行政院國家科學委員會專題研究計畫成果報告

乳癌 *hMSH2* 和 *hMLH1* 基因突變之研究

Mutations of *hMSH2* and *hMLH1* in Breast Cancer

計畫類別： 個別型計畫

計畫編號： NSC87-2314-B-002-136

執行期間： 86 年 8 月 1 日 至 87 年 7 月 31 日

個別型計畫： 計畫主持人： 張金堅

共同主持人： 黃俊升

處理方式：一年後可提供對外參考

執行單位：台大醫學院外科

中華民國 87 **年** 9 **月** 14 **日**

摘　　要

DNA 錯誤配對修補基因 *hMSH2* 和 *hMLH1* 的缺失曾在大腸癌中被偵測到，這兩個基因有缺失的病人在遺傳性非息肉大腸癌約占 60%，散發性大腸癌約占 15%，並且這些突變並未無好發點。雖然此二基因的突變也曾在其他癌症，例如卵巢癌及胃癌被偵測到，然而這兩個基因與乳癌的關係並未被研究過。

乳癌是台灣婦女最常見的惡性腫瘤之一，最近幾年來，罹患乳癌的人數快速增加，並且患者的年齡有年輕化的趨勢。本計畫的目的即在探討 *hMSH2* 和 *hMLH1* 是否為台灣乳癌癌化的潛在遺傳因子。我們分析了 40 位乳癌病人的檢體，其中有 6 位(15%)有 *hMLH1* 的突變，5 位(12.5%)有 *hMSH2* 的突變，整體的總突變率與散發性大腸癌差不多，與家庭遺傳病史無太大關連，然而，大多數的突變是在少於 50 歲的病人檢體中偵測到(有 *hMLH1* 突變的 6 位中有 5 位、有 *hMSH2* 突變的 5 位中有 3 位少於 50 歲)。另外兩位大於 50 歲、有 *hMSH2* 突變者，具有相同的 C 到 A 的置換，並未改變所攜帶的蛋白序列，因此可能只是基因的多形性。雖然突變率在年輕與年長兩個族群中有很明顯的差異(在 *hMLH1* 是 19.2%比 7.1%，在 *hMSH2* 是 11.5%比 0%)，然而因樣品數不夠多，並未達生物統計學上的意義。

總合我們的結果，DNA 錯誤配對修補基因 *hMSH2* 和 *hMLH1* 的突變率在台灣早發的婦女散發性乳癌中偏高(30.7%)，突變的位置也無任何好發點。此二 DNA 錯誤配對修補基因的缺失在早發性乳癌可能扮演重要的角色。

關鍵詞： 乳癌； DNA 錯誤配對修補； *hMLH1*； *hMSH2*； 突變

ABSTRACT

Defects in the DNA mismatch repair genes, *hMLH1* and *hMSH2*, have been detected in colon cancers. Mutations in these two genes combined account for up to 60% in hereditary nonpolyposis colorectal cancer (HNPCC) and 15% in sporadic colon cancers. The mutation spectra show no hot spots. Mutations of these two genes have also been detected in ovarian and gastric cancers. However, the roles of these two genes in breast cancers were not well studied.

In Taiwan, breast cancer is one of the most common malignancies in women. Increasing number of cases and early onset have been noted recently. To find the possible genetic factors involved in the carcinogenesis, we screen 40 breast cancer patients for *hMLH1* and *hMSH2* mutations. Six (15%) and five (12.5%) patients have mutations in *hMLH1* and *hMSH2* genes, respectively. The overall mutation rates are about the same as those of sporadic colon cancers. There is no association between mutations and family history. However, the majority of mutations were found in the patients younger than 50 years old (five out of six for *hMLH1* and three out of five for *hMSH2*). The two *hMSH2* mutations in the older group have the same C to A transversion, which do not change the encoded amino acid ($glycine_{157}$). Therefore, these two mutation events should be considered as polymorphism. Although there are obvious differences between mutation rates of the young and old groups (19.2% vs. 7.1% for *hMLH1*, and 11.5% vs. 0% for *hMSH2*), they are not statistically significant because of low case numbers.

In summary, frequencies of mutation in the mismatch repair genes *hMLH1* and *hMSH2* are high in the Taiwanese sporadic breast cancers with early onset (30.7%) and the mutations have no hot spots. Defects in these two genes may play important roles in tumorigenesis of breast cancers with early onset.

