Sweet Dicer

Impairment of Micro-RNA Processing by Diabetes

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Micro-RNAs (miRNAs) are small noncoding RNAs that control gene expression epigenetically. miRNA genes are transcribed by RNA polymerase II into a primary transcript—the primary miRNA that consists of at least 1 hairpin structure, with the characteristic long stem and a terminal loop. Mature miRNAs will be typically generated from the primary transcripts after 2 cleavage events (Figure): the first cleavage occurs in the nucleus by an RNase III, the Drosha–DGCR8 complex, from primary miRNAs to pre-miRNAs. The second cleavage takes place in the cytosol where pre-miRNAs are further cleaved by Dicer, and the mature miRNAs are loaded into the RNA-induced silencing complex. Although platelets lack a nucleus, there are several lines of evidence suggesting a functional miRNA pathway in platelets: (1) platelets contain miRNAs and express both Dicer (required for cleavage of pre-miRNAs to mature miRNAs) and Argonaute 2 (required for RNA-mediated gene silencing by the RNA-induced silencing complex) and (2) they are also major contributors to the circulating miRNA pool as evidenced by significant positive correlations to platelet expression and activity could account for the observed alterations in the miRNA profile of diabetic patients. In support of their hypothesis, Dicer was decreased in diabetic platelets, whereas Argonaute 2 levels did not differ. Detection of degradation products indicated proteolytic cleavage of Dicer. Truncation of Dicer can alter its activity, that is, proteolytic processing of Dicer in C. elegans converted its RNase III into a DNase activity that initiated apoptotic chromosome fragmentation. On the basis of proteomics findings in platelets from diabetic patients, Fleming et al have previously discovered that diabetes mellitus-induced platelet dysfunction is mediated, at least in part, by calpain activation. In cortical neurons, the calpain family of calcium-dependent cysteine proteases induced cleavage of Dicer. A series of elegant experiments performed in this study demonstrated that Dicer is also a calpain substrate in platelets. Acute calpain activation led to an increase in Dicer RNAse III activity, whereas prolonged calpain activation induced Dicer cleavage and resulted in impaired processing of pre-miRNAs. On incubation of let-7a pre-miRNA with protein extracts from diabetic patients, a 35-nucleotide long transcript with yet unknown function was observed besides the mature miRNA with 21 nucleotides. Because only the pre-miRNA for let-7a was tested, it remains unclear whether this incomplete processing applies to other miRNAs as well. However, several Dicer-dependent miRNAs (miR-142, miR-143, miR-155, and miR-223) but not Dicer-independent miRNAs (miR-451a) were reduced in platelets from diabetic patients and Ins2Akita mice.

To explore the functional consequences of reduced miRNA expression in platelets, the authors focused on miR-223, an abundant platelet miRNA. Decreased levels of miR-223 have previously been associated with increased platelet reactivity in patients on clopidogrel and with a higher incidence of cardiovascular events in the general population. In male miR-223 knockout mice (miR-223−/−), there was no difference in the tail bleeding time, but the in vivo formation of neutrophil–platelet aggregates was increased, the thrombus formation after FeCl3 injury was exaggerated, and more emboli were detected in the microcirculation on laser-induced endothelial injury. In the presence of low concentrations of thrombin and collagen, miR-223−/− platelets spontaneously formed larger aggregates resembling the platelet phenotype observed in streptozotocin-treated mice, a model of type I diabetes mellitus. Similar results were obtained in wild-type mice after inhibition of miR-223 by an antagonir approach. These findings are in contrast to a previous study examining platelet function in miR-223 null mice. Apart from a mild and transient delay in the recovery of platelet numbers after depletion, Leierseder et al observed no differences for platelet activation, adhesion, and aggregation in miR-223 null mice. Likely explanations for these discrepancies are the lower concentration of platelet agonists used in this study. Consistent with the role of miRNAs in other cell types, miR-223 may play a role in fine tuning the platelet response and become dispensable at higher agonist concentrations.

Analysis of the platelet proteome from miR-223−/− mice revealed, among other protein changes, differential expression of kindlin-3, an integrin-binding protein, and factor XIII-A (FXIII-A), a transglutaminase regulating clot stability. Interestingly, kindlin-3 has no apparent miR-223 binding site.
in the 3′ untranslated region, but its altered expression was accompanied by an increase in β1 and α2 integrins. β1 integrin is a direct target of miR-223. The authors hypothesized that the regulation of kindlin-3 may occur through β1 integrin. However, a siRNA for β1 integrin did not affect kindlin-3 expression. It is worth noting that a putative miR-223 binding site is predicted in the coding region of kindlin-3. Although miRNA binding in the coding region tends to be less potent than binding in the 3′ untranslated region,13 it still exerts repression of gene expression. On the other hand, FXIII-A was validated as a direct target of miR-223 thus providing a potential explanation for the increased levels of FXIII-A in diabetic platelets. Similar to miR-223y/− mice, platelets of mice injected with streptozotocin displayed increased expression of kindlin-3 and FXIII-A. Treatment with the calpain inhibitor A-705253 restored Dicer and mature miR-223 levels in platelets and reversed the upregulation of kindlin-3 and FXIII-A. Hence, inhibiting calpain in vivo was sufficient to restore platelet function in diabetic mice.

Diabetes mellitus is a major risk for cardiovascular diseases. Increased platelet aggregability and adhesiveness and impaired fibrinolytic balance are thought to contribute to accelerated atherosclerosis and thrombotic complications in diabetic patients. Diabetic platelets exhibit reduced membrane fluidity, lower nitric oxide and prostacyclin production, and increased expression of adhesion molecules. These changes in platelet reactivity bypass protective mechanisms that inhibit platelet interactions with the endothelial monolayer.14 It remains to be seen whether restoring mature miRNA levels can overcome the platelet dysfunction in diabetic patients. At present, the dual role of calpain, namely to induce Dicer activity in an acute setting and to degrade Dicer on prolonged activation, is poorly understood. Moreover, calpains are expressed ubiquitously and calpastatin acts as an endogenous calpain-specific inhibitor. Combined with the short half life of platelets, these challenges may limit the potential of calpain inhibitors as a novel therapeutic approach to restore platelet function in diabetes mellitus. Nonetheless, this study provides strong evidence for the importance of miRNAs in platelets. As pointed out previously,15 potential effects on platelets have long been ignored when interpreting cardiovascular phenotypes on miRNA manipulation.

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Disclosures
A. Zampetaki is an Intermediate Fellow of the British Heart Foundation. M. Mayr is a Senior Fellow of the British Heart Foundation. King’s College London has filed patent applications on miRNA biomarkers.

References


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