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Selective hyperexpression of c-*jun* oncoprotein by glass fiber- and silica-transformed BALB/c-3T3 cells

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Abstract

Mining and mineral processing are important industries in the United States. A large number of workers are potentially exposed to silica during mining and to glass fibers during manufacturing. There is a concern regarding lung cancer risk among workers exposed to silica and glass fibers. Our previous studies showed that both glass fibers and silica induced transformation of BALB/c-3T3 cells. In order to explore the relationship between silica and glass fiber-induced cell transformation and oncoprotein expression, the protein products of seven proto-oncogenes (c-K-*ras*, c-H-*ras*, c-*sis*, c-*myc*, c-*myb*, c-*erb* B1 and c-*jun*) and one tumor suppressor gene (*p53*) were examined in BALB/c-3T3 cells transformed by glass fibers or silica using immunoblotting with specific monoclonal or polyclonal antibodies. The results showed that all transformants, including eight induced by glass fibers and eight by silica (Min-U-Sil 5), were positive for c-*jun* protein expression; the level of c-*jun* protein was elevated 8–21-fold in these transformants. Other protooncogene proteins in transformed cells were either not detectable or not different from non-transformed cells. These results suggest that the overexpression of c-*jun* is common in BALB/c-3T3 transformed cells induced by glass fibers or silica. It seems, therefore, that the expression of c-*jun* may play an important role in the transformation process. © 1997 Elsevier Science Ireland Ltd. All rights reserved

Keywords: Mineral particles; Cell transformation; BALB/c-3T3; Oncoproteins; Western blotting

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1. Introduction

Glass fibers and silica are widely used in construction, plastics, glass, communication, and decoration industries [1,2]. Since silica is being mined and glass fibers are being manufactured in increasing amounts, more and more workers are potentially exposed to these agents. Concern has been raised regarding whether these substances can induce neoplastic changes and whether there is a relationship between lung cancer risk and exposure to glass fibers and silica. It has been demonstrated that both mineral dusts and fibers are potentially carcinogenic in mammalian systems; however, epidemiologic studies have had inconsistent results [2,3].

Cell transformation is considered a useful in vitro model for studies of tumor induction and neoplastic growth. Glass fibers and silica induced a concentration-dependent increase in morphological transformation in cultured Syrian hamster embryo cells [3–6].

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Our studies have shown that both agents induced BALB/c-3T3 cell transformation [7,8]. Results of DNA transfection and soft agar cloning analyses indicate that the transformants have neoplastic properties [7,8]. However, there has been little information regarding the molecular mechanism and the process of neoplastic change in the transformed cells induced by glass fibers and silica.

Oncoprotein overexpression has often been found in human tumor cells and has been suggested to be an important process in cancer induction [9–11]. In the present study, the relationship between glass fiber- and silica-induced cell transformation and oncoprotein expression of the transformants was investigated.

2. Materials and methods

2.1. Transformed cells, cell culture and media

Transformed BALB/c-3T3 cells induced by glass fibers (Owens-Corning AAA-10 microfiber) or silica (Min-U-Sil 5) were isolated from type III foci [7] and grown in Eagle's minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 100 units penicillin, and 0.1 mg streptomycin/ml, and 10% fetal bovine serum. These transformed cells were then incubated in 5% CO_2 at 37°C. The medium and reagents were obtained from Gibco (Grand Island, NY). Phosphate buffered saline (PBS) was purchased from Microbiology Systems (Cockeysville, MD).

2.2. Antibodies

Eight primary antibodies were used in the study to detect proteins produced by seven proto-oncogenes (c-K-*ras*, c-H-*ras*, c-*sis*, c-*myc*, c-*myb*, c-*erb* B1, and c-*jun*) and one tumor suppressor gene (*p53*). Mouse monoclonal antibodies specific for c-K-*ras*, c-H-*ras*, c-*sis*, c-*myc*, c-*myb* and c-*erb* B1 were obtained from Quality Biotech (Camden, NY); *p53* (Ab-3) mouse monoclonal (IgG_1) antibody and rabbit polyclonal IgG antibody against c-*jun*/Ap-1 (Ab-1) were obtained from Oncogene Science (Uniondale, NY). The secondary antibody (i.e. biotinylated, goat anti-mouse IgG) and alkaline phosphatase (Ap)-conjugated streptavidin were purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL).

