Chapter

MicroRNAs in Platelets: Should I Stay or Should I Go?

Sonia Águila, Ernesto Cuenca-Zamora, Constantino Martínez and Raúl Teruel-Montoya

Abstract

In this chapter, we discuss different topics always using the microRNA as the guiding thread of the review. MicroRNAs, member of small noncoding RNAs family, are an important element involved in gene expression. We cover different issues such as their importance in the differentiation and maturation of megakaryocytes (megakaryopoiesis), as well as the role in platelets formation (thrombopoiesis) focusing on the described relationship between miRNA and critical myeloid lineage transcription factors such as RUNX1, chemokines receptors as CRCX4, or central hormones in platelet homeostasis like TPO, as well as its receptor (MPL) and the TPO signal transduction pathway, that is JAK/STAT. In addition to platelet biogenesis, we review the microRNA participation in platelets physiology and function. This review also introduces the use of miRNAs as biomarkers of platelet function since the detection of pathogenic situations or response to therapy using these noncoding RNAs is getting increasing interest in disease management. Finally, this chapter describes the participation of platelets in cellular interplay, since extracellular vesicles have been demonstrated to have the ability to deliver microRNAs to others cells, modulating their function through intercellular communication, redefining the extracellular vesicles from the so-called "platelet dust" to become mediators of intercellular communication.

Keywords: platelets, megakaryocytes, microRNAs, intercellular communication, microvesicles

1. Introduction

The discovery of noncoding RNAs, apart from raising new questions about the central dogma of molecular biology, has greatly contributed to the basic knowledge of numerous diseases, and to their therapeutic utility [1]. Nowadays, nobody doubts that the so-called "junk genome," a large DNA proportion of the genome [2], undoubtedly participates in the flow of genomic information [3] that gives rise to cellular functions and that frequent diseases are associated with malfunction of noncoding RNAs [4].

Although essential processes are common to all kind of cells, in physiological condition, each tissue or cell has unique features or responses that define their phenotype. These functions will be controlled by the gene expression patterns of that tissue and therefore by the regulatory mechanisms of such expression.

Thus microRNAs (miRNAs), which are a family of small noncoding RNAs, are an important element in gene-expression-regulation [5]. This function of miRNAs, which is exerted through repression of gene expression, is related to the specific miRNA expression, in a spacial and timely manner; in other words, it is tissue and/or developmental stage-specific [6].

This becomes more important in a cell that lacks a nucleus, such as platelets, and therefore lacks the ability to regulate gene expression through canonical mechanisms such as transcription factors (TFs) or epigenetic mechanisms of genome modification, for example, cytosine methylation or histone acetylation. In this scenario, miRNAs may acquire more prominence in the processes of gene regulation at a post-transcriptional level [7], not only during platelet production (thrombopoiesis), but also providing to the platelets with certain capacity to modulate their phenotype and consequently contribute to their ability to respond to external stimuli.

Historically, the study of hematopoiesis has been focused in general on the investigation of cytokine receptors and TFs, which ultimately govern the different membrane marker characteristics of each hematopoietic lineage, in a continuous change in gene expression patterns. The TFs expressed by megakaryocyte (MK) progenitors that enable their engagement with the lineage are increasingly understood. During MK differentiation, the progression to specific hematopoietic pathways is influenced by changes in the levels of these factors. These changes are dynamic, and in this scenario, it is clear that the repressive action exerted by miRNAs on their targets, for example, TFs, may be one more element to pay attention to in hematopoiesis [8, 9].

Finally, as mentioned above, there is a certain specificity of miRNA expression at the tissue-cell level, but in recent years cellular communication mechanisms have also been described in which a transfer of content from cell to cell [10], through extracellular vesicles (EVs), for example, microvesicles or exosomes, can occur. Within the content of EVs, we can find genetic material such as miRNAs [11], thus making possible the regulation of gene expression at a distance. If we add to this the fact that platelets provide a large proportion of the total cellular miRNAs found in blood [12], in addition to the high correlation observed in miRNA profiles expression between plasma and platelets [13] and that most of the EVs that circulate in bloodstream come from platelets [14], their study from a pathophysiological point of view, as well as their possible use as a therapeutic tool, is very relevant.

In summary, in this chapter we intend to discuss different topics, always using the miRNA as the guiding thread of the review, which would cover issues such as their importance in the platelet biogenesis, as well as their participation in their (platelets') physiology and function. This review will also introduce issues such as the use of miRNAs as biomarkers of platelet function, and the detection of pathogenic situations. Finally, this chapter will describe the participation of platelets in cellular interplay, since EVs have been demonstrated to have the ability to deliver their cargo to other cells, modulating their function through intercellular communication.

2. Biogenesis and function of miRNA

2.1 Biogenesis

The synthesis of miRNAs begins in the nucleus, through their transcription by RNA polymerase II, giving rise to a product called pri-miRNA, which has

a size range from several hundred nucleotides (nt), to several kilobases; this may depend on the miRNA gene location, that is, polycistronic, intronic, or introgenic. Still in the nucleus, pri-miRNA is processed into pre-miRNA by the Microprocessor catalytic complex, composed by Drosha and DGCR8. The first component is a nuclease with two RNAse III domains, which are responsible for cutting the pri-miRNAs. DGCR8, on the other hand, contains RNA-binding domains that stabilize the pri-miRNA for being processed by Drosha [15]. The pre-miRNA resulting structure is 3'overhang hairpin-like shape and it has a size of approximately 70 nt. Pre-miRNA will leave the nucleus, mainly by a RanGTP/export 5-dependent mechanism and once in the cytoplasm will be reprocessed, by Dicer, resulting in a new ~22 nt size double-stranded form [16]. Of the two strands that form the mature-duplex pre-miRNA, one is the so-called "guide" strand and will be loaded into Argonaute (AGO), while the other strand may be degraded or even become functional too. Despite the fact that general aspects of the miRNA maturation pathway have been known for a long time, at present and due to structural studies, the functions of the macromolecular complexes involved are increasingly known in detail, giving a deeper understanding of their involvement in miRNA cytoplasmic processing [16, 17].

2.2 Function

The complete functional unit consists of the mature miRNA and the AGO protein, which recognizes by base pairing its target mRNA, mainly in the 3′ untranslated (3'UTR) region, and this union will induce both, an inhibition of mRNA translation in its first stages and a RNA decaying. The repressive action on translation is exerted by the initiation factors 4 A-I (eIF4A-I) and eIF4A-II. On the other hand, the mRNA decay is favored by the 5'mRNA deadenylation, followed by its decapping [18]. Finally, this leads to an indelible genetic silencing, which is often complex to study due to the intricate network and interactions that occur between different miRNAs and mRNAs, since a miRNA can have multiple target mRNAs, and a single mRNA can be cooperatively regulated by multiple miRNAs (**Figure 1**).

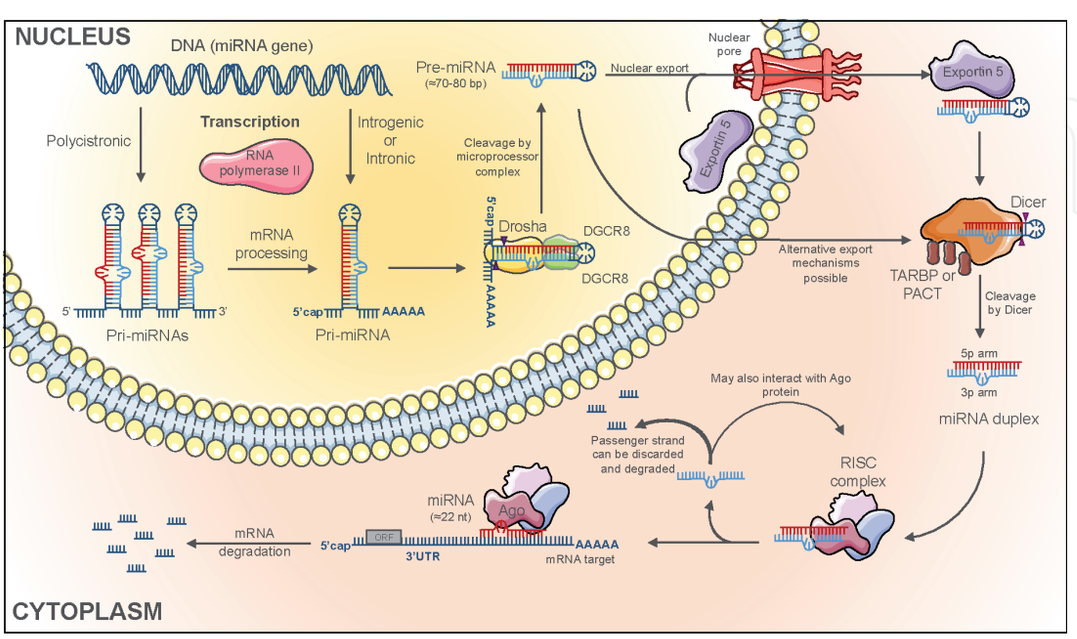

Figure 1.
Outline of the processes involved both in miRNA biogenesis and in its function.

