The 2024 Nobel prize in Medicine: impact on hemostasis and thrombosis research

Loredana Bury, Marco Malvestiti, Paolo Gresele

Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Italy

Introduction

On October 7th 2024, the Nobel Prize in Physiology or Medicine was awarded jointly to Victor Ambros and Gary Ruvkun for the discovery of microRNA and its role in post-transcriptional gene regulation. Their discovery stems from studies of two genes, *lin-4* and *lin-14*, involved in the development of a small worm, *Caenorhabditis elegans*, conducted in the 1980s. It was revealed that one of the two genes, *lin-4*, encoded an RNA of no more than 22 nucleotides that did not code for a protein but was able to shut down the translation of *lin-14*. Indeed, the suppression of the synthesis of the protein encoded by *lin-14* at a certain stage of the worm's development was found to be crucial for the progression beyond larval stages. Later, Gary Ruvkun identified another microRNA, encoded by the gene *let-7*, which was found to be present in all living organisms, demonstrating that miRNA-regulation of gene expression is a general and highly conserved mechanism in the animal kingdom. Since then, at least 1,000 microRNAs have been found in the human genome. This discovery has opened an entirely new field of research with manifold implications, including in hemostasis and thrombosis.

Correspondence: Paolo Gresele, Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Strada Vicinale Via delle Corse, 06126 Perugia, Italy. Tel. +39.075.5783989. E-mail: paolo.gresele@unipg.it

Key words: microRNA; gene expression; platelets.

Received: 29 October 2024.
Accepted: 29 October 2024.

Bleeding, Thrombosis and Vascular Biology 2024; 3:159
doi:10.4081/btvb.2024.159

MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNAs, typically around 22 nucleotides in length, that regulate gene expression by binding to complementary sequences on target messenger RNAs (mRNAs), leading to either their degradation or the inhibition of their translation.[1,2] MiRNAs are involved in the fine-tuning of gene expression, affecting diverse biological processes such as cell development, differentiation, and response to stress. miRNAs are encoded by specific DNA sequences that transcribe primary miRNAs (pri-miRNAs), which are then processed in the nucleus into precursor miRNAs (pre-miRNAs) by the enzyme Drosha. Pre-miRNAs are then exported to the cytoplasm, where Dicer processes them into mature miRNA duplexes: a double-stranded structure consisting of a guide strand (the functional miRNA) and the passenger strand, which is typically degraded. The guide strand is incorporated into the RNA-induced silencing complex (RISC), a multi-protein complex that uses it to recognize complementary mRNA targets and regulate their translation.[3]

MicroRNAs in hemostasis and thrombosis

In platelets, miRNAs play significant roles in both physiological and pathological processes. Although anucleate, platelets have a rich transcriptome, including numerous miRNAs and a functional miRNA processing machinery, with components such as Dicer and Argonaute proteins.[4,5] Platelets from healthy individuals contain approximately 6,000 different protein-coding transcripts[6,7] and at least 178 commonly expressed miRNAs,[8] 11 of which are platelet-specific.[9] Platelet miRNAs are also incorporated into platelet-derived microparticles (PMPs) released by activated platelets, which mediate their transfer to neighboring cells.[10]

Several miRNAs have been implicated in megakaryocyte differentiation and platelet production. For example, miR-150 promotes megakaryopoiesis by downregulating the MYB transcription factor, which negatively regulates megakaryocyte development.[11] Conversely, miR-486-3p is a negative regulator of megakaryopoiesis by suppressing MAF, a transcription factor involved in megakaryocyte differentiation.[12]

Altered miRNA expression profiles in platelets are associated with disorders such as diabetes, ischemic cardiovascular disease, and certain cancers. For example, in diabetes, platelet miR-223, miR-142, and miR-155 are reduced due to the cleavage of Dicer by calpain, leading to platelet hyperreactivity and increased thrombotic risk.[13] In cardiovascular diseases, miR-223 and miR-126 are important biomarkers: lower levels of miR-223 are associated with increased platelet reactivity and a higher risk of myocardial infarction, while elevated levels of miR-126 are as-

sociated with a higher risk of myocardial infarction.[14] MiR-223 is upregulated in platelets from non-small cell lung cancer (NSCLC) patients, promoting tumor cell invasion by targeting the tumor suppressor gene EPB41L3.[15] These miRNAs, due to their stability in blood, especially within PMPs, hold promise as biomarkers for disease diagnosis and prognosis by reflecting changes in platelet function associated with disease progression.[16]

