Do miRNAs Have a Role in Platelet Function Regulation?

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Hamostaseologie 2021;41:217–224.

Abstract

MicroRNAs (miRNAs) are a class of non-coding RNAs known to repress mRNA translation and subsequent protein production. miRNAs are predicted to modulate many targets and are involved in regulating various cellular processes. Identifying their role in cell function regulation may allow circulating miRNAs to be used as diagnostic or prognostic markers of various diseases. Increasing numbers of clinical studies have shown associations between circulating miRNA levels and platelet reactivity or the recurrence of cardiovascular events. However, these studies differed regarding population selection, sample types used, miRNA quantification procedures, and platelet function assays. Furthermore, they often lacked functional validation of the miRNA identified in such studies. The latter step is essential to identifying causal relationships and understanding if and how miRNAs regulate platelet function. This review describes recent advances in translational research dedicated to identifying miRNAs' roles in platelet function regulation.

Keywords

- microRNA
- platelet function
- biomarker

Introduction

Platelets play a key role in maintaining hemostasis[1] and vascular integrity; they are also involved in regulating inflammation,[2] immunity,[3] and tumor metastasis.[4] Platelets' main role is stopping bleeding during vascular lesions by accumulating at the site of vessel injury, thanks to their adhesive properties. Contact with the subendothelial components exposed in damaged vessels promotes platelet activation, aggregation, and thrombus growth. Additionally, platelet activation induces phosphatidylserine exposure at the membrane surface, activating the coagulation cascade and formation of a stable clot.[5] Platelets' ability to promote hemostasis—platelet reactivity (PR)—is a variable phenotype in individuals taking or not taking aspirin,[6,7] and may be associated with bleeding or thrombotic events. The determinants of PR have yet to be fully elucidated, but family-based studies point to a genetic origin.[8]

In recent decades, miRNAs' roles in the regulation of various biological processes and the progression of several diseases have gradually emerged. miRNAs are noncoding sections of RNA approximately 22 nucleotides long; they regulate gene expression at the post-transcriptional level by degrading mRNA or repressing its translation. Increasing numbers of clinical trials have shown correlations between PR and levels of circulating miRNA, revealing several platelet-derived miRNAs as putative biomarkers of platelet function or the recurrence of ischemic events.[9–14] Nevertheless, a causal link between circulating miRNA and platelet function or thrombus formation remains elusive, and mechanisms involved in miRNA-mediated regulation of platelet function are poorly understood. This may be due to the many miRNA–mRNA duplexes potentially involved and the variety of biological pathways that could be either upregulated or downregulated by miRNA levels. Validating candidate miRNAs identified as being regulators of platelet function in

received
November 30, 2020
accepted after revision
April 8, 2021

Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI https://doi.org/10.1055/a-1478-2105.
ISSN 0720-9355.

association studies is a major challenge, and of utmost importance to select them as potential biomarkers and to identify the underlying mechanisms.[15]

miRNA Biogenesis

In humans, miRNA loci are mainly located in intronic regions, in both transcription coding or non-coding units. Approximately 10% of all miRNAs are encoded by exons.[16] The genes that encode for miRNAs are transcribed by either RNA polymerase II or III into primary miRNA transcripts (pri-miRNA), depending on the miRNA loci and their upstream promoters.[17,18] Pri-miRNA consists of a stem and a terminal loop flanked by two single-stranded segments. The single-strand–double-strand junction of the pri-miRNA hairpin is cleaved by Drosha, an RNase III endonuclease coupled to the DiGeorge syndrome critical region 8 (DGCR8) at approximately 11 bp from the pri-miRNA stem, which allows the release of the precursor miRNA (pre-miRNA). Exportin-5 forms a complex with the guanine triphosphate (GTP)-bound form of the small nuclear guanine triphosphatase (GTPase) Ran (RanGTP). This complex recognizes the pre-miRNA structure,[19] protects pre-miRNA from degradation, and promotes the export of properly processed pre-miRNA into the cytoplasm.[19,20] In the cytoplasm, the double-strand pre-miRNA is cleaved by the RNase III endonuclease Dicer, resulting in the generation of two partially complementary strands of an miRNA,[20] each with its own targets and regulating specific biological functions.[21]

Mature miRNA recognizes six to eight nucleotides located on the 3′-untranslated region (3′-UTR) of mRNA.[22] Moreover, one miRNA can target several mRNAs and regulate several biological functions. Conversely, one mRNA can have a binding sequence for multiple miRNAs; therefore, the same gene may be regulated by several miRNAs. The miRNA–mRNA duplex guides the binding of the Ago2 protein in an RNA-induced silencing complex,[23] allowing mRNA cleavage, mRNA decay, and/or translational repression (►Fig. 1).

Platelets contain the necessary machinery for mRNA translation and protein synthesis.[24] In response to their

