

LETTER TO THE EDITOR

Beyond Stanton and Pott hypothesis; carbon nanotubes-induced malignant mesothelioma as a disease of gene loss

Dear Editor

Two excellent review articles on the potential carcinogenic risk of carbon nanotubes (CNTs) have been recently published in your journal.^{1,2} Owing to the unique physicochemical properties and functionality of CNTs, medical diagnostic and therapeutic applications have rapidly increased. However, there is a growing concern regarding the potential hazards of CNTs and their risk to induce lung and mesothelium carcinogenesis much the same way as asbestos. Given both their medical usefulness and health risks, the nature of CNTs has been termed as the two faces of Janus.

Reactive oxygen species (ROS) accumulation generally promotes carcinogenesis synergistically with chronic inflammation. The exposure to multi-walled CNTs (MWCNTs) causes peritoneal mesotheliomas in p53 heterozygous mice,¹ which is consistent with the Stanton and Pott hypothesis describing those biopersistent fibers of particular length and diameter can be carcinogenic simply because they are fibers regardless of their physicochemical characteristics. In contrast, the exposure to single-walled CNTs (SWCNTs) and carbon nanofibers (CNFs) induces the double-strand breaks in DNA, leading to the emergence of the gain-of-functional mutation of oncogene *KRAS* 1 year after the exposure.² Furthermore, as Dr Kobayashi et al mentioned,² SWCNTs exposure is responsible for TGF β -dependent pulmonary fibrosis, which is the precursor lesion of lung cancer.

Importantly, malignant mesothelioma is considered to be a “disease of gene loss” rather than being associated with driver mutations.³ Genetic analyses have clarified the depletions and/or loss-of-functional mutations of *CDKN2A*, *NF2*, *BAP1* genes, and both p53-dependent DNA repair pathway and PI3K/Akt/mTOR signal transduction are involved in mesothelioma development. Alterations in *CDKN2A* locus with inactivation of p16 (INK4a) are reported in more than a half of malignant mesothelioma patients. Recent investigations have revealed that depletion of *p19* (*ARF*) and epigenetic silencing of p16 with *CDKN2A* hypermethylation are frequently associated with mesothelioma progression due to either amosite asbestos or long-shaped CNTs exposure.³ Mechanistically, the degree of genotoxic oxidative stress measured by 8-OHdG and the activation of pro-oncogenic signal pathways regulated by Akt, ERK1/2, and Src kinase

are quite similar in the pleura between asbestos and CNTs exposure. This fact is consistent with Dr Fukushima's review article¹ stating that exposure to MWCNTs results in asbestos-like pathologic alterations including lung carcinoma and pleural mesothelioma. Remarkably, the sustained activation of pro-oncogenic signal pathways in CNTs-induced lesions occurs largely in non-mesothelial stromal cells,³ suggesting that the presence of oxidative DNA damage in the chronically inflammatory microenvironment contributes to the hypermethylation and silencing of *CDKN2A*-coding tumor suppressor molecules (Figure 1).

Chromosome tangling theory instead of Stanton hypothesis, however, has attracted much attention in the field of occupational carcinogenesis due to asbestos exposure. Nagai et al reported that MWCNTs directly pierce mesothelial plasma and nuclear membranes, whereas asbestos fibers are internalized by mesothelial cells via encapsulation by vesicular membranous structures.⁴ For a typical instance, MWNT-7, one of the fibrous straight MWCNTs, induces polyploidy without specific genetic mutations. Animal experiments have shown that MWNT-7 exposure results in the development of pre-neoplastic hyperplasia, adenomas, and bronchioloalveolar carcinoma in the concentration-dependent manner.¹ MWCNTs which remain in the cytoplasm and uncovered by membranes are expected to injure chromosomes during mitosis as compared with asbestos.⁴ Indeed, there are multiple machineries underlying chromosomal instability bringing about carcinogenesis; faulty sister chromatid cohesion, defective centrosome duplication, telomere dysfunction, hyperactive or hypoactive spindle assembly checkpoint, and overly stable attachments of microtubules to chromosomes.⁵ The long latency of malignant mesothelioma, with decades-long chronic inflammation accompanied by the aberrant microenvironment rich in ROS and subsequently oxidative DNA damage, may advocate a multifactorial mechanism of disease progression including epigenetic modifications and loss-of-tumor suppressor genes such as p16 and p19 (Figure 1).

CONFLICTS OF INTERESTS

There are no conflicts of interest to be declared about this letter.

