P53、P21 和 CCND1 基因多形性、8-OHdG 及砷甲基化能力與泌尿道上皮癌之相關

The Relationship among Genetic Polymorphisms of P53, P21 and CCND1, 8-OHdG, Arsenic Methylation Capability and Urothelial Carcinoma.

中文摘要

本病例對照研究為探討調控細胞週期 G1/S 關卡點之 p53、p21 和 CCND1 基因多形性及砷甲基化能力、抽菸、氧化傷害產物 8-hydroxydeoxyguanine（8-OHdG）與泌尿道上皮癌之相關性。研究對象為臺灣大學附設醫院泌尿科門診之泌尿道上皮癌患者 172 位，而對照組為臺北醫學大學附設醫院及台北市立萬芳醫院參與健康檢查之民眾，排除罹患癌症者且與病例之年齡及性別分層頻率配對共 405 位。在研究對象了解本研究目的並取得其同意書後以標準化問卷訪視研究個案，收集基本人口學資料、生活習慣、職業暴露及個人與家族疾病史之外，亦收集其血液和尿液檢體。利用高效能液相層析儀串聯氫化式原子吸收光譜儀（HPLC/HG?AAS）測定尿液中三價砷、五價砷、單甲基砷酸及雙甲基砷酸濃度。以競爭型酵素連結免疫吸附分析（enzyme-linked immunosorbent assay; ELISA）試劑測定尿液中 8-OHdG 含量。由白血球萃取之 DNA，利用聚合?連鎖反應（polymorphism chain reaction; PCR）增幅所需特定序列及限制片段長度多形性（restriction fragment length polymorphism; RFLP）方法分析 p53、p21 和 CCND1 之基因型。結果發現病例組單甲基砷酸、雙甲基砷酸、總砷濃度和單甲基砷酸百分比顯著較對照組高，而雙甲基砷酸百分比及二級甲基化指標則是顯著低於對照組。調整潛在干擾因子之後，攜帶 p21 codon 31 Arg/Arg 基因型者，罹患泌尿道上皮癌的危險性是攜帶 Ser/Ser 和 Ser/Arg 基因型者的 1.59 倍（95%信賴區間 1.00 - 2.53）。p53 codon 72 和 CCND1 G870A 基因多形性與泌尿道上皮癌無關。病例組尿液中 8-OHdG 平均含量顯著高於對照組。隨著雙甲基砷酸及總砷的濃度愈高，尿液 8-OHdG 含量也會顯著增加。以沒有暴露任何一個危險因子為基準，抽菸、砷甲基化能力較差且攜帶 p21 codon 31 Arg/Arg 基因型和尿液中 8-OHdG 含量較高者，罹患泌尿道上皮癌之危險性最高並呈現顯著的劑量效應關係。

英文摘要

The case-control study was conducted to explore the relationships among genetic polymorphisms of p53, p21 and CCND1, arsenic methylation capability, cigarette smoking, oxidative damage 8-OHdG levels and urothelial carcinoma (UC). In total, 172 pathologically proven UC patients were recruited at the Department of Urology, National Taiwan University Hospital between September 2002 and December 2005.

405 age-gender frequency matched control subjects without any sign or evidence of UC were collected from a hospital-based pool including those who received senior citizen health examination at Taipei Medical University Hospital and those who received adult health examination at Taipei Municipal WanFang Hospital. Well-trained personnel carried out standardized personal interviews based on a structured questionnaire after subscribing informed consent. Information obtained included sociodemographic characteristics, lifestyle (consumption habits of alcohol and other beverages, and cigarette smoking), occupational and environmental exposure to possible carcinogens, and personal and familial disease history. Peripheral blood and urine samples were collected simultaneously. Urine samples of these subjects were examined by high-performance liquid chromatography to separate arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA), and dimethyarsinic acid (DMA) and then quantified by hydride generator combined with atomic absorption spectrometry. The 8-OHdG amounts of urine samples were determined by competitive enzyme-linked immunosorbent assays (ELISA) method with an ELISA kit. DNA was extracted from buffy coat to analyze the gene variants in the p53, p21 and CCND1 polymorphism utilizing the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assay. Overall, we found UC cases had a significantly higher MMA, DMA, total arsenic level and MMA percentage than controls. The DMA percentage and secondary methylation capability index were lower in cases than in controls. The odds ratio of p21 codon 31 Arg/Arg genotype versus Ser/Ser and Ser/Arg genotype was 1.59 (95% confidence interval, 1.00 - 2.53) after adjusting other potential confounders. There were no associations among p53 codon 72, CCND1 polymorphisms and UC. The mean urinary concentration of 8-OHdG was significantly greater for cases compared with controls. Amounts of urinary 8-OHdG were significantly increased with MMA and total arsenic level. Study subjects who did not expose risk factors as a reference group, UC risk was highest and presented a significant dose-response relationship when the study subjects have cigarette smoking, worse arsenic methylation capability, p21 codon 31 Arg/Arg genotype and higher urinary 8-OHdG levels.