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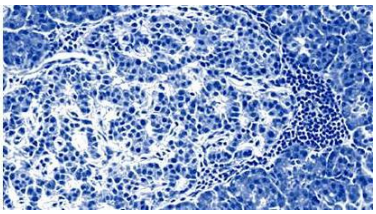

RNA Exosome Content from Insulin-Producing β Cells Incorporates Stress Granule Components and May Serve as Circulating Biomarkers of β Cell Stress in Type 1 Diabetes

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PURPOSE

In early T1D, β cells are subjected to stresses such as inflammation, putative viral infections (or other "danger signals"), and increased endoplasmic reticulum (ER) protein load. Under these conditions, β cells activate a process known as the "integrated stress response," which is highlighted by the phosphorylation of the translation factor eIF2α. Phosphorylation of eIF2α leads to a generalized block in the translational initiation of many mRNA transcripts in an attempt to mitigate the energy-consuming process of translation and allow for cellular recovery. These translationally-inhibited mRNAs (including alternatively-spliced mRNAs and miRNAs) are compartmentalized in an orchestrated process into discrete, non-membranous inclusions known as stress granules (SGs) and processing bodies (PBs). A recent perspective has been emerging that SGs may function as hubs that intercept other intracellular pathways to signal a "state of emergency," and as such, SG content can be deposited into other cellular compartments such as extracellular vesicles, and subsequently released into the circulation. Therefore, the circulating contents of EVs may contain RNA molecules that reflect the state of β cell stress. In this study we sought to identify circulating mRNAs that are sequestered into stress granules (SG) during early β cell ER stress, are released in extracellular vesicles, and that the unique contents of these vesicles might serve as biomarkers of β-cell stress.

METHODS

Min6, INS-832/13, human EndoC BH1 cell line and human pancreatic islets were treated with or without pro-inflammatory cytokines (IL-1β and IFN-γ) to mimic T1D conditions. Exosomes were isolated using either an ExoQuick precipitation method or by serial ultracentrifugation and vesicle derived insulin mRNA levels were determined using RT-PCR. Untreated and cytokine treated human islets were subjected to poly-ribosome profiling (PRP) to assess the cytokine mediated global mRNA translation initiation block. Droplet digital PCR was used to measure the whole blood derived circulating levels of insulin mRNA

from multiple T1D animal models and human subjects. Single molecular FISH was performed to identify the co-localization of INS mRNA with stress granule markers.

SUMMARY OF RESULTS

The treatment of mouse MIN6 cells, rat INS 832/13 cells, human EndoC-BH1 β cells, and human islets with pro-inflammatory cytokines (a mimic of the stress seen in T1D) resulted in a block in mRNA translation initiation, decreased protein synthesis, formation of stress granules and increase in SG markers in RNA granules, as assessed by polyribosomal profiling, puromycin incorporation and immunofluorescence staining, respectively. Islets treated with cytokines exhibited increased formation of stress granules, P-bodies, and sec-bodies, as observed by immunofluorescence and immunoblotting. Moreover, studies using single molecular FISH confirmed that human β cells treated with cytokines have increased colocalization of INS mRNA and G3BP1 (a marker of stress granules). EndoC-BH1 human β cells transfected with fluorescently labeled G3BP1 and CD63 (a marker of exosomes) and treated with cytokines exhibited co-localization of stress granules with exosomes, suggesting that stress granule contents are secreted extracellularly. Human preproinsulin (PPI) mRNA was recoverable almost exclusively in microvesicular fractions isolated from supernatants of stressed islet cultures. Pharmacological inhibition of microvesicle release decreased the PPI mRNA into the culture media, suggesting that PPI mRNA is selectively released from stressed β cells via exosomes. Digital PCR analysis demonstrated that circulating mouse PPI mRNA was detectable in circulation of several diabetic mouse models prior to the onset of diabetes. Likewise, we observed significantly higher levels of PPI mRNA in circulation of new-onset T1D subjects compared to healthy, age-matched controls.

CONCLUSIONS

Taken together, our data suggest a link between SGs and the endosomal pathway that liberates extracellular vesicles. Our studies provide mechanistic underpinning to the observation that RNA species generated or translationally inhibited under stress conditions (such as PPI mRNA) in human β cells can be liberated into the circulation, and thereby serve as circulating nucleic acid biomarkers of β cell stress in early T1D. Further high-depth interrogation of RNA species in SGs and EVs would likely provide new candidate nucleic acid biomarkers for early T1D.