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Methylation analysis for multiple gene promoters in non-small cell lung cancers in high indoor air pollution region in China

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Summary

Aim > The prevalence and mortality rates of lung cancer in Xuanwei, Yunnan, China, are the highest in the world. The severe indoor air pollution caused by smoky coals with high benzo (a) pyrene (BaP) and quartz levels is the main environmental factor. The aim of this study was to investigate methylation profiles of promoters in eight genes in primary non-small cell lung cancers (NSCLC) exposed to smoky coals.

Materials and methods > Candidate genes including CDKN2A, DLEC1, CDH1, DAPK, RUNX3, APC, WIF1 and MGMT were determined for the promoter methylation status using Nested methylation-specific PCR (nMSP) in primary 23 NSCLC tissues and in circulating tumor DNA (ctDNA) isolated from 42 plasma samples (9 matched to tissues) as well as 10 healthy plasma samples, using Sanger sequencing to verify the results.

Results > Seven of the 8 genes, except MGMT, had relatively high methylation frequencies ranging from 39%–74% in tissues. Moreover, methylation frequencies in five genes identified in lung cancer plasma were 45% for CDKN2A, 48% for DLEC1, 76% for CDH1, 14% for DAPK, 29% for RUNX3, with a relatively good concordance of methylation among 9 tissues and paired plasma. However, the genes from all healthy plasma showed no methylation.

Conclusions > A panel of genes including CDKN2A, DLEC1, CDH1, DAPK and RUNX3 may be used as potential epigenetic biomarkers for early lung cancer detection. CDH1 promoter methylation was associated with lung cancer metastasis in areas of air pollution from buring of smoky coals. DLEC1 and CDH1 exhibited specific high methylation frequencies, different from previous reports.

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Introduction

Lung cancer (LC) is the leading cause of cancer-related deaths worldwide[1]. The National Central Cancer Registry of China reported that lung cancer is the most prevalent type of cancers and the major cause of cancer deaths in the country. Especially, southwest China ranks the second highest in incidence and mortality in lung cancer[2], which is likely due to its high prevalence of tobacco use[3], high environmental exposures to such serious air pollution caused by burning smoky coals[4,5] and limited medical resources. Late Permian coal districts including eastern Yunnan province is a main smoky coal-producing regions in Southwest China[6]. Especially in Xuanwei, and Fuyuan regions, Yunnan Province, the use of smoky coals for cooking and heating is common[7], which makes the morbidity and mortality rates of lung cancer in the area be the highest in China and the world[8,9]. Incomplete combustion of smoky coals produces various carcinogens such as quartz, volatile polycyclic aromatic hydrocarbons (PAHs) and combustion generated particles, which are closely linked with lung cancer occurrence[7,8]. Additionally, crops exposed to burning smoky coals also increase lung cancer risk in the regions[10].

Epidemiological and experimental evidence indicated that environmental factor exposure during prenatal and early postnatal period has critical effects on embryonic development and tissue differentiation[11], and may lead to permanent epigenetic modifications, thus increasing the possibility of developing adult-onset disorders including lung cancer[12,13]. DNA methylation, the major epigenetic modification, with the effects of activating oncogenes and inactivating tumor suppressor genes (TSGs) [14], has been reported to be the early event of lung cancers[15], varying in types of lung cancer samples including plasma, urine, semen, lymph node, tissue, and stool[15–17]. Indoor BaP exposure caused by use of smoky coals in Xuanwei region has showed to induce abnormal DNA methylation status in lung cancer tissues, LC-cell lines, bap-treated cells and animal models[18]. This may provided strong evidence that the high incidence of lung cancer in Xuanwei may relate to the abnormal DNA methylation alterations of LC-related gene induced by indoor smoky coal exposure.

Previous reports[19,20] showed that aberrant methylation in some LC-related genes could be detected in circulating tumor (ctDNA) of lung cancer, including tumor suppressor genes such as *CDKN2A, RUNX3, APC, DAPK, WIF1*, and cell-regulation genes including *DLEC1, CDH1*, and *MGMT*. Aberrant methylation of promoters of those genes may become the indications of early lung cancer occurrence. However, studies for those genes were usually performed in smoker population and mainly in western counties. Their promoter methylation profiles in the lung cancers exposed to smoky coals, and the relationship between their methylation status and clinicopathological characteristics are unknown.

We hypothesized that the high rates and clinicopathological characteristics of lung cancers exposed to smoky coals in Xuanwei were associated with DNA methylation in promoters of LC-related genes. In this study, promoter methylation status of candidate genes in lung cancer tissues and plasma samples from patients and healthy controls matching for age, sex, and smoking habits were examined with Nested methylation-specific PCR (nMSP). Additionally, an informative set of multiple genes was validated for developing a non-invasive detection method with epigenetic markers.

Materials and Methods

Patients

Eight-seven patients with lung cancer and 31 healthy subjects from Xuanwei, Yunnan province, China, were recruited and performed a questionnaire for environmental factors exposure status such as smoking status and smoky coal exposure history from 2013 to 2016. Subsequently, age-matched 56 patients and 10 healthy subjects were selected, after excluding subjects who had no smoky coal exposure history or underwent chemo-or radiotherapy.

Finally, plasma samples from a total of 42 (9 matched to tissues) lung cancer and 10 healthy controls were collected, centrifuged, and stored at −80 °C. Furthermore, 13 paraffin and 10 fresh tissues were collected and stored at −4 °C and−80 °C, respectively. All specimens were obtained from the First People Hospital of Yunnan Province, Kunming University of Science and Technology, China. All lung cancer specimens were matched for age, sex, and cigarette smoking habits. If the subjects had smoked < 100 cigarettes or never smoked in a lifetime, they were considered as nonsmokers. All diagnoses were based on pathology evidence. Among the 23 lung tissues, there were 14 adenocarcinomas (ACs), 3 squamous cell carcinomas (SCCs), 6 adenosquamous carcinomas (ADCs). The pathological stages (according to the TNM stage classification of the International Union against Cancer) were stage I-II in 18 patients and stage III-IV in 5 patients. 42 plasma samples included 18 ACs, 15 SCCs, and 9 ADCs. Clinical stages comprised 9 I-II and 19 III-IV, respectively. However, there were 14 without clinical stage information. Ten healthy samples were randomly obtained from the population age-matched with lung cancers. The study was approved by the ethics committees of the medical school of Kunming University of Science and Technology. Informed consent was obtained from all individual participants included in the study.

Bisulphite treatment of DNA and nMSP

Genomic DNA from thirteen paraffin and ten fresh lung cancer tissues was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) and Ezup Post Genomic DNA Extraction Kit (Sangon, Shanghai, China), respectively. 42 lung cancer and 10 healthy plasma DNA were extracted with the QIAamp