3. Platelets miRNA repository

Since Landry et al. showed in 2009 that platelets have abundant and diverse miRNAs [19], numerous studies have analyzed the content of miRNA in platelets. We review the miRNAs detected in platelets, focusing especially on those detected with greater expression. First, we have to keep in mind that there are different technologies, therefore platforms, in which a high throughput miRNA analysis can be performed, such as microarray, nanostring, or RNA-seq [20]. We will not go into details about the technologies used, but we will rather give importance to the experimental conditions used, in terms of the platelets purity. Since the total RNA content in platelets, in absolute terms, is very low, compared to other blood cells [12], that is leucocytes, a possible contamination can alter or bias the results. Thus, we here focus on studies that performed a leukocyte depletion step by filtration or immunoselection. We summarize in a table (**Table 1**), the expression of the 40 most expressed miRNAs from selected studies, in order to outline the miRNAs that coincide between them. To note, all the provided information comes from human

	Landry [19]	**Nagalla [23]**	**Edelstein [21] and Simon [22]**	**Teruel-Montoya [24]**
1	miR-142-5p	miR-223	miR-223	miR-126
2	miR-142-3p	miR-26b	miR-26a	miR-223
3	miR-223	miR-26a	miR-126	miR-142-3p
4	let-7a	miR-23a	miR-142-3p	miR-26a
5	miR-185	miR-126	miR-16	let-7 g
6	let-7c	miR-21	miR-92a	miR-16
7	let-7i	let-7f	miR-21	miR-15b
8	let-7b	miR-22	miR-103a	miR-92a
9	miR-126	miR-24	miR-20a/b	let-7a
10	miR-103a	miR-720	let-7a	let-7d
11	miR-320	miR-16	miR-24	miR-21
12	miR-30c	miR-23b	let-7 g	miR-199a/b
13	miR-130a	miR-142-3p	miR-199a/b	miR-451a
14	miR-26a	miR-142-5p	miR-15a	miR-15a
15	miR-191	miR-191	let-7d	let-7f
16	miR-30b	miR-451a	miR-30b	miR-23a
17	miR-146a	miR-30b	let-7f	miR-103a
18	miR-23b	miR-15b	let-7i	let-7i
19	miR-21	let-7a	miR-15b	miR-20a/b
20	miR-23a	miR-1826	miR-23a	miR-24
21	miR-19a	miR-17	miR-221	miR-191
22	miR-106a	miR-103a	miR-146a	miR-25
23	miR-15b	miR-106a	miR-451a	miR-19b
24	miR-20a	miR-30c	miR-19b	miR-30b
25	miR-107	miR-130a	miR-142-5p	miR-221
26	miR-451a	let-7 g	miR-148b	miR-148b
27	miR-222	miR-30a	miR-191	miR-146a

	Landry [19]	**Nagalla [23]**	**Edelstein [21] and Simon [22]**	**Teruel-Montoya [24]**
28	miR-151-5p	miR-146b	miR-25	miR-27b
29	miR-24	miR-34b	miR-106b	miR-151a
30	miR-625	miR-106b	miR-423-5p	miR-181a
31	miR-671	miR-15a	miR-27b	miR-720
32	miR-29a	miR-374a	miR-340	miR-29a
33	miR-22	miR-20a	miR-181a	miR-142-5p
34	miR-16	miR-185	miR-151-5p	miR-423
35	miR-199a/b	miR-146a	miR-720	miR-125a
36	miR-106b	let-7i	miR-199a-5p	miR-374a
37	miR-146b	let-7c	miR-101	miR-106b
38	miR-151-3p	miR-19a	miR-374b	miR-29c
39	miR-98	let-7d	miR-29a	miR-335
40	miR-148b	miR-27a	miR-148a	miR-148a

Underlined those miRNAs that appear in the top 40 in all the studies.

Table 1.
Comparison of the 40 most expressed miRNA in platelets ranked by expression.Rank.

healthy subjects. While it is true that there may be differences in age, gender, or race [21, 22], we observe that there are 18 miRNA coincidences, that is, miR-223, miR-126, miR-26a, miR-142-3p, miR-16, miR-21, miR-103a, let-7a, miR-24, miR-30b, let-7i, miR-15b, miR-23a, miR-146a, miR-451a, miR-142-5p, miR-191, and miR-106b. With a lower degree of coincidence, there are five miRNA coincidences in four out of the five publications: let-7f, let-7d, miR-15a, let-7 g, and miR-720. In addition, there are 11 miRNA matches in at least 3 of the 5 datasets, that is, miR-103, miR-19b, miR-27b, miR-92a, miR-181a, miR-148a, miR-221, miR-25, miR-20a/b, and miR-151-5p.

The knowledge of the platelets-miRNA-cargo, as it is explained throughout the chapter, is important for understanding not only platelet biogenesis and physiology itself, but also MK function. Thus, platelets represent a faithful reflection of the physiological state of its progenitor with the advantage that platelets are much more accessible than the MKs. A better understanding of the physiological state of the platelet will undoubtedly help us to detect oscillations that may be pathological, and it is here that miRNAs can help us in diagnosis, prognosis, as well as to follow-up the disease progression. In addition to their great accessibility, circulating and platelet miRNAs are relatively easy to measure [25] and their physical-chemical properties make them good candidates for being used as biomarkers [26]. Furthermore, platelets are an important source of circulating miRNAs that can remotely regulate gene expression in other cells, such as macrophages or endothelial cells.

4. Role of miRNA in megakaryopoiesis and thrombopoiesis

Megakaryopoiesis and subsequent thrombopoiesis occur through a series of complex biological processes. First, MK precursors developed from hematopoietic stem cells (HSCs) initially proliferate, and then differentiate into mature polyploid MKs, which eventually release platelets. Thus, we cannot ignore great peculiarities

of platelets, in particular the fact that they are anucleate cells derived from MKs and that their content is the reflection of their parental cells, including the miRNA cargo. Accordingly, we consider it essential to describe the role of miRNAs in platelet and the megakaryopoiesis itself.

4.1 miRNAs in megakaryopoiesis

Megakaryopoiesis takes place primarily in the bone marrow. To understand this process in a simple way, it can be separated into two phases: the proliferative phase, in which the expansion of the MK precursors takes place, and the maturation phase, in which the two main events of this lineage occur. The first is at the nuclear level, the polyploidization by endomitosis, and the second is the cytoplasmic maturation [27].

In the last decade, many miRNAs have been described that can regulate or be regulated by TFs implicated in megakaryopoiesis (**Table 2**). For practical reasons, we only focus on some of the regulatory miRNAs. The miR-144/451 cluster, the miRNA most expressed in erythrocytes [12], can be repressed by RUNX1 during megakaryopoiesis. In myeloid differentiation, RUNX1 takes on a major role, since it represses erythroid lineage-specific genes and at the same time activates the transcription of specific genes of the MK lineage [28], and it is known to regulate MK polyploidization [29] and cytoskeleton rearrangement in the process of MK maturation formation [30]. On the other hand, it has been shown that miR-144/451 locus is activated by GATA1 [31–33], which primarily promotes erythropoiesis, but which has also been described as important for megakaryopoiesis. Another well-studied TF-miRNA interaction is mirR-27a and Runx1. miR-27a can repress Runx1 expression in mice. During megakaryopoiesis, Runx1 and miR-27a are engaged into a feedback positive loop regulation of miR-27a expression by Runx1. Ben-ami et al. also observed in K562 a human immortalized myelogenous leukemia cell line, and upon megakaryocytic differentiation by 12-o-tetradecanoylphorbol-13-acetate (TPA), that RUNX1 binds to a putative miR-27a regulatory region and upregulates its expression [34]. RUNX1-miRNA relationship goes in the direction of miRNA regulation by RUNXI, but RUNXI can also be regulated by miRNA; in this sense, miR-9 can regulate the expression of RUNX1. The increase of this miRNA in human MEG-01 and DAMI cell lines, both megakaryoblast phenotype cell lines, has shown a decrease in RUNX1 at both mRNA and protein levels [35]. Furthermore, an inverse level of expression in RUNX1 and miR-9 has been observed in MKs derived from umbilical cord blood (MKCB) and peripheral blood (MKPB) [35, 36]. We can also mention two more cases: (i) PLZF, a transcription factor whose expression increases during megakaryopoiesis, can downregulate miR-146a, which represses CXCR4 [37]; therefore, PLZF activates CXCR4 translation and the increase in CRCX4 can induce MK migration through bone marrow. (ii) ETS1 and MEIS1, both, are TFs with well-known functions in hematopoiesis; ETS1 is upregulated in megakaryocytopoiesis, regulating MK-specific gene promoters, such as platelet factor 4, GATA-2 or GPIIb, indicating that ETS1 promotes MK differentiation [38], and MEIS1 is vital for megakaryopoiesis and thrombopoiesis from human pluripotent stem cells [39]. MiR-155 downregulates both ETS1 and MEIS1, in HSCs, whereas miR-155 expression rapidly declines during TPO-induced megakaryocytic differentiation [40], thus favoring the process of megakaryocyte differentiation through ETS1 and MEIS1. As we have seen, there is evidence of the possible regulation of megakaryopoiesis by miRNAs, a connection between TF and miRNAs, and that the expression of the miRNA-coding genes is affected [41]. We do not know if this is due to a causal relationship or a consequence of the maturation process, perhaps

