Physicochemical Properties and Factors that Induce Asbestos-Related Respiratory Disease†

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Abstract

It is thought that inhaled dusts such as asbestos and man-made mineral fibers in the lung repeatedly induce persistent inflammation and finally lead to pulmonary fibrosis and respiratory cancer. There have been many studies about whether a variety of factors, such as oxidative stress including free radicals, chemokines, inflammatory cytokines, and fibrosis-related cytokines are related to pulmonary fibrosis, lung cancer and malignant mesothelioma. In this paper, we introduce the relationship between these factors and these diseases. It is important to determine what physicochemical properties of fibrous materials such as asbestos are related to asbestos-related diseases. We show the relationship between the physicochemical properties of not only asbestos but also other fibrous materials and inflammation, fibrosis and biopersistence in the lung.

Keywords: asbestos, lung cancer, malignant mesothelioma, cumulative exposure

Asbestos exposure and respiratory tumor

It is thought that a high concentration of asbestos exposure induces lung cancer1). There are some reports that a cumulative exposure of 25–100 fiber-years induced the onset of lung cancer caused by asbestos, and the Helsinki criteria showed that a cumulative exposure of 25 fiber-years, the minimum cumulative exposure level, is necessary for the onset of lung cancer induced by asbestos2). Cumulative exposure correspond to a 2-fold risk of lung cancer. The indexes of the risk of lung cancer are 1) retained fiber levels of 2 million amphibole fibers (> 5 μm) per gram of dry lung tissue or 5 million amphibole fibers (> 1 μm) per gram of dry lung tissue measured by electron microscopy, (Fig. 1); 2) 5000 asbestos bodies per gram of dry tissue measured by light microscopy, or 5 asbestos bodies per milliliter of bronchoalveolar lavage fluid measured by light microscopy (Fig. 2); and 3) a profusion score of 1 in a chest x-ray finding, which means early asbestosis.

Henderson et al.3) proposed, as a revised version of the Helsinki criteria, that a cumulative exposure of 20 fiber-years for amphibole asbestos, cumulative exposure of 25 fiber-years for asbestos yarn spinning, cumulative exposure of 200 fiber-years for chrysotile asbestos only (work in chrysotile mine quarrying, crushing and friction materials production in Canada), or cumulative exposure of more than 25 fiber-years for the combined exposure of chrysotile and amphibole asbestos is necessary for the onset of lung cancer induced by asbestos.

The onset of malignant mesothelioma is thought to be induced by a low concentration of asbestos exposure, although there is no significant evidence of a relationship between the occurrence of mesothelioma and the amount of exposure to asbestos. Previous studies4) reported that malignant mesothelioma was contracted by workers who were engaged in indirect asbestos exposure in a shipyard or in the vehicle manufacturing industry developed malignant mesothelioma, a person who washed workers’ clothes, to which asbestos was attached, and neighboring inhabitants around asbestos mines and the asbestos product manu-

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Fig. 1 Scanning electron microscope of asbestos bodies
ufacturing industry. Therefore, asbestos exposure other than in occupation induced the disease. For that reason, in examining asbestos-related diseases, it is very important to know not only the occupational history of workers but also the residential history of families. It is thought that from cohort studies the degree of risk of developing mesothelioma by asbestos depends on the kind of asbestos, namely when the strength of chrysotile, amosite and crocidolite is 1: 100: 500, respectively\(^5\). In addition, the onset is unrelated to smoking.

**Relationship between size of asbestos and asbestos-related tumor**

The physicochemical properties of asbestos in the induction of lung cancer are thought to be 1) fibers with low solubility and 2) fibers which are thin and long.

If fibers reach the lung, some of them melt in body fluid and some are divided into small fragments. These fibers, which become small, are phagocytized by alveolar macrophages and are excreted outside the lungs with mucociliary escalator. Even if fibers have low solubility, short fibers are also excreted by alveolar macrophages. However, it is difficult for macrophages to phagocytize the fiber physically, particularly when fibers with low solubility are longer than the diameter of the macrophages. Thus fibers with low solubility and long length are deposited in the lung in the long term and will influence the lung continuously without being excreted. It is thought that fibers which are not excreted from the lung have a physically and chemically harmful effect on the lung.

Solubility tests (in vitro test to examine how fibers dissolve in medium) and inhalation studies of not only asbestos but also man-made mineral fibers have been performed\(^6\). The solubility in the solubility test is associated with pathological lesions in acute and chronic inhalation studies. In the solubility test, fibers with low solubility, such as crocidolite and amosite, induced a long half-life of the fiber in an acute inhalation study and developed pulmonary fibrosis and cancer in a chronic inhalation study. On the other hand, fibers with high solubility, such as slagwool and HT stone wool, reduced the half-life of the fiber and caused no significant pathological lesions.

