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ISLET STUDIES

Coxsackievirus B5 Infection Induces Dysregulation of microRNAs Predicted to Target Known Type 1 Diabetes Risk Genes in Human Pancreatic Islets

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Extensive research has identified enterovirus (EV) infections as key environmental triggers of type 1 diabetes. However, the underlying molecular mechanisms via which EVs contribute to the pathogenesis of type 1 diabetes remain unclear. Given that EVs dysregulate host microRNAs (miRNAs), which function as key regulators of β-cell biology, we investigated the impact of coxsackievirus B5 (CVB5) infection on the cellular expression of miRNAs within human islets. Using high-throughput quantitative PCR nanofluidics arrays, the expression of 754 miRNAs was examined in CVB5-infected human pancreatic islets. In total, 33 miRNAs were significantly dysregulated (≥ threefold difference) in the infected compared with control islets ($P < 0.05$). Subsequently, these differentially expressed miRNAs were predicted to target mRNAs of 57 known type 1 diabetes risk genes that collectively mediate various biological processes, including the regulation of cell proliferation, cytokine production, the innate immune response, and apoptosis. In conclusion, we report the first global miRNA expression profiling of CVB5-infected human pancreatic islets. We propose that EVs disrupt the miRNA-directed suppression of proinflammatory factors within β-cells, thereby resulting in an exacerbated antiviral immune response that promotes β-cell destruction and eventual type 1 diabetes.

Type 1 diabetes is an immune-mediated disease resulting from the complex interplay between genetic and environmental factors. The role of enteroviruses (EVs) as key triggers of this disease has been heavily debated due to the lack of unequivocal proof that EVs serve as causal agents, despite significant associations between EV infection and islet autoimmunity (odds ratio 3.7) and clinical type 1 diabetes (odds ratio 9.8) (1). Among EV strains implicated with this disease, coxsackievirus B (CVB) group has been studied most extensively. Multiple mechanisms by which CVB might initiate and/or accelerate autoimmunity have been proposed, including direct induction of β-cell death, promotion of inflammatory cytokine production, activation of toll-like receptors (TLRs), molecular mimicry, and most recently, dysregulation of host microRNAs (miRNAs) (2,3).

Sized between 20 and 22 nucleotides, miRNAs are noncoding RNAs that function as potent regulators of gene expression (4). Derived from sequential processing of longer precursor transcripts, they load Argonaute proteins to mediate either destabilization or translational inhibition of target mRNAs. Sequence specificity of this control is provided by the complementary base pairing between the miRNA and mRNA, usually within its 3′ untranslated region (3′UTR).

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Growing evidence supports pivotal roles of miRNAs in β-cell biology, including regulation of differentiation, glucose sensing, insulin exocytosis, inflammation, and apoptosis (3,5–7). Moreover, a number of miRNAs have been identified as components of pathways initiated by or contributing to the pathogenesis of both type 1 and type 2 diabetes (3). Therefore, it can be anticipated that changes in miRNA activities elicited by environmental exposures contribute significantly to the initiation and acceleration of islet autoimmunity.

To date, very few studies have examined global miRNA expression in the pancreas and none in CVB-infected human β-cells. In fact, only one study has investigated the effect of CVB infection on pancreatic miRNA expression, using rat β-cells (8). Therefore, the aim of this study was to identify miRNAs dysregulated in CVB5-infected human pancreatic islets and investigate whether they target genes associated with risk of type 1 diabetes.

RESEARCH DESIGN AND METHODS

CVB5 Infection

Human cadaveric pancreatic islets from two anonymous donors were cultured separately and infected with one clinical CVB5 strain (GenBank accession no. GQ126860.1) at 1 multiplicity of infection (MOI), as previously described (9). MOI was optimized to ensure >95% viability of cells in culture after viral exposure using the trypan blue dye exclusion test. Cells were harvested at 1, 4, and 7 days postinfection (dpi) and stored in TRI Reagent (Ambion) at −80°C.

miRNA Profiling

RNA extraction, cDNA synthesis, and preamplification were performed as previously described (10). Human Pool A v2.1 and B v3.0 Megaplex stem-loop RT Primers (Applied Biosystems) were used to produce cDNA. After preamplification, cDNA was diluted (1:40), combined with an equal volume of Master Mix, and loaded onto the TaqMan OpenArray Real-Time PCR Plates containing 754 miRNA assays. Plates were analyzed on the QuantStudio 12k Flex Real-Time PCR System using recommended settings. For each batch of human islets, experiments were repeated at least three times and evaluated in triplicate.