TF	miRNA	Function/effect	Ref.
RUNX1	miR-144/451	miR-144/451 cluster is repressed by RUNX1 during megakaryocytopoiesis	[28]
	miR-9	miR-9 represses RUNX1 during megakaryocytic differentiation	[35]
		miR-9 expression is higher in MKs from cord blood than MKs from peripheral blood, as opposed to RUNX1	[35, 49]
	miR-27a	In megakaryocytic differentiation, miR-27a is stimulated by RUNX1, and it can directly target RUNX1 (negative feedback loop)	[34]
FLI1	miR-145	miR-145 downregulates FLI1 during megakaryocytic differentiation. This increases MKs production compared to erythrocytes	[50]
		FLI1 may target back miR-145 promoter, creating a negative feedback loop	[51]
GATA1	miR-144/451	GATA1 activates miR-144/451 locus	[31–33]
	miR-138	miR-138 expression is increased by GATA1, and miR-138 represses BCR-ABL1 fusion gene	[52, 53]
HOXA1	miR-10a	miR-10a represses HOXA1. miR-10 is downregulated in human CD34+ bone marrow progenitor-derived MKs	[54]
		In cord blood HSCs, miR-10a knockdown increases HOXA1 expression and stimulates megakaryocytic differentiation, even in the absence of TPO	[55]
HOXA11	miR-181a	It is predicted that miR-181a targets HOXA11	[56]
ETS1 MEIS1	miR-155	miR-155 downregulates both ETS1 and MEIS1. In human HSPCs, during TPO-induced megakaryocytic differentiation, miR-155 expression is rapidly declined	[40]
ETV6	miR-181a	It is predicted that miR-181a targets ETV6	[56]
		miR-181a targets ETV6/RUNX1 gene fusion, observed in acute lymphoblastic leukemia	[57]
	miR-320a	miR-320a is downregulated by ETV6/RUNX1 gene fusion, and targets survivin, an antiapoptotic protein	[57, 58]
	miR-494	miR-494 is downregulated by ETV6/RUNX1 gene fusion, and targets survivin, an antiapoptotic protein	[57, 58]
MAFB	miR-130a	miR-130a, which represses MAFB, is downregulated during human megakaryocytic differentiation	[54]
GFI1	miR-22	miR-22 downregulates GFI1, stimulating myeloid and lymphoid differentiation	[59, 60]
		Overexpression of miR-22 increased K562 megakaryocytic differentiation, while miR-22 knockout inhibited this process induced by PMA (and upregulates GFI1)	[60]
		miR-22 knockout mice showed less of both immature and mature MKs in bone marrow and more GFI1 expression	[60]
GFI1B	miR-22	GFI1B, a GFI1 paralog without miR-22 seed sequence, is highly expressed in MK lineage and in K562 cells, and promotes MK and erythrocyte differentiation	[60]
		In this case, miR-22 may regulate GFI1B indirectly through GFI1 because GFI1 and GFI1B could compete for DNA occupancy	[60]

TF	miRNA	Function/effect	Ref.
FOSB	miR-22	FOSB stimulates miR-22 expression in K562 stimulated by PMA, required for megakaryocytopoiesis	[61]
		miR-22 expression could be regulated by opposing activities of FOSB and GFI1	[62]
PLZF (ZBTB16)	miR-146a	ZBTB16 downregulates miR-146, which represses CXCR4.	[37]
		miR-146a and CXCR4 (and its ligand SDF1) regulate HSC homing, MK proliferation, differentiation, and maturation	[63, 64]
EVI1	miR-133	EVI1 overexpression upregulates miR-133, in acute myeloid leukemia cells	[65]
		miR-133 downregulates EVI1, making a negative feedback loop	[66]
	miR-1	EVI1 overexpression upregulates miR-1, which increases cell proliferation, in acute myeloid leukemia cells	[65]
	miR-449a	miR-449a is repressed by EVI1, and this microRNA downregulates NOTCH1 and BCL2. In this way, miR-449a expression decreases cell viability and increases apoptosis of MECOM overexpressing leukemic cells	[67]

ABL1: ABL proto-oncogene 1, non-receptor tyrosine kinase; BCL2: BCL2 apoptosis regulator; BCR: BCR activator of RhoGEF and GTPase; CXCR4: C-X-C motif chemokine receptor 4; ETS1: ETS proto-oncogene 1; ETV6: ETS variant transcription factor 6; EVI1: ecotropic viral integration site 1; FLI1: Fli-1 proto-oncogene, ETS transcription factor; FOSB: FosB proto-oncogene, AP-1 transcription factor subunit; GATA1: GATA binding protein 1; GFI1: growth factor independent 1 transcriptional repressor; GFI1B: growth factor independent 1B transcriptional repressor; HOXA1: Homeobox A1; HOXA11: Homeobox A11; HSCs: hematopoietic stem cells; HSPCs: hematopoietic stem and progenitor cells; MAFB: MAF BZIP transcription factor B; MECOM: MDS1 and EVI1 complex locus; MEIS1: Meis Homeobox 1; MKs: megakaryocytes; NOTCH1: notch receptor 1; PLZF: promyelocytic leukaemia zinc finger; PMA: phorbol myristate acetate; RUNX1: RUNX family transcription factor 1; SDF1: stromal cell-derived factor 1; TPO: thrombopoietin; ZBTB16: zinc finger and BTB domain containing 16.

Table 2.
Transcription factors-miRNA relationship in megakaryocytic differentiation.

both, but what we do know is that it can have a side effect on the expression of many other genes.

Besides the relationships of TFs and miRNAs in megakaryopoiesis, other miRNAs have been described as complex effectors of megakaryopoiesis, for example the miR-146 family, which includes miR-146a and miR-146b. Specifically, miR-146a, a miRNA closely related to inflammatory diseases, seems to play an important role in the development of normal hematopoiesis. Thus, deficient miR-146a mice, stands out for the phenotypic features of abnormal hematopoiesis, highlighted by bone marrow myelofibrosis [42]. Its complex role in megakariopoiesis lies in part in some controversy about the role of this miRNA in this process. It has been described that miR-146a expression is high during induced megakaryocytopoiesis in vivo in murine model and in vitro in human cell culture; however, enforced miR-146a expression has minimal effects on the process [41]. On the other hand, it has been reported that miR-146a expression is downregulated when human cord blood-derived CD34+ cells are induced to differentiate into MKs [37], but miR-146a overexpression impaired megakaryocytopoiesis [41] and knockdown of miR-146a in mouse HSCs resulted in increased MKs in the bone marrow [43, 44]. This disagreement may be due to differences in experimental conditions and the differences between human and murine models that have been used, but this does not detract from the fact that miR-146a may actually have an effect on megakaryopoiesis. The other member of miR-146 family, miR-146b, regulates directly and indirectly,

through GATA-1, the expression of the platelet-derived growth factor receptor α (PDGFRA), in phorbol 12-myristate 13-acetate (PMA)-differentiated K562 cells. In addition, the expression of miR-146b increases in CD34+ hematopoietic stem/progenitor cells undergoing megakaryocytic differentiation; at the same time, the expression of PDGFRA decreases [45]. Finally and also in CD34+ derived MK differentiation assays, other researchers suggest that miR-28 plays a negative role in the differentiation of MK precursors. Through miR-28 transduction experiments on human CD34+ cells, with subsequent differentiation to MKs, using thrombopoietin (TPO), they observed a reduction of more than 50% in the number of MKs with proplatelets. This is, in part, explained by a repression of MPL gene expression, which encodes for the TPO receptor [46]. In relation with TPO, the expression of miR-150 has been described to increase with this hormone [47], and assays consisting in overexpression of miR-150, seem to suggest that elevated levels of miR-150 enhanced both in vitro and in vivo megakaryocyte differentiation at the expense of erythroid differentiation [48].

4.2 miRNAs in thrombopoiesis

Thrombopoiesis is the process by which platelets are generated from MKs During this process, microtubules mediate elongation of the MK extensions (proplatelet, **Figure 2**) and granule trafficking from MKs to nascent platelets [68].

As we have already mentioned, the TFs cited above are not only of great importance in megakaryopoiesis, but also in thrombopoiesis. It is very intuitive to think that the miRNAs that can regulate these TFs will also affect thrombopoiesis. Since many of the mutations described in RUNX1, FLI1, GATA1, GFI1B, ETV6, EVI1, and HOXA11 have been associated with variable thrombocytopenia [69]. The same effect can be expected for both cases, in the repression of TF-coding-gene expression, and when a mutation is observed that causes a loss of function in the same TF. Therefore, as far as possible, we will not be redundant with those relationships of miRNAs and TFs already described above (**Table 2**) that have repercussions on thrombopoiesis. In addition miRNAs can exert a quantitative regulation of