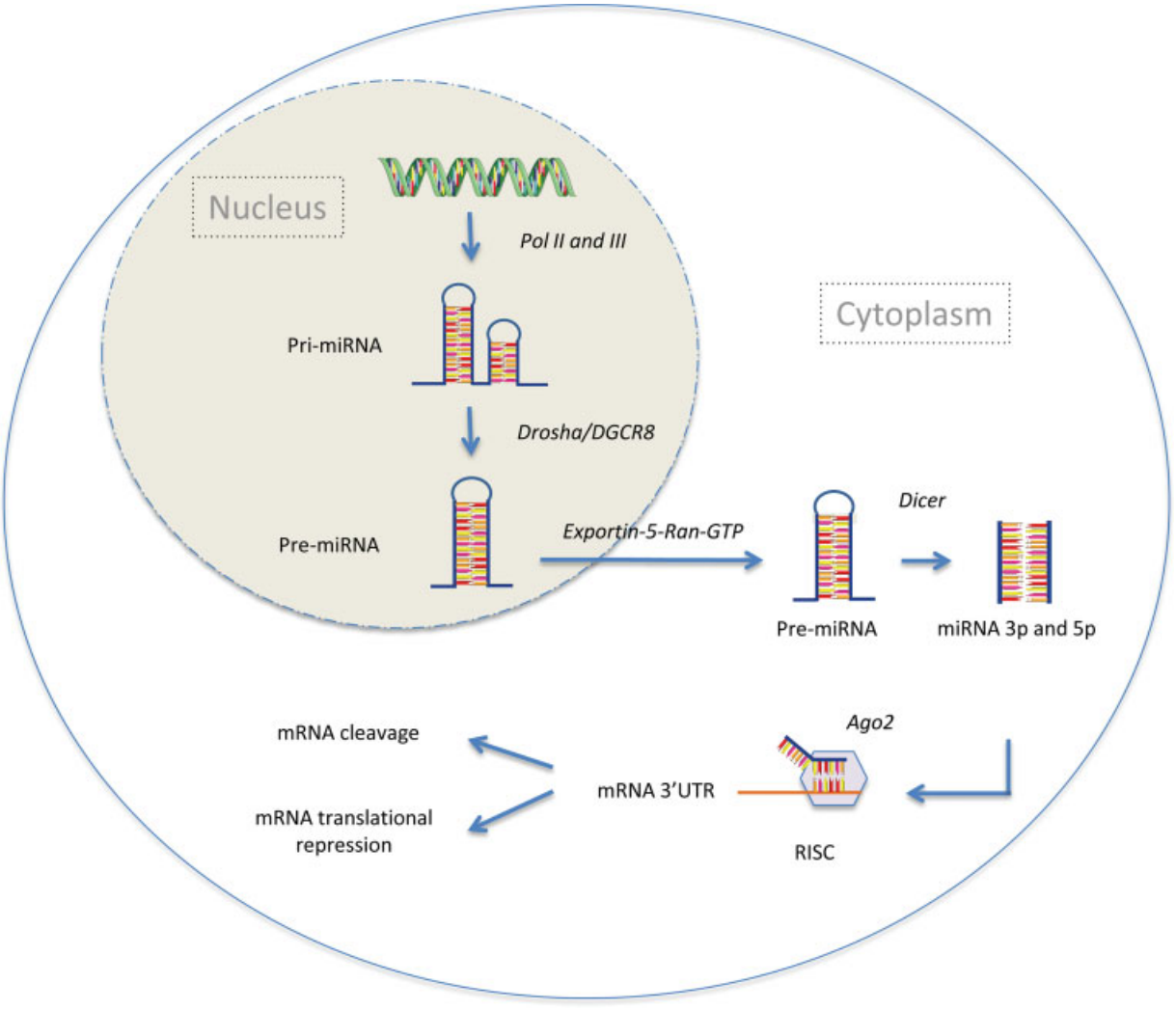

Fig. 1 miRNA biogenesis. miRNAs are mainly transcribed from the intronic region by polymerase II/III. The pri-miRNAs is processed into pre-miRNAs by the Drosha–DGCR8 complex and is then exported to the cytoplasm by exportin-5 for further maturation by the Dicer protein. Mature miRNA binds to a seed sequence by imperfect complementarity on the 3′-untranslated region (3′-UTR) of an mRNA. The miRNA–mRNA duplex is stabilized by Ago2 into an miRNA-induced silencing complex (RISC), thus triggering mRNA translational repression.

activation, platelets synthesize proteins by signal-dependent translation, using newly spliced mRNA and mature mRNA.[25] Platelets contain more than 500 miRNAs inherited from their parent cell, the megakaryocyte.[26] Therefore, it seems reasonable to speculate that these miRNAs have a *fine-tuning* function in the modulation of PR.

Circulating miRNAs

After stimulating various cells, including cell fragments such as platelets, circulating miRNAs release their intracellular content into the extracellular medium. Current understanding points to microvesicles being the main transporters of circulating miRNAs[27] since miRNAs' profiles in the blood are close to those of microvesicles.[28] However, the composition of this pool of extracellular miRNAs also includes miRNAs bound to proteins. A recent study reported that miRNAs are more abundant in non-vesicular than in vesicular fractions,[29] and that these vesicle-free miRNAs were associated with the Ago2 protein.[30] The authors concluded that Ago2 as a miRNA carrier could account for all vesicle-free miRNAs; however, other miRNA complexes can also exist in plasma, such as with nucleoplasmin or high-density lipoproteins.[31,32] The mechanism governing extravesicular biogenesis, transport, and cargo uptake in recipient cells is not entirely understood and is reviewed elsewhere.[33]

Since approximately 45% of microvesicles circulating in the blood are released from platelets,[28] the circulating miRNA profile may reflect the platelet miRNA content and provide information on platelet activation status in real time. This concept is illustrated by the impact of antiplatelet drugs on circulating miRNA,[34] meaning that the profile of circulating miRNA may provide important information on PR in diseases where platelets play major roles, such as in the progression of atherosclerosis,[35] cancer metastasis,[36] and inflammation.[3]

Associations between miRNAs and Platelet Reactivity in Cardiovascular Patients

Several studies have addressed correlations between circulating miRNAs and PR or cardiovascular events,[14,34,37] promoting the concept of using circulating, platelet-derived microRNAs (e.g., miR-223, miR-126, miR-197, miR-150, miR-21, miR-96, or miR-204) as potential biomarkers for the recurrence of cardiovascular events (►Fig. 2).[15,38] It is of note that miR-223–3p and miR-126–3p, among the most abundant platelet-derived miRNAs, have been particularly studied.