Gene Expression Analysis

Total RNA (1 μg) from control and CVB5-infected cells (4 dpi) were DNase treated (Promega) before cDNA synthesis using Superscript III (Invitrogen) and random hexamers. Resulting cDNA was diluted equally (1:10) before quantitative PCR, performed using SYBR FAST Master Mix (Kapa Biosystems) and gene-specific primers (5′-3′): *BACH2* (GCCTCAATGACCAGCGGAAA; CAAACAGGCCAT CCTCACTG), *GAPDH* (TCAAGATCATCAGCAATGCCTCC; ATCACGCCACAGTTTCCCG), *β-ACTIN* (CTGTACGCCAAC ACAGTGCT; GCTCAGGAGGAGCAATGATC), *SH2B3* (AAC CACCAGGTTCCTGCAAC; GGACAGCCAGAAGAACTAAGGTG), *GLIS3* (CAACCAGATCAGTCCTAGCTTACA; GCGAAATAAGG GACCTGGTATC), *CLEC16A* (CATCAAGACGAGTGGGGAGAGT; TCCTCGTCCGTGGTGTTCTG), *TLR7* (CCAGATATAGGAT CACTCCATGCC; CAGTGTCCACATTGGAAACACC), and *IKZF1* (CACAGTGAAATGGCAGAAGACC; GGCCCTTGTCC CCAAGAAAT).

The following cycle was repeated 40 times on the LightCycler 480II (Roche): 95°C 10 s, 60°C 20 s, and 72°C 15 s. Assays were performed in triplicate and confirmed at least three times. *GAPDH* and *β-ACTIN* levels were used to normalize Ct values. Relative mRNA expression for each gene was expressed as a fold difference to control expression, and significance was evaluated using Student *t* tests.

Bioinformatics Analyses

Only miRNAs with Ct values ≤35 and amplification scores ≥1.15 were considered for normalization. Global mean normalization (11) was applied to compare the geometric mean of all miRNA Ct values to individual miRNAs using ExpressionSuite v1.0.3 (Life Technologies). Fold differences between control and infected cells were calculated using the $2^{-\Delta\Delta Ct}$ method (12). When fold difference was <1, the negative reciprocal was taken to express a negative fold change. Only miRNAs with ≥ threefold difference were included for target prediction analysis using miRWalk (13). All identified miRNAs and type 1 diabetes risk genes compiled from T1DBase (14) were assessed by the miRWalk predictor tool using all 10 databases with the following settings: "human species," "restricted to 3′UTR," "2,000 upstream flanked," "transcript = longest," "minimum seed length = 7," and "pValue = 0.05." Genes predicted as targets by three or more databases were functionally annotated using the Database for Annotation, Visualization, and Integrated Discovery (DAVID), version 6.7 (15), which clustered them according to their associated gene ontology annotations and assigned each cluster an enrichment score (−log[*P* value]).

Statistical Analyses

The nonparametric Mann-Whitney *U* test was used to compare normalized Ct values of miRNAs in CVB5-infected versus control cells. Significance was set at an unadjusted P value <0.05 and miRNAs satisfying this were included for further analyses. For DAVID, biological processes with a false discovery rate of ≥0.05 computed by the Benjamini-Hochberg correction were deemed not statistically significant and discarded. Statistical analyses were performed using Prism (version 6; GraphPad Software Inc., San Diego, CA) and SPSS (version 22; IBM, Armonk, NY).