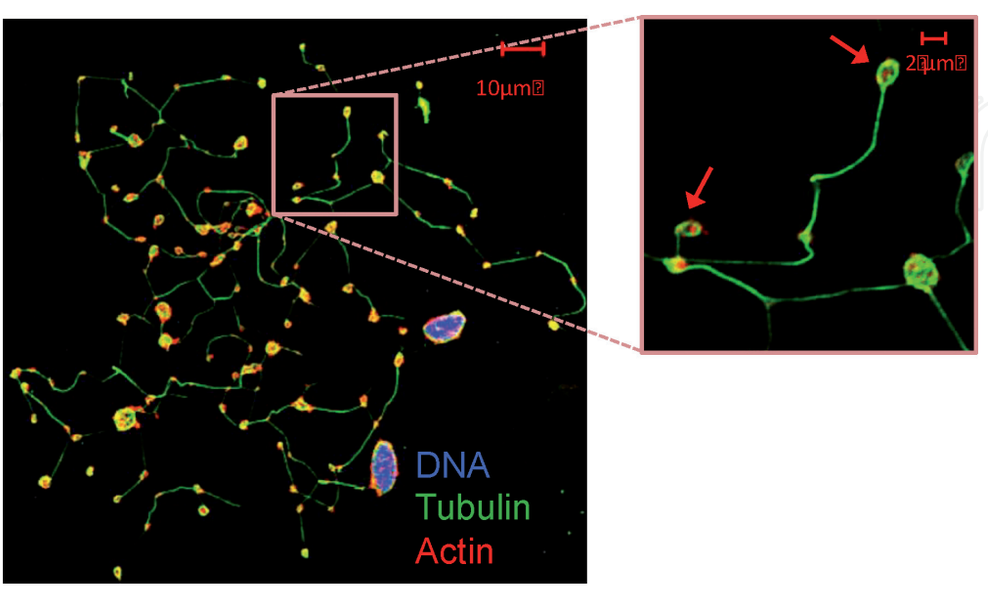

Figure 2.
Megakaryocytes and proplatelets [21]. CD34+ hematopoietic stem/progenitor cells obtained from human cord blood and differentiated to MKs and proplatelets. At day 14 of culture, MKs show proplatelet formation (pointed with red arrows). The color code of the fluorescence staining is as follows: DNA (DAPI, purple-blue), α-tubulin (green), and actin (red).

megakaryopoiesis, which will eminently translate into a regulation, a priori quantitative, of platelet formation.

In this part, we discuss those miRNAs that have been described as regulatory entities for the expression of genes encoding proteins of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, which is a fundamental pathway that controls platelet homeostasis. Indeed, mutations in those genes of this pathway, such as JAK2V617F in JAK2 and mutations in MPL exon 10, that cause a constitutive activation of the pathway, developing a thrombocytosis phenotype, named essential thrombocythemia (ET), have been described. As stated above, it has been reported that miR-28 targets the 3'UTR region of MPL, inhibiting its translation and probably contributing to reduce the number of MKs forming proplatelets [46], and therefore platelets number. The regulation of JAK2 by miRNAs in the context of thrombopoiesis is unknown, but Navarro et al. have described direct regulation of JAK2 by miR-135a by targeting its 3'UTR region in Hodgkin lymphoma [70]. Interestingly, the same author in another study described that SOCS1 and SOCS3, which are negative regulators of the JAK/STAT pathway, are directly regulated by miR-203 and miR-221 (a highly platelet expressed miRNA), respectively [71]. In addition, the levels of expression of these two miRNAs maintain a highly inverse correlation with the levels of SOCS1 and SOCS3 in platelets from patients with ET, suggesting that, in combination with a epigenetic regulation, those miRNAs could explain the SOCS1 and SOCS3 downregulated state in ET JAK2V617-negative patients, activating JAK/STAT pathway [71].

Finally and besides the JAK/STAT pathway, Rowley et al. observed in a specific MK Dicer-deficient murine model reduced levels in most platelet miRNAs, which lead to an altered profile of mRNA that apart from the functional consequences related to the increase of Itga2b (αIIb) and Itgb3 (β3), also provokes a mild thrombocytopenia [72].

5. miRNAs in platelet physiology and function

In previous sections, we dealt with quantitative changes observed in the production of platelets by miRNA. In contrast, in this section we deal with the qualitative changes that these can produce in platelets. The role of miRNAs in hemostasis is not well known, and most of the studies have focused on platelets as the central elements of primary hemostasis. Indeed, the first study reporting the presence of miRNAs in platelets was performed in 2008 in healthy controls and patients with polycythemia vera [73]. Of note, platelet miRNA levels have been recently correlated with platelet maturity and platelet function in patients with essential thrombocythemia [74]. Afterwards, many studies reported the presence of a large number of miRNAs in platelets [75]. Importantly, Landry et al. published a landmark paper that established miRNAs as important effectors of platelet function [19]. The authors showed that miRNAs were not only inherited from megakaryocytes, but platelets were also able to produce mature miRNAs from pre-miRNA since they contain all the machinery permitting the miRNA maturation (i.e., Dicer, TRBP2, and Ago2). Different circumstances make uneasy the study of miRNA function in platelets and until now their role still remains elusive. The main reasons are that platelets do not perform transcription and in vitro experiments with platelets are difficult to develop. Since it has been shown by different reports that pre-mRNA maturation as well as de novo translation occurs in platelets after agonist activation such as thrombin [76, 77], different studies have investigated if the same happens for miRNAs since, as mentioned above, they contain the necessary molecular tools for this process. The results

are controversial since whereas Corduan et al. showed that thrombin does not provoke miRNA maturation [78], other studies have shown that agonists such as thrombin receptor-activating peptide, collagen, and adenosine diphosphate (ADP) regulate miRNA levels [79]; the difference between both studies may reside in the incubation time, 10 min versus 120 min in the latter study. Thus, as suggested by Corduan et al., quantitative changes in miRNA level unlikely modulate de novo protein synthesis in the minutes following platelet activation, the regulation may rather occur during the whole platelet lifetime by mechanisms that are still to be fully characterized [77]. Upon activation, platelets are able to synthesize proteins such as interleukin-1β (IL-1β), plasminogen activator inhibitor-1 (PAI-1), or thrombospondin-1 (TSP-1). Interestingly, Miao et al. found that platelet activation with thrombin for 30 minutes modified the expression of 103 miRNAs [80]. Among these miRNAs, the authors showed that miR-27b levels, that target TSP-1 [81], decreased after thrombin activation, while TSP-1 increased. The authors also showed using a patented platelet transfection reagent (Ribojuice) that transfection of platelets with a miR-27b mimic decreased TSP-1 levels after thrombin activation [80]. Additionally, Corduan et al. suggested that several de novo translated proteins, among them integrins αIIb and β3, may be regulated by platelet-expressed miRNAs [78]. Interestingly, Rowley et al. demonstrated that in a conditional mouse model (MK) of Dicer1 deficiency, which provokes a reduction of certain miRNA expression levels, mice have high levels of platelet αIIbβ3, elevated platelet reactivity, shortened tail-bleeding time, and reduced survival following collagen/epinephrine-induced pulmonary embolism [72]. In particular, the authors suggest that miR-128, miR-326, miR-331, and miR-500 may regulate αIIbβ3 expression (**Table 3**). Recently, Middleton et al. demonstrated that sepsis increased αIIbβ3 translation in a cecal ligation and puncture (CLP) mouse model; it would be of interest to test if miRNAs involved in the regulation of these two proteins are downregulated and may be implicated in αIIbβ3 overexpression [82].

miRNA.	**Target**	**Ref.**
miR-223	$P2Y_{12}$ Factor XIII	[19, 87]
miR-126-3p	ADAM9 PLXNB2	[83]
miR-96	VAMP8	[88]
miR-21	WASP	[89]
miR-27b	TSP-1	[80]
miR-30c	PAI-1	[90]
miR-181a	RAP1B	[91]
miR-128 miR-500 miR-331 miR-326	αIIβ αIIβ,β3 αIIβ,β3 αIIβ	[72]
miR-24	αIIβ	[78]
miR-148a	TULA-2	[92]
miR-376c	PC-TP	[21]
miR-26b miR-140	SELP	[93]

Table 3.
miRNA-targets associated with platelets functionality.

Indeed, Szilágyi et al. recently studied the regulation of miRNAs in a CLP model and observed an important dysregulation of miRNAs in platelets that may explain the pro-thrombotic phenotype caused by this pathology.

Besides pathological conditions, genetic factors may also alter miRNA levels. For example, miR-126 rs4636297 SNP drives the efficiency of miR-126-3p transcription and has been associated with several plasma markers of platelet reactivity such as P-selectin (SELP) or PAF4 suggesting that lower miR-126-3p platelet levels would lead to lower platelet reactivity [83]. Zhou et al. recently showed another potential regulatory pathway for miR-126-3p [84]. The authors discovered that platelets from patients with diabetes mellitus (DM) type 2 had higher levels of long noncoding RNA metallothionein 1 pseudogene 3 (MT1P3) than healthy controls. Interestingly, MT1P3 is able to sponge miR-126-3p and provoke an overexpression of $P2Y_{12}$. This mechanism would additionally explain the high levels of ADP receptor observed in DM2 patients.

As indicated previously, investigating the role of miRNAs in platelet is sometimes an arduous task given the fact that basic cellular assays such as transfection or cell culture are difficult to perform. Investigators are trying to circumvent these problems by developing/adapting transfection reagent [80, 85], but also as nicely shown by García et al. by using alternative approaches [86]. In this paper, the authors demonstrated by using platelet-like structures obtained by differentiating human HSC CD34+ that miR-126-3p is involved in platelet function by regulating Plexin B2 (PLXNB2), an actin dynamics regulator [86]. The use of this kind of technology may help to better define the role of miRNAs in platelets.