Taken together, the physicochemical properties of asbestos which affect the lung tissue are its length and solubility. Long and thin fibers with low solubility induce biopersistence in the lung and finally cause lung disorders. From the clinical point of view, the length of the fiber affects the development of lung cancer. There are regulations based on the length of fiber, such as more than 1 μm or 5 μm, to measure the number of asbestos fibers in lung tissue in order to examine whether or not lung cancer is related to asbestos exposure. This may show that the length of fibers is related to the onset of lung cancer induced by asbestos.

In malignant mesothelioma, the association between physicochemical properties and the development of mesothelioma is unclear, but it has been reported that short fibers induce malignant mesothelioma. In the case of malignant mesothelioma, it may be important that the onset of mesothelioma is associated with the movement of the asbestos to the parietal pleura. The physical limitation of phagocytosis of the fibers by the macrophages may be related to the shortness of the fibers.

**Lung damage by free radicals and inflammation**

Inhalation of fibers can cause inflammation in the respiratory tract and pulmonary alveolar space in not only the acute phase but also in the chronic phase\(^7\). These inflammations, especially continuous inflammation, progress to fibrosis of the lungs and pleura, or lung tumor (malignant mesothelioma and lung cancer)\(^\)\(^7\),\(^8\). We performed intratracheal instillation of different mineral fibers to rat, and examined lung inflammation from 3 days up to 6 months\(^9\). Harmful respirable particles like crystalline silica or crocidolite asbestos, which are kinds of asbestos, caused persistent inflammation from the initial instillation until six months. However, transient inflammation was only observed early in the instillation when less harmful titanium dioxide of micron size was inhaled. In the inhalation exposure examination to rat with chrysotile for 20 days, continuous inflammation and fibrosis containing mainly neutrophils were observed\(^9\). Continuous inflammation causes lung injury, and free radicals play a central role in this injury. There are two types of free radicals, one is reactive oxygen species and the other is reactive nitrogen.
Asbestos is known as a carcinogen, and it can cause malignant pleural mesothelioma and lung cancer. However, the mechanism of carcinogenesis has not been sufficiently clarified. Its oncogenesis might be influenced by complicated factors, such as diversity of the asbestos (type, geometry, dose of the fiber, and so on), individual sensitivity, and synergistic effects with other carcinogens like cigarette. Abnormality of gene expression is broadly classified into genetic abnormality and epigenetic abnormality. Genetic abnormality is a disorder which is directly caused by a mutation in the nucleotide sequence of the gene. On the other hand, epigenetics is a study that reveals the diversity of gene expression inherited even after cell division without a change in the nucleotide sequence. Both genetic abnormality and epigenetic abnormality are associated with not only oncogenesis but also malignant progression. In cancer cells, there are two types of mutations. One is passenger mutations, which are accumulated only by chance, and the other is oncogenic driver mutations, which occur in important genes involved in the phenotype of cancer. Oncogenic driver mutations have been found in the EGF receptor, K-ras, HER2, AKT1, etc. (Table 1). In addition, there is a cancer that is completely dependent on the oncogenic signal associated with cell proliferation and survival of cancer by only one mutated gene. This is called oncogene addiction, and its representative example is L858R mutation in the EGFR gene.

In the genetic abnormality of asbestos-related lung cancer, mutations of the k-ras and TP53 (p53) have been reported. K-ras mutation is an oncogene that plays an important role in the signal transduction of the epidermal growth factor receptor (EGFR). Husafvel-Pursiainen et al. reported that asbestos exposure alone was not significantly associated with an increased occurrence of K-ras mutations. However, a strong and significant association was found between adenocarcinoma and K-ras mutation in a group of combined smoking and asbestos exposure. Nelson et al. suggested that asbestos exposure increases the likelihood of mutation at K-ras codon 12 and that this process occurs independently of the induction of interstitial fibrosis. Mutant p53 proteins acquire oncogenic properties that enable them to promote invasion, metastasis, proliferation and cell survival. Wang X et al. reported that p53 mutations occurred significantly more frequently in patients with a history of occupational exposure to
asbestos. It has also been reported that the mutation of p53 gene is common in asbestos-associated cancers. An analysis of specific gene copy number changes in asbestos-related lung cancer revealed some allelic imbalances. In particular, allelic imbalances of 19p, 9q33, and 2p16 were important in asbestos-related lung cancer. FHIT, a candidate tumor suppressor gene, contains the FRA3B common fragile site and is highly susceptible to carcinogen damage. Deletion of the FHIT gene in the chromosome 3p14.2 and reduced expression of the FHIT protein are correlated with malignant non-small cell lung cancer. It has also been indicated that these mutations are associated with smoking and asbestos exposure.

