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Platelet mRNA: the meaning behind the message

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Abstract

Purpose of review—It is now well appreciated that megakaryocytes invest platelets with a diverse repertoire of messenger RNAs (mRNAs), which are competent for translation. Herein we describe what is currently known regarding the expression, function, and clinical significance of mRNAs in platelets.

Recent findings—Although mRNA was detected in platelets nearly 30 years ago, we are only beginning to understand the roles of mRNA in platelet biology and human disease. Recent studies have shown that megakaryocytes specifically sort, rather than randomly transfer, mRNA to platelets during thrombopoiesis. As a result, platelets are released into the circulation with thousands of mRNAs. The emergence of next-generation RNA sequencing has demonstrated that platelet mRNAs possess classic structural features, which include untranslated regions and open reading frames. There is also growing evidence that platelet mRNA expression patterns are altered in human disease.

Summary—Intense investigation of platelet mRNA has shed considerable light on predicted functions of platelets and identified previously unrecognized attributes of platelets. Lessons learned from platelet mRNA is presented in this review.

Keywords

megakaryocytes; messenger RNA; platelets; splicing; translation

INTRODUCTION

Platelets sprout from megakaryocytes as fragments of cytoplasm that lack genomic DNA. Thus, they are incapable of transcribing nuclear material. This inadequacy generated a central dogma that human platelets are synthetically stagnant during their 9–11 day circulation period. This limited view, however, has taken a backseat as emerging evidence demonstrates that megakaryocytes invest platelets with requisite translational machinery that includes ribosomes, initiation and termination factors, microRNAs (miRNAs), and template messenger RNAs (mRNAs). Recent reviews from our group and others have described the general synthetic activities of platelets [1–4] and others have highlighted the potential roles of miRNAs in platelet function [5,6]. Here, we take a focused look at mRNA in platelets.

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Conflicts of interest

There are no conflicts of interest.

SOURCE OF PLATELET MESSENGER RNA

It is generally believed that transcriptional activity ramps up in individual megakaryocytes as they prepare to generate and release thousands of platelets [7]. As a result, megakaryocytes transcribe thousands of mRNAs that serve as templates for protein synthesis during thrombopoiesis. Although the actual transcriptome of bone marrow megakaryocytes has not been elucidated, a small number of studies have profiled mRNAs in progenitor cells that have been differentiated into megakaryocytes *in vitro* [8–14]. These studies have identified genes important in megakaryocyte and platelet biology and revealed new insight into the diversity and breadth of the megakaryocyte transcriptome.

A key question is, what happens to mRNAs after they are transcribed by megakaryocytes? Are they degraded, sequestered in cytoplasmic compartments that fail to reach platelets, or transferred to platelets? Absolute answers to the fate of megakaryocyte mRNAs are not yet known. Nonetheless, there is compelling evidence that megakaryocytes transfer many of their mRNAs to platelets. This conclusion is based on series of studies over the last 25 years that have either detected candidate mRNAs in platelets by northern blot analysis and PCR amplification or profiled the platelet mRNA pool by using high-throughput array-based technology (reviewed by Harrison and Goodall [4]). The take home is that platelets express thousands of mRNAs, which presumably are megakaryocyte-derived.

Our group has recently used next-generation RNA sequencing (next-gen RNA-seq) to gather more in-depth information regarding the platelet transcriptome [15[■]]. RNA-seq generates one or more short sequencing reads for nearly every transcript present in a sample. Sequence reads are then aligned (mapped) to the genome to identify expressed regions. Like other eukaryotic cells, the majority of sequencing reads in platelets map to annotated exons, whereas the remaining reads map to introns, predicted novel genes and exons, or other uncharacterized intergenic regions [15[■]]. As shown in Fig. 1, we also observed similar preponderance for annotated exons in human CD34⁺-derived megakaryocytes. Thus, mRNA expression in anucleate platelets is similar to nucleated cells in which the majority of messages map to exons but also to predicted novel genes, intergenic regions, or introns. Forthcoming RNA-seq analyses on these and future datasets will hopefully clarify the significance of the hundreds of thousands of reads mapping to intergenic and predicted novel gene regions.

