

中文摘要

目前研究發現，人類 *hMYH* 雙等位基因突變(biallelic germline mutations, G : C→T : A)與大腸直腸癌及其它癌症的產生有著密切的關係。*hMYH* 為腺嘌呤糖基解酶，它是一種基因修復蛋白。本研究是以 allele-specific polymerase chain reaction (AS-PCR)方法來進行 *hMYH* 基因中單核苷酸多態性(single nucleotide polymorphism, 簡稱 SNP)分析。一開始先以質體 DNA(含 *hMYH* 基因)作為模板，建立 AS-PCR 的反應條件，並且發現適當地調整 DNA 模板濃度、引子接合溫度或改變 dNTP 混合比例，將可增加 AS-PCR 檢測 *hMYH* 基因中 SNP 的可信度。此外，我們也證明了在對偶特異性引子的 3' 末端倒數第二位置增加鹼基錯配，可提升 AS-PCR 方法的特異性。最後對 30 位已被台中榮民總醫院診斷患有大腸直腸癌的患者，篩選 *hMYH* 基因上的突變熱點 G382D、Y165C 及 V232F。雖然沒有患者的 *hMYH* 基因被檢測出有突變熱點存在，但此實驗結果與先前由韓國科學家所報導之結果相似。在大腸直腸癌病理學上，本實驗結果暗示 *hMYH* 突變發生率會隨著種族的差異而有所不同。

關鍵字：對偶特異性聚合酶連鎖反應(AS-PCR)，*hMYH* 基因，單核苷酸多態性(SNP)、大腸直腸癌(colorectal cancer)

Abstract

Biallelic germline mutations in *hMYH*, a gene for adenine DNA glycosylase, have been reported to be associated with colorectal cancer. In this study, we have modified the allele-specific polymerase chain reaction (AS-PCR) for single nucleotide polymorphism (SNP) genotyping. In order to make the AS-PCR more practical and to assess its ability in distinguishing the SNPs of *hMYH*, we employed the plasmid DNA containing *hMYH* as the DNA template to establish the AS-PCR conditions, and we found that the reliability of AS-PCR might be improved by optimizing the concentration of DNA template, the annealing temperature, as well as the ratio of dNTP. In addition, we demonstrated that the incorporation of an additional mismatch at the penultimate position near the 3' of allele specific primer could further enhance the specificity of AS-PCR. Finally, we screened for the mutations in *hMYH*, such as G382D, Y165C and V232F, from the genome of 30 Taiwanese patients who had been diagnosed as colorectal cancer by the Veterans General Hospital at Taichung. However, none of patients were detected with these reported mutations in *hMYH*, in which was similar to that reported for Korean population. Our results further indicated that the frequencies of *hMYH* mutations depended on the pathology of colorectal cancer and might also vary among different ethnic groups.

Keywords: allele-specific polymerase chain reaction (AS-PCR), *hMYH* gene, single nucleotide polymorphism (SNP), colorectal cancer