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OR05-3 Mir-21 Contributes to Cytokine-Induced Beta Cell Dysfunction via Inhibition of mRNAs Regulating Beta Cell Identity

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Abstract

A hallmark of diabetes is the loss of physical or functional β cell mass. Alterations in β cell microRNA (miRNA) profiles have been described in diabetes. MiRNAs have also been shown to serve as important regulators of β cell development and function, implicating them in β cell dysfunction during diabetes development. Our lab has previously demonstrated that β cell microRNA 21 (miR-21) is increased in models of diabetes. However, a comprehensive analysis of the β cell effects of miR-21 remain poorly defined, and the effects of miR-21 on *in vivo* glucose homeostasis have never been explored. To this end, we performed a comprehensive *in silico* analysis of bioinformatics databases to identify potential β cell targets of miR-21, which yielded multiple targets in the Transforming Growth Factor Beta 2 (*Tgfb2*) and Fibroblast Growth Factor Receptor 3 (*Fgfr3*) pathways associated with regulation of differentiation. We hypothesize that β cell miR-21 plays a critical role in inhibiting β cell function and inducing loss of β cell identity. To validate targets *in vitro*, we developed a model whereby miR-21 is upregulated using a dose dependent lentiviral Tetracycline-on system in INS1 cells. Overexpression of miR-21 led to a reduction in expression levels of several members of the *Tgfb2* and *Fgfr3* pathways as well as multiple transcription factors associated with β cell function and identity, and an increase in aldehyde dehydrogenase transcripts, consistent with β cell dedifferentiation. To verify direct interactions between miR-21 and candidate target mRNAs, a biotin pulldown experiment was performed using a 3' biotinylated mature miR-21 construct and a 3' biotinylated cel-miR-67 control construct. Several mRNAs associated with β cell identity were enriched in the pulldown, indicating a direct interaction with miR-21. Lineage tracing was performed within an *in vivo* zebrafish model of β cell specific oxidative stress in which β cells expressed a nuclear GFP signal. Whole body knock down of miR-21 by morpholino microinjection showed a protective effect in stressed β cells and rescued against a dedifferentiated phenotype. To test the effect of miR-21 on glucose tolerance *in vivo*, inducible β cell specific knockout (βmiR-21KO) and overexpression (βmiR-21) mice were generated

by crossing *Ins1tm1(CreERT2)*Thor mice with miR-21 floxed mice and miR-21-CAG-Z-EGFP mice, respectively. When compared to littermate controls, intraperitoneal glucose tolerance tests (IPGTT) exhibited hyperglycemia in βmiR-21 mice and euglycemia in βmiR-21KO mice. Metabolic studies, including glucose stimulated insulin secretion (GSIS) and insulin tolerance tests (ITT) are ongoing in our mouse models. Our results implicate miR-21 as a regulator of β cell dedifferentiation during diabetes development.