6. Platelets-derived miRNA as biomarkers in pathophysiological conditions

Interestingly, platelet miRNAs are the major source of the circulating miRNA pool. Therefore, circulating miRNAs may be interesting biomarkers of disease and of diverse pathophysiological conditions [13]. Indeed, platelets have been implicated in pathological processes such as cardiovascular disease (CVD) [94], for example, myocardial infarction (MI), hypertension, stroke, atrial fibrillation, thrombosis or atherosclerosis, and another pathologies, for example, sepsis or diabetes. Platelet activation plays an important role in the development of the different pathological cardiovascular situations mentioned above. On the other hand, in situations such as sepsis, platelet activation may be a consequence of the disease. In one way or another, platelet activation is essential in these pathologies and miRNAs could be used as a molecular biomarker of this process [13].

miRNAs, whose expression in platelets is consensually high, have been described as independent biomarkers in CVD. For example, let-7 g and miR-191 are independent biomarkers of chronic kidney disease among patients with hypertension [95]. Also, miR-22 and miR-223 were reduced in hypertensive patients with cardiovascular complication being negatively correlated with systolic blood pressure. Furthermore, miR-126 levels were indicative of cardiovascular disease in this patient cohort [96]. miR-21 has been demonstrated to inhibit inflammatory responses in the early phase of MI by targeting KBTBD7 and impairing MKK3/6 activation in immune cells, which subsequently prevented excessive scar formation and improving cardiac function in mice [97].

A special mention should be made of miR-223, abundantly expressed in platelets and highly detected in plasma. This miRNA has been involved in the pathogenesis of different cardiovascular diseases through its effect in platelet reactivity and in endothelial cells [98, 99]. Furthermore, miR-223 may become a reporter of the

efficacy of anti-platelet therapy, since it was described that circulating miR-223 may serve as a novel biomarker to assess clopidogrel responsiveness in troponin-negative non-ST elevation acute coronary syndrome patients [100]. Therefore, platelet-derived miRNAs have been related with different cardiovascular diseases and might have important roles as biomarkers not only for cardiovascular disease susceptibility, but also for its prognosis and treatment.

Finally, and as mentioned above, platelets-derived miRNAs can also be helpful from the point of view of biomarkers in other pathological situations such as sepsis or diabetes. Little is known about the regulation of miRNAs in platelets; conditions such as sepsis may play an important role. Indeed, septic platelets showed an altered miRNA profile and reduced platelet miR-26b correlated with sepsis severity and mortality [101]. Therefore, it could become a useful biomarker to indicate the high state of platelet activation in this disease. Other pathological conditions may also alter miRNA levels and impact platelet function. In diabetes, calpain, which is increased in platelets, may cleave Dicer, decreasing the levels of several miRNAs such as miR-223 that may affect functional targets such as the purinergic ADP receptor P2Y12, ultimately promoting the development of thrombosis [19, 87]. Other miRNAs were also found to be altered in diabetes. Fejes et al. showed that miR-26b levels were reduced in DM2 patients with an impact in the levels of SELP [93].

7. Involvement of platelets in intercellular communication

EVs were discovered by Peter Wolf in 1967 and named as "platelets dust" [102]. EVs are basically divided into exosomes, having the smallest diameter size from 30 to 100 nm; apoptotic bodies, which are larger in size (1–5 μm), released during apoptosis; and microvesicles or microparticles ranging in size from 0.1 to 1 μm. Microparticles are cell-derived vesicles that lack the synthetic capacity and may contain proteins, mRNAs, miRNAs, and cytoskeletal components. Platelet-derived microparticles (PMPs) are the most abundant microparticles in circulation and they can be produced from platelet itself or from megakaryocytes. CD42+, CD41+ CD61+/CD31− are some surface markers to identify PMPs, and phosphatidylserine exposure in the surface; whereas CD63 is a marker for platelet exosomes [103]. PMPs have different functions and can be released spontaneously depending on αIIβ3 integrin and cytoskeletal turnover. Platelets activation enhances MPs shedding, and depending on the stimulus or stress, differences in MPs cargo or number have been characterized. Indeed, increased PMPs have been described in myocardial infarction, hypertension, thrombosis, sepsis, diabetes, and other pathologies. Additionally, the platelet activation with different agonists showed that miR-223 was the most abundant miRNA in all samples, and 46 miRNAs were common to all conditions. An enrichment of these 46 miRNAs (in particular, miR-451a and miR-21) was observed in PMPs compared with platelet cargo. Interestingly, platelet activation by CRP (GPVI agonist) generated fourfold more vesicles than the stimulation with ADP, PAR1, or PAR4. Furthermore, platelets stimulated with thrombin shed MPs containing AGO2 and miR-223 complexes that were internalized by endothelial cells (ECs) targeting some genes such as FBXW and EFNA1 [104]. Indeed, miR-223 from PMPs regulated ECs apoptosis targeting insulin-like growth factor 1 receptor [105]. These MPs were also internalized by ECs progenitors in culture [106]. Importantly, PMPs are not only uptaken by endothelial cells. These microparticles play different roles in coagulation and thrombosis, immune response and endothelial senescence and permeability. Thus, PMPs containing miR-126-3p

were internalized by macrophages improving their phagocytic capacity, and regulating mRNAs of cytokines or chemokines [107]. Platelets also have a role beyond hemostasis like cancer. Hence, platelets and PMPs are important in tumor progression. MiR-223 from PMPs increased in lung cancer patients promoting its invasion [108]. However, Michael et al. have described that PMPs were infiltrated in the solid tumor promoting tumor cells apoptosis through inhibition of mitochondrial function by miR-24, one of the most abundant miRNAs in these MPs [109].

Platelets-derived exosomes also have an important role in miRNA transference. Plasma exosomes enriched in miR-223, miR-339, and miR-21 were shed before thrombosis in a stenosis murine model, resulting also increased in platelets from a murine stenosis model before thrombosis. These miRNAs were transferred to vascular smooth muscle cells (VSMCs) inhibiting PDGFRβ, involved in cellular proliferation [110]. The inhibition of PDGFRβ was observed in vivo as well [111]. Nevertheless, Zeng and coworkers found that activated platelet internalization by VSMCs rather than MPs increased miR-223 levels promoting VSMCs differentiation. Platelets from diabetic mice had reduced miR-223 levels and consequently increased VSMCs hyperplasia [112].

The phenomenon of horizontal miRNA transference by platelets and microvesicles internalization by ECs, macrophages, VSMCs, cancer cells, and other cells has been described in hepatocytes regulating their proliferation [113]. Activated platelets due to myocardial infarction lose the expression of specific miRNAs. Thus, some of these miRNAs were transferred to ECs in a microvesicle-dependent manner. In particular, miR-320b was transferred to ECs regulating the expression of ICAM-1 modulating adhesion to their surface [114]. Therefore, platelet miRNA transference has a pivotal role in the influence of intercellular signaling and gene expression reprogramming of different cells and in different pathophysiological situations. Moreover, we cannot exclude the emerging role of other noncoding RNAs such as YRNAs, long noncoding RNAs, and circular RNAs in platelets and in the intercellular communication [115] (**Figure 3**).

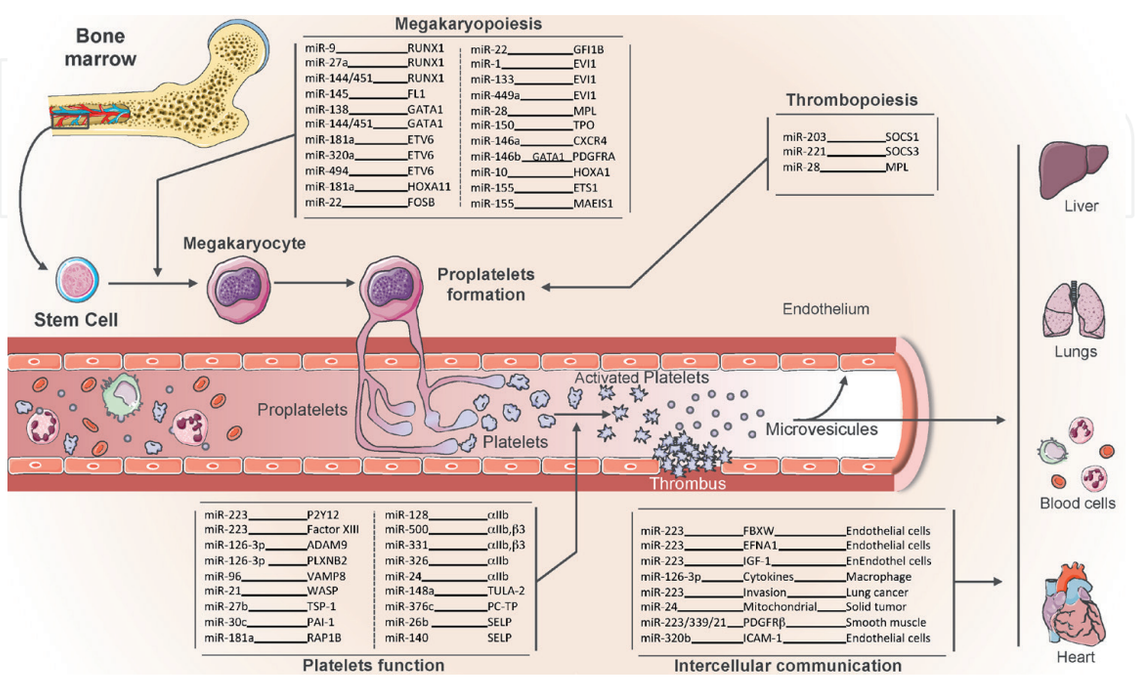

Figure 3.
Graphical abstract.