There are many reports about the genetic abnormality of malignant pleural mesothelioma. Mutations of K-ras and p53 in malignant pleural mesothelioma have been reported in the same way as other malignant tumors. However, association with malignant pleural mesothelioma and the BRCA1 associated protein-1 (BAP1), the cyclin-dependent kinase inhibitor 2A (p16/CDKN2A), neurofibromin 2 (NF2), and EGFR genes have been well investigated. BAP1 binds to BRCA1 and acts as a tumor suppressor gene, contains the FRA3B common fragile site and is highly susceptible to carcinogen damage. Deletion of the FHIT gene in the chromosome 3p14.2 and reduced expression of the FHIT protein are correlated with malignant non-small cell lung cancer. It has also been indicated that these mutations are associated with smoking and asbestos exposure.

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Table 1: Oncogenic driver mutations for lung cancer

| Entrez GeneID | Entrez GeneID |
|---------------|---------------|
| KRAS (Kirsten rat sarcoma viral oncogene homolog) | 3845 |
| EGFR (epidermal growth factor receptor) | 1956 |
| HER2 (v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2) | 2064 |
| BRAF (v-raf murine sarcoma viral oncogene homolog B) | 673 |
| MET (met proto-oncogene, hepatocyte growth factor receptor) | 4233 |
| AKT1 (v-akt thymoma viral oncogene homolog 1) | 207 |
| MAP2K1 (mitogen-activated protein kinase kinase 1) | 5604 |
| PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) | 5290 |
| EML4-ALK | 27436/238 |

* EML4-ALK is a product of the fusion gene lined the N-terminus of EML4 (echinoderm microtubule associated protein like 4, GeneID 27436) and the C-terminus of ALK (anaplastic lymphoma receptor tyrosine kinase, GeneID 238).

The status of DNA methylation, structural changes in the chromatin by chemical modification of histones (Table 2), the action of non-coding RNA, etc., are important in epigenetic abnormality. For the methylation of DNA, guanine and cytosine rich regions as the promoter of the gene (CpG island) are easily transferred by the addition of a methyl group by a specific enzyme. Transcription factor can not bind to the methylated promoter and transcriptional activity is suppressed. For example, when the promoter or cording region of a tumor suppressor gene is methylated, tumor suppressor proteins can not be synthesized and the tumor suppression effect is attenuated. Same reported DNA-hypermethylated tumor suppressor genes are, p16/CDKN2A, mutL homologue 1 (MLH1), epithelial-cadherin (E-cadherin), runt-related transcription factor 3 (RUNX3), adenomatous polyposis coli (APC), O(6)-methylguanine-DNA methyltransferase (MGMT), Ras association domain family 1A (RASSF1A), death-associated protein kinase (DAPK), cell adhesion molecule 1 (CADM1), retinoic acid receptor beta (RARB), metalloproteinase inhibitor 3 (TIMP3), and FHIT.

Hypermethylation of tumor suppressor genes is also involved in malignant mesothelioma and lung cancer caused by asbestos. When Dammann et al. analyzed the methylation from the lung tissue of lung cancer patients, hypermethylation of p16 was observed in 47% of lung cancer tissue and in 14% of normal lung tissue of patients. They also reported that there is a correlation between inactivation of p16 and asbestos exposure. On the other hand, Fujii et al. reported that hypermethylation of p16 was observed in 7.7% of malignant pleural mesothelioma, which was lower than 30.4% of lung cancer. Further
target mRNA and reduces the protein expression by inhibiting translation. An association between miRNA and gene expression is not affected by asbestos exposure. Some reports indicate the target genes of miRNA and the effects on cell transformation. There are few reports of miRNA in asbestos-related lung cancer and biomarkers for early detection of malignant pleural mesothelioma is anticipated.

**Conclusion**

It is thought that asbestos inhaled into the lungs causes inflammation and eventually leads to pulmonary fibrosis and tumors. In this inflammation, free radicals such as those produced by reactive oxygen species and reactive nitrogen oxide species induce not only cell and tissue damage but also the progression of fibrosis and inflammation. These observations are forming a consensus that continuous inflammation is important in the formation of pathogenesis. It is believed that additional mutations in the bronchial-alveolar epithelial cells and pleural mesothelial cells cause the onset of lung cancer and pleural mesothelioma, respectively. The characteristics of the fibers that are related to fibrosis and carcinogenicity are low solubility and thin-long type. It is necessary to elucidate the molecular mechanisms of asbestos-related lung cancer and malignant pleural mesothelioma. The development of biomarkers using serum miRNA is also required. In particular, the development of biomarkers to distinguish between asbestos-related and non-asbestos-related lung cancer and biomarkers for early detection of malignant pleural mesothelioma is anticipated.

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