RESULTS

After CVB5 infection, two miRNAs were significantly dysregulated (≥ threefold difference compared with mock-infected control) in human pancreatic islets at 1 dpi, with miR-155-5p increased fourfold and miR-181a-3p decreased threefold (Table 1). In contrast, 19 miRNAs were significantly decreased at 4 dpi (Fig. 1*A*). At 7 dpi, 12 miRNAs were decreased and 5 were increased (Fig. 1*B*). Overall,

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Table 1—Thirty-three miRNAs differentially expressed in CVB5-infected human islets compared with uninfected control

miRNA	Dysregulation*	Fold-change†	dpi‡	Rank of abundance in β-cells (x/518)§
625-5p	Down	14.35	4	–
365b-3p	Down	9.24	4, 7	353
34a-5p	Up	8.22	4	115
149-5p	Down	7.71	4	334
99b-3p	Down	7.69	4	247
182-5p	Down	7.31	7	16
21-3p	Up	7.16	7	191
93-3p	Down	5.93	4	419
216b-5p	Down	5.07	4	–
376c-3p	Down	4.91	4, 7	–
193b-3p	Down	4.85	4	423
432-5p	Down	4.54	7	62
339-3p	Down	4.53	4	204
181a-2-3p	Down	4.41	7	330
425-3p	Down	4.30	4	174
183-3p	Down	4.17	4, 7	321
155-5p	Up	4.07	1	385
1290	Up	4.01	7	–
493-3p	Down	3.88	7	273
217	Down	3.83	7	167
30a-3p	Down	3.82	4	164
30e-3p	Down	3.48	4, 7	108
345-5p	Down	3.44	7	209
720	Down	3.39	4	335
885-5p	Down	3.38	7	440
29a-3p	Down	3.35	4	57
191-5p	Down	3.28	4	9
663b	Up	3.27	7	–
186-5p	Down	3.22	4	40
10b-3p	Up	3.21	7	–
411-5p	Down	3.19	4, 7	43
181a-3p	Down	3.15	1	–
629-3p	Down	3.03	4	–

miRNAs are listed in the order of highest to lowest fold change. *Direction of dysregulation. †Fold difference in miRNA levels between CVB5-infected and uninfected human islets. ‡The dpi at which they were dysregulated. §Rank (x/518) of abundance (number of reads/total number of reads) among 518 total miRNAs detected by Hi-Seq in healthy human pancreatic β-cells (7).

levels of 33 miRNAs were significantly altered during 7 days of infection, with 6 upregulated and 27 downregulated (Table 1). Greatest upward and downward fold changes were exhibited by miR-34a-5p and miR-625-5p, respectively. All differences measured between control and CVB5-infected islets were statistically significant ($P < 0.05$). Of the 33 CVB5-responsive miRNAs, 6 ranked among the top 100 most abundant miRNAs expressed in purified β-cells of healthy humans (7).

To explore the functional significance of these dysregulations, miRWalk database was used for miRNA target prediction (13). Alignments were restricted to the 3′UTRs of all candidate type 1 diabetes risk genes reported at the time of investigation (14). Remarkably, 57 of the total 72 risk genes were predicted as targets (Table 2). More specifically, miRNAs dysregulated at 1, 4, and 7 dpi were associated with 8, 56, and 47 different target risk genes, respectively. Most miRNAs were predicted to target multiple genes, with many exhibiting significant overlap. *BACH2* displayed the greatest overlap, with 21 miRNAs predicted to target its 3′UTR (Table 2).

Biological processes of the 57 putative targets were investigated using DAVID (15), which grouped them according to their respective gene ontology annotation and provided an enrichment score for each cluster (Fig. 2*A*). No statistically significant biological processes were identified from the predicted targets of miRNAs dysregulated at 1 dpi. In contrast, 67 common processes were identified from the predicted targets of miRNAs differentially expressed at 4 and 7 dpi. The process with the highest enrichment score was "positive regulation of immune processes."

CVB5 infection can induce upregulation of many immune response genes in human islets, including pattern recognition receptors (PRRs) (16). Indeed, mRNA levels of *IFIH1* and *TLR7* were significantly elevated in the CVB5-infected cells compared with controls at 4 dpi (Fig. 2*B*). Examination of other type 1 diabetes risk genes revealed similar increases in the mRNAs of *SH2B3* and *IKZF1*, whereas levels of *BACH2*, *GLIS3*, and *CLEC16A* remained unchanged.

DISCUSSION

Our examination of CVB5-infected human islets revealed significant dysregulation of 33 miRNAs during 7 days of infection (Table 1). This represents 2.2% of all characterized human miRNAs (5) and 6.4% of miRNAs expressed in healthy human pancreatic β-cells (7). Since a single miRNA can potentially regulate hundreds of genes (4), disruption of even a small subset of miRNAs can profoundly impact the transcriptome and biology of cells.

