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Diabetes Mellitus Reveals Its Micro-Signature

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With 285 millions individuals affected worldwide in 2010 and more than 400 millions expected in 2030, diabetes mellitus is a major public health concern and a huge economic burden. This disease, characterized by chronic elevation of blood glucose levels, is frequently associated with micro- and macrovascular complications, putting diabetic patients at risk for heart and renal failure, stroke, lower limb amputations, and blindness.[1] In view of the dramatic impact of diabetes on public health, the definition of new methods predicting the manifestation of the disease and the occurrence of its debilitating complications deserves the highest priority. In this issue of *Circulation Research*, Zampetaki et al report that a group of circulating RNA molecules belonging to the microRNA family is considerably less abundant in the blood of diabetes patients compared to normoglycemic individuals.[2] Interestingly, a reduction in the level of these microRNAs is already detectable years before the appearance of the disease and is correlated with subclinical and manifest peripheral artery disease. These findings suggest that plasma microRNA levels may become valuable tools to assess the probability of diabetes manifestation in high-risk individuals displaying impaired fasting glucose and could assist in predicting the occurrence of micro- and macrovascular complications in diabetic patients.

MicroRNAs are small noncoding RNA molecules discovered in 1993 in *Caenorhabditis elegans*. Later on, they were identified in many other eukaryotes and it became clear that all mammalian cells contain hundreds of them.[3] Many microRNAs are ubiquitously expressed, but some of them are restricted to a limited number of tissues where they play specific roles. Since their discovery, microRNAs have been the focus of intense investigations and have rapidly turned out to form a complementary RNA–directed regulatory network acting in concert with transcription factors to tune gene expression. In most cases, microRNAs function as translational repressors that exert their action by partially pairing to one or more sequences in the 3′ untranslated region of target mRNAs.[3] This mode of interaction permits a single microRNA to inhibit the expression of hundreds of different targets. Hence, collectively, these regulatory RNA molecules can potentially directly control the expression of more than one-third of all human genes.[3] Although we are only beginning to appreciate the immense potential of microRNAs as controllers of gene networks, there is no doubt that these small RNA molecules play a central role in governing a variety of physiological and pathological processes, including tissue differentiation, cell proliferation, apoptosis, and inflammation. Moreover, there is substantial evidence for an involvement of microRNAs in the development of human diseases, including cancer, cardiovascular disorders, and diabetes mellitus. Indeed, the expression of this class of RNAs is very often deregulated in tumor cells, and the level of a set of them is modified in animal models of cardiac hypertrophy and diabetes.[4–6]

Besides their recognized regulatory function accomplished inside the cells, microRNAs have also recently been detected in extracellular fluids, including blood.[7] In contrast to other RNA molecules that are notoriously prone to degradation by RNases, circulating microRNAs are astonishingly stable in frozen plasma samples. In fact, microRNAs are not simply accumulating in the blood because of cell disruption. They are actively secreted via the exosome pathway or released in small microparticles called apoptotic bodies and circulate inside these membrane-delimited organelles that protect them from degradation. The functional role of microRNAs in extracellular fluids is not yet precisely established. However, microRNAs associated with exosomal or apoptotic bodies can be delivered to neighboring cells, where they are capable to regulate gene expression, raising the intriguing possibility of the involvement of these small RNA molecules in a new cell-to-cell communication mode.[8,9] Interestingly, the blood microRNA profile has been found to be modified under different pathophysiological conditions. This prompted several groups to assess the predictive value of changes in plasma microRNAs. Indeed, plasma microRNAs are emerging as powerful biomarkers for the diagnosis of cancer, heart failure, and liver injury.[10–12]

Cardiovascular disease causes most of the excess morbidity and mortality of diabetes mellitus and accounts for up to 80% of premature mortality in diabetic patients.[1] Endothelial dysfunction, smooth muscle cell proliferation, and myocardial fibrosis are regarded as important factors in the pathogenesis of diabetic micro- and macroangiopathy.[13] Alterations in the level of a specific set of microRNAs are emerging as major causes of these pathophysiological processes, pointing to a potential role for these small RNA molecules in vascular complications associated with the diabetic condition.[14–18]

