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Mitochondrial DNA Content and Lung Cancer Risk

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Abstract

Smoky coal contains polycyclic aromatic hydrocarbons (PAHs) and has been strongly implicated in etiology of lung cancer in Xuan Wei, China. While PAHs have been demonstrated to form bulky adducts in nuclear DNA, they have a 90-fold greater affinity for mitochondrial DNA (mtDNA). To compensate for mitochondrial dysfunction or damage, mtDNA content is thought to increase. We conducted a population-based case-control study of lung cancer in Xuan Wei, China hypothesizing that mtDNA content is associated with lung cancer risk. Cases ($n = 122$) and controls ($n = 121$) were individually matched on age (± 2 yrs), sex, village of residence, and type of heating/cooking fuel currently used. Lifetime smoky coal use and potential confounders were determined with questionnaires. mtDNA was extracted from sputum and content was determined with quantitative RT-PCR. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated with unconditional logistic regression. mtDNA content was dichotomized at the median based on the distribution among the controls. mtDNA content > 157 was associated with a 2-fold increase in lung cancer risk (OR = 1.8; 95% CI = 1.0–3.2) compared with those with ≤ 157 copies. Risk was higher among those > 57 years of age compared with those ≤ 57 years (p interaction = 0.01). In summary, mtDNA content was positively associated with lung cancer risk. Furthermore, there was some evidence that mtDNA content was more strongly associated with lung cancer risk among older individuals. However, due to the small sample size, additional studies are needed to evaluate these associations.

Introduction

The incidence rate of lung cancer in Xuan Wei, China is among the highest in China (1). This high incidence has been largely attributed to the use of smoky coal for cooking and heating (2). Smoky coal (bituminous coal) from this region of China generates very high concentrations of polycyclic aromatic hydrocarbons (PAH) during combustion (3,4). In addition to forming bulky DNA adducts in the nucleus, PAHs have been shown to have approximately a 40 to 90-fold greater affinity for mitochondrial DNA (mtDNA) (5–7). Mitochondria have multiple biologic functions, but are primarily responsible for synthesizing adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) (8). However, reactive oxygen species (ROS)

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are toxic by-products of OXPHOS (8) that have been hypothesized to contribute to the development of cancer as well as other diseases (9). More recently, ROS generated by OXPHOS have been hypothesized to have a role in carcinogenesis by propagating mitogenic signals (10,11). In addition to generating ROS and propagating cellular proliferation, mitochondria have an important role in carcinogenesis because of their role apoptosis (12, 13).

Mitochondria are double membrane organelles that possess their own copy of DNA. Mitochondria from normal cells have between 2–10 copies of their genomes and depending on the cell type, each cell may have up to thousands of mitochondria (14). mtDNAs are circular molecules with 16,569 base pairs coding for 37 genes: 13 proteins involved in electron chain transport, 22 transfer RNAs and two ribosomal RNAs. mtDNA lack introns and histones and are located in the mitochondrial matrix in close physical proximity to the electron transport chain and superoxide anions generated during OXPHOS. The lack of introns, protective histones, and the close proximity to the electron transport chain result in mtDNA being more susceptible to oxidative damage than nuclear DNA. In addition, mitochondria have limited DNA repair capacity. Mitochondria have been hypothesized to compensate for damage and dysfunction by increasing the number of copies of mtDNA (15).

Given that PAHs are known carcinogens, have a much greater affinity for mtDNA, we hypothesized that PAH-rich smoky coal exposure may lead to mitochondrial DNA damage and increased mtDNA content and that mtDNA content is associated with the risk of lung cancer.

Methods

This population-based case-control study has been described elsewhere (1,16). Briefly, cases and controls were recruited into the study over a one-year period between March, 1995 and March, 1996. In total, 122 primary, incident cases of lung cancer and 122 controls were enrolled and matched on age (± 2 years), gender, village of residence, and type of fuel currently used for cooking and home heating. The control/case matching ratio was one to one. Information was collected from in-person interviews regarding demographic data, smoking history, family medical history, smoky coal use, and diet.

