粒線體 DNA 突變及氧化性傷害與唐氏症病理相關性之研究

中文摘要

唐氏症（Down’s syndrome）是細胞遺傳學最常發生的染色體變異(chromosomal aberration)。主要變異的形式爲第 21 對染色體多出一條；即爲 trisomy 21。目前對於此疾病無論是臨床症狀、細胞遺傳學或分子遺傳學已有很多相關的研究，但是對於染色體爲何在生殖細胞有絲分裂（mitosis）或減數分裂（meiosis）階段容易形成 trisomy 的原因迄今仍未明瞭。最近有研究報告指出氧化性壓力（oxidative stress）可能與 trisomy 的形成有關，並進而在其細胞內產生氧化壓力。而粒線體是細胞內產生自由基的主要胞器，我們推論在唐氏症患者的細胞內其粒線體功能缺陷，可能與唐氏症病患的病程有關。本研究我們針對唐氏症病患羊水細胞內粒線體 DNA (mtDNA) 的拷貝數目（copy number）、粒線體 DNA 斷損突變（mtDNA deletion）進行研究分析，並進而探討其與氧化性傷害的相關性。我們以 PCR 方法檢測粒線體 DNA 發生的斷損突變，發現於病患羊水細胞內常有 5335 bp deletion。並以 real-time PCR 測定粒線體 DNA 拷貝數。以流式細胞儀測定羊水細胞脂質過氧化物（lipid peroxide）含量，並以螢光染劑 C11-BODIPY lipid probes 檢測過氧化物對唐氏症病患羊水細胞傷害的程度。由實驗結果顯示唐氏症病患羊水細胞粒線體 DNA 拷貝數較正常人羊水細胞粒線體 DNA 拷貝數減少至 0.9 倍，此外也發現以 primer pair L8251-H13845 檢測細胞內有 5335 bp 粒線體 DNA 斷損突變，經 DNA 序列分析其斷點爲核苷酸序列 8273-13607，在 DNA 序列 8263-8272 及 13598-13607 具有”CCTATAGCAC”的 10 個 nucleotide 長的 direct repeat。粒線體的數目減少與粒線體 DNA 斷損突變是否影響粒線體功能進而促成 trisomy cell bio-aging 的形成需進一步探討。病患的羊水細胞中氧化傷害的指標物質脂質過氧化物（lipid peroxides）及 8-OHdG 則較正常人羊水細胞高。我們推測唐氏症病患羊水細胞有較高的 oxidative stress 導致 mtDNA 受損及 copy number 減少。

英文摘要

Down’s syndrome is the most common disease of chromosomal aberration on cytogenetics. The major form of chromosomal aberration is trisomy 21, an extra chromosome of chromosome 21. There are many studies in Down’s syndrome including clinical pathology, cytogenetics and molecular genetics. It is distinct why the germ cells are susceptible to trisomy formation at meiosis or mitosis stage. Recently, some researches reported that oxidative stress might play some roles in the formation of trisomy. Mitochondria are the major organelles that produce reactive oxygen species. In this study, we hypothesized the mitochondrial dysfunction might be contributed to the pathogenesis of Down’s syndrome. We investigated on

mitochondria DNA (mtDNA) copy number, mtDNA deletion and oxidative damages in amniotic cells of Down's syndrome patient. Decreased copy numbers of mtDNA were found in the patients of Down's syndrome. Moreover, a novel 5335 bp mtDNA deletion was identified in amniotic cells from Down's syndrome patient. Analysis of nucleotide sequences flanking the breakpoints of this deletion revealed a 10 nucleotides direct repeat "CCTATAGCAC" flanking the junction site of the 5335 bp deletion at nucleotide position 8263-8272 and 13598-13607. Increased ROS generation and oxidative damages were revealed in Down's amniotic cells. Taken these data together, we suggested that the dysfunctional mitochondria might play a role in the pathogenesis of Down's syndrome.