粒線體內肝醣合成酶激酶 3beta 蛋白質複合體之蛋白質體研究

Proteomic analysis of mitochondrial proteins form complex with glycogen synthase kinase 3beta

中文摘要

肝醣合成酶激酶 3beta，被認為是調控細胞內糖類代謝的關鍵性激酶。近來在研究上發現到肝醣合成酶激酶 3beta 在細胞受到刺激時會進入粒線體中，而粒線體在細胞的能量代謝及生死調控扮演了無比重要的角色，但肝醣合成酶激酶 3beta 於粒線體中執行何種功能並未被研究闡明。由於粒線體中的蛋白質都以蛋白質複合體的形式存在，所以本研究假設肝醣合成酶激酶 3beta 於粒線體內亦是以蛋白質複合體的形式存在。所以實驗設計利用免疫沉澱法結合二維電泳分離粒線體中與肝醣合成酶激酶 3beta 交互作用的蛋白質，在二維電泳膠片上發現到 28 個蛋白質點；而後利用基質輔助雷射吸附飛行時間質譜儀分析鑑別這些蛋白質，經資料庫的分析比對後整理出 12 個蛋白質分子。4 個蛋白質是參與於代謝路徑及克氏循環中的酵素為 glyceraldehydes-3-phosphate dehydrogenase, isocitrate dehydrogenase, glutamine synthetase, 和 dihydrolipoyl dehydrogenase（pyruvate dehydrogenase subunit）4 個蛋白質點是電子傳遞鏈氧化磷酸化複合體 NADH-ubiquinone oxidoreductase MLRQ subunit (complex I)，ATP synthase-alpha, beta and O subunit (complex V)。而最後 4 個蛋白質為被報導過參與細胞生死調控與粒線體增生的蛋白 voltage-dependent anion-selective channel 1 (VDAC-1)，mitochondrial elongation factor Tu，cofilin 和 prohibitin；綜合以上的發現可以為繼續去探索肝醣合成酶激酶 3beta 調控細胞能量代謝與細胞凋亡的機制提供更多線索。

英文摘要

Glycogen synthanse kinase 3beta(GSK3-beta), a kinase considered to mediate glucose metabolism in cytosol of the cell, was found can be stimulated to translocate to mitochondria, but how GSK3-beta exerts its effects in mitochondria remain unclear. Mitochondria play a key role in regulating cell life. For exploring mitochondrial proteins which complex with GSK3-beta, we used immunoprecipitation, two-dimensional electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) to identify proteins spots of the 2D map. More than 31 protein spots were found co-precipitated with anti-GSK3-betaantibodies, 12 of which were identified by MALDI-TOF MS or MALDI-Q-TOF MS/MS. Among the 12 proteins, four proteins involved in metabolism and the Krebs cycle in mitochondria. They were identified as glyceraldehydes-3-phosphate dehydrogenase, isocitrate dehydrogenase 3 (NAD+)-3 , glutamine synthetase, and dihydrolipoyl

dehydrogenase. The other four proteins are involved in electron transfer complex I and V. They are NADH-ubiquinone oxidoreductase MLRQ subunit (complex I), ATP synthase-alpha, beta and O subunit (complex V). The last four proteins, voltage-dependent anion-selective channel 1 (VDAC-1), mitochondrial elongation factor Tu, cofilin, and prohibitin. These findings might provide clues for understanding the mechanism of how GSK3-beta to regulate the metabolism and apoptosis of the cell.