In the study reported in this issue of *Circulation Research*, Zampetaki et al searched for changes in blood microRNA abundance associated with diabetes mellitus.[2] The authors focused on type 2 diabetes, the most common form of the disease accounting for ≈90% of all diabetes cases. Type 2 diabetes is often associated with obesity and results from the

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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(*Circ Res*. 2010;107:686-688.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.110.228841

incapacity of pancreatic β cells to release sufficient insulin to compensate for a diminished insulin sensitivity of target tissues.[19] The authors determined microRNA levels in plasma samples obtained in a large prospective population-based study including more than 800 individuals.[20] Global microRNA profiling in pooled samples from control and diabetic subjects led to the identification of a group of differentially expressed plasma microRNAs. The most relevant differences in plasma microRNAs were confirmed in samples obtained from each individual patient with manifest diabetes and were observed also in blood samples from hyperglycemic Lep^{Ob} mice, a commonly used type 2 diabetes animal model. Except for miR-28-3p, all the other differentially expressed plasma microRNAs were less abundant in diabetic patients. The expression profile of 5 plasma microRNAs, miR-15a, miR28-3p, miR-126, miR-223, and miR-320 formed a unique signature sufficient to correctly distinguish between individuals with prevalent or incident diabetes from healthy controls. Importantly, the expression of several microRNAs including miR-15a, miR-126, and miR-223 was already significantly reduced compared to matched controls years before the manifestation of diabetes, suggesting that the determination of the level of these small RNAs might be a useful predictive tool. Moreover, measurement of the level of miR-126 in the entire cohort revealed that loss of this particular microRNA negatively correlates with subclinical and manifest artery disease and is a good prognostic value for the onset of diabetic vascular complications.

The experiments described above revealed the existence of a particular plasma microRNA pattern enabling the classification of diabetic patients and potentially permitting the identification of individuals at risk for developing the disease and its vascular complications. Besides its potential predictive value, the characteristic microRNA profile found in the samples of diabetic subjects could also furnish precious information about the pathophysiology of the disease. Most circulating microRNAs can be produced by a wide variety of cells, and the amount of these molecules packaged in exosomes or apoptotic particles does not necessarily reflect the total cellular content of the cells of origin. For this reason, the precise source of plasmatic microRNAs is presently unknown. Part of these molecules is probably shed by blood cells and vascular walls, whereas others may be contributed by liver, skeletal muscle, and adipose tissue, all organs whose function is known to be affected in type 2 diabetes. In the latter case, the characteristic loss of microRNAs observed in the plasma of diabetic subjects may reflect functional alterations in insulin target tissues. In contrast to the other microRNAs associated with diabetes, miR-126 is highly enriched in endothelial cells and endothelial apoptotic bodies.[9,14] Thus, the cells lining the vascular walls are probably the main source of this microRNA in the bloodstream. Consistent with this hypothesis, Zampetaki et al demonstrated that in vitro exposure of endothelial cells to prolonged hyperglycemia selectively reduces the amount of miR-126 released in apoptotic bodies without affecting its cellular concentration.[2] Interestingly, miR-126 regulates angiogenic signaling and plays an important role in vascular integrity,[14,15] and delivery of this microRNA by apoptotic bodies has been shown to confer protection against diet-induced atherosclerosis.[9] Moreover, circulating levels of miR-126 were significantly reduced in patients with coronary artery disease.[21] Taking into consideration all of these findings, it is tempting to speculate that the drastic loss of circulating miR-126 can contribute to the development of micro- and macrovascular complications of type 2 diabetes.

The study by Zampetaki et al unveils the existence of a plasma microRNA signature of diabetes. These findings will now need to be tested in larger collectives of prediabetic and diabetic patients to validate the predictive value of this unique microRNA profile and to assess whether it is influenced by pharmacological treatments. Future studies should also determine whether at least part of the changes in circulating microRNAs are observed in type 1 diabetic subjects. This form of the disease is caused by autoimmune destruction of pancreatic β cells but is also associated with micro- and macrovascular complications. The identification of individuals at risk for developing type 1 diabetes is currently performed by searching for the presence of β cell–directed autoantibodies. Detection of early changes in plasma microRNA levels may contribute to refine this procedure, facilitating the prevention of this autoimmune form of the disease.