8. Conclusions

Collectively, the data presented in this chapter indicate that miRNAs have a pivotal role in all platelet states, from their generation regulating megakaryocytes maturation, mainly through transcription factors to their function influencing platelet response under activation (**Figure 3**). Interestingly, miRNAs are important not only for platelet biology, but also because they reflect the platelet status. Thus, platelet miRNAs are used as biomarkers for pathological situations or anti-platelet therapy because they represent the major contribution to circulating miRNA pool. Moreover, miRNAs from platelets may regulate the function of others cells (VSMC, ECs, immune cells, or tumor cells) by intercellular communication mainly through microparticles. Thus, platelet miRNAs have a great potential for future research as therapeutic tools and as biomarkers of disease. Additionally, their role as modulators of platelet function still has to be fully investigated to confirm that their presence in platelets is not a mere inheritance from MKs with any real impact in platelet biology.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Sonia Águila, Ernesto Cuenca-Zamora, Constantino Martínez and Raúl Teruel-Montoya*
Hematology and Medical Oncology Service, HGUJM Morales Meseguer, IMIB-Arrixaca, Transfusion Center, Murcia, Spain

*Address all correspondence to: raulteruelmontoya@hotmail.com

References

[1] Rezaeian A-H, Khanbabaei H, Calin GA. Therapeutic potential of the miRNA-ATM Axis in the management of tumor radioresistance. Cancer Research. 2020;**80**(2):139-150

[2] Gregory TR. Synergy between sequence and size in large-scale genomics. Nature Reviews. Genetics. 2005;**6**(9):699-708

[3] Shu J, Vieira B, Gao T, Xu Z, Cui J. Dynamic and modularized microRNA regulation and its implication in human cancers. Scientific Reports. 2017;**7**:13356. DOI: 10.1038/s41598-017-13470-5

[4] Lekka E, Hall J. Noncoding RNAs in disease. FEBS Letters. 2018 Sep;**592**(17):2884-2900

[5] Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: An overview of nuclear functions. International Journal of Molecular Sciences. 2016;**17**(10):1712

[6] Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, et al. MicroRNA expression in zebrafish embryonic development. Science. 2005;**309**(5732):310-311

[7] Jackson RJ, Standart N. How do microRNAs regulate gene expression? Science's STKE. 2007;**367**:re1

[8] Alsayegh K, Cortés-Medina LV, Ramos-Mandujano G, Badraiq H, Li M. Hematopoietic differentiation of human pluripotent stem cells: HOX and GATA transcription factors as master regulators. Current Genomics. 2019;**20**(6):438-452

[9] Kim M, Civin CI, Kingsbury TJ. MicroRNAs as regulators and effectors of hematopoietic transcription factors. Wiley Interdisciplinary Reviews: RNA. 2019;**10**(5):e1537

[10] Mittelbrunn M, Sánchez-Madrid F. Intercellular communication: diverse structures for exchange of genetic information. Nature Reviews. Molecular Cell Biology. 2012;**13**:328-335

[11] Teruel-Montoya R, Luengo-Gil G, Vallejo F, Yuste JE, Bohdan N, García-Barberá N, et al. Differential miRNA expression profile and proteome in plasma exosomes from patients with paroxysmal nocturnal hemoglobinuria. Scientific Reports. 2019;**9**(1):3611

[12] Teruel-montoya R, Kong X, Abraham S, Ma L, Kunapuli SP, Holinstat M, et al. MicroRNA expression differences in human hematopoietic cell lineages enable regulated transgene expression. PLoS One. 2014;**9**(7):1-12

[13] Willeit P, Zampetaki A, Dudek K, Kaudewitz D, King A, Kirkby NS, et al. Circulating microRNAs as novel biomarkers for platelet activation. Circulation Research. 2013;**112**(4):595-600

[14] Berckmans RJ, Nieuwland R, Böing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. Thrombosis and Haemostasis. 2001;**85**(4):639-646

[15] Denli AM, Tops BBJ, Plasterk RHA, Ketting RF, Hannon GJ. Processing of primary microRNAs by the microprocessor complex. Nature. 2004;**432**(7014):231-235

[16] Liu Z, Wang J, Cheng H, Ke X, Sun L, Zhang QC, et al. Cryo-EM structure of human dicer and its complexes with a pre-miRNA substrate. Cell. 2018;**173**(5):1191-1203.e12

[17] Jin W, Wang J, Liu C-P, Wang H-W, Xu R-M. Structural basis for pri-miRNA

recognition by drosha. Molecular Cell. 2020;**78**:1-11

[18] Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nature Reviews. Molecular Cell Biology. 2019;**20**(1):21-37

[19] Landry P, Plante I, Ouellet DL, Perron MP, Rousseau G, Provost P. Existence of a microRNA pathway in anucleate platelets. Nature Structural & Molecular Biology. 2009;**16**(9):961-966

[20] Chatterjee A, Leichter AL, Fan V, Tsai P, Purcell RV, Sullivan MJ, et al. A cross comparison of technologies for the detection of microRNAs in clinical FFPE samples of hepatoblastoma patients. Scientific Reports. 2015;**5**:10438

[21] Edelstein LC, Simon LM, Montoya RT, Holinstat M, Chen ES, Bergeron A, et al. Racial differences in human platelet PAR4 reactivity reflect expression of PCTP and miR-376c. Nature Medicine. 2013;**19**(12):1609-1616

[22] Simon LM, Edelstein LC, Nagalla S, Woodley AB, Chen ES, Kong X, et al. Human platelet microRNA-mRNA networks associated with age and gender revealed by integrated plateletomics. Blood. 2014;**123**(16):e37–e45

[23] 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):5189-5197

[24] Teruel-Montoya R, Kong X, Abraham S, Ma L, Kunapuli SP, Holinstat M, et al. MicroRNA expression differences in human hematopoietic cell lineages enable regulated transgene expression. PLoS One. 2014;**9**(7):e102259

[25] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(30):10513-10518

[26] Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. Cell Research. 2008;**18**(10):997-1006. Available from: http://www.nature.com/doifinder/10.1038/cr.2008.282

[27] Machlus KR, Italiano JEJ. The incredible journey: From megakaryocyte development to platelet formation. The Journal of Cell Biology. 2013;**201**(6):785-796

[28] Kohrs N, Kolodziej S, Kuvardina ON, Herglotz J, Yillah J, Herkt S, et al. MiR144/451 Expression Is Repressed by RUNX1 During Megakaryopoiesis and Disturbed by RUNX1/ETO. PLoS Genetics. 2016;**12**(3):e1005946

[29] Lordier L, Bluteau D, Jalil A, Legrand C, Pan J, Rameau P, et al. RUNX1-induced silencing of non-muscle myosin heavy chain IIB contributes to megakaryocyte polyploidization. Nature Communications. 2012;**3**:717

[30] Bluteau D, Glembotsky AC, Raimbault A, Balayn N, Gilles L, Rameau P, et al. Dysmegakaryopoiesis of FPD/AML pedigrees with constitutional RUNX1 mutations is linked to myosin II deregulated expression. Blood. 2012;**120**(13):2708-2718

[31] Dore LC, Amigo JD, dos Santos CO, Zhang Z, Gai X, Tobias JW, et al. A GATA-1-regulated microRNA locus essential for erythropoiesis. Proceedings of the National Academy of Sciences. 2008;**105**(9):3333-3338

[32] Zhang L, Sankaran VG, Lodish HF. MicroRNAs in erythroid and megakaryocytic differentiation and megakaryocyte–erythroid progenitor lineage commitment. Leukemia. 2012;**26**(11):2310-2316

[33] Rasmussen KD, Simmini S, Abreu-Goodger C, Bartonicek N, Di Giacomo M, Bilbao-Cortes D, et al. The miR-144/451 locus is required for erythroid homeostasis. The Journal of Experimental Medicine. 2010;**207**(7):1351-1358

[34] Ben-ami O, Pencovich N, Lotem J, Levanon D, Groner Y. A regulatory interplay between miR-27a and Runx1 during megakaryopoiesis. Proceedings of the National Academy of Sciences of the United States of America USA. 2009;**106**:238-243

[35] Raghuwanshi S, Gutti U, Kandi R, Gutti RK. MicroRNA-9 promotes cell proliferation by regulating RUNX1 expression in human megakaryocyte development. Cell Proliferation. 2018;**51**:e12414

[36] Bluteau O, Langlois T, Favale F, Rameau P. Developmental changes in human megakaryopoiesis. Journal of Thrombosis and Haemostasis. 2013;**11**:1730-1741

[37] Labbaye C, Spinello I, Quaranta MT, Pelosi E, Pasquini L, Petrucci E, et al. A three-step pathway comprising PLZF/miR-146a/CXCR4 controls megakaryopoiesis. Nature Cell Biology. 2008;**10**(7):788-801

[38] Lulli V, Romania P, Morsilli O, Gabbianelli M, Pagliuca A, Mazzeo S, et al. Overexpression of Ets-1 in human hematopoietic progenitor cells blocks erythroid and promotes megakaryocytic differentiation. Cell Death and Differentiation. 2006;**13**(7):1064-1074

[39] Wang H, Liu C, Liu X, Wang M, Wu D, Gao J, et al. MEIS1 regulates hemogenic endothelial generation, megakaryopoiesis, and thrombopoiesis in human pluripotent stem cells by targeting TAL1 and FLI1. Stem Cell Reports. 2018;**10**(2):447-460

