SMALLER MITOCHONDRIAL NUCLEOIDS IN PANCREATIC β-CELLS OF DIABETIC GOTO KAKIZAKI RATS VISUALIZED BY BIPLANE FPALM

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Since the Goto Kakizaki rat Langerhans islets (LI) contain much less mitochondrial DNA (mtDNA) – its content is reduced down to ~25%, we attempted to distinguish between the two possible models on the level of mitochondrial nucleoids: i) decreased mtDNA would lead to less nucleoids; or ii) decreased mtDNA would cause a different nucleoids shape, size, etc. Nucleoids are loci of mitochondrial DNA, complexed by TFAM protein. They also contain mt single-stranded-DNA-binding protein, mtSSB, in the replication origins at D-loops of mtDNA or at active replication or transcription locations. We have imaged nucleoids using 3D BiplaneFPALM [1] after lentiviral overexpression of mtSSB conjugated to a photoconvertible fluorescent protein Eos in isolated LI from control Wistar rats as well as of Goto-Kakizaki diabetic rats.

We have found that diabetic (Goto-Kakizaki LI) beta cells contain smaller nucleoids of mtDNA when compared to nucleoids of Wistar rat control β-cells. When nucleoid images were fit by principal component analysis and normalized by the volume derived from the Delauney tetrahedron modeling, thus obtaining symmetrical ellipsoids, their size was analyzed as well as the space density. The space density fit to the similar range for LI of control Wistar rats as well the diabetic Goto-Kakizaki rats. The average ellipsoid nucleoid short diameter was $57 \pm 24$ nm for diabetic Goto-Kakizaki and $75 \pm 24$ nm for Wistar control rat pancreatic beta cells. The long ellipsoid diameter was $105 \pm 44$ nm vs. $127 \pm 67$ nm. When the sphere model was applied, the average diameter was $102 \pm 68$ nm in Goto-Kakizaki rat islet β-cells vs. $141 \pm 75$ nm. Note, that hepatocellular carcinoma HepG2 cell mitochondrial nucleoids have average ellipsoid dimensions of $44\times44\times133$ nm, when visualized by the identical method, i.e., by mtSSB-Eos contouring the space of unfolded DNA, including the third-strand structures, such as the D-loop.

We had to abandon a working hypothesis i) assuming that if 75% reduction of mitochondrial DNA copy number exists in β-cells of Goto-Kakizaki diabetic rats, then the number of nucleoids should be reduced by the identical proportion. We had to prefer the hypothesis ii) and conclude that the reduced mtDNA mass is contained in smaller objects (the nucleoids) in diabetic β-cells. Thus, spherical model volume of diabetic nucleoids was ~2.6-fold lower complying with a similar reduction of mtDNA. In conclusion, the significant contribution to the β-cell pathology in diabetic Goto-Kakizaki rats is exerted by the reduced mitochondrial DNA mass manifested as nucleoids of smaller size.

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