MiR-223 may mediate its effect on platelet function via the regulation of several genes. For example, miR-223 represses $P2Y_{12}$ mRNA translation, the ADP receptor targeted by antiplatelet drugs (thienopyridines and ticagrelor).[24,39] Increased exosomal miR-223 was shown to be associated with the occurrence and severity of acute ischemic stroke and short-term outcomes.[40] In patients with acute coronary syndrome, levels of circulating miR-223 in plasma were statistically correlated with the PR determined using vasodilator-stimulated phosphoprotein phosphorylation flow cytometry after clopidogrel or dual-antiplatelet therapy. Moreover, a lower level of circulating miR-223–3p was the only independent predictor of poorer response to treatment, as determined using a PR index.[39,41] These results indicated that miR-223 might be useful in predicting disease severity and assessing responsiveness to antiplatelet agents.

Zampetaki et al showed that diabetes was associated with a reduced level of miR-126 in plasma,[42] which may reflect a platelet dysfunction in diabetic populations. A study of

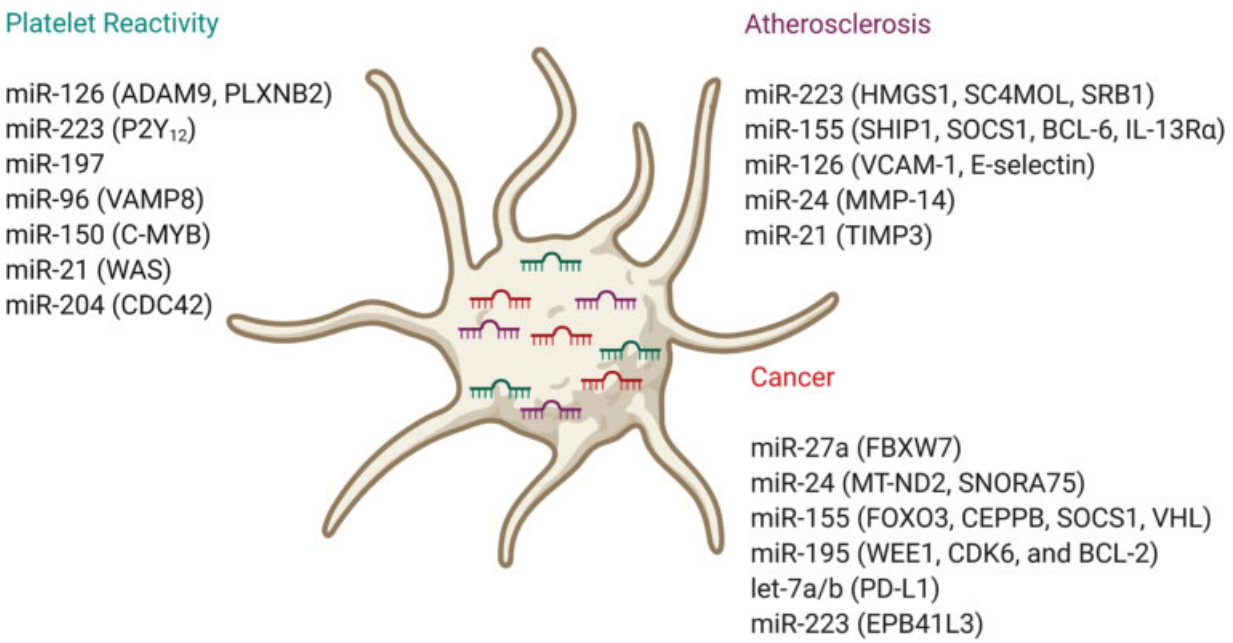

Fig. 2 Platelet-derived miRNA. Selected platelet-derived miRNAs and their experimentally validated mRNA targets (in brackets) associated with atherosclerosis, platelet reactivity, and cancer. For miR-197, no validated target related to these diseases was identified.

patients with type 2 diabetes showed a direct correlation between platelet activation, as assessed by measuring soluble P-selectin, and the level of circulating miR-126. In addition, the administration of aspirin induced a decrease in the level of circulating miR-126 in this population.[43] Another study, of patients with diabetes mellitus, showed that miR-126 exhibited antithrombotic properties by regulating post-transcriptional tissue factor expression. Optimizing antidiabetic treatment using metformin or sulfonylurea was associated with the upregulation of miR-126 in plasma, which correlated with reduced thrombogenicity.[44] However, this association may not be true in nondiabetic patients.[45] In recent years, several hypotheses have emerged regarding the underlying mechanisms mediated by miR-126's impact on platelet function. These include the modulation of ADAM9, a mediator of platelet binding to collagen,[37] and of PLXNB2, a transmembrane protein known to support thrombus formation in mice.[46]

Other miRNA–mRNA duplexes have been described as putative regulators of PR, including miR-96–VAMP8, described as having a role in the heterogeneity of PR.[47]

Platelet-Derived miRNAs and Atherosclerosis

Accumulated evidence has indicated that many miRNAs, including platelet-derived miRNAs, can affect the initiation and progression of atherosclerosis and the development of its complications via endothelial cell (EC) function regulation, including the maintenance of vascular integrity (miR-126), EC proliferation (miR-126, miR-17, miR-18, miR-20a), migration (miR-218, miR-106b-25 cluster), and senescence (miR-34, miR-217, miR-146a; see ►Fig. 2).[48]

miRNAs are important regulators of lipoprotein homeostasis and lipid accumulation. Among them, miR-223 represses genes involved in cholesterol biosynthesis (*HMGS1*, *SC4MOL*) and HDL uptake (*SRB1*).[49] The miR-33 family targets the HDL–reverse cholesterol transport pathway.[50,51]

