OUKA
Osaka University Knowledge Archive

Title	Chronic hyperglycemia reduces the expression of intercellular adhesion molecules and increases intercellular hyperpermeability in the periodontal epithelium
Author(s)	Narukawa, Yuki; Sugiyama, Naoyuki; Miura, Jiro et al.
Citation	Journal of Periodontal Research. 2023, 58(4), p. 813-826
Version Type	AM
URL	https://hdl.handle.net/11094/94622
rights	© 2023 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.
Note	

Osaka University Knowledge Archive : OUKA

https://ir.library.osaka-u.ac.jp/

Osaka University

SUPPLEMENTARY INFORMATION

Supplementary methods

ATP measurements

epi 4 cells were seeded in 96-well black plates (Costar, Corning Inc., NY, USA) and cultured in NG (5.5 mM) and HG (30 mM) D-glucose for two weeks. ATP concentration was determined using the CellTiter-Glo® 2.0 Assay (Promega) based on the luciferase reaction. Luminescence was measured using GloMax® 96 Microplate Luminometer (Promega) (Fig.S2).

Proteomic analysis

The mouse gingival tissues were frozen in liquid nitrogen and then crushed using a mortar and pestle under liquid nitrogen. The samples were further disrupted with a metal cone using Multi-beads shocker (MB400U, Yasui kikai) at 2,000 rpm for 10 sec (2 cycles). The disrupted samples were suspended in 450 mL of methanol, and then 250 mL of water and 500 mL of chloroform. After vortex mixing, the suspension was centrifuged at 4,600 *g* for 5 min, and then both of methanol/water and chloroform phases were removed to yield the intermediate precipitates. The precipitates were dried under vacuum, and then crude proteins extracted from the precipitates using the phase-transfer surfactant method were subjected to reductive alkylation, followed by successive digestion with Lys-C endopeptidase and trypsin as previously described. After

acidification with trifluoroacetic acid, the detergents were removed with ethyl acetate extraction. The peptide solution was desalted using a Stage tip equipped with an SDB-XC Empore disk membrane (3M). The desalted peptides were isotopically labeled with labeling kits (Thermo Fisher Scientific) using a nanoscale solid-phase labeling method and then fractionated with a strong cation exchange chromatography using a combined acid salt gradient method.

The fractionated peptides were analyzed using nanoLC/MS/MS, using an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific) in data-dependent acquisition (DDA) mode, coupled to Ultimate3000 RSLCnano system (CTC Analytics) and HTC-PAL autosampler (CTC). A homemade column containing ReproSil C18 materials (3 mm, Dr. Maisch) was packed into a self-pulled fused silica capillary (150 mm length × 100 mm I.D., 6 mm opening). Synchronous precursor selection (SPS) was employed with a higher-energy collisional dissociation (HCD) at 10 SPS precursors. The other settings for nanoLC/MS/MS were the same as the previously described.

Peak lists were generated from the raw DDA analysis data using ProteoWizard, on the basis of the recorded fragmentation spectra. Peptides were identified via automated database searching, using Mascot v. 2.7.0 (Matrix Science, London), against UniProtKB/ReferenceProteome (2017/11) of *Homo sapiens*. Mass tolerance of 10 ppm and 0.8 Da was applied for precursor and fragment ions, respectively. Trypsin was specified as the reference enzyme, and up to two missed

cleavages were allowed. Carbamidomethylation of cysteine was set as a fixed modification, and oxidation of methionine and TMT-labeling of amino groups at the peptide *N*-terminus and in lysine side chains were allowed as variable modifications.