In-situ hybridization-based strategies have shown that specific mRNAs localize to bulbar pro-platelets that extend from megakaryocytes, consistent with their expression in circulating platelets [16,17]. This type of discrete localization suggests that megakaryocytes transfer mRNA to platelets in an organized fashion. Support for this concept comes from recent work by Cecchetti *et al.* [18[■]] demonstrating that megakaryocytes differentially package mRNAs for matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) into platelets. mRNA for MMP and TIMP family members is typically transferred to platelets with its corresponding protein [18[■]]. However, exceptions exist. One of these includes MMP-2, which is present at both the mRNA and protein level in megakaryocytes, whereas platelets only express protein for MMP-2 [18[■]]. Expression of TIMP-2 also serves as a notable exception. In this regard, megakaryocytes transfer TIMP-2 mRNA, but not its protein, to platelets [18[■]].

Future studies that concurrently examine freshly isolated bone marrow megakaryocytes and platelets from the same donor are required to substantiate conclusions drawn from in-vitro cultures of megakaryocyte differentiation. In addition to confirming the megakaryocytic origin of platelet mRNA, these types of studies may reveal that platelets endocytose mRNA from other cells. It is well known that platelets are capable of internalizing proteins and

recent studies in other cell types have shown that genetic exchange between cells can occur via exosome-mediated transfer of mRNA from cell-to-cell [19,20]. Risitano *et al.* [21[■]] have also recently shown that platelets are capable of transferring functional RNA to monocytes, raising the possibility that platelets may also be recipients of mRNA transfer from other cells.

KEY FEATURES OF PLATELET MESSENGER RNA

When comparisons are made on a cell-to-cell basis (i.e., 1: 1, platelet: leukocyte), estimates of total mRNA in platelets is considerably less than leukocytes [22,23]. This difference remains intact, but is reduced when analyses are normalized to the number of platelets and leukocytes that are commonly observed in a microliter of blood. As one might expect, normalization based on circulating cell numbers is more favorable to platelets because their numbers in the bloodstream far exceed leukocytes. The fact that platelets contain less RNA than other cells is not surprising given the small size of platelets and their anucleate stature, which precludes ongoing transcription of nuclear-derived DNA. Despite limited quantities of total mRNA, several independent studies using serial analysis of gene expression, microarray profiling, or next-gen RNA-seq have demonstrated that platelets have a diverse repertoire of mRNAs [15[■],24–26]. As many as 3000–6000 mRNAs have been predicted to be in platelets [4,25–27], a number that is likely to grow as next-gen RNA-seq and other more sensitive techniques are implemented more frequently.

Recent next-gen RNA-seq results have shed even more light on critical attributes of platelet mRNA. Figure 2, which displays paired-end deep sequencing screenshots of mRNA from unactivated platelets for secreted protein acidic and rich in cysteine (SPARC) and interleukin-1 β (IL-1 β), highlights some of these features. As shown in Fig. 2 and described elsewhere [28], platelet mRNAs contain defined 5'-untranslated and 3'-untranslated regions. The majority of platelet transcripts are spliced. This is represented by the transcript SPARC, in which the bulk of paired reads map within exons or across exon/exon junctions, and very few reads map to introns (Fig. 2, top panel).

Platelets are reported to contain protein for SPARC, which is stored in α -granules and released upon activation [29]. SPARC protein has also been detected in platelet microparticles and its intracellular expression is significantly downregulated in platelets isolated from patients with non-ST segment elevation acute coronary syndrome [30,31].

It is not known whether mRNA for SPARC is translated by platelets in a constitutive or regulated fashion.

In roughly 70% of cases, as with the expression of SPARC, mRNAs and their corresponding proteins are coexpressed in platelets [32]. In contrast to SPARC, sequence reads for IL-1 β fall within exons, introns, and across exon/intron junctions (Fig. 2, bottom panel). This confirms previous reports that unactivated platelets express IL-1 β precursor mRNA (pre-mRNA) [16,33[■],34,35]. IL-1 β is among a small group of pre-mRNAs that are expressed by unactivated platelets (Fig. 1). In response to activating signals, platelets are capable of splicing IL-1 β pre-mRNA into a mature transcript that is competent for translation [16,33[■], 34,35]. Splicing provides platelets with a unique mechanism to synthesize protein *de novo* [16,17,33[■],34–37]. This new function of platelets was unexpected because splicing is typically restricted to the nucleus [38].

