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High frequency of frameshift mutation on p53 gene in Taiwanese with non small cell lung cancer

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Abstract

Extensive researches have found that the mutation of p53 tumor suppressor gene is the most frequent event in many human cancers and associated with a poor clinical outcome in lung cancer patients. Because the p53 molecular mutation involved in tumorigenesis of patients with lung cancer in Taiwan remains poorly defined, the aim of this study was to assess the p53 mutation spectrum and possible etiological factors of Taiwan's patients with Non-Small Cell Lung Cancer (NSCLC). Cancer specimens were obtained surgically from 61 patients with pathologically proven NSCLC. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and direct sequencing were used to study p53 mutations in exon 4-8. We also performed immunohistochemistry (IHC) to detect p53 protein expression. Our results provided that 34 mutations of p53 gene were found in 27 cases with a mutation rate of 44% (27/61). There were six cases having more than two p53 mutations. Among the 34 mutations, 19 were point mutations (56%, 19/34) consisted of a majority of missense mutations including transversion (13/19, 68%) and transitions (6/19, 32%) with four cases (4/6, 67%) occurring in the CpG sequence. One of the most important finding in our study was the high frequency of frameshift (44%, 15/34) which included 11 insertions and 4 deletions of p53 in NSCLC in Taiwan. Surprisingly, our results disclosed distinct novel mutations at codon 181, 185, 208 (Exon 5-6) of p53. Especially, 4 cases with mutation at codon181 and codon 185 seemed to have more advanced clinical outcome with survival time less than 6 months. In addition, there were two recurring mutations at codon 168 and three at condon193. The different

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mutation spectrum in our series, including a high frequency of frameshift mutations and distinctly novel hot spots suggested the heterogenous entity of exogenous mutagens in NSCLC in Taiwan.

Keywords: Non small cell lung cancer; p53; Frameshift mutation; Hot spot

1. Introduction

In Taiwan, lung cancer has been the leading and second-leading cause of cancer deaths in women and men, respectively since 1985 [1]. Epidemiological studies conducted in mainland China and Taiwan have shown that cigarette smoking is the main risk factor for lung cancer [2,3]. In most studies, it is also found that the risk factors of getting lung cancer are related to high percentage of passive smoking [4], cooking oil vapors [5,6] and occupational exposures [7]. Nevertheless, when compared with the epidemiological researches of non-small cell lung cancer (NSCLC) in Asia, United States and many European countries, NSCLC in Taiwan has been shown to have a low male:female ratio of 2:1 for mortality [8–10]. These results suggest that different molecular mechanisms may be involved in lung tumorigenesis of patients with NSCLC in Taiwan.

The multistage process of tumorigenesis generally includes the gain of protooncogenes activity and the loss or inactivity of tumor suppressor genes [11]. Among tumor suppressors, p53 gene abnormalities are the most frequent genetic events illustrated to date [12,13]. For example, p53 mutation and aberrant p53 gene product expression in over half of adult cancers, including lung, breast, colon, esophagus, and skin cancers, are now considered to be one of the most common genetic features in a wide range of human cancers [14–16]. p53 cancer-associated mutation in the highly conserved DNA-binding domain may prevent or inhibit p53-mediated cell cycle arrest, DNA repair, programmed cell death, and other protective responses to cell stress and DNA damage. The causes of p53 mutations include both endogenous factors which contribute to the infidelity of DNA synthesis and exogenous factors such as chemical mutagens and radiation exposure [17]. Various genotoxic compounds have been shown to selectively induce alterations of specific base pairs in p53, that are related to cancer [18]. These alterations can be explained by the presence of regionally distinct carcinogens in both smoke and air, interacting with local environmental cofactors, in the development of lung cancer. Besides, the genetic mistake of p53 in NSCLC, as a result of either p53 protein overexpression [19] or p53 gene mutation [20,21], is found to be strongly correlated with tumor grade and can predict a poor prognosis [22–24]. Another study in Taiwan even finds that the nodal status of NSCLC has a strong association with mutation within exon 8, and that the mutation rate (42%, 15/36) of p53 in lung cancer in this country agrees with other study results. Yet, a distinct spectrum of p53 mutations is also found. The majority of p53 mutations in NSCLC in Taiwan is missense mutation [9].

To our knowledge, few extensive studies on p53 gene with environmental analyses in lung cancer have been conducted in Taiwan. In order to clarify the spectrum, role, and tumor progression of p53 mutations in carcinogenesis and in primary NSCLCs, we performed mutation analyses by using direct sequencing and immunohistochemical staining (IHC) to detect p53 mutations among NSCLC patients in Taiwan.

2. Material and method

2.1. Specimen collections and DNA extraction

Fresh tumors specimens were obtained surgically from 61 patients with pathologically proven primary lung cancer at the Department of Chest Surgery of Kaohsiung Medical University Hospital. All tissue specimens were placed in liquid nitrogen as soon as possible after surgical isolation. Genomic DNA was extracted from fresh-frozen specimens using Puregene DNA isolation protocol (Gentra Systems, Inc., Minneapolis, MN) as described previously [25].