以蛋白質體學法分析短鏈脂肪酸代謝異常 ENU 突變鼠

Proteomic analysis of ENU mice with abnormal defect of short chain fatty acid

中文摘要

本研究目的爲利用二維電泳法 (Two-dimensional electrophoresis ; 2-DE) 以找出ENU (ethyl-nitrosourea)突變小鼠表現差異的蛋白質點並進行質譜分析，以瞭解突變小鼠短鏈脂肪酸代謝異常原因，並且希望能應用此一模式動物於脂肪酸的代謝研究及探討人類相關臨床疾病。我們利用 ENU 注射動物方法藉以誘發大量基因突變小鼠的產生，經由繁殖配對至第三代後，再以串聯式質譜儀篩得血漿短鏈脂肪酸 (C4-OH) 代謝異常突變小鼠。實驗小鼠 (C57BL/6J) 分爲正常小鼠及血漿C4-OH 含量較正常小鼠高 4 倍標準差的 ENU 突變小鼠，兩組小鼠分別犧牲後取其肝臟、肌肉組織，進行粒線體蛋白質萃取後以 SDS-PAGE 進行一維電泳，然而突變小鼠和正常小鼠粒線體蛋白質表現量並無差異。因此，更進一步利用二維電泳法進行突變小鼠和正常小鼠粒線體蛋白質表現的比較。研究結果顯示突變小鼠和正常小鼠的肌肉粒線體存有差異的蛋白質點，經由介質輔助雷射脫附游離-飛行式質譜儀 (Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry; MALDI-TOF) 鑑定出差異蛋白質點的身份。在突變小鼠表現量增加的蛋白質爲磷酸化的 myosin regulatory light chain 2、tropomysin 1 α chain、myosin light chain 1，表現量減少的蛋白質爲非磷酸化的 myosin regulatory light chain 2、calsequestrin-1 precursor、adenylate kinase isoenzyme 1、ATP synthase D chain。然而這些蛋白質表現的消長是否與突變鼠體內 C4-OH 含量上升有關，仍有待更進一步的研究加以驗證。關鍵詞：致突變劑 (ethyl-nitrosourea; ENU)、串聯質譜儀、短鏈脂肪酸、二維電泳法(Two-dimensional electrophoresis ; 2-DE)、介質輔助雷射脫附游離-飛行式質譜儀(Matrix-assisted laser desorption / ionization time-of-flight mass Spectrometry; MALDI-TOF)

英文摘要

The purpose of this study was to develop a 2-DE coupling with MALDI-TOF analysis to find the differential proteins in ENU mouse which could explore the cause of genetic defect of short chain fatty acid in mice. We hope that the C4-OH animal model can provide us to investigate fatty acid metabolism related to human genetic disease. We generated ENU- mutagenized mouse and applied to MS/MS tandem mass spectrometry to screen third generation progeny of ENU-mutagenized mouse for abnormalities in the pathway of fatty acid metabolism. The short chain fatty acid (C4-OH) level in ENU mice was three to four folds than normal mice. After protein evaluation, there was no differential protein in mitochondria fraction between normal

and ENU mice by SDS-PAGE. Surprisingly, there were eleven differential proteins between normal and ENU mice in muscle in 2-DE approach. These protein spots were applied to MALDI-TOF for futher identification. In results, ENU mouse showed higher protein expressions in phosphorylated myosin regulatory light chain 2, myosin light chain 1 and tropomyosin 1αchain and lower expressions in myosin regulatory light chain 2, adenylate kinase isoenzyme, ATP synthase D chain and calsequestrin-1 precursor in ENU mouse to normal control. These differential proteins expression whether involve to increase C4-OH value in mice needs further study to verify.
Key words：Two-dimensional electrophoresis, ethyl-nitrosourea, C4-OH, MS/MS tandem mass, MALDI-TOF