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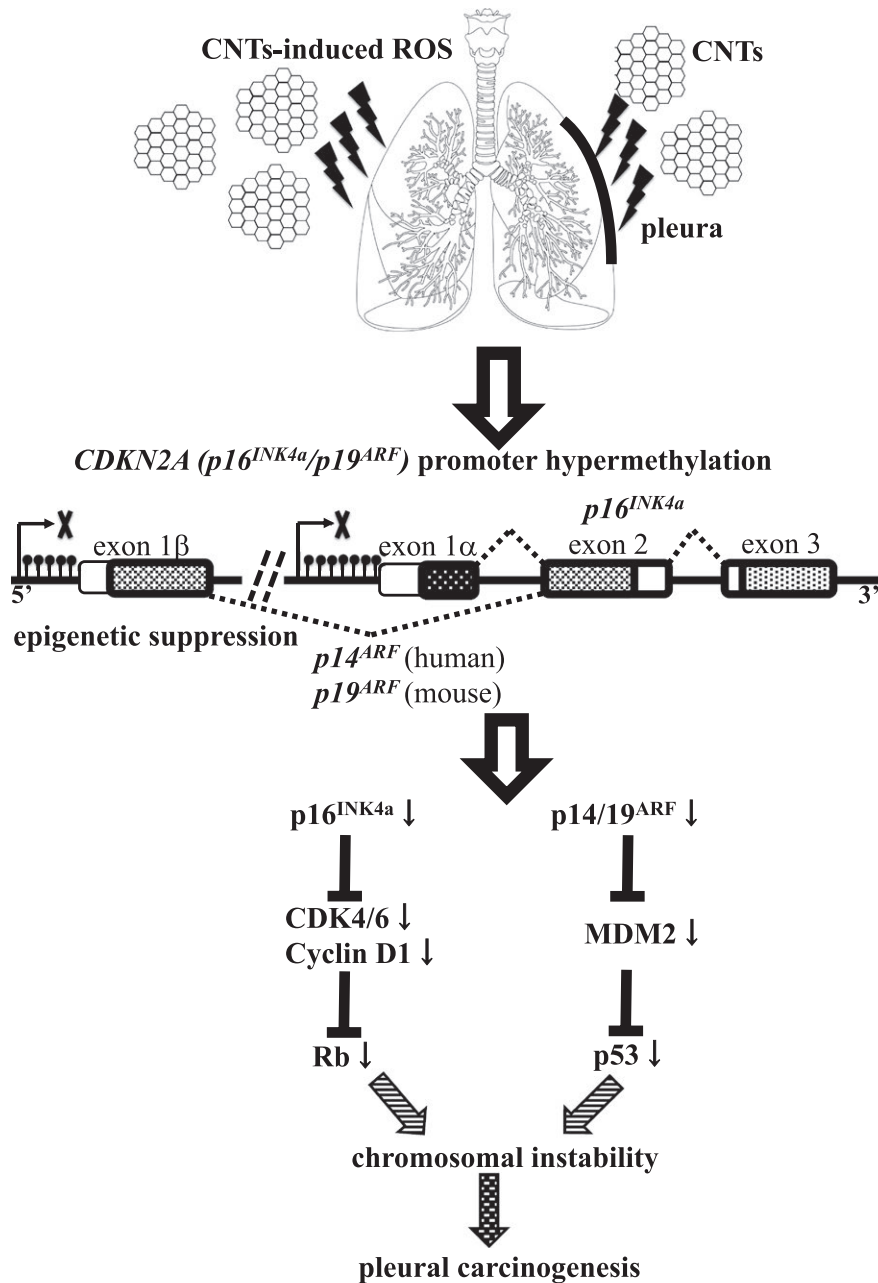


FIGURE 1 The epigenetic silencing of *CDKN2A* gene due to CNTs-induced ROS. The presence of CNTs in the pulmonary tissues provokes a large amount of oxidative stress, thereby leading to the epigenetic suppression of *CDKN2A* gene coding cyclin-dependent kinase (CDK) inhibitors characterized by p16 and p14 (human)/p19 (mouse). The absence of those CDK inhibitors is responsible for the chromosomal instability, causing the malignant transformation of pleural mesothelial cells

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REFERENCES

1. Fukushima S, Kasai T, Umeda Y, Ohnishi M, Sasaki T, Matsumoto M. Carcinogenicity of multi-walled carbon nanotubes: challenging issue on hazard assessment. *J Occup Health*. 2018;60:10-30.
2. Kobayashi N, Izumi H, Morimoto Y. Review of toxicity studies of carbon nanotubes. *J Occup Health*. 2017;59:394-407.

3. Chernova T, Murphy FA, Galavotti S, et al. Long-Fiber Carbon Nanotubes Replicate Asbestos-Induced Mesothelioma with Disruption of the Tumor Suppressor Gene Cdkn2a (Ink4a/Arf). *Curr Biol.* 2017;27:3302-3314.
4. Nagai H, Okazaki Y, Chew SH, et al. Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. *Proc Natl Acad Sci U S A.* 2011;108:E1330-E1338.
5. Bakhoun SF, Compton DA. Chromosomal instability and cancer: a complex relationship with therapeutic potential. *J Clin Invest.* 2012;122:1138-1143.