Overexpression of miR-146a can delay both inflammatory response and oxidized low-density lipoprotein accumulation by inhibiting activation of the toll-like receptor 4–dependent signaling pathway.[52] MiR-30c has been shown to decrease the production of apoB-containing lipoproteins (very low density lipoprotein, low-density lipoprotein) by targeting both the microsomal triglyceride transfer protein (*MTTP*), a protein essential for the lipidation of nascent apoB, and lysophosphatidylglycerol acyltransferase 1 (*LPGAT1*), which reduces de novo lipogenesis.[53]

miRNAs can also play roles in the regulation of proinflammatory response. The inhibition of endogenous miR-125a-5p expression has been correlated with an increased inflammatory cytokines tumor growth factor-β level, tumor necrosis factor-α, interleukin 2 (IL-2), and interleukin 6 (IL-6).[54]

Additionally, other miRNAs, including miR-126–3p, miR-17–3p, and miR-31, regulate the development of vascular inflammation by controlling the expression of adhesion molecules, such as vascular cell adhesion molecule 1, intercellular adhesion molecule, and E-selectin, respectively.[54,55] Other miRNAs facilitate calcification by targeting Ets1 (miR-125b) or, on the contrary, inhibit calcification (miR-29a and miR-29b) by suppressing the expression of ADAMTS-7.[56,57] Furthermore, microRNAs play roles in plaque stability, with either stabilizing effects (miR-222, miR-24, miR-26a, miR-27b, let-7e) or destabilizing effects (miR-21, miR-181, miR-712, miR-29).[58] Finally, several microRNAs, including miR-155–5p, miR-483–5p, and miR-451a, have been reported as potential biomarkers for the early identification of plaque rupture.[59]

Among the microRNAs identified as playing roles in the development of atherosclerosis, several are found in platelets and in platelet-derived microvesicles, including miR-223, miR-126, miR-24, miR-155, and miR-21. Therefore, platelet-derived microRNAs may contribute to the development of atherosclerosis at every stage leading to the formation of atherosclerotic plaques.

Platelet-Derived miRNA and Cancer

Besides their potential role in PR and cardiovascular diseases, activated platelets and platelet-derived microvesicles are suggested to be implicated in the progression of cancers.[60] Cancer patients are also at an increased risk of platelet-driven venous thromboembolism.[61]

Several miRNAs enriched in platelet-derived microvesicles can target both tumor suppressor genes and oncogenes (in multiple cancer types), among them miR-27a, miR-24, miR-155, miR-195, let-7a/b, and miR-223.[60,62–69] The platelets and platelet-derived microvesicles of patients with non-small-cell lung cancer contain higher levels of miR-223 than those of healthy individuals. The incubation of A549 human lung cancer cells with platelet-derived microvesicles has been shown to result in the rapid delivery of miR-223 into those cells, thus inhibiting the EPB41L3 tumor suppressor and promoting A549 cell invasion.[67]

However, although platelet-derived microvesicles are generally considered to participate in cancer progression, recent data suggest that they may have a cancer-suppressive effect. Platelet-derived microvesicles infiltrate solid tumors in humans and mice and transfer their RNA content, including miRNAs promoting tumor cell apoptosis. MiR-24 was a significantly present species in this transfer. In another model, where human platelet-derived microvesicles were transfused to mice, mitochondrial *mt-Nd2* and *Snora75*—two small, noncoding, nucleolar RNAs—were identified as direct targets of platelet-derived miR-24. These target RNAs were suppressed in platelet-derived, microvesicle-treated tumor cells, resulting in mitochondrial dysfunction and tumor growth inhibition.[60]

Analytical Pitfalls in Measuring Circulating miRNAs

miRNAs are highly stable in biological fluids[70,71] and could therefore be interesting for developing a new type of biomarker. Over recent decades, numerous studies have

investigated using circulating miRNAs as potential biomarkers,[37,39,72–74] raising several issues regarding sample types, extraction and quantification procedures, and clinically significant cut-off values.[75]

Sample selection is important. Indeed, serum samples may reflect the total miRNA content in platelets and miRNAs derived from the microvesicles of other blood cells that can be activated during the coagulation process. miRNA quantification using plasma samples is probably more likely to reflect *basal* platelet function status and to be better for evaluating the risk of ischemic event recurrence; thus, plasma samples are preferred.[75,76] Freezing and thawing cycles can lyse residual cells and release all of their miRNA content, biasing miRNA quantification. Therefore, plasma samples should be prepared using a double centrifugation step, and measuring platelet-specific or leukocyte-specific proteins (e.g., ITGA2B or PTPRC, respectively) can validate the absence of any residual cell contamination.[77]

The miRNA extraction procedure is usually performed using a TRIzol-based method followed by (or not) a standardized column extraction process.[39] This only enables a small amount of miRNA to be extracted, however; so, a preamplification step is usually performed to optimize subsequent quantification. The ratio between sensitivity and the number of targets differs according to the quantification technique. Indeed, a complete miRNA profile can be evaluated using high throughput techniques such as RNA sequencing,[37] whereas custom-designed locked nucleic acid (Exiqon)[37,78] and NanoString Technologies[73,74] enable the quantification of approximately 100 miRNAs targeted in one sample. The most sensitive technique remains the TaqMan-based quantitative polymerase chain reaction on complementary DNA,[75,79] which is considered the gold standard. Because only a few miRNAs can be evaluated at once, however, candidate miRNAs must have been identified in a previous study.