Key words: breast cancer; DNA mismatch repair; *hMLH1*; *hMSH2*; mutation

INTRODUCTION

DNA mismatches are induced via different factors, including replication errors, spontaneous deamination and oxidative stress, and repaired by different pathways (Modrich, 1991; Fishel & Kolodner, 1995). Defect in mismatch repair system results in higher mutation rate and may cause serious damage to the cells. In the recent years, defective mismatch repair genes *hMSH2* and *hMLH1* have been detected in the hereditary nonpolyposis colon cancer (HNPCC) and sporadic colon cancer (Bronner et al., 1994; Leach et al., 1994; Papadopoulos et al., 1994; Peltomaki et al., 1993). It is implicated that inherited malfunction of mismatch repair system could cause accumulation of mutations in the genome and eventually lead to development of cancers.

DNA mismatch repair has been studied in *E. coli* system for decades. The identification of similar mismatch repair genes in other organisms including human indicates that the repair system is conserved throughout evolution. *E. coli* possesses multiple mismatch repair pathways that are distinguished on the basis of mismatch specificity and size of the repair tracts (Lieb et al., 1986; Lu & Chang, 1988; Radicella et al., 1988; Su et al., 1988; Tsai-Wu et al., 1991; Tsai-Wu & Lu, 1994; Modrich, 1991; Fishel & Kolodner, 1995). These pathways include the *dam* methylation-directed mismatch repair (the *mutHLS* pathway), and the *mutY* (*micA*)-dependent mismatch repair. The *mutHLS* mismatch repair pathway directed by adenine methylation (*dam*) at the d(GATC) sequence has been shown to correct most DNA replication errors in *E. coli* (Claverys & Lacks, 1986; Radman & Wagner, 1986; Modrich, 1991, 1995; Fishel & Kolodner, 1995). This pathway repairs mismatches on the transiently undermethylated daughter strands according to the parental sequence. It displays broad mismatch specificity (Lu & Chang, 1988a; Su et al., 1988). The human *mutS* and *mutL* homologues have been identified (Fishel et al., 1993; Leach et al., 1993; Bronner et al., 1994; Papadopoulos et al., 1994). The repair mechanism in human is similar but more complicated than that of *E. coli*. Mutations in these mismatch repair genes have been implicated in the hereditary nonpolyposis colon cancer (HNPCC) and sporadic colon cancer (Bronner et al., 1994; Leach et al., 1993; Peltomaki et al., 1993; Papadopoulos et al., 1994). These mutations cause high frequency of replication errors (RER) and render a mutator phenotype. Certain oncogenes or tumor suppresser genes could have higher chance to accumulate mutations in these RER cells. Therefore, mutation of mismatch repair genes should involve in the early stages of tumorigenesis.

Breast cancer is the most common malignancy of women. The etiology of this cancer involves a complicated interaction of genetic and hormonal factors. Breast cancer patients usually do not die of their primary breast lesions; instead they often die of the subsequent metastatic complications. The procedures involved in the invasiveness and metastasis are very important subjects to study. Although there are a lot of research about breast cancer, mutation

of mismatch repair genes in this cancer has not been well characterized. In Taiwan, breast cancer is also one of the most common malignancies of women. Increasing number of cases and early onset have been noted recently. However, we know little about the molecular mechanism of this important disease in Taiwan. We are trying to investigate the linkage between defect in mismatch repair genes and Taiwanese breast cancer and set up the mutation spectra. Here, we report the result of screening among patients with early onset.

MATERIALS & METHODS

Specimen Collection

Forty breast cancer patients from National Taiwan University Hospital were included in this study. Among these 40 patients, 26 were younger and 14 were older than 50 years old at on the surgery dates. The surgical tissues of breast cancers were collected. The demographic data were collected from the hospital charts, the cancer registry and interviews with the patients and treating physicians. The specimens were immediately frozen in liquid nitrogen for further use.

Reverse Transcriptase-based Polymerase Chain Reaction (RT-PCR)

Total RNAs of the specimens were isolated by Trizol (Life Technology) following the manufacture's instruction. According to the published sequences of *hMSH2* and *hMLH1*, 3 sets of primers for each gene were used to amplify DNA fragments by RT-PCR. The products were purified and subjected to mutation detection.

Mutation Detection

The purified RT-PCR products were subjected to direct DNA sequencing by Thermosequenase using ^{33}P-labeled dideoxy terminators (Amersham) or automatic DNA sequencing. All these procedures (PCR and DNA sequencing) were repeated at least twice to prevent mistaking PCR errors as mutations.

