cosis-related) lung cancer. Furthermore, no mutations in codon 12 of K-ras gene (predominant in common lung cancer) were found in LCWS. These findings in the mutational spectrum support a carcinogenic effect of silica dust at the DNA molecular level.

We also observed the differences of the silica induced cytotoxicity and hprt gene mutagenesis between lung fibroblasts and alveolar type II epithelial cells. The proliferation cytotoxicity was detected by MTT (3-[4,5-Dimethylthiazolzyl]-2,5-Diphenyl Tetrazolium Bromide) coloric method. Mutation in the hprt gene was selected by culture in the presence of the toxic purine analog, 6-thioguanine (6-TG). Alveolar type II cells were more sensitive than lung fibroblasts for proliferation inhibition. The median proliferation inhibition concentration (IC50) of silica to epithelial cells was 140 μg/cm², whereas to fibroblasts was 282 μg/cm². The hprt gene mutation frequency in alveolar type II cells was statistically higher than that in fibroblasts ($P<0.05$). We concluded that there were significant differences of both silica-induced cell proliferation inhibition and hprt gene mutation between rat lung fibroblasts and type II epithelial cells. Type II epithelial cells were more sensitive in cytotoxicity and hprt gene mutagenesis to silica dust than lung fibroblasts were. By transferring hTERT gene into human embryo cell, a telomerase positive cell line was established. To compare telomerase positive cells and telomerase negative cells the following experiments were conducted: single-cell gel electrophoresis (SCGE), cell growth curve, karyotype analysis and malignant transformation.

The results showed that there were the same karyotype between these two cell strains, but the ability of DNA damage repair in telomerase positive cells is significantly higher than in the other one. When the cells were treated by silica and asbestos separately, the telomerase positive cell line had higher malignant transformation rate.

59 Activation of protein kinase C is required for silica-induced activation of the MAP kinase-AP-1 pathway

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Silica particles are considered to be fibrogenic agents and have been recently designated as carcinogenic, but the mechanisms for cancer initiation and progression are not well understood. By using an AP-1-luciferase reporter cell line, we demonstrated that activator protein-1 (AP-1) activation by crystalline silica is through mitogen-activated protein kinases (MAPK), i.e., ERKs and p38 kinases. The present study investigates the role of protein kinase C (PKC) in silica-induced activation of the MAPK-AP-1 pathway. Treatment of JB6 cells with freshly fractured silica stimulated translocation of PKC", PKC*, and PKC, from the cytosol to the membrane and activated AP-1 activity. Rotterin (a selective inhibitor of PKC*), RO-32-0432 (a relative specific inhibitor for PKCΔ), and calphostin C and bisindolylmaleimide I (general PKC inhibitors for all the subtypes), inhibited silica-stimulated AP-1 activation. These effects of PKC inhibitors were dose dependent. Dominate negative mutant transfectants of PKCΔ and PKC, markedly blocked AP-1 activation induced by silica. Western blot analysis using phosphor-specific antibodies indicates that PKC inhibitors or dominant negative mutant transfectants of PKCΔ or PKC, also blocked silica-induced phosphor ylation of ERKs and p38 kinase. These results demonstrate that PKCΔ, PKC*, and PKC, mediate silica-induced AP-1 activation through MAP kinase (ERKs and p38 kinases) pathways.

60 Regulation of silica-induced apoptosis by the tumor suppressor gene, p53

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Occupational exposure to mineral dusts, such as silica, has been associated with progressive pulmonary inflammation and fibrosis. Increasing evidence suggests that excessive induction of apoptosis (programmed cell death) by silica may be a contributing factor to the development of silicosis. However, the molecular mechanisms involved in this process are poorly understood. Since the tumor suppressor, p53, is a key transcription factor for the regulation of many important apoptosis-related genes, we hypothesized that p53 may play an important role in silica-induced apoptosis and that abnormal regulation of p53 by silica may contribute to exaggerated induction of apoptosis and subsequent lung disorders. To test this hypothesis, p53 activity and apoptosis in silica-treated cells or experimental animals were investigated. The role of p53 in silica-induced apoptosis was also studied by employing p53-deficient ($p53^{-/-}$) cells or $p53^{-/-}$ knockout mice. Using JB6 cells stably transfected with a p53-luciferase reporter plasmid, we found that exposure of cells to freshly fractured silica caused a dose and time dependent transactivation of p53 activity. Western blot analysis indicated that silica stimulated p53 protein expression and its phosphorylation at the FL.393 position. Freshly fractured silica also induced cell apoptosis in a cell culture system, as determined by TUNEL and DNA ladder assays. In contrast, silica-induced apoptosis was significantly blocked in p53-deficient ($p53^{-/-}$) cells. Similar results were obtained in *in vivo* studies, using experimental animals. Exposure of wild-type ($p53^{+/+}$) mice to silica via intratracheal instillation (3 mg/100 g body weight) resulted in an increase in lung cell apoptosis with minimal necrosis. In contrast, silica-induced apoptotic and necrotic effects on the lung cells were markedly inhibited in $p53^{-/-}$ knockout mice. These results indicate that: (1) silica induces p53 transactivation in cell culture systems; (2) silica-induced p53 activation is via induction and phosphorylation of p53 protein; (3) silica stimulates apoptosis in both *in vitro* and *in vivo* systems; and (4) p53 is required for silica-induced apoptosis. These studies demonstrate for the first time that p53 plays a crucial role in the regulation of silica-induced apoptosis and possibly to silica-induced lung pathogenesis.

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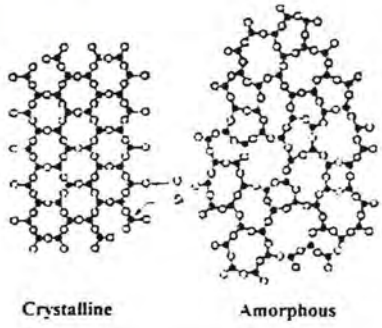

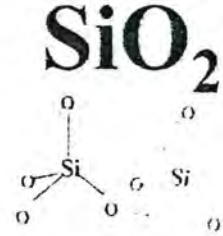

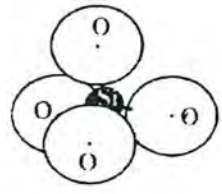

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