行政院國家科學委員會專題研究計畫成果報告

大腸直腸癌之分子遺傳學研究

Molecular genetic study of colorectal cancer

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一、中文摘要

大腸直腸癌近年來皆為為臺灣地區十大癌症死因之一。近來的研究已知有兩種大腸直腸癌與家族的遺傳有關：一是家族性多發性息肉症(FAP)，其乃導因於 *APC* 基因的突變 另一則是遺傳性非息肉性大腸直腸癌(HNPCC)，其則主要是 DNA 錯誤配對修復因基：*hMSH2*、*hMLH1*、*hPMS1*、*hPMS2*和 *hMSH6* 之一發生突變；約有 95% HNPCC 腫瘤可發現微小衛星體不穩定(microsatellite instability)亦稱為複製錯誤(replication error, RER)的現象。腫瘤的發展過程中，某些基因發生雜接合子漏失(loss of heterozygosity, LOH)以及複製錯誤是很重要的現象，其與臨床之診斷和預後亦或有相關。因此，本研究於大腸直腸癌腫瘤中，針對其相關的基因：*APC*、*hMSH2*、*hMLH1*、*DCC*，以及抑癌基因：*P53*、*NM23*和致癌基因 *MET* 等進行 LOH 和 RER 分析，並探討此些基因變化與臨床病理特徵、腫瘤轉移及預後之相關性，進而了解其在臨床上的應用性。

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Abstract

Colorectal cancer is one of the most common malignancies in Taiwan. Recent advances in genetics have more clearly defined the impact of inheritance in the multistep process of the colorectal cancer. Researchers have identified single genes that confer a susceptibility to hereditary colorectal cancer: familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC). FAP is caused by mutation of the adenomatous polyposis coli (*APC*) gene. HNPCC is caused by mutation of one of several DNA mismatch repair genes including *hMSH2*, *hMLH1, hPMS1, hPMS2* and *hMSH6*. Additionally, microsatellite instability, also referred to as replication error (RER), is observed in most cancers from HNPCC patients. Loss of heterozygosity (LOH) and RER are important phenomena in tumor development, with diagnostic and prognostic relevance. Therefore, screening for LOH and RER is a desirable first step in the molecular analysis of tumors. This study was conducted to determine LOH and RER with a set of 10 microsatellite loci linked to *APC*, *hMSH2, hMLH1, DCC, P53, NM23, HPC1* and *MET* genes as well as tumor suppressor gene(s) on 8p22 in colorectal tumors and to ascertain the significance of the genetic alteration at specific loci in the clinical pathology, chronological progression and prognosis of colorectal cancer.

Keywords: colorectal cancer, replication error, loss of heterozygosity

一、緣由與目的

Colorectal carcinogenesis is a multistep process in which the majority of carcinomas arise from adenomatous polyps through many consecutive steps. This involves the accumulation of genetic alterations: the mutational activation of oncogenes (e.g. *K-ras*) and the mutational inactivation of tumor

suppressor genes (e.g. *p53*) [1,2]. About 80% of patients with colorectal cancer have sporadic disease, with no evidence of having inherited the disorder. In the other 20% of patients, there appears to be a genetic link. There are two well known inherited colorectal cancer: familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) [3]. The recent identification of specific genes associated with a predisposition to colorectal cancer has provided new insight, not only regarding inherited colorectal cancer, but also about the underlying mechanisms of sporadic tumor formation.

Microsatellite analysis is emerging as an important tool in the study of cancer. The addition of novel microsatellite alleles is referred to as replication error (RER) or microsatellite instability, indicating possible mutations in the cell's DNA repair mechanisms. Another phenotype, exhibited by chromosome deletions, often referred to as allelic loss or loss of heterozygosity (LOH), is commonly believed to represent the second genetic inactivation step consistent with the "two-hit" theory of carcinogenesis. The detection of either of these genetic changes demonstrates the presence of a clonal population of cells that share altered genetic information, which is characteristic of cancer cells.

The presence of RER has been shown in colorectal cancers, notably HNPCC and sporadic tumors in other tissues such as bladder and stomach [4,5]. Studying RER and LOH phenomena in tumor vs. normal tissues will help to elucidate which types of cancer exhibit these phenotypes and their possible relevance for future use as a diagnostic tool. Tumors of the proximal colon, which display RER, have been reported to have a better clinical prognosis [6]. Studies to date have also indicated that LOH of microsatellite markers near specific loci in colorectal cancer can have some prognostic significance [7]. Research in these areas is ongoing. The main aim of this study was to determine the incidence of LOH and RER at specific loci, its associations, and significance in the chronological course of tumor progression in colorectal cancer.

三、結果與討論

Thirty-nine patients with sporadic colorectal cancer were accumulated. The stored frozen tissues were retrieved for simultaneous analyses of RER and LOH at 10 distinct chromosomal loci via an automated fluorescent microsatellite assay. RER was observed in 8/39 (20.5%) of the cases at one or more chromosomal loci. Five of RER^+ tumors showed LOH at one to three microsatellite loci linked to *APC*, *hMSH2, hMLH1, P53, NM23* and *HPC1* genes as well as tumor suppressor gene(s) on 8p22. RER^+ phenotype was associated with LOH at the *hMLH1* gene (P = 0.035). On the other hand, LOH was detected at all microsatellite loci studied, with the exception of that linked to *MET* oncogene. More than one third of informative tumors showed LOH at *P53*, *DCC* and *APC* genes with the frequency 57.9%, 35.3% and 33.3%, respectively. LOH at *hMSH2* and *hMLH1* genes was present in 12.1% and 9.1% of informative tumors. Of 22 informative tumors, six showed LOH at marker D8S254 that was suspected near one or more tumor suppressor genes. The frequencies of LOH at *NM23* and *HPC1* genes were 18.5% and 7.4%, respectively.

四、計畫成果自評

We have established the automated procedures of LOH and RER assay system via ABI 377 automated sequencer as well as GeneScan and Genotyper software. The majority of sporadic colorectal cancer studied showed RER and/or LOH at the microsatellite loci linked to *APC*, *hMSH2, hMLH1, DCC, P53, NM23* and *HPC1* genes as well as tumor suppressor gene(s) on 8p22. The significance of the genetic alterations at specific loci in the clinical pathology, chronological progression, tumor recurrence and prognosis of colorectal cancer is being ascertained.

五、參考文獻

1. Fearon ER and Vogelstein B (1990): A genetic model for colorectal tumorigenesis. Cell 61:759-767.
2. Cho KR and Vogelstein B (1992): Genetic alterations in the adenoma-carcinoma sequence. Cancer 70:1727-1731.
3. Giardiello FM (1995): Gastrointestinal polyposis syndromes and hereditary nonpolyposis colorectal cancer. In: Rustgi AK ed. Gastrointestinal Cancers: Biology, Diagnosis, and Therapy. Philadelphia, Pa: Lippincott-Raven.
4. Peltomaki P, Lothe RA, Aaltonen LA et al. (1993): Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. Cancer Res 53:5853-5855.
5. Aaltonen LA, Peltomaki P, Leach FS et al. (1993): Clues to the pathogenesis of familial colorectal cancer. Science 260:812-816.
6. Thibodeau SN, Bren G, Schaid D et al. (1993): Microsatellite instability in cancer of the proximal colon. Science 260:816-819.
7. Jen J, Kim H, Piantadosi S et al. (1994): Allelic loss of chromosome 18q and prognosis in colorectal cancer. N Engl J Med 331:213-221.