MicroRNA-7 Control of β-Cell Replication

Juan Domínguez-Bendala, Dagmar Klein, and Ricardo L. Pastori

The study of the insulin-producing pancreatic β-cells transcends the realm of basic biology because of their importance for the maintenance of glucose homeostasis. As their autoimmune-mediated destruction or the impairment of their function cause diabetes, the pursuit of strategies for β-cell replenishment and/or replication is a major objective of regenerative medicine. Owing to their slow turnover in humans, the pancreatic β-cells have been traditionally considered postmitotic (1). However, new evidence supports the notion that β-cells can dynamically adapt their mass and number. This is supported, for instance, by the observation of a perinatal burst of β-cell proliferation (2) or the fact that residual β-cells are found in type 1 diabetic patients decades after diagnosis (3). Although most factors behind this adaptation are pathological (e.g., obesity or hyperglycemia), others are physiological (e.g., pregnancy) (4). Animal models offer us a plethora of examples of β-cell regeneration associated with specific interventions, including duct ligation, β-cell ablation approaches, or partial pancreatectomy (4). In this issue of *Diabetes*, Wang et al. (5) describe the proliferation of β-cells induced by regulation of the mTOR pathway through microRNA-7 (miR-7). MicroRNAs (miRNAs) are noncoding gene products that posttranscriptionally regulate gene expression (6). miRNAs recognize and bind to partially complementary sequences on the RNA's 3′UTR, inhibiting its expression by translation repression or degradation. miR-7 is a representative islet miRNA (7), highly conserved across species and preferentially expressed in the human embryonic and the adult endocrine pancreas (8,9).

The mechanistic target of rapamycin (mTOR) is a highly conserved serine/threonin protein kinase that controls cell proliferation and cell survival in response to a variety of cellular signals such as levels of energy, growth factors, nutrients, hypoxia, and stress (10). The mTOR signaling pathway has a critical role in metabolic diseases such as diabetes and cancer development (10). mTOR exists in two complexes, mTORC1 and mTORC2, both sharing the catalytic component but with different biological activities regulated by distinct scaffold proteins. Both complexes are sensitive to rapamycin, although mTORC2 is only affected by prolonged exposure (10). Several studies show that β-cell number, size, and/or physiology are affected by signaling from both mTOR pathways, which was also confirmed in a mouse pregnancy model with rapamycin (11). Constitutive activation of mTORC1 in mouse β-cells increased their number and size and correlated with decreased blood glucose levels and hyperinsulinemia. Conversely, deficiency in RPS6KB1 (S6K1), an mTORC1-dependent downstream kinase promoting protein synthesis, or the inhibition of mouse mTORC2 by deletion of the scaffold protein Rictor, produced the opposite effects (10).

The mTOR pathway positively controls cell cycle progression and cell proliferation by regulating S6K1 and the eukaryotic translation initiation factor 4E binding protein (4E-BP1). Phosphorylation of 4E-BP1 by mTOR disrupts binding to eIF4E, activating cap-dependent translation. Both S6K1 and 4E-BP1/eIF4E pathways mediate mTOR-dependent G1 phase transition (12).

Wang et al. (5) show that in vitro inhibition of miR-7a (the major murine isoform corresponding to human miR-7) in islets upregulates expression of mTORC1 components S6K1 and eIF4E, as well as the mTORC2-specific scaffold protein Mapkap1 (mSn1) and two downstream ERK threonine/serine protein kinases (MNK1/2), which phosphorylate eIF4E (13). This effect was detected only at the protein level, suggesting translational repression. In vitro targeting of reporter genes supported the specificity of this effect. Upregulation of S6K61, Mapkap1, and MNK1/2 was paralleled by an increase in phosphorylation of their respective substrate targets S6, Akt, and eIF4E. The increase in S6 and Akt phosphorylation, as well as elevated eIF4E protein levels, indicate a bona fide stimulation of the mTOR pathway activity. The biological significance of the MNK1/2-mediated increased phosphorylation of eIF4E, and its effect on translation is not completely understood (13). The activation of mTOR resulted in β-cell proliferation, confirmed by colocalization of insulin expression with replication markers. The effect was abrogated by the mTOR inhibitor rapamycin, substantiating the mTOR involvement and ruling out the possibility of other miR-7 targets controlling cell proliferation.

The miRNA-mediated regulation of mTOR and the subsequent effect on cell proliferation has been studied mostly in the context of cancer. Several "tumor suppressor" miRNAs are known to target mTOR or its components, thus controlling cell cycle progression and proliferation (14–17). The role of miR-7 at inhibiting hepatocarcinoma by targeting the phosphoinositide 3-kinase catalytic subunit delta (PIK3CD), as well as mTOR and S6K1, has been recently proposed (18). Interestingly, Wang et al. (5) did not observe miR-7–mediated changes of mTOR expression at either the RNA level or the protein level. Collectively, these results indicate that miR-7 impedes β-cell replication via downregulation of the mTOR signaling pathway. This is the first study showing miRNA control of β-cell replication. From a basic biological perspective—and as discussed by the authors of the article—it is intriguing that miR-7–dependent mTOR activation may have conflicting functions in embryonic development and in mature cells. This is an observation that certainly warrants additional research.

Given the unique therapeutic value of β-cells, it is important to understand the role of miRNAs in islet biology

From the Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, Miami, Florida.
Corresponding author: Ricardo L. Pastori, rpastori@med.miami.edu.
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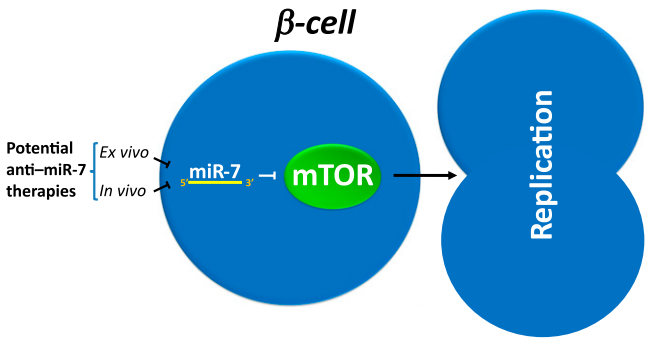

FIG. 1. Negative control of miR-7 on proliferation of mature β-cells. Ex vivo inhibition of miR-7 in pancreatic human and mouse islets results in the activation of the mTOR pathway, leading to β-cell replication without induction of apoptosis. This finding may be applied to in vivo or ex vivo induction of β-cell proliferation for potential therapeutic purposes.

and also identify their translational potential (Fig. 1). We might foresee approaches involving the direct delivery of anti–miR-7 to β-cells either in vivo to induce their regeneration or ex vivo to expand them in culture. The proliferation of human β-cells induced by this straightforward strategy was increased by more than 30-fold, which is comparable (~40-fold) to what was reported in islets transduced with recombinant adenoviruses expressing the cell cycle proteins Cdk-6 and cyclin D1 (19). Given the transient nature of mTOR activation using this method, the risk of cancer induction is negligible. An additional advantage is the absence of a negative effect on the insulin secretory machinery and the lack of apoptosis, which was previously reported during β-cell replication (20). Further studies on physiology and the life span of newly formed β-cells will determine if this strategy could be applicable in a clinical setting.

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