MnSOD、Catalase、UCP2、hOGG1、COMT 和 GSTM1 基因多型性與子宮內膜異位症相關性之研究

MnSOD, Catalase, UCP2, hOGG1, COMT and GSTM1 Polymorphisms in Endometriosis

中文摘要

子宮內膜異位症(Endometriosis)是指原應生長在子宮內膜的組織轉而生長在身體的其他部位，到目前爲止其真正的致病機轉並未清楚明瞭。近來許多研究證實基因的變異和氧化壓力都與子宮內膜異位症的發展形成相關。然而體內約85-90%活性氧化物質（reactive oxygen species, ROS）主要來源是經由粒線體的電子傳遞鏈產生。隨著氧化壓力(oxidative stress)的增加，粒線體內的抗氧化機制對於保護組織細胞避免產生氧化性傷害有著非常重要的角色。因氧化壓力的產生與粒線體抗氧化功能缺損及粒線體基因完整性(mitochondrial genome integrity)有關。本研究收集了 122 個不孕婦女檢體(其中 67 位來自病人的血液檢體以及 55 位病人中取得的子宮內膜組織樣本)及 48 個正常生育婦女血液檢體，我們將檢體分爲三組，分別爲 endometriotic group(group A)、all female factor infertile group(group B)及 normal fertile control(group C)組。探討 manganese superoxide dismutase （MnSOD）、catalase（CAT）、uncoupling protein 2 (UCP2)、human 8-oxoguanine glycosylase 1（hOGG1）、catechol-O-methyltransferase（COMT）與 glutathione S-transferase M1（GSTM1）的基因多型性及 mitochondrial DNA（mtDNA）T8993G 突變與子宮內膜異位症的相關性，並分析這些基因的基因多型性(gene polymorphism)與女性不孕的相關性。研究利用聚合?連鎖反應（polymerase chain reaction, PCR）、Restriction Fragment Length Polymorphism（RFLP）的方法確認基因型態，並以 HPLC-ECD 分析 8-OHdG 含量及 Long-PCR 分析 mtDNA 斷損突變(4977 bp and 5756 bp)，並將結果以卡方檢定運算其是否具有統計上的顯著性差異。結果顯示 group A 和 group B 與 group C 比較後有高比率的 MnSOD CT+TT type (分別爲 25.1%, 33.6%及 16.7%) (p=0.02, group A vs. group C; p=0.029, group B vs. group C)。並且發現 catalase TT type 僅存於 group A (p=0.029)。此外分析比較 group A 和 group B 與 group C 其 hOGG1 的基因多型性有低比率 CC type (分別爲 15.7% ,15.6%及 31.3%) (p=0.034, group A vs. group C ; p=0.021, group B vs. group C)，且帶有 G allele 的組織其對 8-OHdG 修復能力較 C allele 差，我們進而分析罹患子宮內膜異位患者的病灶處有大量 8-OHdG，並帶有 CC 基因型相較於 CG+GG 基因型的 8-OHdG 含量(分別爲 0.39±0.11 及 0.76±0.24 8-OHdG/105 dG)有顯著差異(p=0.01)。在本研究中並無發現到 UCP2、COMTLL 及 GSTM1 null type 基因型在子宮內膜異位患者中有顯著差異，且也無發現有任何 mtDNA T8993G 突變。綜合以上結果，我們研究發現 hOGG1、

MnSOD、catalase 與子宮內膜異位症有相關性，由此可推論粒線體內的氧化壓力與子宮內膜異位症及女性因素的不孕症確實有關。

英文摘要

Endometriosis is characterized by the implantation and growth of endometrial tissue outside the uterine cavity. The aetiological factor of endometriosis is still remain poorly understood. Somatic genetic alterations have been found in endometriotic lesions and these may contribute to initiation and progression of endometriosis. Approximately 85～90% of reactive oxygen species (ROS) generation was proposed during the mitochondrial electron transport chain. The intramitochondrial antioxidant enzymes play the pivotal role on the maintenance of mitochondrial genome integrity and intracellular normal function. In this study, we collected 122 infertile women and 48 normal fertile women. Women were divided into three groups: endometriosis (group A, n = 89), infertility patients (group B, n = 122) and normal control (group C, n = 48). The aim of this study was to test whether the manganese superoxide dismutase (MnSOD), catalase (CAT), uncoupling protein 2 (UCP), human 8-oxoguanine glycosylase 1 (hOGG1), catechol-O-methyltransferase (COMT), glutathione S-transferase M1 (GSTM1) polymorphisms and mtDNA T8993G mutation contributed to the risk of endometriosis pathogenesis. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were performed to analyze the genotype and genotype frequencies in the three groups and made statistic analysis by using chi-square test. In addition, we measured the content of 8-OHdG by HPLC-ECD detection and the occurrences of mtDNA deletions (4977 bp and 5756 bp) by long-PCR analysis. The frequency of the MnSOD CC+CT type was significantly higher in group A (25.1%:16.7%, p=0.02) and group B (33.6%:16.7%, p=0.029) compared with group C (16.7%). The proportion of catalase TT type was found higher in group A (25.1%:0%, p=0.029) compared group C (0%). Furthermore, the lower incidence of the hOGG1 CC genotype was observed in the patients with group A (15.7%:31.3%, p=0.034) and group B (15.6%:31.3%, p=0.021) compared with group C (31.3%). In addition, the hOGG1 CC genotype endometriotic tissues have lower 8-OHdG production than CG with GG genotype group (respectively 0.39±0.11 and 0.76±0.24 8-OHdG/105 dG, p=0.01). We supposed the C allele exhibited substantially the higher DNA repair activity than the CG variant and the G allele. No significant difference was found that the frequencies of UCP2, COMTLL, GSTM1 null type in cases and controls. Similarly, no association was observed in mtDNA T8993G mutation of endometriosis. In summary, hOGG1, MnSOD and CAT polymorphisms may play an important role in the risk for endometriosis. Our results suggest that the mitochondrial oxidative stress was related

to the susceptibility of endometriosis and female infertility.