RESULTS

Mutations in *hMLH1* Gene

Six out of 40 patients (15%) have changes in their hMLH1 gene sequence. Among these six patients, only one patient was older than 50 years old, the others were between 26 to 47 years old at diagnosis. Most of the changes were heterozygous (representative DNA sequencing gel patterns were shown in Figs. 1 and 2) except for patient 28 was homozygous. The mutation patterns are summarized in Table 1.

Table 1. Mutation patterns of *hMLH1*.

Patient number	Age	Nucleotide change	Amino acid change
1	26	C_{649}→T	Arg_{217}→Cys
4	33	exon 6 deleted	frameshift
19	60	A_{655}→G	Ile_{219}→Val
28	46	A_{1273}→T	Arg_{425}→Trp
		C_{2101}→A	Gln_{701}→Lys
29	47	exon 12 deleted	frameshift
30	45	exon 12 deleted	frameshift

Mutations in *hMSH2* Gene

Changes in *hMSH2* sequence were detected in five out of 40 patients (12.5%). Among these 5 patients, two were older than 50 years old (60 and 76, respectively) and had the same sequence change that do not change the encoded amino acid (Table 2). In addition, two of the younger patients also had the same missense mutations that change the glutamic acid at position 809 to a lysine. To confirm if this mutation is a polymorphism among Taiwanese population, we screened 80 normal samples for sequence changes at this site. However, we din not detect any change at this position. Sequence changes in patients 2 and 19 were homozygous (Fig. 3). Changes in other patients were heterozygous.

Table 2. Mutation patterns of *hMSH2*.

Patient number	Age	Nucleotide change	Amino acid change
2	33	G_{2425}→A	Glu_{809}→Lys
17	43	G_{2425}→A	Glu_{809}→Lys
		A_{1886}→G	Gln_{629}→Arg
19	60	C_{471}→A	Gly_{157}, no change
23	39	1A insertion at $A_{2642\text{-}2647}$	frameshift
27	76	C_{471}→A	Gly_{157}, no change

DISCUSSION

Breast cancer is the third most common tumors in the world and represents 9% of the global cancer burden. This percentage varies considerably around the world. Although Taiwan is considered as a low-risk area, the incidence and mortality of breast cancer are increasing rapidly. It has become the second leading cause in incidence and the fourth leading cause in mortality of cancers among Taiwanese women. Furthermore, the peak age at diagnosis is about 45 to 50 years old which is 5 to 10 years younger than that of the western countries. Etiology of breast cancer involves complex interaction of genetic and hormonal factors as well as exposure to environmental carcinogens. Analyses of *p53* mutations in breast cancer have led to the discovery of substantial diversity of the mutation spectrum among cohorts from various areas in the world (Hartmann et al., 1997). The differences reflect an intrinsic pattern of mutations plus exposure to particular environmental carcinogens. Thus, it is important to investigate the molecular mechanism of tumorigenesis and find the differences.

Unlike mutations in many oncogenes which play their roles late in the tumorigenesis process, mutations in the mismatch repair genes should lie early in the tumorigenesis process. It is conceivable that the mutator phenotype generated by defects in the repair genes could cause accumulation of mutations in many other oncogenes that eventually cause cancers. Defects in the mismatch repair genes have been implicated in the pathogenesis of hereditary nonpolyposis colorectal cancer (HNPCC) as well as several sporadic cancers that exhibited microsatellite instability. In this study, we investigated mutations of *hMSH2* and *hMLH1* genes in Taiwanese breast cancers. Mutation frequency of these two genes in breast cancers (15% and 12.5% for *hMLH1* and *hMSH2*, respectively) are about the same as reported in other sporadic cancers. There is no linkage between the mutations and patients' family history, tumor size, ER and PR status, lymph node metastasis, or distal metastasis. However, mutation frequencies of these two genes among breast cancer patients with early onset (younger than 50 years old) are higher than those of older patients. Among 26 patients younger than 50 years old, 5 (19.2%) and 3 (11.5%) had mutations in *hMLH1* and *hMSH2* genes, respectively. In the group of older than 50 years old, only one patient had *hMLH1* mutation (7.1%). Patients 19 and 27 had the same C_{471} to A change that did not change the encoded amino acids. Therefore, no meaningful mutation in *hMSH2* was detected among older patients. However, the differences between mutation frequencies of these two genes in young and old groups are not statistically significant because of small sample sizes. More samples should be included to clarify if defects in *hMLH1* and *hMSH2* genes play roles in early onset of Taiwanese breast cancers.

