glucose condition. Although, PMI-5011 greatly increased GLUT2 abundance at low doses (1 and 2.5 µg/ml), the effects of PMI-5011 on insulin signaling were reduced at high concentration of glucose. IRS-2, PI-3 and GLUT2 content decreased with increase of PMI-5011 concentrations. Furthermore, silent IRS-1 using IRS-1 siRNA transfections resulted in reduction of insulin secretion, IR β and GLUT2 abundance in INS-1 cells as well as blocked the effects of PMI-5011 on insulin release.

This study suggests that PMI-5011 increases insulin secretion in cultured pancreatic β-cell by enhancing insulin signaling pathway.

2260-P

High Fidelity Taq DNA Polymerase Leads to Erroneous Methylation Analysis of the Human Insulin Gene

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Differential DNA methylation of CpG dinucleotides (CpG-DNs) can maintain or repress gene transcription in a cell specific manner. Insulin-producing β-cells show reduced methylation of the insulin gene. Identification of differentially methylated CpG-DNs is used for the study of insulin gene regulation and for the detection of β-cell death in the blood of diabetic patients. Gene methylation can be detected by bisulfite treatment of genomic DNA resulting in the conversion of unmethylated cytosines (C) to thymidines (T), while methylated Cs remain intact. Bisulfite treated DNA is then amplified in a PCR reaction using Taq DNA polymerase (Taq-pol) followed by sequencing of the PCR amplicon. Native Taq-pol lacks proofreading capacity, presenting with a higher probability of DNA mutations during polymerization. Recently engineered Taq-pols offer DNA proofreading and are known as high fidelity (HF) Taq-pols. We tested whether HF Taq-pols can be used for the amplification and analysis of methylated or demethylated human insulin DNA treated with bisulfite. Synthetically methylated or demethylated human insulin DNA was treated with bisulfite followed by a PCR reaction using HF and low fidelity (LF) Taq-pols. Amplicons were gel purified, cloned and sequenced. Surprisingly, HF Taq-pol showed higher rates of nucleotide substitution and deletion of demethylated insulin DNA when compared with low fidelity (LF) Taq-pol. These errors reduced the ability to detect demethylated CpG-DNs, impairing the correct analysis of DNA methylation. Methylated insulin DNA, in which CpG-DNs are spared following bisulfite treatment, was less prone to mutation by HF Taq-pols, suggesting that unconverted CpG-DNs can be detected by HF Taq-pol. In conclusion, our data show an increase in the rate of mutations of bisulfite treated insulin DNA when using HF Taq-pols, highlighting the importance of using LF Taq-pols for the analysis of gene methylation in diabetes.

Supported by: JDRF (17-2012-588)

ISLET BIOLOGY—SIGNAL TRANSDUCTION

Guided Audio Tour: Beta Cell Signal Transduction *(Posters: 2261-P to 2268-P), see page 15.*

2261-P

Identification of Circulating MicroRNAs inHNF1A-MODY Carriers

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HNF1A-MODY is caused by mutations in the *TCF1/hepatocyte nuclear factor 1-alpha* (*hnf1a)* gene, the most common being Pro291fsinsC. MicroRNAs (miRNAs) have emerged as potent regulators of gene function in numerous diseases including diabetes. miRNAs are typically 19-25 nucleotide non-coding RNA molecules, which suppress gene expression by repression of protein production or mRNA degradation. To date, there have not been any studies investigating the potential involvement of miRNAs in the pathogenesis of HNF1A-MODY.

In this study, a miRNA array assay was carried out in INS-1 cells inducibly expressing Pro291fsinsC-HNF1A, which acts as a dominant-negative mutant *in vitro*. Among several other miRNAs, inducible expression of Pro291fsinsC-HNF1A caused a significant upregulation of miR-103 expression. Elevated miR-103 levels upon induction of Pro291fsinsC-HNF1A was validated by real-time PCR. miRNAs have been shown to be secreted from cells, and present in exosomes in the serum of humans. We next determined serum levels of miR-103 in n=31 HNF1A-MODY carriers and n=10 MODY-negative family members by absolute real-time PCR analysis. Mutations in the *HNF1A* gene included L17H, P291finsC, S352fsdelG, F426X, P379T, and IVS7-6G>A. Strongly elevated levels of miR-103 were detected in the serum of HNF1A-MODY carriers when compared to controls (copy number mean 3837±1162; median=1030, IQR=458-3318 vs. mean 81.3±48.2; median=14, IQR=0-126; p=0.00001). miR-103 correlated negatively with triglycerides (p=0.02) and systolic blood pressure (p=0.01), but did not correlate with HbA_{1c}, age, or type of mutation (transactivation vs. dimerization domain).

