粒線體生合成調控對胰島β-細胞功能的影響

Effects of mitochondrial biogenesis regulation on pancreatic beta–cell function

中文摘要

爲了瞭解胰島β-細胞中粒線體生合成對胰島素分泌的機轉，本研究探討粒線體功能異常是否會影響β-細胞胰島素的分泌。本實驗利用大鼠胰島β-細胞，RIN-m5F 細胞，運用 RNA 干擾技術 (RNA interference，RNAi)，以短暫轉染(transient transfection)方式干擾粒線體轉錄因子 A mRNA (mitochondrial transcription factor A, TFAM)，以期降低粒線體 DNA 複製與轉錄，進而影響粒線體呼吸傳遞鏈功能，使 ATP 生成降低，影響胰島素分泌。經過兩次轉染，以同步定量聚合?鏈鎖反應 (real-time PCR) 進行分析，轉染後第 4 小時起，開始抑制 TFAM mRNA 的表現，最高可抑制 99 %，在第 48 ~ 72 小時，也觀察到 TFAM mRNA 下降 53.3 %，並影響粒線體轉錄之 ND6 mRNA（NADH-uibiquinone oxidoreductase subunit 6 , ND6）表現量下降，僅剩原本之 15 %；同時也觀察到粒線體 DNA 拷貝數的下降。粒線體轉譯之呼吸傳遞鏈複合體四中次單位 COX I (cytochrome c oxidase, subunit I)也顯著降低 27 %，可能導致粒線體呼吸傳遞鏈功能缺失，進而使胰島素分泌降低，可用於模擬第二型糖尿病人粒線體功能不全的情形。本研究利用 100 ng/mL 溴化乙錠 (ethidium bromide, EtBr)破壞 RIN-m5F 細胞粒線體 DNA 作爲另一研究模式，25 天後觀察到粒線體 DNA 拷貝數僅剩 0.13 %。以 EtBr 處理五個月後，明顯觀察到細胞因粒線體 DNA 被破壞後，生長速度趨於緩慢，進一步利用葡萄糖、白胺酸(Leucine)、精胺酸(Arginine)來刺激經 EtBr 處理的 RIN-m5F 細胞，ATP 的生成明顯降低，且胰島素分泌趨於減少，推測應爲粒線體功能異常，ATP 無法正常生成進而影響胰島素分泌。本研究顯示粒線體 DNA 套數減少及粒線體基因的表現受到抑制皆會導致粒線體功能異常進而影響β-細胞胰島素分泌情形。

英文摘要

To examine the impact of mitochondrial dysfunction on insulin secretion of pancreatic b–cell, multiple RIN-m5F cell clones with mitochondrial DNA (mtDNA) depletion by RNA interference (RNAi) or ethidium bromide (EtBr) treatment were created. The mitochondrial transcription factor A (TFAM) were presented to regulate mtDNA replication and transcription process. It could affect the ATP production from respiratory chain reaction, and insulin secretion in the pancreatic b–cell. In this study, related genes mRNA expression and mtDNA copy number were determined by real-time quantitative PCR analysis. The mRNA expression levels of TFAM and mtDNA-encoded NADH-uibiquinone oxidoreductase subunit 6 (ND6) were significantly decreased after transient transfection of siRNA 4 to 72 hours. TFAM gene expression was disrupted up to 99 % and decreased to 53.3 % of TFAM after twice siRNA transfection. Both of mtDNA copy number (10 % of control after 64 h transfection) and mtRNA expression (reduced 85 % of ND6 mRNA) were swayed by defective mitochondrial biogenesis. RIN-m5F cells characterized with slow growth rate, reduced nutrients (glucose, leucine, arginine)-induced insulin secretion and ATP synthesis after EtBr treatment over 5 months. We suggested that both mtDNA depletion and reduced mitochondrial genes expression may compromise mitochondrial function and disrupt insulin secretion by pancreatic b–cell.