA-122 as a potential biomarker for liver injury. Mol Med Rep. 2012;5(6):1428–32. PMID: 22427142. Available from: 10.3892/mmr.2012.838.

59. Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, et al. MiR423-5p as a circulating biomarker for heart failure. Circ Res. 2010;106(6):1035–9. PMID: 20185794. Available from: 10.1161/CIRCRESAHA.110.218297.

60. Yang S, Zhao J, Chen Y, Lei M. Biomarkers Associated with Ischemic Stroke in Diabetes Mellitus Patients. Cardiovascular toxicology. 2016;16(3):213–222.

61. Santilli F, Vazzana N, Liani R, Guagnano MT, DavìG. Platelet activation in obesity and metabolic syndrome. Obes Rev. 2012;13(1):27–42. PMID: 21917110. Available from: 10.1111/j.1467-789X.2011.00930.x.

62. Santilli F, Vazzana N, Liani R, Guagnano M, Davì G. Platelet activation in obesity and metabolic syndrome. . doi:. Obes Rev. 2012;13(1):27–42. Available from: 10.1111/j.1467-789X.2011.00930.x.

63. Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G. Dicer Is Required for Embryonic Angiogenesis during Mouse Development. Journal of Biological Chemistry. 2005;280(1):9330–

9335. Available from: 10.1074/jbc.M413394200.

64. Duan R, Pak C, Jin P. Single nucleotide polymorphism associated with mature miR-125a alters the processing of primiRNA. Human molecular genetics. 2007;16(9):1124–1131. Available from: 10.1093/hmg/ddm062.

65. Albinsson S, Suarez Y, Skoura A, Offermanns S, Joseph M, Sessa WC. differentiation and function. 2011;30(6):1118-1126. Available from: 10.1161/ATVBAHA.109.200873.miRNAs.

66. Altuvia Y, Landgraf P, Lithwick G, Elefant N, Pfeffer S, Aravin A, et al. Clustering and conservation patterns of human microRNAs. Nucleic Acids Res. 2005;33(8):2697–706. PMID: 15891114. Available from: 10.1093/nar/gki567.

67. Zhu N, Zhang D, Chen S, Liu X, Lin L, Huang X, et al. Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. Atherosclerosis. 2011;215(2):286–93. PMID: 21310411. Available from: 10.1016/j.atherosclerosis.2010.12.024.

68. Hartmann D, Thum T. MicroRNAs and vascular (dys)function. Vascul Pharmacol. 2011;55(4):92–105. PMID: 21802526. Available from: 10.1016/j.vph.2011.07.005.

69. Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta N, et al. MicroRNA-145 targeted therapy reduces atherosclerosis. Circulation. 2012;126(11):81–90. PMID: 22965997. Available from: 10.1161/CIRCULATIONAHA.111.084186.

70. Lu M, Zhang Q, Deng M, Miao J, Guo Y, Gao W, et al. An analysis of human microRNA and disease associations. PLoS One. 2008;3(10):e3420. PMID: 18923704. Available from: 10.1371/journal.pone.0003420.

71. Nieuwdorp M, Stroes ESG, Meijers JCM, Büller H. Hypercoagulability in the metabolic syndrome. Curr Opin Pharmacol . 2005;5(2 Spec. Iss):155–159. Available from: 10.1016/j.coph.2004.10.003.

72. a Zimmerman G, Weyrich AS. Pathways to Altered Phenotype and Function. Arterioscler Thromb. 2008;28(3). Available from: 10.1161/ATVBAHA.107.160218.Signal- Dependent.

73. Schulte C, Molz S, Appelbaum S, Karakas M, Ojeda F, Lau DM, et al. MiRNA-197 and miRNA-223 predict cardiovascular death in a cohort of patients with symptomatic coronary artery disease. PLoS One. 2015;10(12):e0145930. PMID: 26720041. Available from: 10.1371/journal.pone.0145930.

74. JA W, N E, R P, JE F, JF K, K T, et al. Circulating cell and plasma microRNA profiles differ between non-ST-segment and ST-segment-elevation myocardial infarction. Family medicine & medical science research. 2014;2(2):108. Available from: 10.4172/2327-4972.1000108.

75. Goren Y, Meiri E, Hogan C, Mitchell H, Lebanony D, Salman N, et al. Relation of reduced expression of MiR-150 in platelets to atrial fibrillation in patients with chronic systolic heart failure. Am J Cardiol. 2014;113(6):976–81. PMID: 24462065. Available from: 10.1016/j.amjcard.2013.11.060.

76. Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, et al. Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature. 2001;409(6817):202–7. PMID: 11196645. Available from: 10.1038/35051599.