Both miR-155-5p and -181a-3p are important modulators of the immune response, directing suppression of proinflammatory cytokines and NF-kB pathway activators (17,18). In this study, miR-155-5p was elevated in CVB5-infected cells and predicted to target the antiapoptotic gene *GLIS3*. Loss of *GLIS3* sensitizes cells to cytokine-mediated apoptosis (19). Therefore, increased miR-155-5p may reduce *GLIS3* expression and promote β-cell apoptosis, whereas the concurrent reduction of miR-181a-3p is expected to produce the opposite effect. Although it seems contradictory that two miRNAs negate each other's effects, apoptosis is highly disadvantageous for the virus,

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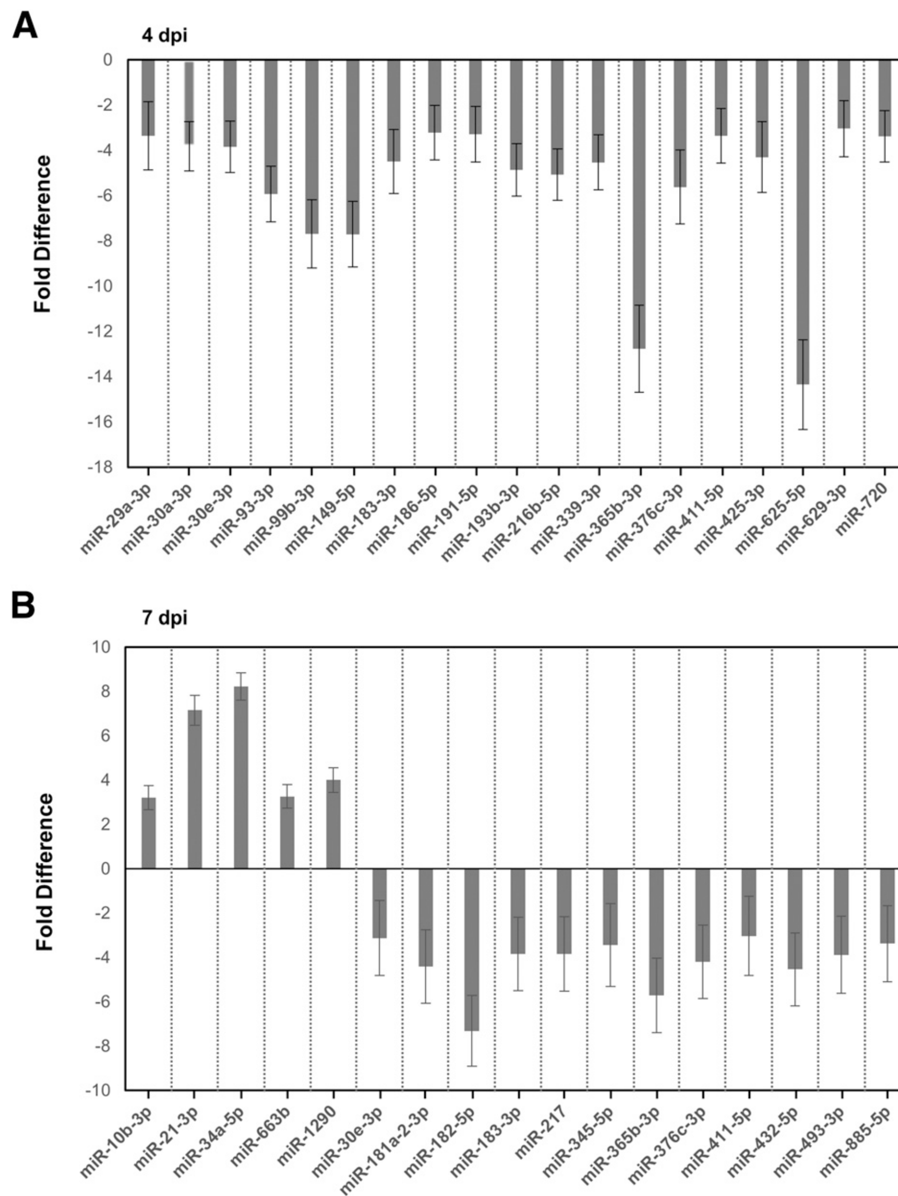

Figure 1—Dysregulation of miRNAs in CVB5-infected human pancreatic islets (at MOI = 1). *A*: Significant downregulation of 19 miRNAs at 4 dpi expressed as the negative fold difference compared with the mock-infected control. *B*: Significant dysregulation of 17 miRNAs at 7 dpi. Elevation of 5 miRNAs and the reduction of 12 miRNAs expressed as positive and negative fold difference compared with control, respectively. Each column represents results from six independent experiments, and the error bars correspond to the SEM.

and therefore CVB5 may directly decrease miR-181a-3p to nullify the host's promotion of apoptosis via miR-155-5p.