In conclusion, we have identified miR-103 to be negatively controlled by HNF1A and strongly upregulated in the serum of HNF1A-MODY carriers. The pathophysiology of HNF1A-MODY may be associated with the overexpression of miR-103 which has been previously linked to diabetes and obesity.

2262-P

Hydrogen Peroxide Is a Metabolic Secondary Signal in Pancreatic Beta-Cells

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Islet β-cells are thought to be susceptible to oxidative damage, in part due to low levels of antioxidant enzymes. We examined which of these enzymes were lacking in normal isolated rat islets relative to hepatocytes at the mRNA, protein, and enzyme activity levels. Essentially, we found that the levels of superoxide dismutases (SOD1 and SOD2), which convert superoxide into H2O2, were comparable to that in liver. However, glutathione peroxidase (GPX1) and catalase (CAT), which dispose of H2O2 to water, were lacking in islets (GPX1 activity was <20% and CAT activity was negligible) relative to hepatocytes ($p \leq 0.01$). As such, normal β-cells are wired for H2O2 production from oxidative metabolism-generated superoxide.

We postulate that H2O2 may act as a metabolic secondary signal in β-cells. Using Amplex Red fluorometry, we found endogenous H2O2 production in isolated islets to rapidly increase within 2 min of raising glucose concentrations from basal 2.8 mM to stimulatory 16.7 mM (4-fold; $p \leq 0.04$) and remain elevated until reintroduction of basal glucose returned H2O2 to resting levels. Adenoviral-mediated expression of CAT (AdV-CAT) in islets prevented a glucose-induced increase in H2O2 (>90%; $p \leq 0.04$). Elimination of H2O2 by AdV-CAT significantly inhibited glucose-induced insulin secretion, especially its amplification under hypercalcemic clamp conditions ($p \leq 0.05$). Moreover, glucose-induced activation of mammalian target of rapamycin (mTOR), specifically mTOR complex-1 (mTORC1) and not mTORC2, was significantly inhibited in AdV-CAT islets ($p \leq 0.03$). Conversely, enhancing H2O2 production with AdV-SOD2 increased glucose activation of mTORC1 ($p \leq 0.008$). In addition, glucose-induced H2O2 production in islets was capable of inhibiting protein tyrosine phosphatases (PTPs), particularly PTPN11. Thus, the antioxidant system of islet β-cells is coordinated to generate H2O2 as a normal metabolic secondary signal that amplifies specific cellular functions including regulated insulin secretion.

Supported by: NIH (DK055267)

2263-P

Regulation of Pancreatic Islet Beta-Cell Functions by Nrf2 In Vivo and In Vitro

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Pancreatic islet beta cells secrete insulin in response to blood glucose to maintain body's metabolic fuel homeostasis. On the other hand, beta cells are susceptible to a range of toxic signals such as high glucose, free fatty acids, oxidative stress, and inflammation, which are major contributing factors to the development of diabetes. The nuclear factor erythroid 2-related factor 2 (Nrf2) is an emerging regulator of cellular defense against oxidative and inflammatory insults. We have previously found that mice with targeted knockout (KO) of Nrf2 were sensitive to streptozotocin (STZ) induced diabetic lesions. In the present study, islet beta cells from Nrf2 wild-type (WT) and KO mice were analyzed in vivo and in vitro. Treatment with STZ induced diabetic conditions that were significantly more severe in Nrf2 KO than WT mice (blood glucose of 558±38 mg/dl for KO and 380±17 for WT). In addition to reducing pancreatic islet size, STZ induced islet oxidative stress and inflammation that were significantly more apparent in KO than WT. Characterization of Nrf2 KO mice revealed that loss of Nrf2 induced a sub-diabetic condition with significantly reduced glucose tolerance and reduced levels of serum insulin. To examine if Nrf2 directly affects islet functions, pancreatic islets were isolated. Islet cells from KO mice showed increased oxidative stress and apoptosis, and reduced survival in the presence of high glucose. Furthermore, Nrf2 KO islets exhibited significantly decreased insulin secretion upon stimulation with glucose in comparison with WT. Taken together the findings revealed that Nrf2 directly regulates islet beta cell functions by modulating glucose stimulated insulin secretion and by boosting protection against oxidative stress and STZ toxicity.

Supported by: NIOSH