Finally, although the normalization procedure remains challenging, it is of utmost importance for comparing samples and studies. A wide range of strategies has been developed,[80] including using exogenous spike-in (e.g., UniSP6[14] and cel-miR-39[81]), endogenous, small, non-coding RNA (e.g., RNU6[82]) or endogenous miRNA(s). The spike-in is useful for evaluating the quality and reproducibility of the extraction and quantification process, whereas endogenous RNA or miRNAs are more adapted to normalize the level of quantified miRNA. However, endogenous normalizers from the same class of nucleotide sequence should be preferred. Endogenous miRNA normalizers differ according to the sample used—for example, miR-638, miR-93, and miR-484 are used for plasma,[83–85] whereas miR-23a, let7a, and miR-1260 are preferred for serum.[78,86,87] Using a combination of stable normalizers, determined using dedicated tools (e.g., GeNorm[88] and NormFinder[89]), enhances the efficiency of the normalization.[87]

Functional Validation of miRNAs

Although several miRNAs have been pinpointed as potential biomarkers of PR or the recurrence of ischemic events, the functional validation of candidate miRNAs as true regulators of platelet function remains a crucial step. It will be the cornerstone criterion formally linking a specific miRNA and platelet function regulation.[15] Platelets have a very short lifespan and only remain functional for a few hours after blood sampling. This precludes the modulation of their miRNA content via transfection; thus, alternative strategies should be used. Several in vitro models are available using megakaryocyte lines (MEG01, DAMI, or K562) or megakaryocytes from hematopoietic stem cells (CD34) differentiated into platelet-like structures (PLSs). The latter model has the advantage of producing PLSs that are functionally close to human platelets.[46,90] However, this model's main limitation is the small number of cells recovered, which means that it is not compatible with all of the conventional methods dedicated to the study of platelet function. Modulation of the miRNA content in these in vitro models can be done using lipofection or nucleofection to transfect the megakaryocytes with synthetic miRNA mimics or miRNA inhibitors. An alternative method uses lentiviral transduction, where the lentiviral vector genome is integrated into the host genome, leading to stable miRNA expression.

Several in vivo models are available, including zebrafish and mouse models that have hemostatic systems close to that of humans. The zebrafish model is widely used. The transparency of zebrafish embryos and larvae between 24 hours and 5 days after fertilization facilitates the visualization of their vessels and real-time thrombocyte accumulation after laser injury.[15,45] Modifications in thrombocyte miRNA content can be obtained by genetic modifications. Inserting a sequence coding for the miRNA of interest, under the control of CD41 promoter, results in the generation of a transgenic line that only overexpresses miRNA in thrombocytes without affecting the levels of other miRNAs.[45]

The mouse is a model of choice for studies related to hemostasis. It allows the performance of aggregation tests, monitoring the exposure of CD62P and the expression of activated GPIIb–IIIa on platelets using flow cytometry, and the assessment of tail bleeding time. Several gene-editing strategies can be used, including transgenesis, homologous recombination using the Cre/loxP,[91–93] and the TALEN[94] and CRISPR[95] systems that can successfully induce miRNA deletions. The transduction of a lentiviral vector specifically designed to stably overexpress a specific miRNA can be injected into mice.[96] Bidirectional miRNA reporter lentiviral vectors have shown promising results for post-transcriptional (miRNA-restricted) repression in hematopoietic stem cells in mice via the insertion of target sequences for hematopoietic stem cell–specific miRNA into the lentiviral cassette.[97]

In contrast, a transient modulation of a miRNA level can be obtained by systemic injection of locked nucleic acid, a chemical inhibitor.[37]

Generating transgenic lines has obvious advantages for the functional investigation of a miRNA's impact on PR and hemostasis. However, animal models must be used cautiously due to the possible non-conservation of miRNA–mRNA duplexes, which can differ from miRNA's function in humans.

Identification and Validation of miRNA Targets

The in silico identification of target miRNAs using databases such as Targetscan, MiRanda, or DIANA-microT represents the first step in deciphering the mechanisms linking miRNA and platelet function. However, different databases may identify variable numbers of putative targets. For example, the Targetscan database (Release 7.2) finds 423 putative targets for miR-223, whereas the MiRanda database finds 3,485. These databases are also known to provide high numbers of false-positive results[98]; therefore, any miRNA–mRNA duplexes predicted in silico must be validated. The reporter gene assay is one of the most widely used methods to identify an miRNA's binding site on the target gene's 3′-UTR and to validate the direct interaction between an miRNA and its target. In addition to P2Y12, calpain-2 (known to regulate the platelet signaling pathway)[99] has also been validated as a direct target of miR-223–3p.[100] Several other predicted targets of miR-223–3p have been validated using this method, including PDGFRβ,[101,102] EPB41L3,[67] Septin-2, and Septin-6[103]; however, their direct role in platelet function remains poorly understood.

Conclusion

There is increasing evidence of associations between levels of specific miRNAs and PR and the recurrence of ischemic events.

The significant methodological differences between different clinical association studies, in terms of their biological samples, miRNA extraction methods, quantification, and clinical settings, make functional validation of the utmost importance when attempting to provide a causal link and a mechanistic insight into the role of candidate miRNAs in regulating PR.[15] These functional validation data are more limited but are critical to selecting the most promising miRNAs as biomarkers for diseases where platelets play a major role.

Taken together, in the near future, the profile of a patient's circulating miRNA could be evaluated for the biomarkers of various platelet-related diseases and contribute to the emergence of personalized medicine.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the Private Foundation of the Geneva University Hospitals (Grant RC04–05).