2.3. Preparation of cell lysate

Transformed BALB/c-3T3 cell lines, eight induced by glass fibers and eight by silica [7], were used in this study. Exponentially growing cells were harvested and resuspended in cold lysis buffer (1.5×10^7 cells/ml) which contained 50 μg soybean trypsin inhibitor/ml, 0.5 μg aprotinin/ml, 0.1% deoxycholic acid, 0.1% sodium azide, and 5 mM EDTA in 0.1 M Tris buffer (pH 8). Cell suspensions were kept on ice for 20 min and then sonicated three times, 20 s each, at approximately 0°C. The lysates were centrifuged at 13 000 rpm for 12 min at 4°C, and the protein concentrations of the supernatant were measured by the Lowry method using Bio-Rad reagents (Bio-Rad, Richmond, CA) and bovine gamma globulin (Bio-Rad) as a standard.

2.4. Western blot analysis

The procedure followed the method described by Niman et al. [12] with slight modification. Briefly, 5 μg protein from each cell lysate was mixed with 10 μl of loading buffer (6.25% sodium dodecyl sulfate, 6.25% glycerol, 2.5% 2-mercaptoethanol) in deionized and distilled water, and placed in a 95°C Dri-bath for 3 min. Samples were then loaded in a 5–17% polyacrylamide gradient gel, electrophoretically separated, and transferred to Immobilon™ PVDF transfer membranes (Millipore, Bedford, MA). Pre-stained SDS-PAGE molecular weight standards were used to monitor the electrophoresis and subsequent transfer. After blocking with PBS containing 3% bovine serum and 0.1% Triton X-100, the blotted membranes were incubated overnight at 4°C with primary antibodies (diluted 1:2000 for those from Quality Biotech and 1:100 for those from Oncogene Science) and directed against synthetic peptides representing fragments or whole oncoprotein molecules. The membranes were washed three times for 10 min each and incubated for 1 h at room temperature with the biotinylated secondary antibody. Again the membranes were washed three times, 10 min each, and incubated for 1 h at room temperature with alkaline phosphatase-conjugated streptavidin. After washing, the antibody complexes were visualized using an avidin-biotin-alkaline phosphatase detection system. A background control was also run without the primary antibody. The levels of protein expression were measured by comparing the integrated intensity (I.I.) of target bands on membranes using a Bio-Image Whole Band Analyzer (Millipore Corporation, Ann Arbor, MI).

Increased levels of protein expression in the cell lysates were also determined by the serial dilution of samples until there was no difference observed in protein expression from that of the non-transformed cell sample. Protein levels of five-fold or higher, relative to control, were considered to be significantly elevated [13].

3. Results and discussion

Carcinogenesis is a multi-step process involving initiation, promotion, conversion, and progression [14]. The activation of proto-oncogenes and the inactivation of tumor suppressor genes are generally considered to be important in these events [15]. To date, more than 100 proto-oncogenes have been identified and classified into several groups. In order to understand the possible mechanism of the transformation process induced by glass fibers and silica, the oncoprotein expression in glass fiber- and silica-transformed cells was studied. Seven representative proto-oncogenes and one tumor suppressor gene, in which protein products range from the growth factor (c-*sis*), growth factor receptor (c-*erb* B1), and signal transducers (c-H-*ras* and c-K-*ras*) to transcription factors (c-*jun*, c-*myc*, c-*myb*, and *p53*), were selected for the detection of protein expression in transformed cells. Under the conditions used, all the transformants studied were found to have a higher level of c-jun protein (Figs. 1–3). The level of increase in the transformed cells was 8–21-fold higher than that of non-transformed cells. It is interesting to note that no increase in c-*jun* protein was found in either of two spontaneously transformed cell lines (date not shown), suggesting that the elevated c-*jun* expression is not simply a consequence of transformation in BALB/c-3T3 cells and that it may be related to trans-

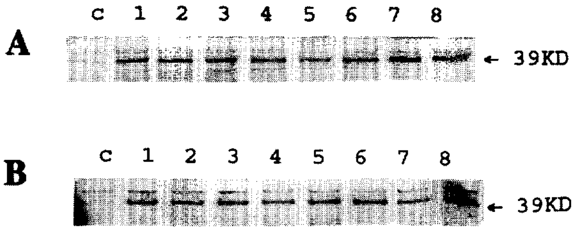

Fig. 1. Immunoblotting for c-*jun* protein in BALB/c 3T3 cells transformed by glass fibers (A) and silica (B). 1–8, transformed cell lines; c, non-transformed BALB/c-3T3 cell.

