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Risk Assessment of Nickel Carcinogenicity and Occupational Lung Cancer

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Recent progress in risk assessment of nickel carcinogenicity and its correlation with occupational lung cancer in nickel-exposed workers is reviewed. Epidemiological investigations provide reliable data indicating the close relation between nickel exposure and high lung cancer risk, especially in nickel refineries. The nickel species-specific effects and the dose-response relationship between nickel exposure and lung cancer are among the main questions that are explored extensively. It is also suggested that some confounding factors such as cigarette smoking cannot be neglected. The determination of nickel concentration in lung tissue may be conducive to estimating the nickel exposure level, but it is uncertain whether the high nickel content in lung tissue indicates high lung cancer risk in nickel-exposed workers. Immunologic studies suggest that the suppressive effect of nickel on NK cell activity and interferon production may also be involved in the mechanisms of nickel carcinogenesis. As a potential mutagen, nickel can cause chromosome damage both *in vitro* and *in vivo*; and on a molecular basis, nickel is found to induce DNA damage (DNA strandbreaks and crosslinks, infidelity of DNA replication, inhibition of DNA repair, and the helical transition of B-DNA to Z-DNA) by binding of nickel ions to DNA and nuclear proteins. The discovery of oncogene promises both a challenge and an opportunity for nickel carcinogenesis research. It can be predicted that, with the rapid development of molecular biology and oncology, new approaches will be established for both understanding and controlling nickel-induced occupational lung cancer.

Introduction

Nickel, a metal used widely in modern industry, has been shown to exert many adverse effects on the human body (*1,2*). Among them, the carcinogenic effect and the correlation between nickel exposure and occupational lung cancer have attracted particular interest. Epidemiological investigations have shown a high incidence of lung cancer in nickel-exposed workers. Mechanisms of nickel carcinogenesis are understood more clearly than ever before with the development of molecular biology and oncology. In this paper, the recent advances in knowledge of risk assessment of nickel carcinogenicity and occupational lung cancer are reviewed.

Epidemiological Investigations

Because epidemiological investigation is a primary and potential tool to evaluate the risk of nickel-induced occupational lung cancer, numerous epidemiological studies have been carried out since the first recognition of high lung cancer risk in workers from a nickel refinery in Wales (*3-9*). Most of them were historical perspective studies and the high lung cancer risk was observed, especially in nickel refinery (*4,7,8,9*).

However, the epidemiological investigations performed in the past could not answer many important questions with regard to the relation between nickel exposure and high lung cancer risk. Grandjean et al. (*10*) reviewed these studies and pointed out some deficiencies in general, including insufficient information on past exposure, misclassification of exposure groups, and insufficient follow-up periods. Because of the considerable latency time of nickel carcinogenic effects and the extreme complexity of nickel exposure conditions, these deficiencies may result in erroneous outcomes in some investigations.

Two of the most important problems that remain in epidemiological studies are the nickel-species-specific effects on increased lung cancer risk and the nickel-dose-specific models of its carcinogenicity. The former is understood more clearly with the recent publication of a special report (*11*).

In this comprehensive report (*11*), which was organized by the International Committee on Nickel Carcinogenesis in Man, 10 epidemiological cohorts carried out in different countries were summarized: three in the United States (Hanna Nickel Smelting Company, Huntington Alloy Inc. (INCO), and Oak Ridge Gaseous Diffusion Plant), two in Canada (Falconbridge Nickel Mines, Ontario, and INCO, Ontario), one in South Wales (Mond/INCO Nickel Reifinery, Clydach), one in Norway (Falconbridge Nickel Refinery, Kristiansand), one in Finland (Outokumpu Oy Refinery), one in New Caledonia (Societé Le Nickel), and one in England (Henry Wiggin Alloy Company). A total of 140,888 nickel-exposed workers were included with the minimum employment period from 6 months to 5 years. For each studied cohort, the principal nickel process techniques, together with the differences in airborne concentrations of different nickel compounds (metallic, oxidic, sulfidic, and soluble nickel) by

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Table 1. Nickel concentration in lung tissue of nickel-exposed workers and controls.

Group	No. of samples	Nickel concentration, μg/g dry lung tissue	References
Controls	16	0.76 ± 0.39[b]	Andersen et al. (*19*)
Nickel refinery	39	150 ± 280	
Roasting and smelting	15	330 ± 380	
Electrolysis	24	34 ± 48	
Controls	30	0.02 ± 0.04[a]	Raithel et al. (*20*)
Nickel refinery	10	285 ± 308	
Stainless-steel workers	2	28.6 ± 30.7	
Random autopsies	16	0.043 ~ 0.361[a]	Kollmeier et al. (*21*)
Random autopsies	35	0.04 ± 0.025[a]	Bartsch et al. (*22*)
Random autopsies	15	0.0163 ~ 0.2422[a]	Raithel et al. (*23*)
Patients with no occuational nickel exposure	9	173 ± 94	Rezuke et al. (*24*)
Lung cancer	15	0.60 ± 0.81	Raithel et al. (*25*)
Nonlung cancer patients	19	0.47 ± 0.57	
Lung cancer	274	2.09 ± 5.73	Adachi et al. (*26*)
Nonlung cancer cases	1715	2.13 ± 7.96	

