Abscisic acid (ABA) enhances beta cell function under glucolipotoxicity conditions

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Overview

Background: In type 2 diabetes, hyperglycemia in combination with high levels of free fatty acids, a condition known as glucolipotoxicity, affects the mass viability and function of beta cells. In plants, the phyto-hormone abscisic acid (ABA) prevents cell apoptosis and DNA degradation¹ whereas ABA in humans reportedly induces insulin secretion², reduces insulin resistance³, lowers fasting blood glucose and improves glucose tolerance⁴.

Objectives: Here, we hypothesized that ABA might have similar protective effects in mammalian beta cells under glucolipotoxic conditions. Thus, we have investigated the effects of ABA on the viability and function of mammalian beta cells treated with high levels of glucose and palmitic acid (PA).

Results: The abundance of key proteins involved in insulin signaling was modulated by ABA in the presence of high levels of glucose and PA. The expression of the transcription factors PDX1, RICTOR, and MAP4K4 was increased under treatment with ABA, while the expression of pathways downstream of ANGPT2 and of pirinixic acid was inhibited under these conditions. Interestingly, canonical signaling pathways such as those involving EIF2, PKA, and the NRF2-mediated Oxidative Stress Response were also clearly modulated by ABA.

Material & Methods

Cell culture and treatment:

The rat insulinoma INS-1 832/13 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin, 1mM Sodium pyruvate, 10mM HEPES and 0.05 mM 2-Mercabtoethanol at 37°C in a humidified atmosphere containing 95% air and 5% CO₂. When reached confluence, INS-1 cells were washed with PBS pH 7.2, treated with 0.05% trypsin for 4 min then suspended in fresh media. 2.5x10⁵ cells were seeded in 10 mm dishes supplied with normal media (11.1 mM glucose) for 48 hr for adhering then incubated in modified medium with and without (10nM ABA) under the following conditions:

- 16.7mM Glucose.
- 400 µM Palmitic acid.
- 16.7mM Glucose and 400 µM PA (Glucolipotoxicity).

PA was conjugated to bovine serum albumin FFA-free (BSA) (Millipore) at a 2:1 molar ratio. Control cells were incubated with media containing FFA-free BSA at the same concentration as exposed cells. Cells were maintained for 14 days with refreshing the media on days 4, 8, and 12 and finally harvested for protein extractions.

Cell viability using MTT assay:

Cell viability experiments were conducted in 24-well plates (1.0x10⁴ were seeded per well) using TACS MTT assay (Trevigen, Inc.).

Protein extraction and digestion for proteomics analysis:

Proteins were extracted from cells, fractionated and digested on SCX beads and then analyzed by LC- Orbitrap Velos platform and label-free quantified. Briefly, 3 independent repeats of cell cultures were pelleted and lysed in 200 µL of buffer (2M urea, 4% CHAPS) with a mini-complete protease inhibitor cocktail for 30 min at 4°C. 20 µg of proteins were added to 10 µL of strong cationic exchange (BcMag™ SCX Magnetic bead slurry, Bioclone) in 1ml of 5% formic acid, centrifuged at 10,000xg, the pellet was resuspended in 1ml of 0.5% formic acid and then, reduced with 20mM DTT and then alkylated with 100mM iodoacetamide. The alkylation reaction was stopped by adding 1ml of 0.5% formic acid. The beads were centrifuged and proteins were digested with Trypsin overnight at 37°C and then, eluted with 300 µL of Ammonia (pH 12).

NanoLC-MS/MS Analysis:

Peptide digest analysis was done using an Easy nano-LC (C18-A1 pre-column, and C18-A2 analytical column) coupled to an LTQ-Orbitrap Velos. Peptides were eluted with a gradient of 5 to 35% acetonitrile with 0.1% formic acid over 80 min. Full MS spectra scan was performed at a resolution of 60,000. MS/MS spectra were acquired in a DDA mode. Data were analyzed using Sequest and Mascot search engines. MaxQuant software was used for quantification of identified proteins and IPA software for pathways analysis.

References:

[1]. Fath et al. Plant J. 1999
[2]. Bruzzone et al. J Biol Chem. 2008
[3]. Guri et al. Clin Nutr. 2010
[4]. Guri et al. Clin Nutr. 2007

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Results

Figure 1: The influence of various treatment conditions on INS-1 cells growth w/wo 10nM ABA, 16.7mM Glucose, 400µM PA. Cells were seeded in 24-well plates at density 1.0x10⁴ per well for 48 hours in normal medium to adhere then subjected to treatment conditions for 14 days.