REFERENCES

1. Bronner C.E., Baker, S.M., Morrison, P.T., Warren, G., Smith, L.G., Lescoe, M.K., Kane, M., Earabino, C., Lipford, J., Lindblom, A., Tannergard, P., Bollag, R.J., Godwin, A.R., Ward, D.C., Nordenskjold, M., Fishel, R., Kolodner, R., and Liskay, R.M. (1994). Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. Nature 368:258-261.
2. Claverys, J.-P. and Lacks, S. A. (1986) Heteroduplex deoxyribonucleic acid base mismatch repair in bacteria. Microbiol. Rev. 50:133-165.
3. Fishel, R., Lescoe, M.K., Rao, M.R.S., Copeland, N.G., Jenkins, N.A., Garber, J., Kane, M., and Kolodner, R. (1993). The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell 75:1027-1038.
4. Fishel, R. and Kolodner, R. (1995). Identification of mismatch repair genes and their role in the development of cancer. Curr. Opion. Genet. Develop. 5:382-395.
5. Hartmann, A., Blaszyk, H., Kovach, J. S. and Sommer, S. S. (1997) The molecualr epidemiology of *p53* gene mutations in human breast cancer. TIG 13: 27-33.
6. Leach, F.S., Nicolaides, N.C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L.A., Nystrom-Lahti, M., Guan, X.Y., Zhang, J., Meltzer, P.S., Yu, J.-W., Kao, F.-T., Chen, D.J., Cerosaletti, K.M., Fournier, R.E.K., Todd, S., Lewis, T., Leach, R.J., Naylor, S.L., Weissenbach, J., Mecklin, J.-P., Jarvinen, H., Petersen, G.M., Hamilton, S.R., Green, J., Jass, J., Watson, P., Lynch, H.T., Trent, J.M., de la Chapelle, A., Kinzler, K.W., and Vogelstein, B. (1993). Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. Cell 75:1215-1225.
7. Lieb, M., Allen, E., and Read, D. (1986) Very short patch mismatch repair in phage lambda: repair sites and length of repair tracts. Genetics 114:1041-1060.
8. Lu, A-L. and Chang, D.-Y. (1988). Repair of single base-pair transversion mismatches of *Escherichia coli in vitro*: correction of certain A/G mismatches is independent of *dam* methylation and host *mutHLS* gene functions. Genetics 118:593-600.
9. Modrich, P. (1991). Mechanisms and biological effects of mismatch repair. Annu. Rev. Genet. 25:229-253.
10. Modrich, P. (1995). Mismatch repair, genetic stability and tumor avoidance. Phil. Trans. R. Soc. Lond. B. 347:89-95.
11. Papadopoulos, N., Nicolaides, N.C., Wei, Y.-F., Ruben, S.M., Carter, A.C., Rosen, C.A., Haseltine, W.A., Fleischmann, R.D., Fraser, C.M., Adams, M.D., Venter, J.C., Hamilton, S.R., Petersen, G.M., Watson, P., Lynch, H.T., Peltomaki, P., Mecklin, J.-P., de la Chapelle, A., Kinzler, K.W., and Vogelstein, B. (1994). Mutation of a *mutL* homolog in hereditary colon cancer. Science 263:1625-1629.

12. Peltomaki, P., Aaltonen, L.A., Sistonen, P., Pylkkanen, L., Mecklin, J.-P., Jarvinen, H., Green, J.S., Jass, J.R., Weber, J.L., Leach, F.S., Peterson, G.M., Hamilton, S.R., de la Chapelle, A., and Vogelstein, B. (1993). Genetic mapping of a locus predisposing to human colorectal cancer. Science 260:810-812.
13. Radicella, J. P., Clark, E. A., and Fox, M. S. (1988) Some mismatch repair activities in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 85:9674-9678.
14. Radman, M. and Wagner, R. (1986) Mismatch repair in *Escherichia coli*. Annu. Rev. Genet. 20:523-528.
15. Su, S.-S., Lahue, R. S., Au, K. G., and Modrich, P. (1988) Mispair specificity of methyl-directed DNA mismatch correction *in vitro*. J. Biol. Chem. 263: 6829-6835.
16. Tsai-Wu, J.-J. and Lu, A-L. (1994) *Escherichia coli mutY*-dependent mismatch repair involves DNA polymerase I and a short repair tract. Mol. Gen. Genet. 244:444-450.
17. Tsai-Wu, J.-J., Radicella, J. P. and Lu, A-L. (1991) nucleotide sequence of the *Escherichia coli micA* gene required for A/G-specific mismatch repair: identity of MicA to MutY. J. Bacteriol. 173: 1902-1910.