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Minireview

p53 and K-ras mutations in lung tissues and sputum samples of individuals exposed to smoky coal emissions in Xuan Wei County, China

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ABSTRACT

Lung cancer mortality in Xuan Wei County (XWC) is among the highest in China. Lung cancer in XWC is associated with exposure, in poorly vented homes, to coal smoke containing high levels of polycyclic aromatic hydrocarbons (PAHs). We have previously investigated mutations in the p53 tumor suppressor gene and the K-ras oncogene in lung carcinomas and in sputum samples from individuals exposed to smoky coal emissions in XWC. This paper summarizes the results concerning p53 and K-ras mutations from these studies, in relation to mutations found in lung cancer patients not exposed to smoky coal emissions.

1. Introduction

Lung cancer remains the most common cause of cancer death worldwide, with 1,600,000 deaths annually, including 490,000 deaths among women [1]. In some regions, the lung cancer rate is particularly high. In Xuan Wei County (XWC), Yunnan Province, China, the lung cancer rate is about 5-fold greater than the Chinese national average, and, in some communes, 24-fold greater [2]. While tobacco smoke exposure is a well-established lung cancer risk factor [3], the high lung cancer mortality rate in XWC women cannot be attributed to tobacco smoke or occupational exposure, since women in this region, who are mostly non-smokers, have the highest lung cancer rate in China, similar to rate in men, who are mostly smokers. This suggests that some other factors, particularly domestic ones, may be responsible [2]. Household fuel surveys indicate that lung cancer is highly correlated with the use (for generations) of “smoky coal” for domestic combustion [2]. Smoky coal is a low-sulfur (0.2%) medium-volatile bituminous coal used for cooking and heating in XWC homes without chimneys. Characterization of the indoor air from homes using smoky coal showed that XWC residents are exposed to high concentrations of submicron particles that contain mostly organic matter, including large amounts of mutagenic/carcinogenic polycyclic aromatic hydrocarbons (PAHs) [4]. These results point to a strong etiologic link between exposure to smoky coal combustion and the high lung cancer rate in women in XWC.

Previous studies showed a high prevalence of mutations in the p53 tumor suppressor gene in lung tumors from both smokers and non-smokers [5–9], while in the K-ras oncogene, mutations were frequent in lung tumors from smokers, but less so in those from non-smokers (mostly women) [10]. It has been suggested that, in lung adenocarcinomas from smokers, these mutations were primarily induced by tobacco smoke carcinogens [11].

The tumor suppressor gene p53 encodes a 53-kDa multifunctional DNA sequence-specific nuclear transcription factor (p53) that is expressed at very low levels, under normal circumstances. In response to genotoxic stress, p53 protein is induced to accumulate in the cell nucleus through posttranslational modifications that convert the protein from a latent to an active form. The active p53 protein trans-activates several target genes involved in cell cycle arrest, repair of DNA damage, cell differentiation, and programmed cell death, preventing cells with severe DNA damage from proliferating and initiating tumorigenesis [12].

The K-ras oncogene belongs to the ras gene family and encodes a membrane-bound guanosine nucleotide-binding protein with intrinsic GTPase activity. When activated by external stimuli, wild type K-ras switches from an inactive GDP-bound to an active GTP-bound conformation, activating multiple downstream effectors in signaling pathways involved in the regulation of cell growth/differentiation and apoptosis. In K-ras mutated cells, oncogenic mutant K-ras remains blocked in its active GTP-bound state and, thus, constitutively activates its downstream signaling pathways, thereby increasing the risk of tumorigenesis [13–15].

We have previously investigated p53 and K-ras mutations in lung

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tumors and sputum samples from XWC male and female lung cancer patients exposed to smoky coal emissions. We also investigated these mutations in sputum samples from individuals with a history of long-term exposure to smoky coal emissions but who had no evidence of lung cancer. This article reviews published data from these studies and discusses the findings in comparison with those from lung cancer patients who had not been exposed to smoky coal emissions, from regions in China, the USA, and Europe.

2. Materials

2.1. Lung tissue and sputum samples

Specimens used for these studies included lung tumors and sputum samples from lung cancer patients, and sputum samples from individuals without evidence of lung cancer from XWC [16]. The lung cancer cases were diagnosed based on a minimum of clinical symptoms and chest x-ray analysis at the XWC hospital. The lung cancer tissues consisted of surgically removed fresh-frozen or paraffin-embedded lung tumors. Clinical and demographic information of patients (histologic type of tumor, gender, age, fuel use history, tobacco smoke history, diet and occupation) was obtained from case history records from the XWC hospital [16]. However, for some patients, information on the histologic types of lung cancer could not be obtained, because these individuals did not undergo surgical removal of lung tumor and were able to provide only sputum samples. The comparison group of patients was from Beijing and Zhengzhou, the capital city of Henan Province, where natural gas is mainly used in homes. In addition, individuals exposed to smoky coal emissions in XWC but who showed no evidence of lung cancer (based on a minimum of clinical symptoms and chest x-ray analysis at the XWC hospital) provided sputum samples for mutation analysis. These studies were conducted according to the recommendations of the World Medical Association *Helsinki Declaration* (1989) [17]. The research protocol met the requirements for protection of human subject certification by the EPA, USA.