Five morning sputum was collected from all cases and controls, preserved in Saccomanno fluid, and stored at 4° C. DNA (genomic and mitochondrial) was extracted with phenol/chloroform extraction. mtDNA content was determined with Quantitative-PCR (Q-PCR) as previously described (17). Briefly, ND1, a mitochondrial gene, and β -globin, a nuclear encoded gene, were amplified and the threshold cycle number (Ct) were determined for both amplicons and standard regression analyses were used to derive the mtDNA content.

Matching between cases and controls was not retained for the analyses because some matched case-control pairs were missing mtDNA content. Unconditional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (95% CIs). The unconditional logistic models were adjusted for age, gender, pack-years of smoking, current type of fuel used for cooking and heating (smoky coal, smokeless coal or wood), and lifetime smoky coal use. Stratified analyses were conducted to assess potential effect measure modification by age (≤ 57 years and >57 years), sex, smoking status (ever and never smokers), and lifetime smoky coal use (<130 tons and ≥ 130 tons). Because none of the female participants reported smoking tobacco, analyses stratified by smoking status were restricted to male participants. A log transformation was used to normalize the mtDNA content variable. The p for trend statistics was determined by the p -value for the coefficient of the log transformed mtDNA content as a continuous variable, while adjusting for covariates. P for interaction was determined with

unconditional logistic regression by the p -value of the coefficient for the product term of mtDNA content and selected variables, while adjusting for other covariates.

Results

Descriptive characteristics (e.g., age and sex) were similar between cases and controls, with a few notable exceptions (Table 1). For instance, lifetime smoky coal use was significantly higher among lung cancer cases (mean = 173.6 tons) compared with controls (mean = 129.8 tons). Pack-years of smoking was not substantially higher among lung cancer cases (mean pack-years smoking = 18.2) than among controls (mean pack-years smoking = 14.9).

We examined the relationship between mtDNA content and several hypothesized determinants of mtDNA content, including, age, smoking, and lifetime smoky coal use, among the controls. Weak inverse correlations were observed between age and mtDNA content and between pack-years of smoking and mtDNA content; the Spearman correlation coefficients were -0.25 ($p = 0.0097$) and -0.20 ($p = 0.0401$) for age and mtDNA content and pack-years of smoking, respectively. No correlation was observed between lifetime smoky coal use and mtDNA content.

The risk of lung cancer was positively associated with mtDNA content. Nearly a two-fold increase in the OR was observed for individuals with an mtDNA content value greater than the median compared with individuals below the median (OR_{adjusted} = 1.8; 95% CI = 1.0–3.2). However, the response gradient was not monotonic when mtDNA content was categorized into tertiles (1st tertile: OR = 1.0 (ref); 2nd tertile: OR = 2.1 (1.0–4.2); 3rd tertile: OR = 1.6 (0.8–3.4)); the p for linear trend was not significant (p trend = 0.41).

The association between mtDNA content and lung cancer risk were not meaningfully different between males and females (Table 3), although the risk estimates were slightly higher among females than males. When stratified by lifetime smoky coal use (higher vs. low), the stratum specific risk estimates were similar. Conversely, the association between mtDNA content and lung cancer risk was modified by an age. For those above the median age of the controls (>57 years), the risk of lung cancer was positively associated with mtDNA content, while lung cancer risk was not associated with mtDNA content for those below the median. We selected several other cut points (e.g., 50, 65, and 70) to dichotomize age for the stratified analyses and observed similar risk estimates to those using 57 years of age as the cut off point (data not shown). We stratified by smoking status among the male participants, however, the number of never smoking cases and controls were small and the resulting risk estimate was difficult to interpret due to large confidence interval (data not shown).

Discussion

We found a positive association between mtDNA content and the risk of lung cancer, although there was little evidence of an exposure-response gradient. The association between mtDNA content and lung cancer was limited to those over the age of 57 years. It is unlikely that the association between mtDNA content and lung cancer among the older age stratum was an artifact due to the subjective selection of the cut point of 57 year because, regardless of the cutpoints selected, an association was most evident in the older age stratum. In addition, the association between mtDNA content and lung cancer risk was more prominent among women than among men, although the p for interaction was not significant (p interaction = 0.508). Similarly, the risk estimates were slightly more pronounced among those with relatively high lifetime smoky coal use. Nonetheless, none of these interactions were statistically significant. Therefore, we cannot rule out the possibility that the divergent odds ratios occurred by chance, with the exception of age.