In addition to platelets, miRNAs play crucial regulatory roles in coagulation, natural anticoagulation, and fibrinolysis. Several miRNAs directly target coagulation factors: for instance, miR-409-3p regulates fibrinogen by targeting its β-chain (FGB), while miR-29c modulates its α-chain (FGA).[17] Similarly, miR-19b and miR-20a inhibit the expression of tissue factor (TF), and their expression was found to be significantly lower in monocytes from patients with antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE), suggesting a role for these miRNAs in the hypercoagulable state of these patients.[18] Among natural anticoagulants, miR-18a and miR-19b regulate antithrombin III (ATIII) expression,[17] while miR-494 targets protein S (PS), reducing PS levels, possibly under conditions where estrogen levels are high, such as during pregnancy, potentially increasing thrombotic risk.[19] Regarding fibrinolysis, diverse miRNAs, such as miR-421 and miR-30c, inhibit plasminogen activator inhibitor-1 (PAI-1).[17] Let-7g also modulates PAI-1 expression, and in patients with lacunar infarction plasma levels of let-7g are inversely correlated with those of PAI-1,[20] suggesting its potential protective role in vascular diseases.

The exploitation of miRNAs for therapeutic purposes

The discovery of non-coding RNAs (ncRNAs) paved the way for the development of the antisense approach, i.e., the generation of synthetic small interfering RNAs (siRNAs) targeting pre-mRNA, mRNA, or ncRNAs to suppress the production of proteins responsible for disease. Unlike traditional small molecule therapies that directly target proteins, antisense drugs prevent their synthesis by binding to the target RNA sequence.[21]

Two strategies have been developed: 13-mer antisense synthetic single-stranded oligonucleotides (ASOs), which bind to RNA, forming ASO-RNA heteroduplexes that serve as substrates for RNase enzymes in the cytoplasm, leading to their degradation *via* RNase H1; or exogenous small interfering RNAs (siRNAs), i.e., double-stranded synthetic RNA sequences, 22 nucleotides in length, which associate with Argonaute 2 (Ago 2) to form the RNA-induced silencing complex (RISC) where the complementary sequence in the targeted mRNA is degraded.[22]

The first example of the therapeutic use of antisense technology in the field of cardiovascular diseases is Mipomersen, an ASO developed by Genzyme. Mipomersen specifically targets the mRNA encoding apolipoprotein B-100 (apo B-100), thereby inhibiting its synthesis. This approach has been developed for the treatment of homozygous familial hypercholesterolemia (HoFH), an autosomal dominant genetic disorder characterized by elevated levels of low-density lipoprotein (LDL) and early-onset atherosclerotic cardiovascular disease caused by mutations in the genes encoding the LDL cholesterol receptor, proprotein convertase subtilisin/kexin type 9 (PCSK9), or apolipoprotein B.[23]

In 2014, clinical development began for the first siRNA targeting PCSK9 mRNA, inclisiran, which can be administered subcutaneously at 3- or 6-month intervals.[24] Inclisiran was approved for clinical use by the European Medicines Agency in 2020, followed by approval from the U.S. Food and Drug Administration in 2021.[25] To date, several ASOs and siRNAs targeting PCSK9, Apo(a), ANGPTL3, APOC3, APOB, and AGT have either been approved or are in clinical trials for the treatment of dyslipidemia and arterial hypertension, respectively.[26]

Fitusiran is a subcutaneously administered prophylactic small interfering RNA (siRNA) therapy for individuals with hemophilia A or B, designed to reduce antithrombin synthesis, thus rebalancing hemostasis. This novel, non-replacement therapy works by enhancing thrombin generation to improve clot formation and reduce bleeding. Early phase 1 and 2 trials showed dose-dependent reductions in antithrombin levels and increases in thrombin generation, leading to improved bleeding outcomes. Phase 3 trials suggest that Fitusiran could become the first subcutaneous prophylactic therapy for hemophilia B, offering effective once-monthly dosing, reducing treatment burden, and improving the quality of life for patients with hemophilia.[27]

Conclusions

The Nobel Prize for the discovery of miRNA by Victor Ambros and Gary Ruvkun reinforces the current enormous interest in RNA research across many fields of biology and therapy. Since the discovery of mRNA in 1961, great attention has been given to this molecule, leading to the hypothesis that life on our planet began with RNA well before the emergence of DNA- and protein-based life, in what has been called the "RNA era".[28]

Recently, the application of mRNA in vaccine development has revolutionized and exponentially accelerated this crucial therapeutic field, with the spectacular results obtained from the rapid and effective development of COVID-19 vaccines, which saved well over 20 million lives worldwide. The breakthrough of miRNAs in the RNA field represents another leap forward in biomedical research, with very promising future therapeutic developments, including in hemostasis, thrombosis, and vascular biology.