[40] Romania P, Lulli V, Pelosi E, Biffoni M, Peschle C, Marziali G. MicroRNA 155 modulates megakaryopoiesis at progenitor and precursor level by targeting Ets-1 and Meis1 transcription factors. British Journal of Haematology. 2008;**143**(4):570-580

[41] Opalinska JB, Bersenev A, Zhang Z, Schmaier AA, Choi J, Yao Y, et al. MicroRNA expression in maturing murine megakaryocytes. Blood. 2010;**116**(23):128-138

[42] Ferrer-Marín F, Arroyo AB, Bellosillo B, Cuenca EJ, Zamora L, Hernández-Rivas JM, et al. miR-146a rs2431697 identifies myeloproliferative neoplasm patients with higher secondary myelofibrosis progression risk. Leukemia. 2020. DOI: 10.1038/s41375-020-0767-3

[43] Starczynowski DT, Kuchenbauer F, Argiropoulos B, Sung S, Morin R, Muranyi A, et al. Identification of miR-145 and miR-146a as mediators of the 5q– syndrome phenotype. Nature Medicine. 2010;**16**(1):49-58

[44] Starczynowski DT, Kuchenbauer F, Wegrzyn J, Rouhi A, Petriv O, Hansen CL, et al. MicroRNA-146a disrupts hematopoietic differentiation and survival. Experimental Hematology. 2011;**39**(2):167-178.e4

[45] Zhai P-F, Wang F, Su R, Lin H-S, Jiang C-L, Yang G-H, et al. The regulatory roles of microRNA-146b-5p and its target platelet-derived growth factor receptor α (PDGFRA) in erythropoiesis and megakaryocytopoiesis. The Journal of Biological Chemistry. 2014;**289**(33):22600-22613

[46] Girardot M, Pecquet C, Boukour S, Knoops L, Ferrant A, Vainchenker W, et al. miR-28 is a thrombopoietin receptor targeting microRNA detected in a fraction of myeloproliferative neoplasm patient platelets. Blood. 2010;**116**(3):437-445

[47] Barroga CF, Pham H, Kaushansky K. Thrombopoietin regulates c-Myb expression by modulating micro RNA 150 expression. Experimental Hematology. 2008;**36**(12):1585-1592

[48] Lu J, Guo S, Ebert BL, Zhang H, Peng X, Bosco J, et al. MicroRNA-mediated control of cell fate in megakaryocyte-erythrocyte progenitors. Developmental Cell. 2008;**14**(6):843-853

[49] Raghuwanshi S, Dahariya S, Musvi SS, Gutti U, Kandi R, Undi RB, et al. MicroRNA function in megakaryocytes. Platelets. 2019;**30**(7):809-816

[50] Kumar MS, Narla A, Nonami A, Mullally A, Dimitrova N, Ball B, et al. Coordinate loss of a microRNA and protein-coding gene cooperate in the pathogenesis of 5q− syndrome. Blood. 2011;**118**(17):4666-4673

[51] Liu T, Xia L, Yao Y, Yan C, Fan Y, Gajendran B, et al. Identification of diterpenoid compounds that interfere with Fli-1 DNA binding to suppress leukemogenesis. Cell Death & Disease. 2019;**10**(2):117

[52] Xu C, Fu H, Gao L, Wang L, Wang W, Li J, et al. BCR-ABL/GATA1/miR-138 mini circuitry contributes to the leukemogenesis of chronic myeloid leukemia. Oncogene. 2014;**33**(1):44-54

[53] Undi RB, Kandi R, Gutti RK. MicroRNAs as haematopoiesis regulators. Advances in Hematology. 2013;**2013**:1-20

[54] Garzon R, Pichiorri F, Palumbo T, Iuliano R, Cimmino A, Aqeilan R, et al. MicroRNA fingerprints during human megakaryocytopoiesis. Proceedings of the National Academy of Sciences. 2006;**103**(13):5078-5083

[55] Zarif MN, Soleimani M, Abolghasemi H, Amirizade N, Arefian E, Rahimian A. Megakaryocytic differentiation of CD133+ hematopoietic stem cells by down-regulation of microRNA-10a. Hematology. 2013;**18**(2):93-100

[56] Zhou S-F, He S-M, Zeng S, Zhou Z-W, He Z. Hsa-microRNA-181a is a regulator of a number of cancer genes and a biomarker for endometrial carcinoma in patients: A bioinformatic and clinical study and the therapeutic implication. Drug Design, Development and Therapy. 2015;**9**:1103

[57] Yang Y-L, Yen C-T, Pai C-H, Chen H-Y, Yu S-L, Lin C-Y, et al. A double negative loop comprising ETV6/RUNX1 and MIR181A1 contributes to differentiation block in t(12,21)-positive acute lymphoblastic leukemia. Lausen J, editor. PLoS One. 2015;**10**(11):e0142863

[58] Diakos C, Zhong S, Xiao Y, Zhou M, Vasconcelos GM, Krapf G, et al. TEL-AML1 regulation of survivin and apoptosis via miRNA-494 and miRNA-320a. Blood. 2010;**116**(23):4885-4893

[59] Möröy T, Vassen L, Wilkes B, Khandanpour C. From cytopenia to leukemia: The role of Gfi1 and Gfi1b in blood formation. Blood. 2015;**126**(24):2561-2569

[60] Weiss CN, Ito K. MicroRNA-22 promotes megakaryocyte differentiation through repression of its target, GFI1. Blood Advances. 2019;**3**(1):33-46

[61] Limb J-K, Yoon S, Lee KE, Kim B-H, Lee S, Bae YS, et al. Regulation of megakaryocytic differentiation of K562 cells by FosB, a member of the Fos family of AP-1 transcription factors.

Cellular and Molecular Life Sciences. 2009;**66**(11-12):1962-1973

[62] Kim M, Civin CI, Kingsbury TJ. MicroRNAs as regulators and effectors of hematopoietic transcription factors. Wiley Interdisciplinary Reviews: RNA. 2019;**10**(5):e1537

[63] Asri A, Sabour J, Atashi A, Soleimani M. Homing in hematopoietic stem cells: Focus on regulatory role of CXCR7 on SDF1A/CXCR4 axis. EXCLI Journal. 2016;**15**:134-143

[64] Pang L, Weiss MJ, Poncz M. Megakaryocyte biology and related disorders. The Journal of Clinical Investigation. 2005;**115**(12):3332-3338

[65] Gómez-Benito M, Conchillo A, García MA, Vázquez I, Maicas M, Vicente C, et al. EVI1 controls proliferation in acute myeloid leukaemia through modulation of miR-1-2. British Journal of Cancer. 2010;**103**(8):1292-1296

[66] Yamamoto H, Lu J, Oba S, Kawamata T, Yoshimi A, Kurosaki N, et al. miR-133 regulates Evi1 expression in AML cells as a potential therapeutic target. Scientific Reports. 2016;**6**(1):19204

[67] De Weer A, Van der Meulen J, Rondou P, Taghon T, Konrad TA, De Preter K, et al. EVI1-mediated down regulation of MIR449A is essential for the survival of EVI1 positive leukaemic cells. British Journal of Haematology. 2011;**154**(3):337-348

[68] Cuenca-zamora EJ, Ferrer-mar F, Rivera J. Tubulin in platelets: When the shape matters. International Journal of Molecular Sciences. 2019;**20**(14):3484

[69] Songdej N, Rao AK. Hematopoietic transcription factor mutations: Important players in inherited platelet defects. Blood. 2017;**129**(21):2873-2881

[70] Navarro A, Diaz T, Martinez A, Gaya A, Pons A, Gel B, et al. Regulation of JAK2 by miR-135a: Prognostic impact in classic Hodgkin lymphoma. Blood. 2009;**114**(14):2945-2951

[71] Navarro A, Pairet S, Álvarez-Larrán A, Pons A, Ferrer G, Longarón R, et al. miR-203 and miR-221 regulate SOCS1 and SOCS3 in essential thrombocythemia. Blood Cancer Journal. 2016;**6**(3):e406

[72] Rowley JW, Chappaz S, Corduan A, Chong MMW, Campbell R, Khoury A, et al. Dicer1-mediated miRNA processing shapes the mRNA profile and function of murine platelets. Blood. 2016;**127**(14):1743-1751

[73] Bruchova H, Merkerova M, Prchal JT. Aberrant expression of microRNA in polycythemia vera. Haematologica. 2008;**93**(7):1009-1016

[74] Tran JQD, Pedersen OH, Larsen ML, Grove EL, Kristensen SD, Hvas A-M, et al. Platelet microRNA expression and association with platelet maturity and function in patients with essential thrombocythemia. Platelets. 2020;**31**(3):365-372

[75] 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

[76] Denis MM, Tolley ND, Bunting M, Schwertz H, Jiang H, Lindemann S, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. Cell. 2005;**122**(3):379-391

[77] Weyrich AS, Schwertz H, Kraiss LW, Zimmerman GA. Protein synthesis by platelets: Historical and new perspectives. Journal of Thrombosis and Haemostasis. 2009;**7**(2):241-246

[78] Corduan A, Plé H, Laffont B, Wallon T, Plante I, Landry P, et al. Dissociation of SERPINE1 mRNA from the translational repressor proteins Ago2 and TIA-1 upon platelet activation. Thrombosis and Haemostasis. 2015;**113**(5):1046-1059