LESSONS LEARNED FROM PLATELET MESSENGER RNA

Characterization of platelet mRNAs has provided a wealth of information regarding the function of platelets in health and disease. From a practical standpoint, our group has used

mRNA as a guide to predict whether the expression of specific proteins is likely to be conserved between mouse and human platelets [15[■]]. Because RNA fragments are randomly sequenced, the number of sequencing reads generated for an expressed transcript is generally proportional to the overall abundance of the transcript. Thus, differential expression of transcripts within and between samples, and even across species, can be inferred based on the number of sequencing reads generated. Next-gen RNA-seq data confirmed previous studies demonstrating differential expression of protease-activated receptor 1 and 3, the receptor for platelet activating factor, and factor V between mouse and human platelets. We also identified CD68 mRNA and protein as being expressed by human, but not mouse, platelets [15[■]]. We have also assessed individual candidates, such as tissue factor, via traditional PCR to demonstrate differential expression across species. Unlike human platelets, mouse platelets do not express pre-mRNA or mRNA for tissue factor [39]. We have additionally verified by PCR analysis several candidate mRNAs including small nuclear ribonucleoprotein polypeptide N, lipocalin-2, and TIMP-1 that are highly expressed in human but are very low or not detected in mouse platelets (unpublished observations). Thus, analyses of mRNA expression are useful in determining the utility of knocking-in or knocking-out genes in mouse platelets, especially when high-quality antibodies for target proteins are unavailable.

mRNA expression analyses have also provided detailed insight into the molecular signature and phenotype of platelets in disease. Healy *et al.* [40] used mRNA profiling to show increased expression of myeloid-related protein-14 (MRP-14) in platelets isolated from patients with acute ST-segment elevation myocardial infarction. Critical roles for MRP-14 have subsequently been validated in the PROVE IT-TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy – Thrombolysis in Myocardial Infarction 22) trial [41]. Gnatenko *et al.* [42] used microarray expression analyses to show that transcript profiles distinguish essential thrombocytopenic patients from age-matched healthy individuals. In a follow-up study, the same group identified a small set of transcripts that predicted JAK2 V617F-negative essential thrombocytopenic individuals with 85% accuracy [43]. Other groups have shown that platelets differentially express transcripts in patients with cardiovascular disease, sickle cell anemia, and systemic lupus erythematosus [44–46].

Kahr *et al.* [47[■]] recently used mRNA expression analyses to facilitate the discovery of *neurobeachin-like 2* (*NBEAL2*), the gene responsible for gray platelet syndrome (GPS). Specifically, next-gen RNA-seq was used to sequence the entire transcriptome of platelets isolated from a patient with GPS. RNA-seq revealed abnormal intronic retention in *NBEAL2* (Fig. 3, top panel) [47[■]]. Identification of this region led to the identification of a splice site mutation and an indel in the coded mRNA (Fig. 3, bottom panel). These mutations were subsequently verified by DNA sequencing and defects in the *NBEAL2* gene were found in other patients with GPS [47[■]].

Importantly, two independent groups simultaneously identified *NBEAL2* as the causative gene for GPS [48,49]. The relevance of changes in transcript expression or sequences to disease extends beyond markers of disease. Platelet mRNAs are translatable and can, therefore, directly alter the protein composition and function of platelets. When used as a template in in-vitro translation systems, platelet mRNA is translated into protein [50]. Consistent with this finding, mRNAs in platelets contain a 7-methylguanosine cap and a poly(A) tail at their 5' and 3' ends, respectively, which is required for efficient translation. Our group routinely assesses global mRNA expression patterns in freshly isolated platelets by capturing polyadenylated mRNA from platelets [15[■],18[■]]. We observe similar expression patterns when a high-affinity variant of the cap-binding protein eukaryotic initiation factor 4E is used to purify mRNA from platelets (data not shown) [15[■],18[■],47[■]]. This demonstrates that the majority of platelet mRNAs are capped and polyadenylated.