3. Results and discussion

3.1. p53 mutations in lung tumors from XWC women

p53 mutations were detected frequently in lung tumors from male and female smokers [5,6]. These mutations were also found in lung tumors from non-smokers, but the predominant types and/or spectra of mutations differed between smoking and non-smoking women [5,7,9,18]. The increased lung cancer incidence in female non-smokers in XWC led to investigation of these mutations in lung tumors from these women. The first study analyzed lung tumors that had stained positive for TP53 protein and were expected to harbor p53 mutations [19]. These tumors were adenocarcinomas of bronchioalveolar and acinar origins. The mutations were analyzed by using multiplex PCR for exons 4–9, followed by sequence determination of the amplified exons in both directions. The mutations identified are summarized in Table 1.

Among the 24 tumors, 17 (70.8%) showed at least one p53 mutation, including four tumors (16.7%) each with two different p53 mutations and 13 tumors (54.2%) each with one p53 mutation, while seven tumors (29.2%) showed no detectable p53 mutations within the exons analyzed. There is a high prevalence of G → T transversions in lung tumors from non-smoking XWC women since 16 (76.2%) of the 21 p53 mutations identified were of this mutation type.

Table 1
Mutation characteristics of lung tumors from XWC women.

Mutation status	Tumor numbers (%)	Mutation numbers and types
p53 only	12 (50.0%)	3 (2 different p53 mutations) + 9 (one p53 mutation)
p53 + K-ras	5 (20.8%)	4 (one p53 + one K-ras mutations) 1 (2 p53 + one K-ras mutations)
K-ras only	2 (8.4%)	2 (each with one K-ras mutation)
Mutation-negative	5 (20.8%)	
Total	24 (100%)	

One-third of the mutations were found clustered within the GC-rich region of codons 153–158. The high frequency of G → T transversion and its occurrence at a specific GC-rich region is consistent with exposure to PAH and with the fact that most PAHs produce DNA adducts primarily at guanines [20]. Furthermore, these mutations show an extreme strand bias, as all of the guanines involved in G → T transversions occurred at the non-transcribed strand (NTS). These results are in agreement with those of several previous studies of lung adenocarcinomas from smoking lung cancer patients [5–8]. For instance, in a population of lung cancer patients from Western Pennsylvania, G → T transversions accounted for 52.5% of all mutations, while G → A transitions, rare in lung tumors from XWC women (Fig. 1), accounted for 20.0% [6]. This transition mutation is caused primarily by tobacco smoke nitrosamines [21,22]. Therefore, mutations in the p53 gene in smokers and in patients exposed to smoky coal emissions resulted from DNA damage by tobacco smoke carcinogens, such as PAHs. Adducts formed between guanines and PAH metabolites, such as benzo[*a*]pyrene dihydrodiol epoxide, on NTS of DNA were repaired 2–4 times more slowly than those in the transcribed strand by transcription-coupled repair [23]. These slowly repaired damage hotspots in NTS correspond to mutational hotspots observed in human lung cancer [24].

C → T transitions at CpG sites occurred in about 10% of the tumors from XWC women and are considered to result from an endogenous mutational mechanism. C bases in CpG sites are prone to methylation *in vivo*. The resulting 5-methyl C is preferentially involved in spontaneous mutation; deamination results in a C → T transition [25]. C → T transitions at CpG sites had been also reported previously among p53 mutations in lung tumors of nonsmoking women, but not among those of smoking women, with NSCLC [7]. Therefore, most mutations in smoky coal-exposed individuals and smokers appear to result from unrepaired, exogenous damage on the NTS rather than from DNA damage that is endogenous in origin.

3.2. K-ras mutations in lung tumors from XWC women

In lung cancer, K-ras mutations are associated with tobacco smoking, as they are more frequent in lung tumors from both male and female smokers but less so in those of non-smokers, who are primarily women [7,10,11]. However, investigation of K-ras mutations in the same 24 tumors from XWC women [19] revealed that seven of them each showed a K-ras mutation in codon 12 (Table 1). G → T transversion predominated: six (85.7%) of the mutations were of this type. Therefore, in XWC women K-ras mutations, like p53 mutations, were presumably caused by carcinogens, such as PAHs, in smoky coal emissions to which these women were exposed.

In another study [26], K-ras mutations were compared between lung cancer patients from XWC and those from Beijing and Henan (B&H) regions of China, where the residents were not exposed to unvented smoky coal emissions and the lung cancer rate approximated the average rate for China [2]. The specimens, either lung tumor tissues or sputum samples, were obtained from 152 lung cancer patients, including 102 patients from XWC (41 non-smoking women and 61 smoking men) and 50 patients from B&H (14 non-smoking women and 36 men, most of whom were smokers).

Among the XWC patients (Table 2), K-ras mutant frequencies are similar between non-smoking women and smoking men [21.9% (9 of 41) vs. 22.9% (14 of 61), respectively; $P = 1.00$], with predominant G → T transversions in both patient groups (66.7 vs. 85.7%, respectively). Among the patients from B&H, the K-ras mutant frequency is 18.2% in smoking men, with G → T transversions accounting for 66.7%,