Among miRNAs dysregulated at later time points, miR-10b-3p, -182-5p, -186-5p, -345-5p, -376c-3p, -425-3p, -432-5p, -493-3p, and -629-3p were predicted to target multiple PRRs: *IFIH1*, *TLR7*, and *TLR8* (Table 2). These encode proteins that promote generation of type 1 interferons and proinflammatory cytokines (20). They also activate downstream caspases that trigger apoptosis (21). All PRR-targeting miRNAs were downregulated in response to CVB5 infection, except miR-10b-3p. Therefore, the likely outcomes are the overexpression of PRRs and enhanced immune response against CVB5. Although this seems beneficial for viral defense, such elevation could induce exacerbated inflammation and prolonged apoptosis of β-cells, promoting type 1 diabetes development.

BACH2 is an important regulator of cytokine-induced apoptosis in pancreatic β-cells (22). In rodent and human pancreatic islets, *BACH2* inhibition exacerbates cytokine-induced β-cell destruction. Conversely, its overexpression has protective effects. Therefore, it is plausible that the reduction of miRNAs targeting *BACH2* at 4 and 7 dpi results in *BACH2* overexpression, protecting CVB5-infected cells from apoptosis. This could be a strategy imposed by CVB5 to promote its propagation. If so, the increase of several *BACH2*-targeting miRNAs observed at

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Table 2—Fifty-seven candidate type 1 diabetes risk genes predicted by miRWalk as putative targets of the 33 CVB5-responsive miRNAs

