行政院國家科學委員會專題研究計畫成果報告

不同胃癌突變表現型的基因變化特徵(3/3)

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中 文 摘 要

由微衛星體不穩定性(MSI)和微衛星體有關的突變來判定腫瘤的突變表現型，在癌症的發生扮演重要角色。目前已知有部份的胃癌具有突變表現型，為了更進一步了解此變化在胃癌的意義，吾人依據 10 個微衛星體標記變化的情形將 100 個散發性胃癌患者分成高頻率微衛星體不穩定性(MSI-H)、低頻率微衛星體不穩定性(MSI-L)和微衛星體穩定性(MSS)三種表型，進一步分析 TGFβ RII, IGFIIR, BAX, MSH3, MSH6, E2F4, MSH2, MLH1 和 p53 等基因突變和 MLH1/MSH2 基因，甲基化及蛋白表現的情形，結果發現 27%的胃癌至少有一個微衛星體不穩定性，其中 14%為 MSI-H,13%為 MSI-L。比較 MSI-H, MSI-L 和 MSS 的臨床病理特徵發現 MSI-L 和 MSS 並無差別,但 MSI-H 則有較高頻率為竇部、腸道型、幽門螺旋桿菌血清陽性和較低比率的淋巴結轉移。在相關基因變化方面,MSI-H 尚具有較高比率的 TGFρ RII, IGFIIR, BAX, MSH3 和 E2F4 等基因的移位突變，以及較低比率的 p53 突變。此外，14 位 MSI-H 中有 13 位有 MLH1 基因啟動子的高度甲基化及蛋白質表現減少的情形。吾人初步結論為 MSI-H 胃癌有特殊的表現型及基因變化，其中 MLH1 基因的甲基化在此種腫瘤的癌化過程中扮演重要角色。相對於 MSI-H, MSI-L 及 MSS 則有不同的臨床病理特徵和較高頻率的 p53 突變,意謂在胃癌發生過程中可能有不同的致病機轉。

利用比較基因雜交法分析 53 例胃癌檢體其 DNA 倍數體異常(copy number aberrations – CNAs)和 p53 突變及 MSI 間的關係,結果發現每例腺癌 CNAs 為 6.8 (gain 5.3 loss 1.5)，而且其變化在進行性胃癌、具 p53 突變和 MSS 者有意義的高於早期胃癌(7.7 vs. 3.0)、無 p53 突變 (12.4 vs. 4.8)、和 MSI-H 者 (8.2 vs. 2.6)。其中較常見的染色體異常包括 8q (43%)、6q (26%)、11q (26%)、7p (23%)、17q (23%)和 20q (23%)的 gain，以及 16q (26%)、19p (23%)、5q (19%)、3p (15%)、4q (15%)和 1p (15%)的 loss，進一步分析這些變化顯示在不同期別、不同組織型態和不同腫瘤位置各有不同的特殊染色體變化。

由於最近在大腸癌發現所謂突變表現型和甲基化表現型(methylator phenotype)和 methyltransferase 及 demethylase 變化有關，因此吾人以 real-time PCR 分析 DNA methyltransferase(DNMT)及 demethylase(MBD)在胃癌腫瘤和非腫瘤部組織的 mRNA 變化情形，結果發現 DNMT1 及 MBD 在腫瘤部位明顯高於非腫瘤部位，但是 DNMT3A 和 DNMT3B 則沒有明顯差別。

關鍵語: 胃癌，突變表現型，甲基化表現型，比較基因雜交法

Abstract

Mutator phenotype judged by microsatellite instability(MSI) and its associated mutations plays an important role in gastric carcinogenesis. A subset of sporadic gastric cancers (GC) exhibits MSI. To define the precise role of MSI in GC, a total of 100 patients with sporadic GC were classified into three groups, i.e., high-frequency MSI (MSI-H), low-frequency MSI (MSI-L) and microsatellite stable (MSS) based on ten microsatellite markers. Mutational analyses of TGFâRII, IGFIIR, BAX, MSH3, MSH6, E2F4, MSH2, MLH1 and TP53 genes, and methylation and protein expression of MLH1 and MSH2 were performed and correlated. Twenty-seven percent of GC showed MSI at least in one locus and could be further graded as MSI-H (14%) and MSI-L (13%). No clinicopathologic difference was noted between GC with MSI-L and MSS. Compared with GC with MSI-L or MSS, GC with MSI-H had a significantly higher frequency of antral location, intestinal subtype, H. pylori seropositivity, but a lower incidence of lymph node metastasis, and displayed a higher frequency of frameshift mutations of TGFâRII, IGFIIR, BAX, MSH3, and E2F4 genes but a lower incidence of TP53 mutations. Furthermore, hypermethylation of MLH1 promoter was responsible for the loss of protein function in 13 of 14 MSI-H tumors. It was concluded that a specific phenotype and a distinct profile of genetic alterations exist in MSI-H GC. We speculate that epigenetic inactivation of MLH1 by methylation plays a crucial role in initiating such a pathway of carcinogenesis. In contrast, GCs with MSS and MSI-L exhibit clinicopathologic features that are distinct from MSI-H tumors and have a higher frequency of TP53 mutations, suggesting that they may evolve through entirely different pathway.

DNA copy number abnormalities(CNAs) were assessed in 53 GC using comparative genomic hybridization (CGH) and correlated with clinicopathogical characteristics and status of TP53 and microsatellite instability (MSI). The number of CNAs per tumor was 6.8 (gain 5.3 loss 1.5) and the number of changes was higher in tumors with advanced stage, Tp53 mutations and without MSI than in those with early stage (7.7 vs. 3.0), no TP53 mutations (12.4 vs. 4.8) or MSI phenotype (8.2 vs. 2.6). Frequent abnormalities included gasins on chromosomal arms 8q (43%), 6q (26%),

11q (26%), 13q (24%), 7p (23%), 17q (23%), and 20q (23%) and losees on chromosomal arms 16q (26%), 19p (23%), 5q (19%), 3p (15%), 4q (15%), and 1p (15%). Advanced GC demonstrated a higher prevalence of gains of 8q (51% vs. 10%, p< 0.05) and loss of 16q (33% vs. 0%, p<0.05) than early GC. Gains n 8q (64% vs. 20%, p<0.05), 17q (39% vs. 4%, p<0.05) and losses oon 3p (25% vs. 4%, p=0.05) and 5q *32% vs. 4%, p<0.05) were higher in intestinal GC than in diffuse GC. On theother hand, gains on 13q were more common in the diffuse type (40% vs. 11%, p<0.05). As compared with noncardia cancer, cardia cancer showed more gains on 7p (58% vs. 12%, p<0.05) and 20q (58% vs. 12%, p<0.05) and more losses on 4q (50% vs. 5%, p<0.05). The finding of histology-related aberrations and the combination of CGH and molecular data thus provide aditional evidence suggesting genetic heterogeneity of GC.

Recently, CpG island methylator phenotype and mutator phenotype in colorectal cancer have been ascribed to alterations of methyltransferase and demethylase. We analyzed expression of DNA methyltransferan (DNMT) and demethylase (MBD) by real-time PCR in tumorous and nontumortumorous portion of GC. Our results revealed overexpression of DNMT1 and MBD2, rather than DNMT3A and DNMT3B, was associated with GC.

Key Words: Gastric cancer, mutator phenotype, methylator phenotype, comparative genomic hybridization.

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