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ORAL ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

SEC61B Regulates Calcium Flux and Platelet Hyperactivity in Diabetes Mellitus

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Background/Aim

Platelets in individuals with diabetes mellitus show increased activity at baseline and in response to stimuli, which contributes to the increased cardiovascular events in this population. Endoplasmic reticulum (ER) stress, induced by misfolded proteins, has recently been identified as a mechanism of platelet hyperactivity in diabetes. Newly synthesized proteins enter the ER via the Sec61 translocon for folding. The Sec61 translocon is a heterotrimeric channel comprising of the pore-forming α, and peripheral β and γ subunits. In addition to its roles in proteostasis, it also acts as a leak channel to allow calcium efflux from the ER into the cytosol. However, a function for the Sec61 complex has not been previously described in platelets.

Study design/Methods

Platelets were isolated from 42 patients with and 34 patients without type 2 diabetes mellitus undergoing coronary angiography for suspected or known coronary artery disease. Patients were matched for age, sex, and coronary artery disease burden. Platelet proteins were identified by untargeted LC-MS/MS and analyzed by MaxQuant. Platelet RNA was isolated from a separate cohort of 5 patients with and 12 matched patients without type 2 diabetes. RNA-seq libraries were prepared with TruSeq V2, Illumina, and analyzed with the Deseq2 analysis package. Platelets and megakaryocytes were isolated from 2 mouse models of diabetes (Apoe-/- mice with induction of diabetes by streptozotocin injection) and outbred mice fed a high fat diet. HEK293 cells were generated with knockout or overexpression of SEC61B using CRISPR/Cas9 or cDNA transfection. Proteins of the ER stress response, including eukaryotic translation initiation factor 2A (EIF2A), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6), were determined by immunoblotting or immunostaining. Protein synthesis was measured using the Click-iT ™ AHA Alexa Fluor 488 assay. Calcium flux was measured using fura-2-AM and expressed as normalised fluorescent 340/380 nm ratio. Calcium efflux from the ER was measured in the presence of translation inhibitors puromycin and eeyarestatin I and the sarcoendoplasmic reticulum calcium-ATPase inhibitor thapsigargin.

Results

Proteomics analysis identified an enrichment of proteins involved in response to oxidative stress in platelets of patients with diabetes. From the over 2600 intracellular proteins identified consistently in >50% of human platelet samples, SEC61B was the only protein significantly and positively correlated with serum fructosamine, which is a marker of glycaemic control (Spearman's rho = 0.33, p = 0.029). SEC61B protein abundance was increased in platelets with high fructosamine (>290 µmol/L) compared with normal fructosamine (**panel A**). There were no differences between SEC61 subunits in patients with or without diabetes at the transcriptomic level. Increased SEC61B was confirmed in platelets and megakaryocytes of hyperglycemic mice.

Induction of ER stress in healthy human platelets by incubation with thapsigargin significantly increased the phosphorylation of IRE1 and the expression of SEC61B but not of ATF6 or phosphorylated EIF2A. In HEK293 cells with SEC61B overexpression

or knockout, protein synthesis rate was decreased (58-72% reduction knockout vs control, $p<0.0001$; 22% reduction overexpression vs control, $p = 0.0005$). SEC61B overexpression or knockout resulted in increased ER calcium efflux after treatment with puromycin or eeyarestatin I (which maintain the Sec61 translocon in an open, calcium permeable configuration (**panel B**). Puromycin induced ER-mediated calcium leak in healthy human platelets (22% increase in maximal calcium efflux after puromycin treatment, $p = 0.039$).

Conclusion

Our data identify Sec61 translocon as a novel functional ER calcium leak channel in platelets. Diabetes mellitus is associated with upregulated megakaryocyte and platelet SEC61B. Dysregulated SEC61B expression is associated with increased calcium efflux from the ER. We propose a mechanism whereby ER stress-induced upregulation of platelet SEC61B leads to increased cytoplasmic calcium, potentially contributing to the platelet hyperactivity seen in diabetes mellitus.

Disclosures No relevant conflicts of interest to declare.

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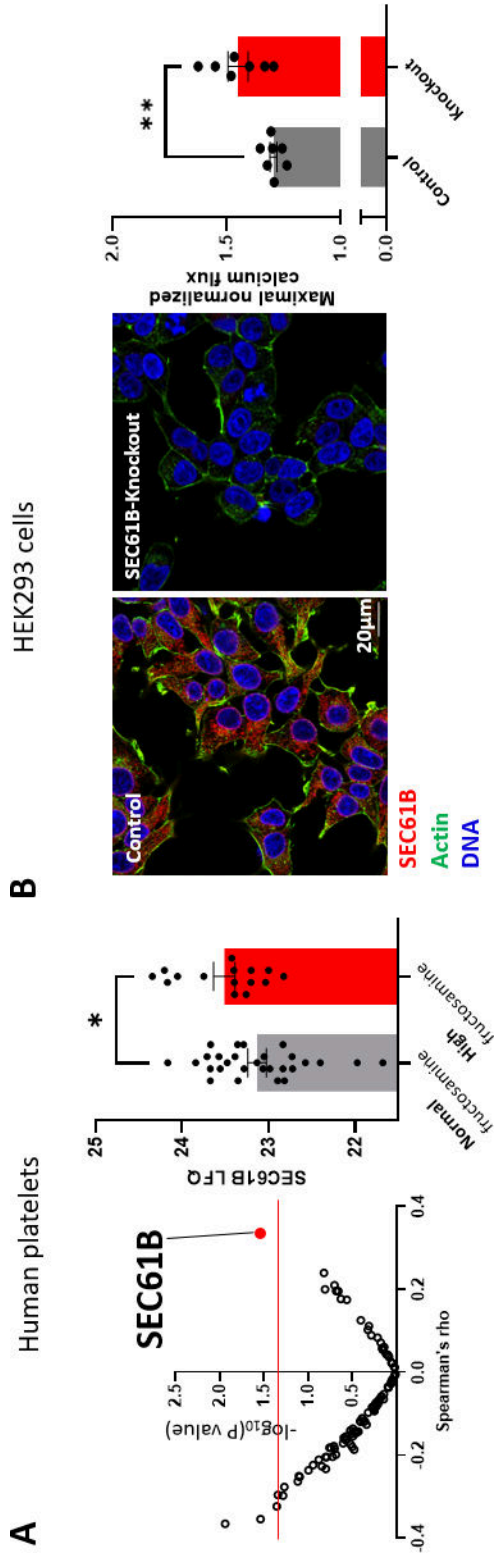

Figure 1