Asbestos, a group of naturally occurring mineral fibers, is a significant lung carcinogen. Exposure to asbestos fibers induces the formation of reactive oxygen species in cells and, subsequently, DNA double-strand breaks (DSBs), the most serious form of DNA damage related to transformation of cells. As a part of the DNA damage response, H2AX histone variant molecules are phosphorylated at ser139, producing γH2AX foci at the site of DSBs, and these serve as a docking site for DNA repair molecule complexes. Thereby, γH2AX foci are considered a sensitive marker for DSBs. DNA damage response has not thoroughly been investigated in particle carcinogenesis. We studied fiber-induced DSB formation in two human lung epithelial cell lines, the cancer cell line A549 and the transformed cell line BEAS-2B to provide further knowledge on DNA damage associated with fiber exposure as a risk factor for lung cancer.

A sub-lethal asbestos fiber concentration of 2 µg /cm^2 was determined, by cell viability tests, as suitable for working with the A549 and BEAS-2B cells. Then, we studied the kinetics of fiber induced DSB formation in terms of H2AX phosphorylation. Foci of γH2AX can be detected using specific antibody in immunocytochemistry assays. A549 and BEAS-2B cells were exposed to asbestos fibers for 4, 24 and 48 h, and γH2AX foci were counted. Concurrent non-exposed control cultures were settled at each time point. At 4 h, the control group displayed remarkable endogenous DSB formation, possibly due to replication stress. In exposed cultures, the formation of γH2AX foci peaked at 24 h. We also explored whether it is possible, by using γH2AX antibody in a chromatin immunoprecipitation followed by next generation sequencing, to find out the distribution of DSBs at 24 h in the fiber-exposed A549 cells. The sequences involved in γH2AX foci were genome-widely distributed. To conclude, the kinetics of fiber-induced γH2AX foci formation in the A549 and BEAS-2B cells was partly interfered by endogenously formed DSBs. This study revealed sequences that may be implicated within γH2AX foci for further elucidation on their contribution to lung tumor progression in particle carcinogenesis (Kettunen et al 2013).

Kettunen E, Tuominen P, Suhonen S, Catalán J, Anttila S, Nymark P, Norppa H. Fiber-induced DNA damage response in human lung epithelial cells. Proceedings - American Association for Cancer Research Annual Meeting Vol 54, 2013, #627, page 153.