Transformation of Human Osteoblasts to Anchorage-independent Growth by Insoluble Nickel Particles

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Nickel compounds are well established by epidemiologic studies as human carcinogens. Although the carcinogenicity of nickel compounds has been studied in experimental animals and in a variety of cultured mammalian cells, there are only sporadic reports of nickel-induced transformation of human cells. In attempts to study the mechanisms of nickel-induced carcinogenesis in human cells, an immortalized human osteoblastic cell line (HOS) that could not grow in soft agar or form tumors in athymic nude mice was repeatedly treated with a water-soluble nickel compound (NiCl₂) or a less water-soluble nickel compound crystalline (NiS). After three rounds of NiS treatments, there was an increase in anchorage-independent (AI) colony formation. This was not found in untreated or NiCl₂-treated cells. Ten Al colonies obtained from NiS-treated cells were isolated. All of these clones showed changes in cell morphology, including the appearance of uniform polygon shape, growth in multilayers, and heavy staining with Giemsa. Most of these clones were retested for their ability to grow in soft agar and showed growth efficiencies of 5 to 50%. It has been shown by other investigators that aggregate growth is well correlated with tumorigenic potential in viral or chemical transformants of HOS cells. Four of seven tested NiS-transformed clones were able to form large aggregates compared to their untransformed counterparts, and continued to proliferate in aggregate form when they were plated on 0.9% agar. Current investigations focus on the molecular and genetic changes induced by nickel compounds in these human cells. — Environ Health Perspect 102(Suppl 3):289–292 (1994).

Key words: nickel, transformation, human osteoblast, anchorage independent growth

Introduction

The inhalation of nickel compounds leads to the formation of lung, sinonasal, and laryngeal carcinomas in nickel refinery workers (1,2). Nickel compounds are also carcinogenic in experimental animals (3). Inhalation is not the only administration route for tumor induction by nickel compounds. When particulate nickel was administered to animals intrathecally, intramuscularly, and intravenously, it caused tumors at these injection sites (4,5). In addition to tumor induction in whole animals, nickel compounds also induced morphologic and neoplastic transformation in various rodent cell systems such as Syrian hamster embryo (SHE) cells (6), C3H1/10T1/2 mouse embryo fibroblasts (7), and Chinese hamster embryo (CHE) cells (8). In spite of their potent carcinogenicity, nickel compounds are not effective in inducing gene mutations in most mammalian and bacterial cell systems, but do cause DNA strand breaks (9), DNA–protein cross-links (9–11), and chromosomal aberrations (12,13). In studies of nickel-induced chromosomal aberrations it was shown that nickel preferentially damages heterochromatin (14).

While the carcinogenicity of nickel compounds has been extensively studied in experimental animals and in a variety of cultured mammalian cells, there are only sporadic reports of nickel carcinogenesis in cultured human cells. Recently, Kumar and collaborators have successfully induced neoplastic transformation of the immortal human osteoblastic cell line (HOS) by treatment with the water-soluble nickel compound nickel sulfate (NiSO₄) (15). However, the most potent carcinogenic nickel compounds are the water-insoluble particulate nickel compounds such as crystalline nickel subsulfide and crystalline nickel sulfide (4,16,17). Water-soluble nickel compounds usually fail to produce tumors in experimental animals (17). In the present study, we utilized an immortal, nontumorigenic HOS cell line to study the capability of particulate nickel sulfide to induce transformation and compared the activity of a particulate compound with water-soluble nickel chloride.

Materials and Methods

Chemicals

Nickel chloride (NiCl₂) and crystalline NiS were purchased from Alfa Inorganics (Danvers, MA). NiS was ground and then passed through a 5-µm screen and sterilized by washing with acetone. Suspensions of particulate nickel were prepared in tissue culture media by agitation in a bath-type sonicator. Measured volumes of the suspension were applied to the cells and gently mixed into the media in a tissue-culture flask.