References

1. Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science 2001;294:862-4.
2. Lee R, Feinbaum R, Ambros V. A short history of a short RNA. Cell 2004;116):S89-92.
3. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010;11:597-610.
4. Landry P, Plante I, Ouellet DL, et al. Existence of a microRNA pathway in anucleate platelets. Nat Struct Mol Biol 2009;16:961-6.
5. Bury L, Gresele P. The amazing genetic complexity of anucleated platelets. Bleeding Thromb Vasc Biol 2022;1:33.
6. Rowley JW, Oler AJ, Tolley ND, et al. Genome-wide RNA-seq analysis of human and mouse platelet transcriptomes. Blood 2011;118:e101-1.
7. Bray PF, McKenzie SE, Edelstein LC, et al. The complex

transcriptional landscape of the anucleate human platelet. BMC Genomics 2013;14:1.
8. Edelstein LC, Simon LM, Montoya RT, et al. Racial differences in human platelet PAR4 reactivity reflect expression of PCTP and miR-376c. Nat Med 2013;19:1609-16.
9. Londin E, Loher P, Telonis AG, et al. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. Proc Natl Acad Sci USA 2015;112:E1106-15.
10. Risitano A, Beaulieu LM, Vitseva O, Freedman JE. Platelets and platelet-like particles mediate intercellular RNA transfer. Blood 2012;119:6288-95.
11. Barroga CF, Pham H, Kaushansky K. Thrombopoietin regulates c-Myb expression by modulating micro RNA 150 expression. Exp Hematol 2008;36:1585-92.
12. Bianchi E, Bulgarelli J, Ruberti S, et al. MYB controls erythroid versus megakaryocyte lineage fate decision through the miR-486-3p-mediated downregulation of MAF. Cell Death Differ 2015;22:1906-21.
13. Elgheznawy A, Shi L, Hu J, et al. Dicer cleavage by calpain determines platelet microRNA levels and function in diabetes. Circ Res 2015;117:157-65.
14. Zampetaki A, Willeit P, Tilling L, et al. Prospective study on circulating microRNAs and risk of myocardial infarction. J Am Coll Cardiol 2012 60:290-9.
15. 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.
16. Krammer TL, Mayr M, Hackl M. microRNAs as promising biomarkers of platelet activity in antiplatelet therapy monitoring. Int J Mol Sci 2020;21:3477.
17. Teruel-Montoya R, Rosendaal FR, Martínez C. MicroRNAs in hemostasis. J Thromb Haemost 2015;13:170-81.
18. Teruel R, Pérez-Sánchez C, Corral J, et al. Identification of miRNAs as potential modulators of tissue factor expression in patients with systemic lupus erythematosus and antiphospholipid syndrome. J Thromb Haemost 2011;9):1985-92.
19. Tay JW, Romeo G, Hughes QW, Baker RI. Micro-ribonucleic Acid 494 regulation of protein S expression. J Thromb Haemost 2013;11:1547-55.
20. Liao YC, Wang YS, Guo YC, et al. Let-7g improves multiple endothelial functions through targeting transforming growth factor-beta and SIRT-1 signaling. J Am Coll Cardiol 2014;63:1685-94.
21. Kaczmarek JC, Kowalski PS, Anderson DG. Advances in the delivery of RNA therapeutics: From concept to clinical reality. Genome Med 2017;9:60.
22. Dhuri K, Bechtold C, Quijano E, et al. Antisense oligonucleotides: an emerging area in drug discovery and development. J Clin Med 2020;9:2004.
23. Raal FJ, Santos RD, Blom DJ, et al. Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. Lancet 2010;375:998-1006.
24. Ray KK, Wright RS, Kallend D, et al. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med 2020;382:1507-19.
25. Lamb YN. Inclisiran: first approval. Drugs 2021;81:389-395.
26. Makhmudova U, Steinhagen-Thiessen E, Volpe M, Landmesser U. Advances in nucleic acid-targeted therapies for cardiovascular disease prevention. Cardiovasc Res 2024;120:1107-25.
27. Young G, Srivastava A, Kavakli K, et al. Efficacy and safety of fitusiran prophylaxis in people with haemophilia A or haemophilia B with inhibitors (ATLAS-INH): a multicentre, open-label, randomised phase 3 trial. Lancet 2023;401: 1427-37.
28. Joyce GF. The antiquity of RNA-based evolution. Nature 2002;418:214-21.