

Insight miR-223 plays a remarkably modest role in thrombopoiesis and platelet function does not depend upon miR-223

miR-223: sailing to terra incognita for microRNAs in platelets

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Few historical dates were more influential on the course of world history than October 12, 1492. It is on this morning that the Italian explorer *Cristoforo Colombo* spotted land after a five-week voyage across the Atlantic Ocean. The finding of uncharted shores was as much the fruit of Colombo's earlier lobbying with private Italian investors and at the Spanish court, which finally succeeded to conquer the last Almo-had Kingdom of Granada in 1492 and ending an eight-century long Muslim civilisation on the Iberian Peninsula, as well as geopolitical changes that coincided with the fall of Constantinople to Ottoman Turks in 1453, rendering the land route to Asia insecure. The discovery of the *New World* was the result of Colombo's gravely inaccurate estimates of the size of the Earth, the size of the Eurasian landmass, and geographical estimates where Japan was located vis-à-vis the coast of China. Ironically, his miscalculation of the Earth's circumference by roughly 10,000 kilometers would likely have been corrected by any contemporary Islamic scholar, who had superior geometrical knowledge, but all of whom were expelled from the European continent by Colombo's financiers at the very year of his voyage. Equally ironic is the realisation that his miscalculations em-

powered Colombo to dare such a voyage while most contemporary navigators would never have attempted a westward voyage from Europe to Asia as 15th century ships were not built to carry enough food and fresh water for a 12,000 kilometer voyage. It was not until a decade later that his successor *Amerigo Vespucci* realised that Brazil and the West Indies did not represent Asia's eastern outskirts, but rather constituted an entirely separate *terra incognita*, a super continent that was henceforth termed America, honouring his first name.

In many aspects, science bears a similar romantic excitement and hopes of finding uncharted tropical shores, discovering new worlds invisible to the layman's eye. But, quite reminiscent to Colombo's quest, few scientists fully realise that often the greatest breakthrough discoveries catch them off-guard and occur secondarily to an original hypothesis to find answers to a seemingly valid objective. One such breakthrough for the cardiovascular system occurred several years ago, with our realisation that microRNAs (miRNAs, miRs) function as crucial, novel regulators of cellular morphology and function. MiRNAs are ~22 nucleotide long, evolutionary conserved noncoding RNA species encoded by our genome and designed to play a role in post-transcriptional gene regulation by imperfect Watson-Crick base pairing to the 3'UTR region of protein coding messenger RNAs (mRNAs), leading to translational suppression and/or the induction of mRNA degradation. Initially thought to act as subtle 'fine tuners' of gene expression, we increasingly realise that individual microRNAs have the ability to coordinately regulate target mRNAs that encode proteins with related functions, thereby redirecting complete interconnected gene programs and acting as regulators of complete biological pathways. Equally exciting is our

realisation that extracellular miRNAs circulate in the bloodstream in a remarkably stable form, raising the possibility that miRNAs can be probed in the circulation and serve as novel diagnostic markers for cardiovascular diseases.

Bearing this in mind, Leierseder et al. studied platelet function in miR-223-deficient mice because miR-223 is one of the most abundantly expressed miRNAs in megakaryocytes and in platelets (1). miR-223 was earlier described as potential circulating marker for platelet activation due to its virtue as most differentially expressed miRNA in platelet-rich plasma and decreased presence in plasma of individuals following anti-platelet therapy (2). Additionally, activated platelets secrete microparticles containing higher levels of miR-223, allowing the platelet-derived microparticles to target endothelial cells and modulate endothelial cell gene expression patterns (3). Surprisingly, in this issue of *Thrombosis and Haemostasis*, Leierseder et al. report that in miR-223-deficient mice platelet number, volume and lifespan were unaltered. Moreover, the weight of the spleen and its capacity to sequester platelets were unchanged. Flow cytometry analysis indicated that platelet surface receptors as well as platelet activation markers were expressed at the same level in miR-223-deficient platelets. Moreover, loss of miR-223 did not affect platelet properties for ADP-induced aggregation, speed of clot retraction, platelet adhesion and tail bleeding times. Megakaryocytes were unaffected based upon the analysis of their spreading potential, pro-platelet formation, their maturation and numbers in bone marrow. Only a slightly delayed recovery of

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platelet numbers following platelet-depleting antibody-induced thrombocytopenia was observed in miR-223-deficient mice compared to wild-type mice, but this did not depend on miR-223 expression in platelet-producing megakaryocytes as bone marrow chimera experiments demonstrated that platelet numbers remained unchanged in wild-type mice transplanted with miR-223 deficient bone marrow. In view of this overwhelming evidence, the authors were forced to conclude that miR-223 plays a remarkably modest role in thrombopoiesis and that platelet function does not depend upon miR-223.

Why is miR-223 highly expressed in megakaryocytes and platelets but has no detectable cellular function? Earlier studies inferred a role for miR-223 in lineage decision of megakaryocyte-erythroid progenitors for miR-223 due to its fluctuating levels during megakaryocytic differentiation and by overexpressing miR-223 in K562 cells (4, 5). The findings by Leierseider et al. hopefully offer a cautionary warning about assigning functionality solely based on supraphysiological overexpression methods and correlations. In contrast, the data by Leierseider et al. are not in appar-

ent contradict with the diagnostic and prognostic potential of plasma miR-223 as it is inversely associated with type II diabetes mellitus and the risk of myocardial infarction (6). Since platelets serve as a major source and vehicle of miR-223, the findings open the intriguing possibility that certain abundantly expressed miRNAs are designed to act independent from cellular miRNA pathways and are present in vesicular bodies with the sole purpose to serve as secreted molecules and act in target cells other than the ones where they are produced. In keeping with this hypothesis, its deficiency would need to have detectable functional effects in targeted cells rather than platelets themselves. Future studies on miR-223-deficient mice will need to demonstrate whether this intriguing *terra incognita* may hold true and could serve as template for other miRNA fields.

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Conflicts of interest

None declared.

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