Cell Culture and Transformation Protocols

The HOS TE 85, Clone F-5 cell line was obtained from American Type Culture Collection (Rockville, MD) and grown in plastic flasks (Corning Glass Company, Corning, NY) in minum essential medium α-(MEM) containing 10% fetal bovine serum. For transformation, 1 × 10⁶ HOS cells were plated in 75 cm² plastic flasks and treated 24 hr later with 10 mg/ml NiS or 100 mM NiCl₂ for 24 hr. The nickel containing medium was removed and the
cells were rinsed three times with serum-free medium. Fresh α-MEM was added and the cells were allowed to grow to confluence. The cultures were split 1:10 and were then treated for a second time with 2 mg/ml NiS or 100 mM NiCl₂, for 24 hr. They were again allowed to recover in fresh medium until the cultures reached 100% confluence. After a second subculture the cells were treated for a third time with 2 mg/ml NiS or 100 mM NiCl₂, for 24 hr, and were allowed to proliferate. After the three separate nickel treatments, the cells were plated in soft agar and ten individual colonies were cloned and expanded into mass culture (HOS1-3/SA-N).

**Assay for Anchorage Independence Growth**

In the assay for anchorage-independence growth, $1 \times 10^5$ or $1 \times 10^3$ cells were plated in 5 ml 0.3% agarose in α-MEM, 10% fetal bovine serum overlaid on a solid layer of 0.6% agarose in α-MEM with 10% fetal bovine serum. The cultures were maintained for 2 weeks and colonies larger than 0.1 mm (small) or >0.5 mm (large) were counted. The plating efficiency in agar was determined by dividing the number of colonies obtained per plate by the number of cells plated.

**Aggregation Assay**

A 2-ml suspension of cells ($1 \times 10^7$/ml) in α-MEM containing 10% fetal bovine serum was plated on top of 2 ml of 0.9% agarose in α-MEM, 10% fetal bovine serum in each well of a six-well tissue-culture plate. The plates were incubated in 5% CO₂, 95% air, at 37°C for 3 days. On the third day of culture, the cell suspension was collected and washed three times with serum-free medium. The collected cell aggregates were resuspended into single cell suspensions in 0.05% trypsin and 0.02% EDTA for 5 min. The dispensed aggregates were resuspended in an appropriate volume and the cell numbers were determined.

**Results**

After repeated treatments with insoluble nickel compounds over a period of one month, no alterations in morphology and growth pattern were observed in the cul-

### Table 1. Plating efficiency of NiS treated HOS cells in soft agar.

| Clone        | PE in soft agar % | Colony size |
|--------------|-------------------|-------------|
| HOS TE-85    | 9.6               | Small       |
| HOS1-3/SA-1  | 5.8               | Small       |
| HOS1-3/SA-2  | 17.8              | Small       |
| HOS1-3/SA-3  | 50.2              | Large       |
| HOS1-3/SA-4  | 26.0              | Large       |
| HOS1-3/SA-5  | 5.0               | Large       |
| HOS1-3/SA-6  | 30.4              | Large       |
| HOS1-3/SA-7  | 32.8              | Large       |

* $1 \times 10^5$ cells were plated in dishes containing 0.3% agar, then incubated for 2 weeks. Colonies 0.1 mm but <0.5 mm (small) or ≥0.5 mm (large) were counted, and the plating efficiencies in soft agar were determined by dividing the number of colonies obtained per plate by the number of cells plated.
tured HOS cells. However, when these cells were plated in soft agar, NiS-treated cells had a plating efficiency of about 0.1%, whereas the control and NiCl₂-treated cells did not form any true colonies (larger than 0.1 mm in soft agar). Ten of the NiS-treated colonies were cloned from the soft agar and expanded to mass culture (HOS1-3/S-A-1, HOS1-3/S-A-2, etc.). All these clones exhibited altered morphology, including dark staining with Giemsa and the acquisition of a uniform polygon shape in contrast to the flat epithelial like morphology of their parental cells (Figure 1). These clones also exhibited an alteration in their growth patterns, such that they grew in multilayers at high density and in aggregates at low cell density, whereas the parental cells grew in a sporadic manner (Figure 1). The eight tested clones exhibited high plating efficiency (5 to 50%) when regrown in soft agar, and most of them formed large colonies (> 0.5 mm) (Table 1). When the nickel-treated and parental cells were plated in liquid medium above a 0.9% agar layer, cell attachment was prevented. Cell aggregates appeared within 24 hr after seeding. Four of the five clones that formed large colonies in soft agar also formed large cell aggregates, and these cell populations proliferated in the aggregate form over a 3 day period (Figure 2).