A number of reports have examined mtDNA content in tumor tissue from various sites, including hepatocellular carcinoma (18), and cancers of the breast (19), thyroid (19), head and neck (20,21), endometrium (22), stomach (23), and kidney (24,25). A common feature of these studies has been to compare mtDNA content in tumor cells with matched normal cells from the same subject. Collectively, these studies suggest that mtDNA content is altered in tumor cells compared with the matched normal cells, however increases as well as decreases have been observed and it is unclear whether alterations in mtDNA content contributes to tumorigenesis or is a secondary consequence of the carcinogenic process (14,24).

Our study, on the other hand, examined mtDNA content from cells expectorated in sputum and our results reflect changes in the target tissue rather than a surrogate tissue such as blood. In addition we compared mtDNA content between lung cancer cases and individuals without lung cancer; consequently, our results are not directly comparable with these previous studies. Nonetheless, our study provides preliminary evidence that alterations mtDNA content may play a role in lung cancer.

While it is unclear whether mtDNA content has an etiologic function in carcinogenesis, mitochondria have been hypothesized to have a role in carcinogenesis dating back to 1931 when Otto Warburg observed that cancer cells catabolize glucose to lactate while consuming oxygen. This is known as the Warburg effect or aerobic glycolysis. In contrast, normal cells breakdown glucose to CO₂ and H₂O by cellular respiration in presence of oxygen (26). More recently, a role in apoptosis has been demonstrated by mitochondria via the release of cytochrome c from the mitochondrial membrane (27). Other mechanisms though which mitochondria or mtDNA damage may play a role in carcinogenesis have been hypothesized, including the production of ROS that can result in nuclear DNA damage (28,29). Moreover, ROS generated by mitochondria have been demonstrated to be important cell signaling molecules that affect cell proliferation signals (11,30).

Several limitations require consideration when interpreting our findings. First, mtDNA from lung cancer cases may have been contaminated by tumor mtDNA and the association we observed may have been an artifact of the carcinogenic process that was exaggerated by a comparison between cases and lung cancer free controls. However, histological review of the sputum samples indicated that tumor cells constituted a small proportion of the total number of cells from which DNAs were extracted. Second, the sample size was relatively small and we had limited power to detect interactions. Nevertheless, we observed that mtDNA content in cells derived from sputum of lung cancer cases was increased compared with non-cases. While our results are necessarily preliminary, they do provide evidence that mtDNA content alterations may exist between lung cancer cases and controls in addition to alterations between tumor tissue and matched normal tissue.

In conclusion, we found a suggestion that mtDNA content was associated with lung cancer risk, although it remains unclear whether this response is involved in the etiology of lung cancer or is an artifact of the disease process. These results provide additional evidence that mtDNA alterations may be related to carcinogenesis and more research is warranted to determine the temporal relation between mtDNA content and lung cancer. Furthermore, epidemiologic and experimental investigations focusing on mtDNA mutations as well as mitochondrial dysfunction could provide important information on the roles that mitochondria and mtDNA may have in carcinogenesis.

References

1. Lan Q, He X, Costa DJ, et al. Indoor coal combustion emissions, GSTM1 and GSTT1 genotypes, and lung cancer risk: a case-control study in Xuan Wei, China. *Cancer Epidemiol Biomarkers Prev* 2000;9:605–608. [PubMed: 10868696]