References

1 Broos K, Feys HB, De Meyer SF, Vanhoorelbeke K, Deckmyn H. Platelets at work in primary hemostasis. Blood Rev 2011;25(04): 155–167
2 Bakogiannis C, Sachse M, Stamatelopoulos K, Stellos K. Platelet-derived chemokines in inflammation and atherosclerosis. Cytokine 2019;122:154157
3 Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. Nat Rev Immunol 2011;11(04):264–274
4 Schlesinger M. Role of platelets and platelet receptors in cancer metastasis. J Hematol Oncol 2018;11(01):125
5 Versteeg HH, Heemskerk JW, Levi M, Reitsma PH. New fundamentals in hemostasis. Physiol Rev 2013;93(01):327–358
6 Faraday N, Yanek LR, Mathias R, et al. Heritability of platelet responsiveness to aspirin in activation pathways directly and indirectly related to cyclooxygenase-1. Circulation 2007;115 (19):2490–2496
7 Zufferey A, Reny JL, Combescure C, de Moerloose P, Sanchez JC, Fontana P. Platelet reactivity is a stable and global phenomenon in aspirin-treated cardiovascular patients. Thromb Haemost 2011;106(03):466–474
8 Bray PF, Mathias RA, Faraday N, et al. Heritability of platelet function in families with premature coronary artery disease. J Thromb Haemost 2007;5(08):1617–1623
9 Nagalla S, Shaw C, Kong X, et al. Platelet microRNA-mRNA coexpression profiles correlate with platelet reactivity. Blood 2011;117(19):5189–5197
10 Cimmino G, Tarallo R, Nassa G, et al. Activating stimuli induce platelet microRNA modulation and proteome reorganisation. Thromb Haemost 2015;114(01):96–108
11 Lindsay CR, Edelstein LC. MicroRNAs in platelet physiology and function. Semin Thromb Hemost 2016;42(03):215–222
12 Osman A, Fälker K. Characterization of human platelet microRNA by quantitative PCR coupled with an annotation network for predicted target genes. Platelets 2011;22(06):433–441
13 Becker KC, Kwee LC, Neely ML, et al. Circulating microRNA profiling in non-ST elevated coronary artery syndrome highlights genomic associations with serial platelet reactivity measurements. Sci Rep 2020;10(01):6169
14 Jakob P, Kacprowski T, Briand-Schumacher S, et al. Profiling and validation of circulating microRNAs for cardiovascular events in patients presenting with ST-segment elevation myocardial infarction. Eur Heart J 2017;38(07):511–515
15 Garcia A, Dunoyer-Geindre S, Fish RJ, et al. Methods to investigate miRNA function: focus on platelet reactivity. Thromb Haemost 2021;121(04):409–421
16 Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. Genome Res 2004;14(10A):1902–1910
17 Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 2004;23(20):4051–4060
18 Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 2006;13(12): 1097–1101
19 Okada C, Yamashita E, Lee SJ, et al. A high-resolution structure of the pre-microRNA nuclear export machinery. Science 2009;326 (5957):1275–1279
20 Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol 2009;11(03):228–234
21 Desvignes T, Batzel P, Berezikov E, et al. miRNA nomenclature: a view incorporating genetic origins, biosynthetic pathways, and sequence variants. Trends Genet 2015;31(11):613–626
22 Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009; 19(01):92–105
23 Pratt AJ, MacRae IJ. The RNA-induced silencing complex: a versatile gene-silencing machine. J Biol Chem 2009;284(27): 17897–17901
24 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(09):961–966
25 Weyrich AS, Schwertz H, Kraiss LW, Zimmerman GA. Protein synthesis by platelets: historical and new perspectives. J Thromb Haemost 2009;7(02):241–246

