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COMMENTARY

Carcinogens that induce the A:T > T:A nucleotide substitutions in the genome

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Abstract **Recently, Ng *et al.* reported that the A:T > T:A substitutions, proposed to be a signature of aristolochic acid (AA) exposure, were detected in 76/98 (78%) of patients with hepatocellular carcinoma (HCC) from the Taiwan Province of China, and 47% to 1.7% of HCCs from the Chinese mainland and other countries harbored the nucleotide changes. However, other carcinogens, e.g., tobacco carcinogens 4-aminobiphenyl and 1,3-butadiene, air toxic vinyl chloride and its reactive metabolites chloroethylene oxide, melphalan and chlorambucil, also cause this signature in the genome. Since tobacco smoke is a worldwide public health threat and vinyl chloride distributes globally and is an air pollutant in Taiwan Province, the estimation of the patients' exposure history is the key to determine the "culprit" of the A:T > T:A mutations. Apparently, without estimation of the patients' exposure history, the conclusion of Ng *et al.* is unpersuasive and misleading.**

Keywords genomic signature; carcinogen; aristolochic acid; tobacco smoke; vinyl chloride; hepatocellular carcinoma

Introduction

Recently, Ng *et al.* sequenced the whole exomes of 98 hepatocellular carcinomas (HCC) from the Taiwan Province of China and reported that the A:T > T:A substitutions that were shown to be a signature of aristolochic acid (AA) exposure, were detected in 78% of the patients. They searched for the A:T > T:A substitutions in 1400 HCCs from diverse geographic regions, and showed that 47% to 1.7% of HCCs from the Chinese mainland, South-east Asia, Korea, Japan, North America and Europe harbored the nucleotide changes [1]. They concluded that AA is responsible for the A:T > T:A nucleotide changes in the genome and is a causal factor for HCC, though they did not estimate the dosages that the patients were exposed to AA.

Aristolochic acid: nephrotoxicity, carcinogenesis, and the A:T > T:A nucleotide substitutions

AA was considered as a nephrotoxin and a carcinogen in the 1970s for the findings that AA in flour from wheat contaminated with seeds of *Aristolochia clematis* was associated with Balkan endemic nephropathy (BEN) in rural populations along the River Danube, and an AA-containing herbal remedy administered by a weight loss clinic in Belgium was associated with a high risk of nephrotoxicity (designated as AA nephropathy, AAN) and upper urinary tract urothelial cell carcinoma [2]. Confounding evidence was that only 2%–5% of the residents in an endemic area developed BEN and only 10%–20% of patients in the slimming clinic in Brussels developed AAN [3,4]. In addition, AA had been used for 25 years in Germany by thousands of patients as an immunomodulatory drug, without reports of patients with AAN or upper urothelial tumors [2].

AA induces a nephrotoxic effect in experimental animals and causes tumors in rats [3,5]. AA was considered as the "culprit" of BEN by the findings that AA exposure induced tumors and caused the A:T → T:A transversions in *c-Ha-ras*, *c-Ki-ras*, and *N-ras* genes [5], and patients with BEN and related upper urinary tract transitional cell cancers harbored the A:T → T:A transversions in *TP53* tumor suppressor [6]. The A:T → T:A nucleotide substitutions were also seen in other genes by whole-genome and exome sequencing of AA-associated upper urothelial cancer [3].

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Table 1 Carcinogens causing the A:T>T:A nucleotide substitutions

Agents/carcinogens	Sites of related cancers	Affected genes	References
4-aminobiphenyl	Liver, bladder, urinary	*H-Ras*	[9]
1,3-Butadiene	Bone marrow	*HPRT*	[10]
AA	Upper urothelial cancer	*RAS, TP53,* exosome wide	[1,5,6,15,16]
Ethylene oxide	Stomach, marrow	*H-Ras*	[17]
Melphalan and chlorambucil	Breast, ovarian, marrow	*TP53, N-RAS, Hprt*	[13,14]
Vinyl chloride	Liver	*TP53*	[12]

Carcinogens that cause the A:T > T:A nucleotide substitutions

As compared with counterpart normal controls, cancer genomes usually have six types of nucleotide changes, G > A, G > T, G > C, A > G, A > T, and A > C. Some environmental factors cause characteristic changes—signature—in the genome, exemplified by polycyclic aromatic hydrocarbons from tobacco smoke and air pollution induce G > T genomic mutations [7]. Of note, one type of nucleotide changes can be induced by different environmental factors or carcinogens. AA causes the A:T > T:A transversions in cells, animals, and patients. Indeed, other carcinogens also induce this type of nucleotide substitutions in the genome (Table 1). For example, tobacco carcinogens 4-aminobiphenyl and 1,3-butadiene [8] cause the A:T > T:A transversions in *H-Ras* and *HPRT* genes [9,10]. Vinyl chloride, which was shown to be associated with liver cancer [11], and its reactive metabolites chloroethylene oxide [8], induce A:T > T:A transversions in *TP53* [12]. The chemotherapeutic agents melphalan and chlorambucil also induce the A:T > T:A nucleotide substitutions in *TP53*, *N-RAS*, and *Hprt* genes [13,14].

Conclusions

The A:T>T:A transversions had been reported previously in HCCs from Chinese mainland [18]. AA is able to induce the A:T > T:A nucleotide substitutions in the genome. However, other carcinogens also cause this signature. Since tobacco smoke is a worldwide public health threat and vinyl chloride distributes globally and is an air pollutant in Taiwan Province [19], the estimation of the patients' exposure history is the key to determine the "culprit"of the A:T > T:A mutations. Apparently, without estimation of the patients' exposure history, the conclusion of Ng *et al.* is unpersuasive and misleading.

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Compliance with ethics guidelines

Guangbiao Zhou and Xinchun Zhao declare no conflicts of interest. This article does not involve a research protocol requiring approval by a relevant institutional review board or ethics committee.

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