2. He XZ, Chen W, Liu ZY, Chapman RS. An epidemiological study of lung cancer in Xuan Wei County, China: current progress. Case-control study on lung cancer and cooking fuel. *Environ Health Perspect* 1991;94:9–13. [PubMed: 1954946]
3. Chapman RS, Mumford JL, Harris DB, He ZZ, Jiang WZ, Yang RD. The epidemiology of lung cancer in Xuan Wei, China: current progress, issues, and research strategies. *Arch Environ Health* 1988;43:180–185. [PubMed: 3377554]
4. Mumford JL, He XZ, Chapman RS, et al. Lung cancer and indoor air pollution in Xuan Wei, China. *Science* 1987;235:217–220. [PubMed: 3798109]
5. Allen JA, Coombs MM. Covalent binding of polycyclic aromatic compounds to mitochondrial and nuclear DNA. *Nature* 1980;287:244–245. [PubMed: 7432460]
6. Backer JM, Weinstein IB. Mitochondrial DNA is a major cellular target for a dihydrodiol-epoxide derivative of benzo[a]pyrene. *Science* 1980;209:297–299. [PubMed: 6770466]
7. Backer JM, Weinstein IB. Interaction of benzo(a)pyrene and its dihydrodiol-epoxide derivative with nuclear and mitochondrial DNA in C3H10T 1/2 cell cultures. *Cancer Res* 1982;42:2764–2769. [PubMed: 6282450]
8. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 2005;39:359–407. [PubMed: 16285865]
9. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;82:47–95. [PubMed: 11773609]
10. Kamata H, Hirata H. Redox regulation of cellular signalling. *Cell Signal* 1999;11:1–14. [PubMed: 10206339]
11. Nemoto S, Takeda K, Yu ZX, Ferrans VJ, Finkel T. Role for mitochondrial oxidants as regulators of cellular metabolism. *Mol Cell Biol* 2000;20:7311–7318. [PubMed: 10982848]
12. Cheng WC, Berman SB, Ivanovska I, et al. Mitochondrial factors with dual roles in death and survival. *Oncogene* 2006;25:4697–4705. [PubMed: 16892083]
13. Mignotte B, Vayssiere JL. Mitochondria and apoptosis. *Eur J Biochem* 1998;252:1–15. [PubMed: 9523706]
14. Penta JS, Johnson FM, Wachsmann JT, Copeland WC. Mitochondrial DNA in human malignancy. *Mutat Res* 2001;488:119–133. [PubMed: 11344040]
15. Lee HC, Lu CY, Fahn HJ, Wei YH. Aging- and smoking-associated alteration in the relative content of mitochondrial DNA in human lung. *FEBS Lett* 1998;441:292–296. [PubMed: 9883902]
16. Lan Q, Mumford JL, Shen M, et al. Oxidative damage-related genes AKR1C3 and OGG1 modulate risks for lung cancer due to exposure to PAH-rich coal combustion emissions. *Carcinogenesis* 2004;25:2177–2181. [PubMed: 15284179]
17. Liu CS, Tsai CS, Kuo CL, et al. Oxidative stress-related alteration of the copy number of mitochondrial DNA in human leukocytes. *Free Radic Res* 2003;37:1307–1317. [PubMed: 14753755]
18. Yin PH, Lee HC, Chau GY, et al. Alteration of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma. *Br J Cancer* 2004;90:2390–2396. [PubMed: 15150555]
19. Mambo E, Chatterjee A, Xing M, et al. Tumor-specific changes in mtDNA content in human cancer. *Int J Cancer* 2005;116:920–924. [PubMed: 15856456]
20. Jiang WW, Rosenbaum E, Mambo E, et al. Decreased mitochondrial DNA content in posttreatment salivary rinses from head and neck cancer patients. *Clin Cancer Res* 2006;12:1564–1569. [PubMed: 16533782]
21. Kim MM, Clinger JD, Masayesva BG, et al. Mitochondrial DNA quantity increases with histopathologic grade in premalignant and malignant head and neck lesions. *Clin Cancer Res* 2004;10:8512–8515. [PubMed: 15623632]
22. Wang Y, Liu VW, Xue WC, Tsang PC, Cheung AN, Ngan HY. The increase of mitochondrial DNA content in endometrial adenocarcinoma cells: a quantitative study using laser-captured microdissected tissues. *Gynecol Oncol* 2005;98:104–110. [PubMed: 15921730]
23. Wu CW, Yin PH, Hung WY, et al. Mitochondrial DNA mutations and mitochondrial DNA depletion in gastric cancer. *Genes Chromosomes Cancer* 2005;44:19–28. [PubMed: 15892105]
24. Meierhofer D, Mayr JA, Foetschl U, et al. Decrease of mitochondrial DNA content and energy metabolism in renal cell carcinoma. *Carcinogenesis* 2004;25:1005–1010. [PubMed: 14764459]