Figure 2: Effect of ABA on INS-1 proliferation. Cells were cultured in up normal conditions in the presence or absence of 16.7mM glucose, 400uM PA, 10nM ABA for 14 days (to reach 80% confluence in control plate).

Altered Regulator	Control		Glu		PA		Glu+PA	
	z-score	p-value	z-score	p-value	z-score	p-value	z-score	p-value
ANGPT2	-1.914	0.0391	NS		-1.287	0.000235	NS	
ATF4	-2.401	0.0402	-1.484	0.0393	NS		NS	
CNR1	-1.633	0.0314	-1.342	0.0291	NS		NS	
CREB1	-2.206	0.00219	NS		-1.091	0.0158	NS	
CST5	1.213	0.00000271	NS		NS		2.121	0.0108
E2f	NS		-1.972	0.0179	NS		NS	
E2F1	-1.135	8.46E-09	-2.178	0.000721	NS		1.914	0.00202
FOXO1	-2.598	0.0289	NS		NS		NS	
GATA6	2.236	0.151			NS		-1.6	0.0118
Ins1	-1.988	0.0215	-1.432	0.0299	-1.105	0.00223	NS	
INSR	-2.622	3.08E-10	1.234	0.00818			1.561	0.000151
Insulin	-1.886	0.118			NS		NS	
IRF4	1.414	0.00261	1.342	0.0323	NS		1	0.0209
KLF15	-2.232	0.000743	-1.616	0.0000146	NS		NS	
KLF3	NS		NS		NS		1.387	0.0000782
KLF5	-1.131	0.0117	NS		NS		NS	
MAP4K4	3.148	0.0000154	1.066	0.0000107	NS		-2.121	0.0000341
MEL T1	1.342	0.00000389	-2	0.0000346	NS		NS	
MYCN	-1.961	0.000571	2	0.0185	NS		NS	
NFE2L1	-2	0.00206	NS		NS		NS	
NFE2L2	-4.69	3.57E-09	NS		NS		NS	
NFKBIA	NS		NS		-1.213	0.0461	NS	
NRF1	-1.114	0.001	NS		NS		NS	
NUPR1	2.449	1	2.121	0.273	NS		NS	
PDX1	2.121	0.00000125	NS		1.091	0.0412	NS	
PGR	-2.897	0.000552	-1.622	0.0067	NS		NS	
PML	1.4	0.0161	1.414	0.00000308	NS		NS	
PPARG	-1.991	0.147	-1.748	0.00417	1.281	0.0000397	NS	
PTEN	-1.051	0.00364	NS		-1.7	0.0223	NS	
RBL1	1	0.00129	1.725	0.00026	NS		-1.929	0.00309
RBL2	1.961	0.0234	NS		NS		NS	
RICTOR	3.68	4.81E-18	1.06	1.96E-17	NS		NS	
SP1	NS		-1.19	0.00303	NS		-1.982	0.0476
SPP1	NS		NS		1.732	0.0429	1.941	0.463
SREBF1	-1.774	0.00721	-1.44	0.00309	NS		2	0.189
SREBF2	-2.366	0.00129	-1.284	0.000262	NS		-2.236	0.0163
TCF3	1.982	0.481			NS		NS	
TGFB1	NS		-1.795	0.000959	NS		NS	
TFEB	-1.997	0.00377	NS		-1.154	0.000801	NS	
TP53	NS		1.996	0.00000242			NS	
TP63	-2.588	0.0424	NS		NS		NS	
TRAP1	2.236	0.000195	-1.463	0.00000395	NS		NS	
TSC2	1.069	0.0098	-1.067	0.0479	NS		NS	
UCP1	-1.572	0.00585	1	0.0262	NS		NS	
VEGFA	-2.557	0.0137	-1.154	0.00517	NS		NS	
XBP1	-1.558	0.00000137	NS		NS		-1.011	0.000395

Conclusion

Specific pathways and proteins modulated by ABA and identified in the present study provide insight into the potential beneficial effects of ABA in protecting beta cells against glucolipotoxic conditions and might promote the use of ABA in the prevention and management of diabetes.

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