26 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
27 Provost P. The clinical significance of platelet microparticle-associated microRNAs. Clin Chem Lab Med 2017;55(05): 657–666
28 Diehl P, Fricke A, Sander L, et al. Microparticles: major transport vehicles for distinct microRNAs in circulation. Cardiovasc Res 2012;93(04):633–644
29 Jeppesen DK, Fenix AM, Franklin JL, et al. Reassessment of exosome composition. Cell 2019;177(02):428–445.e18
30 Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A 2011;108 (12):5003–5008
31 Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011; 13(04):423–433
32 Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res 2010;38(20):7248–7259
33 O'Brien K, Breyne K, Ughetto S, Laurent LC, Breakefield XO. RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat Rev Mol Cell Biol 2020;21(10):585–606
34 Willeit P, Zampetaki A, Dudek K, et al. Circulating microRNAs as novel biomarkers for platelet activation. Circ Res 2013;112(04): 595–600
35 De Meyer GR, Hoylaerts MF, Kockx MM, Yamamoto H, Herman AG, Bult H. Intimal deposition of functional von Willebrand factor in atherogenesis. Arterioscler Thromb Vasc Biol 1999;19 (10):2524–2534
36 Leblanc R, Houssin A, Peyruchaud O. Platelets, autotaxin and lysophosphatidic acid signalling: win-win factors for cancer metastasis. Br J Pharmacol 2018;175(15):3100–3110
37 Kaudewitz D, Skroblin P, Bender LH, et al. Association of microRNAs and YRNAs with platelet function. Circ Res 2016;118(03): 420–432
38 Pordzik J, Pisarz K, De Rosa S, 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;9:74
39 Shi R, Ge L, Zhou X, et al. Decreased platelet miR-223 expression is associated with high on-clopidogrel platelet reactivity. Thromb Res 2013;131(06):508–513
40 Chen Y, Song Y, Huang J, et al. Increased circulating exosomal miRNA-223 is associated with acute ischemic stroke. Front Neurol 2017;8:57
41 Zhang YY, Zhou X, Ji WJ, et al. Decreased circulating microRNA-223 level predicts high on-treatment platelet reactivity in patients with troponin-negative non-ST elevation acute coronary syndrome. J Thromb Thrombolysis 2014;38(01):65–72
42 Zampetaki A, Kiechl S, Drozdov I, et al. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 2010;107(06):810–817
43 de Boer HC, van Solingen C, Prins J, et al. Aspirin treatment hampers the use of plasma microRNA-126 as a biomarker for the progression of vascular disease. Eur Heart J 2013;34(44): 3451–3457
44 Witkowski M, Weithauser A, Tabaraie T, et al. Micro-RNA-126 reduces the blood thrombogenicity in diabetes mellitus via targeting of tissue factor. Arterioscler Thromb Vasc Biol 2016; 36(06):1263–1271
45 Zapilko V, Fish RJ, Garcia A, et al. MicroRNA-126 is a regulator of platelet-supported thrombin generation. Platelets 2020;31(06): 746–755
46 Garcia A, Dunoyer-Geindre S, Zapilko V, Nolli S, Reny JL, Fontana P. Functional validation of microRNA-126-3p as a platelet reactivity regulator using human haematopoietic stem cells. Thromb Haemost 2019;119(02):254–263
47 Kondkar AA, Bray MS, Leal SM, et al. VAMP8/endobrevin is overexpressed in hyperreactive human platelets: suggested role for platelet microRNA. J Thromb Haemost 2010;8(02): 369–378
48 Chamorro-Jorganes A, Araldi E, Suárez Y. MicroRNAs as pharmacological targets in endothelial cell function and dysfunction. Pharmacol Res 2013;75:15–27
49 Vickers KC, Landstreet SR, Levin MG, et al. MicroRNA-223 coordinates cholesterol homeostasis. Proc Natl Acad Sci U S A 2014;111(40):14518–14523
50 Marquart TJ, Allen RM, Ory DS, Baldán A. miR-33 links SREBP-2 induction to repression of sterol transporters. Proc Natl Acad Sci U S A 2010;107(27):12228–12232
51 Rayner KJ, Suárez Y, Dávalos A, et al. MiR-33 contributes to the regulation of cholesterol homeostasis. Science 2010;328 (5985):1570–1573
52 Yang K, He YS, Wang XQ, et al. MiR-146a inhibits oxidized low-density lipoprotein-induced lipid accumulation and inflammatory response via targeting toll-like receptor 4. FEBS Lett 2011; 585(06):854–860
53 Soh J, Iqbal J, Queiroz J, Fernandez-Hernando C, Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. Nat Med 2013;19(07):892–900
54 Suárez Y, Wang C, Manes TD, Pober JS. Cutting edge: TNF-induced microRNAs regulate TNF-induced expression of E-selectin and intercellular adhesion molecule-1 on human endothelial cells: feedback control of inflammation. J Immunol 2010;184 (01):21–25
55 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 U S A 2008;105(05): 1516–1521
56 Wen P, Cao H, Fang L, et al. miR-125b/Ets1 axis regulates transdifferentiation and calcification of vascular smooth muscle cells in a high-phosphate environment. Exp Cell Res 2014;322(02): 302–312
57 Du Y, Gao C, Liu Z, et al. Upregulation of a disintegrin and metalloproteinase with thrombospondin motifs-7 by miR-29 repression mediates vascular smooth muscle calcification. Arterioscler Thromb Vasc Biol 2012;32(11):2580–2588
58 Kowara M, Cudnoch-Jedrzejewska A, Opolski G, Wlodarski P. MicroRNA regulation of extracellular matrix components in the process of atherosclerotic plaque destabilization. Clin Exp Pharmacol Physiol 2017;44(07):711–718
59 Li S, Lee C, Song J, et al. Circulating microRNAs as potential biomarkers for coronary plaque rupture. Oncotarget 2017;8(29): 48145–48156
60 Michael JV, Wurtzel JGT, Mao GF, et al. Platelet microparticles infiltrating solid tumors transfer miRNAs that suppress tumor growth. Blood 2017;130(05):567–580
61 Lazar S, Goldfinger LE. Platelet microparticles and miRNA transfer in cancer progression: many targets, modes of action, and effects across cancer stages. Front Cardiovasc Med 2018; 5:13
62 Chen Z, Ma T, Huang C, Hu T, Li J. The pivotal role of microRNA-155 in the control of cancer. J Cell Physiol 2014;229(05):545–550
63 Jurkovicova D, Magyerkova M, Kulcsar L, et al. miR-155 as a diagnostic and prognostic marker in hematological and solid malignancies. Neoplasma 2014;61(03):241–251
64 Vimalraj S, Miranda PJ, Ramyakrishna B, Selvamurugan N. Regulation of breast cancer and bone metastasis by microRNAs. Dis Markers 2013;35(05):369–387
65 Gao Y, Liu Y, Du L, et al. Down-regulation of miR-24-3p in colorectal cancer is associated with malignant behavior. Med Oncol 2015;32(01):362