Risk genes	Upregulated	Downregulated	*n*
BACH2	10b-3p, 34a-5p, 663b, 1290	29a-3p, 149-5p, 182-5p, 183-3p, 186-5p, 193b-3p, 216b-5p, 217, 339-3p, 345-5p, 376c-3p, 425-3p, 432-5p, 493-3p, 625-5p, 629-3p, 885-5p	21
SH2B3	10b-3p, 34a-5p, 663b, 1290	30a-3p, 30e-3p, 93-3p, 149-5p, 186-5p, 193b-3p, 216b-5p, 217, 345-5p, 411-5p, 432-5p, 629-3p	16
CLEC16A	663b	149-5p, 182-5p, 186-5p, 193b-3p, 339-3p, 345-5p, 365b-3p, 376c-3p, 411-5p, 432-5p, 493-3p, 625-5p, 629-3p	14
GLIS3	663b, 1290	29a-3p, 93-3p, 149-5p, 155-5p, 186-5p, 193b-3p, 216b-5p, 217, 376c-3p, 425-3p, 432-5p	13
IKZF1	34a-5p, 1290	149-5p, 182-5p, 186-5p, 193b-3p, 216b-5p, 217, 345-5p, 432-5p, 493-3p, 625-5p, 629-3p	13
ZMIZ1	10b-3p, 34a-5p, 663b	29a-3p, 149-5p, 186-5p, 217, 345-5p, 432-5p, 493-3p, 625-5p, 885-5p	12
TNFAIP3	34a-5p	29a-3p, 149-5p, 186-5p, 193b-3p, 345-5p, 376c-3p, 432-5p, 425-3p, 625-5p, 720	11
CUX2	34a-5p, 663b, 1290	29a-3p, 149-5p, 182-5p, 216b-5p, 217, 432-5p, 493-3p	10
AFF3	34a-5p	30a-3p, 30e-3p, 186-5p, 216b-5p, 217, 376c-3p, 432-5p, 625-5p, 629-3p	10
C1QTNF6	10b-3p, 663b	29a-3p, 191-5p, 193b-3p, 217, 365b-3p, 376c-3p, 625-5p, 629-3p	10
SKAP2	1290	149-5p, 182-5p, 186-5p, 193b-3p, 217, 411-5p, 432-5p, 625-5p	9
ZFP36L1	10b-3p, 1290	29a-3p, 182-5p, 186-5p, 493-3p, 625-5p, 629-3p, 885-5p	9
GAB3	–	93-3p, 149-5p, 155-5p, 182-5p, 186-5p, 365b-3p, 376c-3p, 629-3p	8
PDE4A	10b-3p, 34a-5p	149-5p, 186-5p, 425-3p, 432-5p, 625-5p	7
IL17D	663b	29a-3p, 182-5p, 186-5p, 193b-3p, 432-5p, 625-5p	7
LMO7	–	30a-3p, 30e-3p, 182-5p, 186-5p, 216b-5p, 217, 493-3p	7
TLR7	–	182-5p, 186-5p, 345-5p, 425-3p, 432-5p, 493-3p, 629-3p	7
SLC11A1	10b-3p	93-3p, 182-5p, 339-3p, 365b-3p, 625-5p, 720	7
CCR5	21-3p	29a-3p, 149-5p, 345-5p, 432-5p, 629-3p	6
ERBB3	34a-5p, 1290	149-5p, 182-5p, 217, 345-5p	6
PTPN2	10b-3p	155-5p, 186-5p, 217, 345-5p, 411-5p	6
IL2RB	34a-5p	149-5p, 345-5p, 625-5p, 629-3p	5
GCA	–	183-3p, 186-5p, 376c-3p, 425-3p, 629-3p	5
IL2RA	–	29a-3p, 186-5p, 191-5p, 345-5p, 629-3p	5
PRKCQ	34a-5p, 1290	186-5p, 193b-3p, 411-5p	5
PTPN22	34a-5p	155-5p, 186-5p, 193b-3p, 629-3p	5
CD226	–	155-5p, 182-5p, 216b-5p, 217, 411-5p	5
FUT2	–	186-5p, 191-5p, 411-5p, 625-5p	4
CD55	–	182-5p, 186-5p, 216b-5p, 629-3p	4
RASGRP1	10b-3p	182-5p, 186-5p, 885-5p	4
RGS1	–	29a-3p, 193b-3p, 345-5p, 425-3p	4
CTLA4	–	155-5p, 376c-3p, 432-5p, 493-3p	4
PGM1	34a-5p	182-5p, 432-5p, 625-5p	4
COBL	–	182-5p, 625-5p, 629-3p, 885-5p	4
ORMDL3	34a-5p, 663b	93-3p, 625-5p	4
DEXI	–	149-5p, 216b-5p, 625-5p	3
HLA-DQB1	–	186-5p, 411-5p, 625-5p	3
C19orf10	–	30a-3p, 30e-3p, 376c-3p	3
TAGAP	21-3p	155-5p, 365b-3p	3

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Table 2—Continued

Risk genes	Upregulated	Downregulated	*n*
SIRPG	–	149-5p, 182-5p	2
IL2	–	186-5p, 376c-3p	2
IL21	–	186-5p, 376c-3p	2
IL10	–	186-5p, 411-5p	2
CYP27B1	–	186-5p, 625-5p	2
SUMO4	–	186-5p, 625-5p	2
CD69	–	182-5p, 186-5p	2
GCG	663b	186-5p	2
HORMAD2	–	155-5p, 216b-5p	2
TLR8	10b-3p	376c-3p	2
FAP	–	345-5p, 629-3p	2
CTSH	–	186-5p	1
IFIH1	–	186-5p	1
STAT4	–	186-5p	1
UMOD	–	193b-3p	1
AIRE	–	629-3p	1
IL7R	–	629-3p	1
CEL	663b	–	1

Genes are listed in the order of highest to lowest number of miRNAs predicted to target their 3′UTR (*n*). The associated miRNAs are shown for each gene, categorized separately into those that were upregulated and downregulated.

7 dpi (Table 2) might reflect the host's efforts to counter CVB5 and restore normal apoptosis of infected β-cells.

Interleukin-2 (IL-2), IL-10, IL-17D, and IL-21 were among the predicted targets of miRNAs downregulated in CVB5-infected cells. Reduced miRNA-directed suppression of these cytokines will likely enhance inflammation and β-cell death, potentiating islet autoimmunity. Consistent with this hypothesis, we previously identified significant correlations between increased levels of proinflammatory cytokines (including IL-2, IL-17, and IL-21) and islet autoimmunity in children (23). Furthermore, expression of many type 1 diabetes risk genes are modified by cytokine exposure in human pancreatic islets, including *PTPN2*, *IFIH1*, *SH2B3*, *STAT-4*, *GLIS-3*, *CD55*, *RASGRP1*, and *SKAP2* (24), which are all predicted targets of CVB5-dysregulated miRNAs identified in this study.

