行政院國家科學委員會補助專題研究計畫成果報告

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※ 非小細胞肺癌之微衛星體複製突變與核酸修補基因(*hMSH2* 基因及 *hOGG1* 基因)發生基因變異之相關連性 ※

※ Microsatellite Instability and Genetic Alteratin of Two DNA Repair Genes- *hMSH2* Gene and *hOGG1* Gene in Non-Small Cell Lung Cancer ※

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計畫類別：■個別型計畫　□整合型計畫

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共同主持人：

本成果報告包括以下應繳交之附件：

- □赴國外出差或研習心得報告一份
- □赴大陸地區出差或研習心得報告一份
- □出席國際學術會議心得報告及發表之論文各一份
- □國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學醫學院內科

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40 pairs of tissues. Analysis of MMR genes is undergoing. Keywords：microsatellite, mutation, mismatch repair, oxidative DNA damage, hMSH2, hOGG1, lung cancer

計劃背景與緣由

Malignant tumors develop in humans for the most part in individuals of 55 years of age or more, and consist of enormous cloning expansion of cells that have accumulated mutations in defined sets of genes [1]. Mutation accumulates throughout life and is the underlying causes of cancer [2]. Statistically, it has been estimated that tumors require four to seven mutations to develop. Ionizing radiation, viral, chemical, or other physical factors are causative events to DNA mutations and are also risk factors for carcinogenesis.

Microsatellites are short (1-4 bp), repetitive, non-codifying, and highly polymorphic DNA sequences, distributed within the genome [3]. Approximately 100,000 microsatellites repeats are scattered throughout human genome. Instability of microsatellite sequence reflects malfunction in the replication or repair of DNA, so called replication error (RER) phenomenon [4]. RER can be witnessed as a change in the length of microsatellite sequences (expansions or contractions) in tumor DNA compared with constitutional DNA, but also as the complete loss of one or both alleles of the repeat locus (LOH). Microsatellite instability serves as a useful marker of a "mutator" phenotype characteristic of hereditary nonpolyposis colorectal cancer (HNPCC) and sporadic colorectal cancers [5,6]. About 90% of HNPCC tumors had microsatellite instability, and approximately 15% of apparently sporadic colorectal carcinomas and other types of cancers also show this abnormality [7,8]. RERs are not uncommon in lung cancer. But different from those found in colon cancer, the RERs occur in few loci, mostly localized on chromosome 3p, but can also be found on chromosome 2p, 9p and 1p [9-11].

The existence of bacteria mismatch repair (MMR) enzymes has been known for decades [12]. In *Escherichia coli*, HLS (*mutH*, *mutL*, *mutS*) system provide main repair pathways for DNA replication error. The human MMR system is believed to operate in similar fashion. The mismatch binding factor (like *mutS* in bacteria) includes, *MSH2* and G/T mismatch binding protein (*GTBP*). There are at least 16 *mutL*-like proteins in human, *MLH1*, *PMS1-8*, *PMSR1-7*, showing a high genetic redundancy. No human counterpart protein for *mutH* has been identified so far [13]. Tumors from HNPCC patients showed inactivation of one of four MMR genes, *MLH1*, *MSH2*, *PMS1*, *PMS2*.

Apart from being responsible for the correction of biosynthetic errors in newly synthesized DNA, the MMR system is also presumed to repair exogenous chemical damage, such as lesions caused by oxidation. Oxidative damaged DNA product, 8-oxo-7,8-dihydro-guanine (8-oxoG) has gained greatest current interest. Evidence also suggests the

critical role of 8-oxoG in mutagenesis and carcinogenesis [13]. *HOGG1* gene, mapped to chromosome 3p25, a region with high frequency of RER in lung cancer, is the repair enzyme that mediate the removal of 8-oxo-G oxidative damages from DNA [14,15]

So far, only few studies concerning microsatellite instability in lung cancer have been reported. These studies demonstrated frequent RER in lung cancer. However, data regarding the alteration of MMR genes in lung cancer are still lacking. This two-year study was proposed to evaluate the frequency of microsatellite instability in non-small cell lung cancer, and the association between RER and genetic alteration of a MMR prototype protein (*hMSH2*) and *hOGG1*.

研究材料與方法

Patients and tumor tissues

Surgical specimens of tumors and the adjacent uninvolved lung tissue were obtained from 40 patients at the time of resection. All patients should have NSCLC confirmed by histological diagnosis.

DNA and RNA extraction

Genomic DNA and total cellular RNA were extracted from tissues using the phenol-chloroform and guanidinium thiocyanate-phenol-chloroform extraction method, respectively.

Microsatellite analysis

Microsatellite sequences were analyzed using PCR. Microsatellite markers for the analysis for each sample were obtained as MAPPAIRS (Research Genetics, Huntsville, AL, USA): D2S123 (2p14), D2S136 (2p14-p13), and D2S162 (2p25-p22) on chromosome 2p, and D3S1339 (3p13-p14), and D3S1403 (3p14) on chromosome 3p; D17S250 on chromosome 17p, as well as two monocucleotide markers: BAT-40 (1q13.1) and BAT-26 (2p). PCR were performed by 35 cycles of amplification in a final volume of 25 μl. The PCR products were electrophoresed on 8% polyacrylamide gels containing 8M urea, dried at 80℃, and exposed to X-ray film from 24 to 72 h. The band pattern will be compared between tumorous and non-tumorous tissues for each patient. The RER (+) tumors were defined by the presence of microsatellite alterations in at least two different loci.

Reverse-transcription polymerase chain reaction of *hMSH2* and *hOGG1*

RNA of tissues obtained from those patients with microsatellite instability were used to determine *hMSH2* and *hOGG* alteration. Two μg of total RNA is reverse transcribedand subjecterd to PCR amplifications in 50 μl reactions.

結果

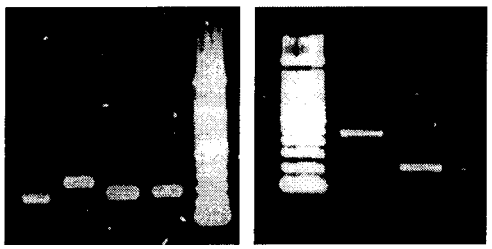
After evaluating several microsatellite markers in 40 pairs of lung cancers , the results showed that : 4 mutants out of 40 were detected in BAT-40 (Fig. 1), 4/40 in BAT-26, 2/40 in D2S123, 2/40 in D2S162, 15/40 in D3S1339 (10 were LOH, 5 instability), 11/40

in D17S250(LOH 6, 5 instability). Twelve were RER (+) according to definition.

Fig. 1 Alteration of microsatellites

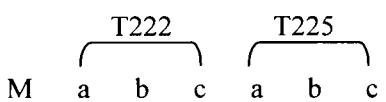
The PCR products for *hOGG1* and *hMSH2* were generated with the fragments size described as following:

Fig.2 PCR products of *hOGG1* exon 1-7



E 1 2 3 4 M M 5-6 7

Fig 3. RT-PCR products of *hMSH2*



a: codon 1-247, b: codon 241-507, c: codon 381-934.

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