**Discussion**

Neoplastic transformation of cells in culture is one of the few systems that can be used to study the mechanisms of human carcinogenesis. To date most of the neoplastic transformation studies have been carried out using rodent cells such as Syrian hamster embryo cells and C3H/10T1/2 cells. Although we do not understand the details, it is known that significant differences exist between rodent and human cells in their responses to carcinogens. In general, cultured human cells are more resistant than rodent cells to neoplastic transformation by carcinogens.

We have selected an immortal non-tumorigenic human osteoblastic cell line, HOS TE 85 to study neoplastic transformation by nickel compounds. The HOS TE 85 cells originated from a human osteosarcoma (18). HOS TE 85 are immortal cells, which grow at high cell density, form minute colonies (less than 20 cells per colony) of less than 0.1 mm in soft agar, exhibit very low plating efficiency, and do not induce tumors when injected into nude mice. It is assumed that HOS TE 85 was epithelial in origin. This cell line has been used in transformation studies because it is easily transformed by viruses and other chemical carcinogens (19,20). Our results show that after NiS treatment HOS cells acquired AI growth and morphologic changes that were similar to those of HOS cells treated with NiSO₄ and hexavalent chromium (15,22). Some isolated clones even exhibited growth in liquid medium. These results indicated that crystalline NiS can induce transformation of the HOS cells.

Previous studies have shown that there is a striking difference in carcinogenic potency between crystalline and water-soluble nickel compounds. This may be the result of differences in the uptake of these two types of nickel compounds. It was found that crystalline nickel compounds such as NiS and Ni₃S₄ were taken up by cells through phagocytosis (23,24) whereas water-soluble nickel salts passed through cell membrane ion channels or were taken up by cells along with other molecules such as proteins and amino acids. Once nickel ions enter cells they bind to intracellular ligands, i.e., proteins. Since nickel ions have high binding constants for certain amino acids, the binding to protein may reduce the accessibility of these ions to the nuclei. In the case of crystalline nickel sulfide, phagocytized particulate nickel dissolves in the cellular vacuoles because of the acidic environment. Relatively high concentrations of these nickel ions were distributed to all cellular compartments including nuclei. The intracellular delivery of a significant nickel ion concentration may be one reason why crystalline nickel sulfide compounds are more carcinogenic than the water-soluble compounds. In our study, human HOS cells were treated repeatedly in culture with insoluble nickel compounds. Under these conditions, only crystalline nickel but not NiCl₂, produced transformation of the HOS cells. Extensive treatment of HOS cells with NiSO₄ for long periods has been shown to induce anchorage-independent growth (22). The present study did not confirm this result, perhaps because the treatment time was insufficient. Future studies will focus on the genetic changes associated with nickel-induced neoplastic transformation of human cells.