77. Gambaryan S, Kobsar A, Rukoyatkina N, Herterich S, Geiger J, Smolenski A, et al. Thrombin and collagen induce a feedback inhibitory signaling pathway in platelets involving dissociation of the catalytic subunit of protein kinase A from an NFkappaB-IkappaB complex. J Biol Chem. 2010;285(24):18352–63. PMID: 20356841. Available from: 10.1074/jbc.M109.077602.

78. Choi YC, Yoon S, Jeong Y, Yoon J, Baek K. Regulation of vascular endothelial growth factor signaling by miR-200b. Mol Cells. 2011;32(1):77–82. PMID: 21544626. Available from: 10.1007/s10059-011-1042-2.

79. Somanath PR, Podrez EA, Chen J, Ma YI, Antoch M, Byzova TV. Deficiency in core circadian protein Bmal1 is associated with a prothrombotic and vascular phenotype. Journal of cellular physiology. 2011;226(1):132–40. Available from: 10.1002/jcp.22314.Deficiency.

80. Shan SW, Lee DY, Deng Z, Shatseva T, Jeyapalan Z, Du WW, et al. MicroRNA MiR-17 retards tissue growth and represses fibronectin expression. Nat Cell Biol. 2009;11(8):1031–8. PMID: 19633662. Available from: 10.1038/ncb1917.

81. Fichtlscherer S, Rosa SD, Fox H, Schwietz T, Fischer A, Liebetrau C, et al. Circulating microRNAs in patients with coronary artery disease. Circ Res. 2010;107(5):677–84. PMID: 20595655. Available from: 10.1161/CIRCRESAHA.109.215566.

82. Peng H, Zhong M, Zhao W, Wang C, Zhang J, Liu X, et al. Urinary miR-29 correlates with albuminuria and carotid intima-media thickness in type 2 diabetes patients. PLoS One. 2013;8(12):e82607. PMID: 24349318. Available from: 10.1371/journal.pone.0082607.

83. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci USA. 2008;105(35):13027–32. PMID: 18723672. Available from: 10.1073/pnas.0805038105.

84. Shiffman D, Rowland CM, Louie JZ, Luke MM, Bare LA, Bolonick JI, et al. Gene variants of VAMP8 and HNRPUL1 are associated with early-onset myocardial infarction. Arterioscler Thromb Vasc Biol. 2006;26(7):1613–8. PMID: 16690874. Available from: 10.1161/01.ATV.0000226543.77214.e4.

85. Ezzell RM, Kenney DM, Egan S, Stossel TP, Hartwig JH. Localization of the domain of actin-binding protein that binds to membrane glycoprotein Ib and actin in human platelets. J Biol Chem. 1988;263(26):13303–9. PMID: 3138234.

86. Elgheznawy A, Fleming I. Platelet-enriched microRNAs and cardiovascular homeostasis. Antioxid Redox Signal. 2017;2017(9). Available from: 10.1089/ars.2017.7289.

87. Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci USA. 2008;105(5):1516–21. PMID: 18227515. Available from: 10.1073/pnas.0707493105.

88. Cavarretta E, Chiariello GA, Condorelli G. Platelets, endothelium, and circulating microRNA-126 as a prognostic biomarker in cardiovascular diseases: per aspirin ad astra. Eur Heart J. 2013;34(44):3400–2. PMID: 23391580. Available from: 10.1093/eurheartj/eht032.

89. Fasanaro P, D'Alessandra Y, Stefano VD, Melchionna R, Romani S, Pompilio G, et al. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem. 2008;283(23):15878–83. PMID: 18417479. Available from: 10.1074/jbc.M800731200.

90. Guduric-Fuchs J, O'Connor A, Cullen A, Harwood L, Medina RJ, O'Neill CL, et al. Deep sequencing reveals predominant expression of miR-21 amongst the small non-coding RNAs in retinal microvascular endothelial cells. J Cell Biochem. 2012;113(6):2098–111. PMID: 22298343. Available from: 10.1002/jcb.24084.

91. Fleissner F, Jazbutyte V, Fiedler J, Gupta SK, Yin X, Xu Q, et al. Short communication: asymmetric dimethylarginine impairs angiogenic progenitor cell function in patients with coronary artery disease through a microRNA-21-dependent mechanism. Circ Res. 2010;107(1):138–43. PMID: 20489163. Available from: 10.1161/CIRCRESAHA.110.216770.

92. Plé H, Landry P, Benham A, Coarfa C, Gunaratne PH, Provost P. The repertoire and features of human platelet microRNAs. PLoS One. 2012;7(12):e50746. PMID: 23226537. Available from: 10.1371/journal.pone.0050746.

Scan me