66 He JF, Luo YM, Wan XH, Jiang D. Biogenesis of MiRNA-195 and its role in biogenesis, the cell cycle, and apoptosis. J Biochem Mol Toxicol 2011;25(06):404–408
67 Liang H, Yan X, Pan Y, et al. MicroRNA-223 delivered by platelet-derived microvesicles promotes lung cancer cell invasion via targeting tumor suppressor EPB41L3. Mol Cancer 2015;14:58
68 Tak H, Kang H, Ji E, Hong Y, Kim W, Lee EK. Potential use of TIA-1, MFF, microRNA-200a-3p, and microRNA-27 as a novel marker for hepatocellular carcinoma. Biochem Biophys Res Commun 2018;497(04):1117–1122
69 Yu D, Liu X, Han G, et al. The let-7 family of microRNAs suppresses immune evasion in head and neck squamous cell carcinoma by promoting PD-L1 degradation. Cell Commun Signal 2019;17 (01):173
70 Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18(10):997–1006
71 El-Hefnawy T, Raja S, Kelly L, et al. Characterization of amplifiable, circulating RNA in plasma and its potential as a tool for cancer diagnostics. Clin Chem 2004;50(03):564–573
72 Ding T, Zeng X, Cheng B, et al. Platelets in acute coronary syndrome patients with high platelet reactivity after dual antiplatelet therapy exhibit upregulation of miR-204-5p. Ann Clin Lab Sci 2019;49(05):619–631
73 Zufferey A, Ibberson M, Reny JL, et al. New molecular insights into modulation of platelet reactivity in aspirin-treated patients using a network-based approach. Hum Genet 2016;135(04): 403–414
74 Simon LM, Edelstein LC, Nagalla S, et al. Human platelet microRNA-mRNA networks associated with age and gender revealed by integrated plateletomics. Blood 2014;123(16):e37–e45
75 Sunderland N, Skroblin P, Barwari T, et al. MicroRNA biomarkers and platelet reactivity: the clot thickens. Circ Res 2017;120(02): 418–435
76 Wang K, Yuan Y, Cho JH, McClarty S, Baxter D, Galas DJ. Comparing the microRNA spectrum between serum and plasma. PLoS One 2012;7(07):e41561
77 Fejes Z, Póliska S, Czimmerer Z, et al. Hyperglycaemia suppresses microRNA expression in platelets to increase P2RY12 and SELP levels in type 2 diabetes mellitus. Thromb Haemost 2017;117 (03):529–542
78 Blondal T, Jensby Nielsen S, Baker A, et al. Assessing sample and miRNA profile quality in serum and plasma or other biofluids. Methods 2013;59(01):S1–S6
79 Zampetaki A, Willeit P, Tilling L, et al. Prospective study on circulating microRNAs and risk of myocardial infarction. J Am Coll Cardiol 2012;60(04):290–299
80 Schwarzenbach H, da Silva AM, Calin G, Pantel K. Data normalization strategies for microRNA quantification. Clin Chem 2015; 61(11):1333–1342
81 Jansen F, Schäfer L, Wang H, et al. Kinetics of circulating microRNAs in response to cardiac stress in patients with coronary artery disease. J Am Heart Assoc 2017;6(08):6
82 Gee HE, Buffa FM, Camps C, et al. The small-nucleolar RNAs commonly used for microRNA normalisation correlate with tumour pathology and prognosis. Br J Cancer 2011;104(07): 1168–1177
83 Tanaka M, Oikawa K, Takanashi M, et al. Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients. PLoS One 2009;4(05):e5532
84 Zalewski K, Misiek M, Kowalik A, et al. Normalizers for microRNA quantification in plasma of patients with vulvar intraepithelial neoplasia lesions and vulvar carcinoma. Tumour Biol 2017;39 (11):1010428317717140
85 Mompeón A, Ortega-Paz L, Vidal-Gómez X, et al. Disparate miRNA expression in serum and plasma of patients with acute myocardial infarction: a systematic and paired comparative analysis. Sci Rep 2020;10(01):5373
86 Li Y, Xiang GM, Liu LL, et al. Assessment of endogenous reference gene suitability for serum exosomal microRNA expression analysis in liver carcinoma resection studies. Mol Med Rep 2015;12 (03):4683–4691
87 Kok MG, Halliani A, Moerland PD, Meijers JC, Creemers EE, Pinto-Sietsma SJ. Normalization panels for the reliable quantification of circulating microRNAs by RT-qPCR. FASEB J 2015;29(09): 3853–3862
88 Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002;3(07): H0034
89 Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 2004;64(15):5245–5250
90 Strassel C, Brouard N, Mallo L, et al. Aryl hydrocarbon receptor-dependent enrichment of a megakaryocytic precursor with a high potential to produce proplatelets. Blood 2016;127(18):2231–2240
91 Kleinhammer A, Wurst W, Kühn R. Constitutive and conditional RNAi transgenesis in mice. Methods 2011;53(04):430–436
92 Park CY, Jeker LT, Carver-Moore K, et al. A resource for the conditional ablation of microRNAs in the mouse. Cell Rep 2012;1(04):385–391
93 Tiedt R, Schomber T, Hao-Shen H, Skoda RC. Pf4-Cre transgenic mice allow the generation of lineage-restricted gene knockouts for studying megakaryocyte and platelet function in vivo. Blood 2007;109(04):1503–1506
94 Takada S, Sato T, Ito Y, et al. Targeted gene deletion of miRNAs in mice by TALEN system. PLoS One 2013;8(10):e76004
95 Chang H, Yi B, Ma R, Zhang X, Zhao H, Xi Y. CRISPR/cas9, a novel genomic tool to knock down microRNA in vitro and in vivo. Sci Rep 2016;6:22312
96 Zeng LL, He XS, Liu JR, Zheng CB, Wang YT, Yang GY. Lentivirus-mediated overexpression of microRNA-210 improves long-term outcomes after focal cerebral ischemia in mice. CNS Neurosci Ther 2016;22(12):961–969
97 Chiriaco M, Farinelli G, Capo V, et al. Dual-regulated lentiviral vector for gene therapy of X-linked chronic granulomatosis. Mol Ther 2014;22(08):1472–1483
98 Fridrich A, Hazan Y, Moran Y. Too many false targets for microRNAs: challenges and pitfalls in prediction of miRNA targets and their gene ontology in model and non-model organisms. BioEssays 2019;41(04):e1800169
99 Kuchay SM, Chishti AH. Calpain-mediated regulation of platelet signaling pathways. Curr Opin Hematol 2007;14(03):249–254
100 Siuda D, Randriamboavonjy V, Fleming I. Regulation of calpain 2 expression by miR-223 and miR-145. Biochim Biophys Acta Gene Regul Mech 2019;1862(10):194438
101 Zhang Y, Wang Y, Zhang L, et al. Reduced platelet miR-223 induction in Kawasaki disease leads to severe coronary artery pathology through a miR-223/PDGFRβ vascular smooth muscle cell axis. Circ Res 2020;127(07):855–873
102 Zeng Z, Xia L, Fan X, et al. Platelet-derived miR-223 promotes a phenotypic switch in arterial injury repair. J Clin Invest 2019;129 (03):1372–1386
103 Chattopadhyay M, Dahiya N, Atreya C. MicroRNA-223 regulates Septin-2 and Septin-6 in stored platelets. MicroRNA 2018;7(03): 223–228