[79] Cimmino G, Tarallo R, Nassa G, De Filippo MR, Giurato G, Ravo M, et al. Activating stimuli induce platelet microRNA modulation and proteome reorganisation. Thrombosis and Haemostasis. 2015;**114**(1):96-108

[80] Miao X, Rahman MF-U, Jiang L, Min Y, Tan S, Xie H, et al. Thrombin-reduced miR-27b attenuates platelet angiogenic activities in vitro via enhancing platelet synthesis of anti-angiogenic thrombospondin-1. Journal of Thrombosis and Haemostasis. 2018;**16**(4):791-801

[81] Wang J-M, Tao J, Chen D-D, Cai J-J, Irani K, Wang Q, et al. MicroRNA miR-27b rescues bone marrow-derived angiogenic cell function and accelerates wound healing in type 2 diabetes mellitus. Arteriosclerosis, Thrombosis, and Vascular Biology. 2014;**34**(1):99-109

[82] Middleton EA, Rowley JW, Campbell RA, Grissom CK, Brown SM, Beesley SJ, et al. Sepsis alters the transcriptional and translational landscape of human and murine platelets. Blood. 2019;**134**(12):911-923

[83] Kaudewitz D, Skroblin P, Bender LH, Barwari T, Willeit P, Pechlaner R, et al. Association of MicroRNAs and YRNAs with platelet function. Circulation Research. 2016;**118**(3):420-432

[84] Zhou M, Gao M, Luo Y, Gui R, Ji H. Long non-coding RNA metallothionein 1 pseudogene 3 promotes p2y12 expression by sponging miR-126 to activate platelet in diabetic animal model. Platelets. 2019;**30**(4):452-459

[85] Hong W, Kondkar AA, Nagalla S, Bergmeier W, Jin Y, Herman JH, et al. Transfection of human platelets with short interfering RNA. Clinical and Translational Science. 2011;**4**(3):180-182

[86] Garcia A, Dunoyer-Geindre S, Zapilko V, Nolli S, Reny J-L, Fontana P. Functional validation of microRNA-126-3p as a platelet reactivity regulator using human haematopoietic stem cells. Thrombosis and Haemostasis. 2019;**119**(2):254-263

[87] Elgheznawy A, Shi L, Hu J, Wittig I, Laban H, Pircher J, et al. Dicer cleavage by calpain determines platelet microRNA levels and function in diabetes. Circulation Research. 2015;**117**(2):157-165

[88] Kondkar AA, Bray MS, Leal SM, Nagalla S, Liu DJ, Jin Y, et al. VAMP8/endobrevin is overexpressed in hyperreactive human platelets: Suggested role for platelet microRNA. Journal of Thrombosis and Haemostasis. 2010;**8**(2):369-378

[89] Barwari T, Eminaga S, Mayr U, Lu R, Armstrong PC, Chan MV, et al. Inhibition of profibrotic microRNA-21 affects platelets and their releasate. JCI Insight. 2018;**3**(21):e123335

[90] Luo M, Li R, Ren M, Chen N, Deng X, Tan X, et al. Hyperglycaemia-induced reciprocal changes in miR-30c and PAI-1 expression in platelets. Scientific Reports. 2016;**6**:36687

[91] Dahiya N, Atreya CD. MiR-181a reduces platelet activation via the inhibition of endogenous RAP1B. MicroRNA. 2020;**9**(3):240-246

[92] Zhou Y, Abraham S, Andre P, Edelstein LC, Shaw CA, Dangelmaier CA, et al. Anti-miR-148a regulates platelet FcγRIIA signaling and decreases thrombosis in vivo in mice. Blood. 2015;**126**(26):2871-2881

[93] Fejes Z, Póliska S, Czimmerer Z, Káplár M, Penyige A, Gál Szabó G, et al. Hyperglycaemia suppresses microRNA expression in platelets to increase P2RY12 and SELP levels in type 2 diabetes mellitus. Thrombosis and Haemostasis. 2017;**117**(3):529-542

[94] McManus DD, Freedman JE. MicroRNAs in platelet function and cardiovascular disease. Nature Reviews Cardiology. 2015;**12**(12):711-717. Available from: http://www.nature.com/doifinder/10.1038/nrcardio.2015.101

[95] Berillo O, Huo K-G, Fraulob-Aquino JC, Richer C, Briet M, Boutouyrie P, et al. Circulating let-7g-5p and miR-191-5p are independent predictors of chronic kidney disease in hypertensive patients. American Journal of Hypertension. 2020;**33**(6):505-513

[96] Marketou M, Kontaraki J, Papadakis J, Kochiadakis G, Vrentzos G, Maragkoudakis S, et al. Platelet microRNAs in hypertensive patients with and without cardiovascular disease. Journal of Human Hypertension. 2019;**33**(2):149-156

[97] Yang L, Wang B, Zhou Q, Wang Y, Liu X, Liu Z, et al. MicroRNA-21 prevents excessive inflammation and cardiac dysfunction after myocardial infarction through targeting KBTBD7. Cell Death & Disease. 2018;**9**(7):769

[98] Chu M, Wu R, Qin S, Hua W, Shan Z, Rong X, et al. Bone marrow-derived microRNA-223 works as an endocrine genetic signal in vascular endothelial cells and participates in vascular injury from Kawasaki disease. Journal of the American Heart Association. 2017;**6**(2):e004878

[99] Chyrchel B, Totoń-Żurańska J, Kruszelnicka O, Chyrchel M, Mielecki W, Kołton-Wróż M, et al. Association of plasma miR-223 and platelet reactivity in patients with coronary artery disease on dual antiplatelet therapy: A preliminary report. Platelets. 2015;**26**(6):593-597

[100] Zhang Y-Y, Zhou X, Ji W-J, Shi R, Lu R-Y, Li J-L, et al. Decreased circulating microRNA-223 level predicts high on-treatment platelet reactivity in patients with troponin-negative non-ST elevation acute coronary syndrome. Journal of Thrombosis and Thrombolysis. 2014;**38**(1):65-72

[101] Szilágyi B, Fejes Z, Póliska S, Pócsi M, Czimmerer Z, Patsalos A, et al. Reduced miR-26b expression in megakaryocytes and platelets contributes to elevated level of platelet activation status in sepsis. International Journal of Molecular Sciences. 2020;**21**(3):866

[102] Wolf P. The nature and significance of platelet products in human plasma. British Journal of Haematology. 1967;**13**(3):269-288

[103] Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: Microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. Blood. 1999;**94**(11):3791-3799

[104] Laffont B, Corduan A, Plé H, Duchez A-C, Cloutier N, Boilard E, et al. Activated platelets can deliver mRNA regulatory Ago2·microRNA complexes to endothelial cells via microparticles. Blood. 2013;**122**(2):253-261

[105] Pan Y, Liang H, Liu H, Li D, Chen X, Li L, et al. Platelet-secreted microRNA-223 promotes endothelial cell apoptosis induced by advanced glycation end products via targeting the insulin-like growth factor 1 receptor. Journal of Immunology. 2014;**192**(1):437-446

[106] Prokopi M, Pula G, Mayr U, Devue C, Gallagher J, Xiao Q, et al.

Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. Blood. 2009;**114**(3):723-732

[107] Laffont B, Corduan A, Rousseau M, Duchez A-C, Lee CHC, Boilard E, et al. Platelet microparticles reprogram macrophage gene expression and function. Thrombosis and Haemostasis. 2016;**115**(2):311-323

[108] Liang H, Yan X, Pan Y, Wang Y, Wang N, Li L, et al. MicroRNA-223 delivered by platelet-derived microvesicles promotes lung cancer cell invasion via targeting tumor suppressor EPB41L3. Molecular Cancer. 2015;**14**:58

[109] Michael JV, Wurtzel JGT, Mao GF, Rao AK, Kolpakov MA, Sabri A, et al. Platelet microparticles infiltrating solid tumors transfer miRNAs that suppress tumor growth. Blood. 2017;**130**(5):567-580

[110] Tan M, Yan H-B, Li J-N, Li W-K, Fu Y-Y, Chen W, et al. Thrombin stimulated platelet-derived exosomes inhibit platelet-derived growth factor receptor-beta expression in vascular smooth muscle cells. International Journal of Experimental Cellular Physiology, Biochemistry and Pharmacology. 2016;**38**(6):2348-2365

[111] Silverman-Gavrila R, Silverman-Gavrila L, Bendeck MP. Cell division fidelity is altered during the vascular response to injury: its novel role in atherosclerosis progression. The American Journal of Pathology. 2013;**182**(3):628-639

[112] Zeng Z, Xia L, Fan X, Ostriker AC, Yarovinsky T, Su M, et al. Platelet-derived miR-223 promotes a phenotypic switch in arterial injury repair. The Journal of Clinical Investigation. 2019;**129**(3):1372-1386

[113] Kirschbaum M, Karimian G, Adelmeijer J, Giepmans BNG, Porte RJ, Lisman T. Horizontal RNA transfer mediates platelet-induced hepatocyte proliferation. Blood. 2015;**126**(6):798-806

[114] Gidlöf O, van der Brug M, Ohman J, Gilje P, Olde B, Wahlestedt C, et al. Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. Blood. 2013;**121**(19):3908-3917. S1-26

[115] Sunderland N, Skroblin P, Barwari T, Huntley RP, Lu R, Joshi A, et al. MicroRNA biomarkers and platelet reactivity: The clot thickens. Circulation Research. 2017;**120**(2):418-435