Summary of JIFSAN meeting presentation "Importance of Mineral Type, Form and Dimensions in Carcinogenic Responses" Brooke T. Mossman, Department of Pathology and Laboratory Medicine, University of Vermont College of Medicine, Burlington, VT

Many properties of minerals are important in their toxicity and carcinogenicity. For example, over a dozen different mineralogical features have been considered in developing a general model for predicting the toxicity and pathogenicity of mineral fibers (Gualtieri, Mossman and Roggli, 2017). Of these many properties, dimensions have been studied most extensively. Beginning with the classical experiments of Stanton et al. (1981), it has been recognized that long, thin fibers (> 8 microns in length and <.25 microns in diameter) are associated with chronic inflammation, pulmonary fibrosis and mesotheliomas in rodents and humans (reviewed in Barlow et al., 2018; Roggli 2015).

Mechanisms of mesothelioma development have been studied extensively because it is a rare tumor associated primarily with human exposures to the durable rod-like amphibole asbestos fibers, amosite and crocidolite asbestos (Garabrant and Pastula, 2018). This tumor arises in mesothelial cells that line the lung and peritoneal cavities. Unlike short fibers (< 5 microns) or short, thick cleavage fragments, long asbestos fibers can align themselves with airways and penetrate the deep lung to get to the pleura. Because of their large size, they are not effectively removed by normal clearance mechanisms including alveolar macrophages that cannot engulf or remove long fibers. At low concentrations, long fibers may pass through stomata to lymphatic channels for elimination. Stomata are approximately 10 microns in diameter and exist between mesothelial cells. At high concentrations of fibers, a bottleneck-like phenomenon occurs whereby these channels are blocked, and fibers remain at sites of tumor development (Moalli et al. 1987; Murphy et al., 2011). In contrast, smaller fragments of minerals may drain out through the lymphatic system.

In addition to the role of fiber dimensions, other properties have been linked to mesothelioma development by amosite or crocidolite asbestos (Guthrie and Mossman,1993; Mossman, 2018). These

include: 1) high iron content driving production of oxidant species that cause oxidation of DNA and stimulation of cell signaling pathways after fiber contact or cell uptake (Janssen et al., 1994). These pathways may arise from receptor stimulation or oxidant liberation by asbestos fibers outside or within the cell when normal cell defense mechanisms are overloaded at high concentrations of fibers. Chains of signaling molecules may also impact transcription factors interacting with genes to cause aberrant cell growth and altered function that are hallmarks of the cancer process; 2) surface availability of iron in a form that drives redox reactions. In this regard, Fe^{+3} drives Fenton-type reactions producing toxic oxidants. Conversely, forms of iron such as ferritin which comprises ferruginous bodies in the lung do not drive these reactions and may be protective when fibers are coated (Gualtieri, Mossman and Roggli, 2017). Gunter and colleagues (2011) have shown differences in iron redox potential between asbestiform and nonasbestiform amphiboles, and Guthrie (1997) emphasizes the significant replacement of iron by magnesium in nonasbestiform riebeckite as compared to crocidolite asbestos; 3) crystal structure and growth. As emphasized in the presentation of Dr. Wylie at this meeting, cleavage fragments including talc do not break parallel to their length and do not have narrow widths. In contrast, crocidolite and amosite asbestos fibers break lengthwise into thinner fibers that have different clearance mechanisms or interactions with cells compared to nonasbestiform fragments; 4) fiber geometry. Studies comparatively with synthetic nanofibers have shown that needle-like shape, as opposed to curly or tangled fibers, is critical to tumor development (Nagai, 2013). Fiber rigidity is also linked to carcinogenicity (Nagai, 2011); 5) surface features of asbestiform fibers vs. nonasbestiform particles are also different in that the latter has surface defects that may affect their reactivity with cells, availability of iron, and chemical composition. 6) fiber durability has been linked to the increased risk of human mesotheliomas after exposures to crocidolite or amosite asbestos that persist in human lungs for decades. Durability may be governed by fiber chemistry, crystallinity, surface defects, and dimensions.

We have explored the roles of crocidolite asbestos comparatively with nonasbestiform riebeckite (Woodworth et al. 1983; Marsh and Mossman, 1988; Janssen et al., 1994; Janssen et al., 1997; Goldberg et al., 1997; Taylor et al., 2013), fibrous talc (Wylie et al., 1997) and nonfibrous talc (Shukla et al., 2009; Hillegass et al., 2010) in several studies examining mechanisms of cancer development in mesothelial and lung epithelial cells. In addition, we have used nonpathogenic particulates (glass beads, fine titanium dioxide, polystyrene beads, etc.) as negative controls in these experiments. These studies have demonstrated that markers for the development of cancers are not observed with talcs and nonasbestiform particles. Our studies are bolstered by chronic studies in animals that do not demonstrate mesotheliomas after inhalation or injections of these materials (Smith et al., 1979; Stanton et al., 1981; Stenback and Rowland, 1978; Wehner et al., 1977; Wagner et al., 1982; Coffin et al, 1992; Cyphert et al., 2016).

Future studies should focus on collaboration between mineralogists and cancer biologists to gain a comprehensive understanding of why nonasbestiform cleavage fragments do not exhibit carcinogenicity. Comparative experiments using asbestos fibers and their nonasbestiform counterparts, i.e. tremolite asbestos vs. nonasbestiform tremolite, are encouraged with negative controls to include glass beads or other nonpathogenic particulates. Characterization of minerals should employ methods that distinguish between asbestiform and nonasbestiform amphiboles and their size dimensions. Lastly, dose response experiments are important in assessing numbers or concentrations of asbestos fibers with carcinogenic potential. In this regard, nonpathogenic minerals at high or “overload” levels can give rise to indicators of oxidant stress and release of oxidants as a consequence of cell death.

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