Direct evidence for translation within platelets is best shown by incorporation of radiolabelled methionine into platelet proteins, which is used as an index of global protein synthesis. Gel electrophoresis of methionine-labeled products demonstrates that platelets synthesize numerous proteins from their mRNA pool [50–52]. mRNAs reported to be translated into protein by platelets include α IIB β 3 integrin, B-cell lymphoma-3, cyclooxygenase 1, IL-1 β , the ascorbate acid receptor SVCT2, plasminogen activator inhibitor 1, tissue factor, TIMP-2, and others [16,17,34,35,50,51,53–62]. Mechanisms and circumstances that control the synthesis of these proteins and their functional significance are reviewed in detail elsewhere [1,2,53]. Of note, recent data suggest that lipopolysaccharide is far more potent than thrombin in inducing the synthesis of IL-1 β [33²², 35]. α -Toxin also induces protein synthesis by platelets [62]. These data suggest that infection may alter the synthetic activities of platelets, which in turn may contribute to the host's response to infection.

CONCLUSION

The last decade has witnessed an explosion of work that characterizes transcript expression in platelets. We now know that platelets possess a diverse repertoire of mRNAs, whose features mirror mRNAs expressed by nucleated cells. Platelets translate several of their mRNAs into protein. They also transfer mRNAs to other cells and these recipients use the mRNA as a template for translation. Future studies will undoubtedly build on these concepts and shed new insight into other functional roles of platelet mRNA. We are also poised to learn how megakaryocytes transfer mRNAs to platelets and how mRNA sorting changes in disease situations. Tackling these issues and others will provide a greater understanding of the meaning behind the platelet message.

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- of special interest
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KEY POINTS

- Megakaryocytes differentially transfer messenger RNAs (mRNAs) to platelets.
- Platelets express a diverse repertoire of mRNAs, including intronic-rich transcripts that can be spliced into mature mRNA.
- Platelet mRNAs are capped, polyadenylated, and, in some cases, translated into protein either constitutively or in a signal-dependent fashion.
- Platelet-derived mRNAs often predict protein expression in human disease and have been used to facilitate the discovery of genetic mutations that are linked to abnormal platelets.

FIGURE 1.

Distribution of next-generation RNA-sequencing reads in human CD34-derived megakaryocytes and platelets. Pie charts represent the percentage of sequencing reads from CD34-derived megakaryocytes (left) and freshly isolated human platelets (right) that map to the specified genomic regions. Only high-quality alignments following de-novo alignment are represented. As shown, the majority of sequence reads map to known exonic regions (combined *Refseq*, *UCSC*, and *ENSEMBL* annotations) of genes (light blue, annotated exons). The remaining reads map to annotated introns (dark green) or intergenic regions (light green). Reads that map to intergenic regions probably mark novel genic regions expressed in platelets.

FIGURE 2.

Interleukin (IL)-1 β messenger RNA (mRNA) expression in platelets. mRNA was collected from freshly isolated, unactivated platelets and processed as recently described. This figure is a visualization using the *Integrated Genome Browser (IGB)* of paired-end next-generation RNA-sequencing (next-gen RNA-seq) reads that are aligned to genes for secreted protein acidic and rich in cysteine (SPARC, top panel) or IL-1 β (bottom panel) (genes are in a 3' to 5' orientation). Gene maps (bottom portion of each figure) are represented by thick (exons) and thin (introns) horizontal lines. The bars immediately above the gene represent sequencing reads from platelet transcripts that were fragmented, sequenced, and aligned to IL-1 β . The lines at the top represent paired-end sequence reads that align within exons only, introns only, or span exon/exon or exon/intron junctions. As shown, exon/exon junction spanning sequences are common for SPARC and rare for IL-1 β , whereas intronic and intron/exon junction spanning reads are common for IL-1 β and rare for SPARC.

FIGURE 3.

Abnormal sequence reads in transcripts from platelet RNA facilitate the discovery of *neurobeachin-like 2* (*NBEAL2*) as the causative gene for gray platelet syndrome (GPS). The top panel displays a snapshot of *NBEAL2* transcripts expressed in platelets from a healthy (control) and GPS patient. The red box outlines a region in which introns are abnormally retained in *NBEAL2* transcripts isolated from the GPS patient. The bottom panel shows exon/intron spanning RNA sequencing (RNA-seq) reads from the GPS patient, which contain the c.1029+1G>A mutation [47■■]. The red box on the right shows the zoomed region where the G>A mutation is located. Reproduced with permission from [47■■].