The number of studies exploring the prognostic value of plasma microRNAs in different settings is increasing exponentially. We presently do not know whether circulating microRNAs simply reflect pathophysiological alterations in the activity of the releasing organs or fulfill precise functions in the blood. Indeed, changes in the microRNA profile could not only signal the development of the disease but may also contribute to its manifestations. Should this be the case, therapeutic interventions permitting to restore appropriate levels of circulating microRNAs may pave the way for the development of new treatments for diabetes mellitus and its vascular complications. With other recently published studies, the article by Zampetaki et al announces the beginning of a new era in biomedical research in which microRNAs promise to play a central role.

Sources of Funding

Supported by grants from the Swiss National Science Foundation (31003A-127254) and the European Foundation for the Study of Diabetes.

Disclosures

None.

References

1. Winer N, Sowers JR. Epidemiology of diabetes. *J Clin Pharmacol*. 2004;44:397–405.
2. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, Shah A, Willeit J, Mayr M. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res*. 2010;107:810–817.
3. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009;136:215–233.
4. Deng S, Calin GA, Croce CM, Coukos G, Zhang L. Mechanisms of microRNA deregulation in human cancer. *Cell Cycle*. 2008;7:2643–2646.
5. Catalucci D, Gallo P, Condorelli G. MicroRNAs in cardiovascular biology and heart disease. *Circ Cardiovasc Genet*. 2009;2:402–408.
6. Kolfschoten IG, Roggli E, Nesca V, Regazzi R. Role and therapeutic potential of microRNAs in diabetes. *Diabetes Obes Metab*. 2009; 11(Suppl 4):118–129.

7. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*. 2008;105:10513–10518.
8. Simons M, Raposo G. Exosomes–vesicular carriers for intercellular communication. *Curr Opin Cell Biol*. 2009;21:575–581.
9. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M, Köppel T, Jahantigh MN, Lutgens E, Wang S, Olson EN, Schober A, Weber C. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal*. 2009;2:ra81.
10. Wittmann J, Jack HM. Serum microRNAs as powerful cancer biomarkers. *Biochim Biophys Acta*. In press.
11. Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, Pinto YM. MiR423–5p as a circulating biomarker for heart failure. *Circ Res*. 2010;106:1035–1039.
12. Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, Hu Z, Hood LE, Galas DJ. Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc Natl Acad Sci U S A*. 2009;106:4402–4407.
13. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clin Sci (Lond)*. 2005;109:143–159.
14. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, Ivey KN, Bruneau BG, Stainier DY, Srivastava D. miR-126 regulates angiogenic signaling and vascular integrity. *Dev Cell*. 2008;15:272–284.
15. Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. *Proc Natl Acad Sci U S A*. 2008;105:1516–1521.
16. Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, Deitch EA, Huo Y, Delphin ES, Zhang C. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. *Circ Res*. 2009;105:158–166.
17. Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, Herias V, van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE. miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res*. 2009;104:170–178.
18. Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res*. 2009;104:476–487.
19. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest*. 2006;116:1802–1812.
20. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Meigs JB, Bonadonna RC, Muggeo M; Bruneck study. Population-based incidence rates and risk factors for type 2 diabetes in white individuals: the Bruneck study. *Diabetes*. 2004;53:1782–1789.
21. Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, Weber M, Hamm CW, Röxe T, Müller-Ardogan M, Bonauer A, Zeiher AM, Dimmeler S. Circulating microRNAs in patients with coronary artery disease. *Circ Res*. 2010;107.

KEY WORDS: microRNA ■ diabetes mellitus ■ blood ■ biomarker ■ endothelium

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Circ Res. 2010;107:686-688
doi: 10.1161/CIRCRESAHA.110.228841
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571