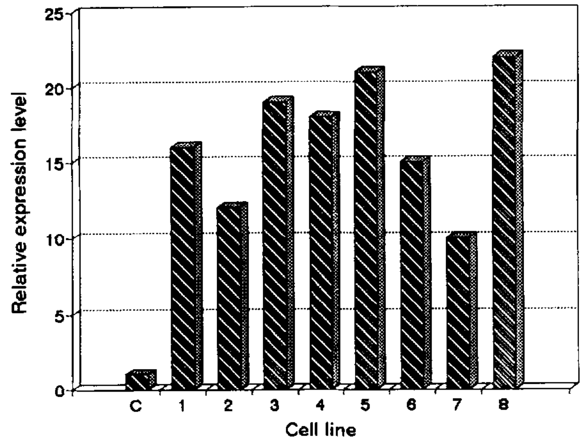

Fig. 2. c-*jun* protein expression in BALB/c-3T3 cells transformed by glass fibers. The relative level of expression determined by Bio-Image analysis is based on the non-transformed control cell as 1. 1–8, transformed cell lines; c, non-transformed BALB/c-3T3 cell.

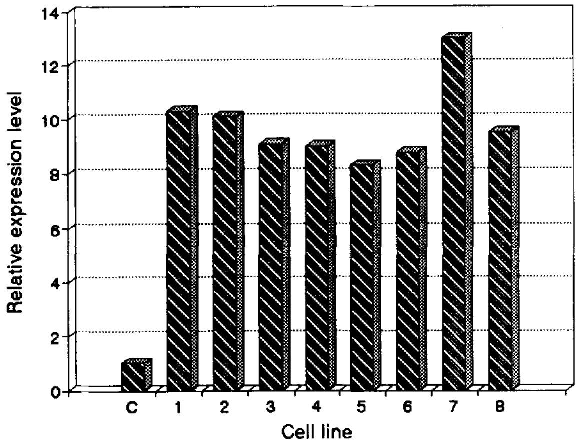

Fig. 3. P39 *jun* protein expression in BALB/c-3T3 cells transformed by silica. The relative level of expression determined by Bio-Image analysis is based on the non-transformed control cell as 1. 1–8, transformed cell lines; c, non-transformed BALB/c-3T3 cell.

formation induced specifically by glass fibers and silica.

Although background bands were present in the membranes, no protein products of c-K-*ras*, c-*myc*, and c-*sis* were detectable in any of the transformed or non-transformed cell lysates. It is not known whether these genes did not produce detectable proteins or whether the assay system used could not detect these gene products. The protein level of the remaining proto-oncogenes and tumor suppressor genes (c-H-*ras*, *p53*, c-*erb* B1, and c-*myb*) was not significantly different between the transformed and non-transformed cells (data not shown).

Results of our study suggest that the glass fiber- and silica-induced cell transformation in BALB/c-3T3 cells may be related to c-*jun* overexpression. However, whether c-*jun* proto-oncogene overexpression is the consequence of BALB/c-3T3 cell transformation or whether c-*jun* proto-oncogene activation plays an important role in cell transformation induced by glass fibers and silica is not known. C-*jun* protein is a known transcription activator which forms a homo- or heterodimer and binds to the AP-1 site in DNA [16]. Based on their studies, Castellazzi and coworkers [17] suggest that c-*jun* proto-oncogene may trigger a subset of the transforming functions which are related to certain neoplastic properties. The overexpression of c-*jun* may stimulate cell growth, because it appears to act as a positive mitotic signal [18]. Furthermore, as an immediate early gene, c-*jun* exerts its expression in response to a variety of growth stimuli, such as hormones, growth factors, and other proto-oncogene products [18]. It receives extracellular stimuli signals, mediates the long-term response of the cell to these signals, and moderates changes in expression of many genes [19]. Therefore, c-*jun* protein overexpression may play an important role in the neoplastic changes of the cell transformation process and may contribute to the morphological transformation induced by glass fibers and silica. Nevertheless, the molecular changes induced by glass fibers and silica, as related to c-*jun* protein overexpression, need to be further elucidated.

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