25. Simonnet H, Alazard N, Pfeiffer K, et al. Low mitochondrial respiratory chain content correlates with tumor aggressiveness in renal cell carcinoma. *Carcinogenesis* 2002;23:759–768. [PubMed: 12016148]
26. Wallace DC. Mitochondria and cancer: Warburg addressed. *Cold Spring Harb Symp Quant Biol* 2005;70:363–374. [PubMed: 16869773]
27. Spierings D, McStay G, Saleh M, et al. Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. *Science* 2005;310:66–67. [PubMed: 16210526]
28. Bandy B, Davison AJ. Mitochondrial mutations may increase oxidative stress: implications for carcinogenesis and aging? *Free Radic Biol Med* 1990;8:523–539. [PubMed: 2193852]
29. Shay JW, Werbin H. Are mitochondrial DNA mutations involved in the carcinogenic process? *Mutat Res* 1987;186:149–160. [PubMed: 3306359]
30. Singh KK, Kulawiec M, Still I, Desouki MM, Geradts J, Matsui S. Inter-genomic cross talk between mitochondria and the nucleus plays an important role in tumorigenesis. *Gene* 2005;354:140–146. [PubMed: 15979824]

Table 1

Selected Characteristics of lung cancer cases and controls in Xuan Wei, China

Characteristic	Cases (n=113)	Controls (n=107)	p-value
	Number (%)		
Sex			
Male	73 (65%)	68(64%)	0.0.871 ^a
Female	40 (35%)	39(36%)	
mtDNA Content			
≤157	41 (36.3%)	53 (50%)	0.047 ^a
>157	72 (63.7%)	54 (50%)	
	Mean (SD)		p-value
Age (years)	54.9 (11.5)	54.5 (12.8)	0.795 ^b
Smoky Coal	173.6 (107.6)	129.8 (77.6)	0.0006 ^b
Smoking (pack years) ^{\$}	18.2 (21.3)	14.9 (21.0)	0.247 ^b

^a *p* based on chi-squared test^b *p* based on *t*-test^{\$} restricted to males only

Table 2

Mitochondrial DNA Content and Lung Cancer; Xuan Wei, China

mtDNA Content	Cases (n=113)	Controls (n=107)	OR (95% CI) *
≤157	41	53	1.0
>157	72	54	1.8 (1.0–3.2)
<i>p</i> trend			0.41

* adjusted for age (continuous), type of current fuel use, sex, pack-years of smoking, and lifetime smoky coal use.

mtDNA Content and Lung Cancer Risk by Sex, Age, and Smoky Coal Use; Xuan Wei, China

Table 3

mtDNA Content	Females			Males		
	Cases	Controls	OR (95% CI)*	Cases	Controls	OR (95% CI)*
≤157	10	17	1.0	31	36	1.0
>157	30	22	2.3 (0.8–6.8)	42	32	1.6 (0.8–3.2)
p trend			0.51			0.82
p interaction			0.25			
	≤ 57 Years Old			> 57 Years Old		
≤157	21	22	1.0	20	31	1.0
>157	39	35	1.1 (0.5–2.6)	33	19	3.2 (1.3–7.7)
p trend			0.17			0.01
p interaction			0.01			
	Smoky Coal Use < 130 tons			Smoky Coal Use ≥ 130 tons		
≤157	18	31	1.0	23	22	1.0
>157	29	34	1.7 (0.7–3.8)	43	20	2.0 (0.9–4.9)
p trend			0.85			0.64
p interaction			0.69			

* Adjusted for age (continuous), type of current fuel use, sex, pack-years of smoking, and lifetime smoky coal use. The stratifying variable was not included in their respective models.