The greatest fold increase was exhibited by miR-34a-5p (eightfold) (Table 1). Previously, its expression increased three- to fourfold after exposure to palmitate and proinflammatory cytokines in rodent and human pancreatic islets (6,25). Moreover, similar elevation of miR-34a was present in β-cells of *db/db* and NOD mice during development of prediabetic insulitis (6,25). Two targets of miR-34a-5p have been verified:

1) *VAMP2*, essential for β-cell exocytosis, and
2) antiapoptotic *BclII*.

Accordingly, miR-34a-5p overexpression reduces both targets in MIN6 cells, diminishing maximum insulin secretion capacity and increasing apoptosis (25). Thus, the sharp increase of miR-34a-5p in CVB5-infected islets may enhance secretory dysfunction and destruction of β-cells, which are hallmarks of type 1 diabetes.

When interpreting fold changes in miRNA expression between test and control samples, it is crucial to consider the natural miRNA abundance. For example, miR-625-5p exhibited the greatest fold change between control and CVB5-infected cells. However, the 14-fold reduction of this miRNA is unlikely to impact β-cell function, as its normal expression in pancreatic β-cells is already very low (Table 1) (7). This also applies to miR-216b-5p, -376c-3p, -181a-3p, and -629-3p. In contrast, sudden increases of low-abundant miRNAs can lead to new miRNA-target interactions and regulatory cascades, which could be the case for miR-34a-5p, -21-3p, -155-5p, -1290, -663b, and -10b-3p.

Six miRNA-target interactions identified in this study (*BACH2*-miR-29a-3p, *BACH2*-miR-34a-5p, *SH2B3*-miR-193b-3p, *TNFAIP3*-miR-29a-3p, *ZFP36L1*-29a-3p, and *RGS1*-miR-29a-3p) have been validated in pancreatic β-cells of healthy adults (7). However, others require future experimental verification. To fully evaluate the significance of our findings, it is important to determine whether the dysregulation of 33 miRNAs in CVB5-infected human islets produces concomitant changes in protein abundances of predicted targets. One high-throughput approach would be to compare global peptide profiles of CVB5-infected and uninfected human pancreatic islet cells using proteomics.