**REFERENCES**

1. Heinberg S. Incidence of cancer in populations with exceptional exposure to metal. In: Origins of Human Cancer, Book A: Incidence of Cancer in Humans, Vol 4 (Hiatt HH, Watson JD, Winston JA, eds). Cold Spring Harbor:NY: Cold Spring Harbor Laboratory, 1977:159-167.
2. IARC. Some Metals and Metal Compounds. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 23. Lyon: International Agency for Research on Cancer, 1980; 39-141.
3. Sunderman FW Jr, ed. Carcinogenicity of nickel compounds in animals. In: Nickel in the Human Environment. New York: Oxford University Press, 1984:127-142.
4. Damjanov I, Sunderman FW Jr, Mitchell GM, Allpass PR. Induction of testicular sarcomas in Fischer rats by intratesticular injection of nickel sulphide. Cancer Res 38:268-276 (1978).
5. Sunderman FW Jr. Carcinogenic effects of metals Fed Proc 37:40-46 (1978).
6. Costa M, Simmons-Hansen J, Bedrossian CWM, Bonura J, Caprioli R. Phagocytosis cellular distribution, and carcinogenic activity of particulate nickel compounds in tissue culture. Cancer Res 41:2868-2876 (1981).
7. Miura T, Patierno SR, Sakuramamoto T, Landolph JR. Morphological and neoplastic transformation of C3H/10T1/2 Cl 8 mouse embryo cells by insoluble carcinogenic nickel compounds. Environ Mol Mutagen 14:65-78 (1989).
8. Conway K, Costa M. Nonrandom chromosomal alterations in nickel-transformed Chinese hamster embryo cells. Cancer Res 49: 6032-6038 (1989).
9. Ciccarelli RB, Hampton TH, Jennette KG. Nickel carbonate induces DNA-protein cross-links and DNA strand breaks in rat kidney. Cancer Lett 12:349-354 (1980).
10. Patierno RS, Costa M. DNA-protein cross-links induced by nickel compounds in intact cultured cells. Chem Biol Interact 55:75-91 (1985).
11. Lin X, Zhuang Z, Costa M. Analysis of residual amino acid-DNA cross-links induced in intact cells by nickel and chromium compounds. Carcinogenesis 13:1763-1768 (1992).
12. Nishimura M, Umeda M. Induction of chromosomal aberrations in cultured mammalian cells by nickel compounds. Mutat Res 68:337-349 (1979).
13. Larramendy ML, Popescu NC, DiPaolo JA. Induction by inorganic metal salts of sister chromatid exchanges and chromosome aberration in human and Syrian hamster cell strains. Environ Mutagen 3:597–606 (1981).

14. Sen P, Costa M. Induction of chromosomal damage in Chinese hamster ovary cells by soluble and particulate nickel compounds: Preferential fragmentation of the heterochromatic long arm of the X-chromosome by carcinogenic crystalline NiS particles. Cancer Res 45:2320–2325 (1985).

15. Kumar S, Rani AS, Qu DQ, Sidhu MK, Panagakos F, Shah V, Klein KM, Brown N, Pathak S. Transformation of immortal, non-tumorigenic osteoblast-like human osteosarcoma cells to the tumorigenic phenotype by nickel sulfate. (in press)

16. Ottolenghi AD, Haseman JK, Payne WW, Salk HL, MacFarland HM. Inhalation studies of nickel sulfide in pulmonary carcinogenesis of rats. J Natl Cancer Inst 54:1165–1172 (1974).

17. Kasprzak KS, Gabryel P, Jarczewska K. Carcinogenicity of nickel(II) hydroxide and nickel(II) sulfate in Wistar rats and its relation to the in vitro dissolution rates. Carcinogenesis 4:275–279 (1983).

18. McAllister RM, Gradner MB, Greene AE, Bradt C, Nichols WW, Landing BH. Cultivation in vitro of cells derived from a human osteosarcoma. Cancer 27:397–402 (1971).

19. McAllister RM, Filbert JE, Nicolson MO, Rongey RW, Gradner MB, Gilden RV, Huebner RJ. Transformation and productive infection of human osteosarcoma cells by a feline sarcoma virus. Nature (New Biol). 230:279–284 (1971).

20. Rhim JS, Park DK, Arnstein P, Huebner RJ, Weisburger EK, Nelson-Rees WA. Transformation of human cells in culture by N-methyl-N’-nitro-N-nitrosoguanidine. Nature 256:751–753 (1975).

21. Rhim JS, Kim CM, Arnstein P, Huebner RJ, Weisburger EK, Nelson-Rees WA. Transformation of human osteosarcoma cells by a chemical carcinogen. J Natl Cancer Inst 55:1291–1294 (1975).

22. Rani AS, Kumar S. Transformation of immortal human osteoblasts by hexavalent chromates: Alteration of morphology, induction of anchorage-independence and proteolytic function. Carcinogenesis 13:2021–2027 (1992).

23. Costa M, Mollenhauer HH. Carcinogenic activity of particulate nickel compounds is proportional to their cellular uptake. Science 209:515–517 (1980).

24. Evans RM, Davies PJ, Costa M. Video time-lapse microscopy of phagocytosis and intercellular fate of crystalline nickel sulfide particles in cultured mammalian cells. Cancer Res 42:2729–2735 (1982).