Role of MicroRNA in Pancreatic β-Cells

Where More Is Less

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One of most intriguing discoveries in biology in recent years is unquestionably the identification of the family of small, noncoding RNAs known as microRNAs (miRNAs). Originally described in worms (1), miRNAs are now known to be virtually ubiquitous among vertebrates, including humans. Over 400 genes encoding miRNAs are dispersed throughout the human genome, many of them unexpectedly located within the borders of conventional, protein-coding genes (2). miRNA genes are transcribed primarily by RNA polymerase II into long precursor molecules that are processed via the RNase III enzymes Drosha and Dicer into the mature, ~22-nucleotide miRNA (3,4).

Functional studies are beginning to reveal the involvement of miRNAs in a remarkable array of key cellular activities including differentiation, proliferation, and metabolic integration (5). Each miRNA targets multiple mRNA species through recognition of complementary sequences, typically located at multiple sites within the 3′ untranslated region. This results in reduction in the level of the corresponding protein, either through destabilization of the mRNA or inhibition of translation (3). The effects of each such interaction may be subtle, complicating the process of dissecting out these effects. Nevertheless, it is becoming abundantly clear that the miRNAs have a profound effect on controlling and integrating programs of gene expression. For example, miRNAs can act both upstream and downstream of key cellular transcription factors (6), thereby exerting broadly pleiotropic effects. Furthermore, evidence is now accumulating that links miRNA to diseases including cancer, neurological disorders, and a variety of developmental defects (7). Although miRNAs are notably absent from the rapidly expanding list of genes associated with type 2 diabetes risk (8), a link between miRNAs and diabetes now seems increasingly likely. What is the evidence for such a connection?

In 2004, Poy et al. (9) identified several miRNAs expressed selectively in pancreatic endocrine cell lines: among these was miR-375, overexpression of which resulted in suppressed glucose-stimulated insulin secretion and whose inhibition enhanced insulin secretion. One of the targets of miR-375 was identified as myotrophin, a protein implicated in exocytosis. Indeed, inhibition of myotrophin production mimicked the effects of miR-375 on insulin secretion. These results establish miR-375 as a potentially important modulator of β-cell function, but what are the other targets of miR-375? A study by El Ouaamari et al. (10) in this issue of *Diabetes* presents a tantalizing new candidate: PDK1 (3′-phosphoinositide–dependent protein kinase 1). Well known as an important component of the PI3K/protein kinase B signal cascade that plays a key role in mediating action of insulin on cell growth and development (11), the central role of PDK1 in β-cells was demonstrated recently through ablation of PDK1 in β-cells, which leads to diabetes concomitant with reduced β-cell mass (12). Using computational algorithms, El Ouaamari et al. identified a single miR-375 binding site in the PDK1 3′ untranslated region. Indeed, overexpression of miR-375 in β-cell lines led to reduced levels of PDK1 protein, accompanied by reduced downstream signaling, including protein kinase B and glycogen synthase kinase-3 phosphorylation. In accord with the known actions of the PI3K signaling pathway, miR-375 led to reduced β-cell number and viability, in parallel with reduced potency of glucose in mediating increases in insulin mRNA. Conversely, inhibition of miR-375 expression led to increased PDK1 levels, as well as elevated glucose-dependent insulin mRNA and β-cell proliferation. El Ouaamari et al. report that glucose is a potential negative modulator of miR-375, and consistent with this, glucose leads to substantial increase in PDK1 protein levels. Finally, glucose is shown to reduce miR-375 in isolated rat islets, and miR-375 levels appear to be reduced in islets of the diabetic Goto-Kakizaki (GK) rat.

The study of El Ouaamari et al. represents a substantial advance because it provides a plausible connection between miRNAs and a critical β-cell signaling pathway, linked to cell proliferation and apoptosis. Yet, there are significant caveats: e.g., much of the evidence presented by El Ouaamari et al. is based on experiments involving substantial overexpression (~500 fold) of miR-375 in β-cell lines. It will therefore be essential to confirm these results in a more physiological setting, ideally in mice bearing selective ablation of the miR-375 gene in β-cells. In fact, German and colleagues (13) recently generated mouse models in which miRNA generation is globally blocked through deletion of a conditional Dicer allele. When Dicer was deleted at an early stage of pancreas development (using the Pdx-1 promoter), serious developmental defects were observed in all pancreatic cell types, consistent with a critical role for miRNAs during pancreas development. When Dicer was deleted later in development (using the insulin promoter), little effect was seen either on pancreas morphology or on β-cell maintenance, which is perhaps surprising in view of the fact that these β-cells are expected to lack all miRNAs. Although these

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results clearly underscore the importance of miRNAs during development (14), they leave open the precise role in the adult islet and emphasize the need for detailed studies on mice lacking miR-375. Unpublished results (cited in ref. 15), indicate that mice lacking miR-375 are hyperglycemic, perhaps as a result of reduced β-cell mass. Whether this is a result of a developmental defect or an impairment in the function of mature β-cells remains to be established.

How is miR-375 expression controlled ? El Ouaamari et al. propose glucose as one factor, an intriguing idea given the central role of glucose in β-cell function and the notion that glucose may play an important role in driving the compensatory β-cell hyperplasia characteristic of the insulin-resistant state (16). Thus, miR-375 may be involved in mediating the β-cell proliferation observed in pregnancy and obesity. Additional regulators of miR-375 expression are suggested by the work of Keller et al. (17), who recently identified conserved sites near the miR-375 gene as potential targets of the critical pancreas transcription factors Pdx-1 and NeuroD1, hinting that miR-375 may be an important player in the network of interacting transcription factors that control pancreas development and maintenance (18).

Clearly, the study of El Ouaamari et al. represents one piece of a large and complex emerging puzzle. It will be important to identify additional targets of miR-375 and other miRNAs expressed in β-cells (19,20) and to establish how they function in developing and mature cells. The interactions of miRNAs with transcription factor networks are likely to provide valuable clues. Finally, fueled by the links between miRNAs and human disease, powerful tools are being developed to improve detection and manipulation of miRNAs (7). Though a "smoking gun" directly linking miRNAs to diabetes has yet to be uncovered, it seems likely that further research in this area may lead to significant new diagnostic and therapeutic approaches to diabetes.

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