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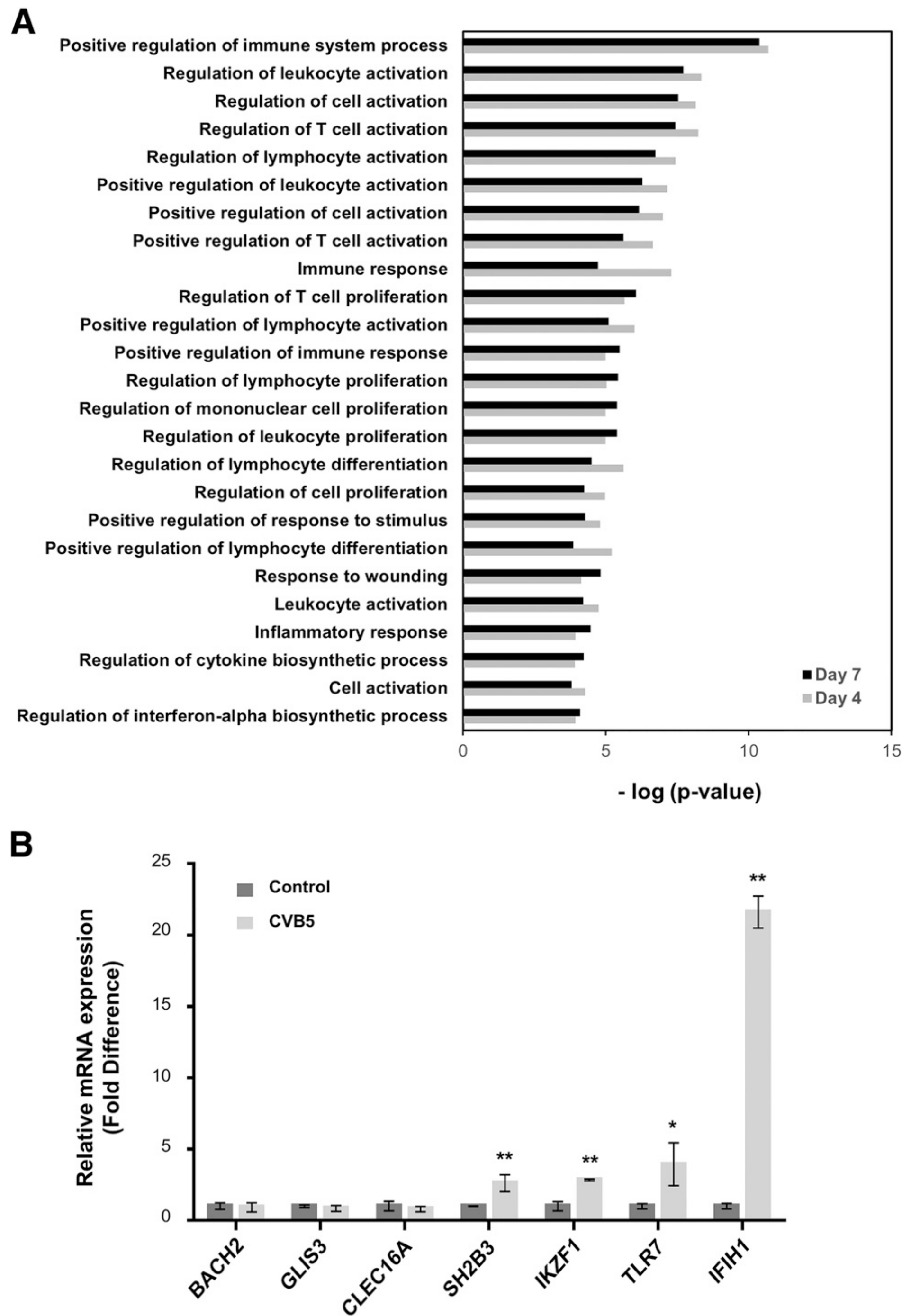

Figure 2—Biological processes of type 1 diabetes risk genes predicted as miRNA targets and their mRNA expression levels in CVB5-infected human islets. *A:* Biological processes are clustered according to their DAVID gene ontology annotation. Each cluster is plotted against their respective enrichment score (−log[*P* value]). Clusters generated from putative target genes of miRNAs dysregulated at 4 and 7 dpi are represented in gray and black, respectively. *B:* Relative mRNA expression of human *BACH2*, *GLIS3*, *CLEC16A*, *SH2B3*, *IKZF1*, *TLR7*, and *IFIH1* as measured by quantitative PCR, normalized to *GAPDH* and *β-ACTIN*. Bars represent fold difference compared with uninfected control at 4 dpi. Mean ± SD of triplicate measurements. $^{*}P < 0.05$; $^{**}P < 0.01$.

In this study, we measured the mRNA levels of seven predicted targets and found significant elevations for *IFIH1*, *TLR7*, *SH2B3*, and *IKZF1* in CVB5-infected islets (Fig. 2*B*). This is consistent with the decreased levels of miRNAs targeting these genes, which could subsequently reduce the destabilization of their mRNAs. In contrast, genes that exhibited no change at the mRNA level may only be affected at the protein level.

In summary, our study represents the first global miRNA expression profiling of CVB5-infected human

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pancreatic islets. Collectively, the 33 dysregulated miRNAs identified were predicted to target mRNAs of 57 candidate type 1 diabetes risk genes. Given that the majority of these miRNAs were significantly downregulated, and their targets function as key regulators of the proinflammatory response, it is plausible that the disrupted activities of these miRNAs induced by CVB5 may exacerbate the antiviral immune response in genetically susceptible individuals, initiating a pathway that leads to the destruction of β-cells and eventually type 1 diabetes.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. K.W.K. researched data and wrote the manuscript. A.H. and A.A.H. researched data and contributed to discussion. A.A.-A. researched data. T.W.H.K. provided the human pancreatic islets used in this study. W.D.R. and M.E.C. researched data, contributed to discussion, and reviewed and edited the manuscript. M.E.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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