[a]Nickel concentration as μg/g wet lung tissue.
[b]+SD.

work department, work area, and time period, were described extensively. It was found that exposure to very high (>10 mg/m³) concentrations of oxidic and/or sulfidic nickel was confined to refinery operations at Mond/INCO and two sinter plants of INCO, Ontario. In contrast, workers at Huntington Alloys, Societé Le Nickel, and in mining and smelting operations at Hanna Mining and the Falconbridge Mines in Ontario had much lower nickel exposure (<2 mg/m³). The concentration of soluble nickel in the mining and smelting operations at Huntington Alloys was extremely low, as was the metallic nickel in nickel-using industries.

The main progress made by this report (*11*) may be the determination of different respiratory cancer risks with different nickel species exposure in nickel processes. From this report, it may be concluded that much of the respiratory cancer risk in nickel-refinery workers was associated with exposure to a mixture of oxidic and sulfidic nickel at high concentration, or to a high level of oxidic nickel alone. Low levels of soluble nickel exposure were also related to high cancer risk, whereas metallic nickel showed no evidence of enhanced lung cancer risk.

In this report (*11*), the ranges of nickel concentration and the percentages of each nickel species were estimated mainly on the basis of knowledge of nickel processes, subjective judgment of relative dustiness, and a limited number of measurement records; so the dose–response relationship between nickel exposure and lung cancer risk remains uncertain. To determine this dose–response relationship will be a challenging task in the future because of its significance for setting up the exposure limit of nickel compounds. At the present time, the exposure limits used in different countries vary considerably. For example, in the United States, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends 1.0 mg/m³ as the threshold limit value (TLV) of nickel metal and insoluble inorganic nickel compounds, and in Norway the exposure limit for all nickel compounds is 0.1 mg/m³ (*10*). In China, the maxmium allowable concentration (MAC) of nickel carbonyl is 0.001 mg/m³. With the consideration of different effects of various nickel species, it is recommended that the exposure limits for some more potent nickel compounds (sulfidic, oxidic, and soluble nickel) must be lower than that of some less potent nickel compounds (metallic nickel).

One of the main difficulties in estimating the carcinogenic effects of nickel in the epidemiological investigations is to exclude the influences of some confounding exposure factors. Several hazardous factors in the nickel-producing occupational environments may play a role in nickel-induced high lung cancer risk (*1,7,11*). Among them, cigarette smoking, a well-known lung carcinogen, attracted much interest. Though the detailed data concerning smoking habits and nickel exposure were absent, investigations carried out in New Caledonia indicated the significance of cigarette smoking in nickel-induced occupational lung cancer (*7,8*), which was consistent with the results of a survey carried out by Kreybery (*12*). Magnus et al. (*13*) assessed the interaction between smoking and occupational nickel exposure and found that the interaction was closer to being additive than multiplicative. In view of the high percentage of smokers among nickel-exposed workers and the undoubted carcinogenic effect of cigarette smoking on lung cancer, it may be important to evaluate the relationship between cigarette smoking and nickel-induced high lung cancer risk further.

Additionally, Langer et al. (*14*) suggested asbestos as a cofactor in nickel-induced carcinogenesis. It was also noted that nickel ions might block the production of enzymes that degradate the carcinogenic breakdown products of benzo[*a*]pyrene and therefore enhanced its carcinogenicity (*5*).

In nickel-using industries, where exposure to nickel is low, other coexposure factors may have a greater influence on the nickel-related high cancer risk. Sorahan et al. (*15,16*) carried out mortality studies in nickel–cadmium battery and nickel–chromium platers and found a significant association of lung cancer with cadmium and chromium exposure. Langard et al. (*17*) reviewed some epidemiological studies on high-alloy and stainless steel welders and came to the same conclusion.

Since the first recognition of occupationally induced cancer among London chimney sweeps in 1775, numerous occupational carcinogens have been identified with occupational epidemiological investigations and experimental research. The carcinogenic effect of nickel in occupationally exposed workers has been studied for more than half a century. With the recent development of molecular cancer epidemiology (*18*), it can be predicted that on the basis of traditional epidemiology, new epidemiological methods will bring out new concepts and

progress in the risk assessment of nickel exposure and occupational lung cancer.

Accumulation of Nickel in Human Lungs

Because workers are exposed to nickel mainly by inhalation, determining the nickel accumulation in human lungs might be helpful for evaluating the exposure magnitude and the lung cancer risk in nickel-exposed workers. Some results concerning nickel content in different populations are summarized in Table 1.

Anderson et al. (*19*) determined the nickel content in 16 normal controls and 39 nickel refineries and demonstrated that the nickel concentration in the pulmonary tissue of nickel-refinery workers was increased significantly over controls, which coincided with the results of Raithel et al. (*20*). They also found that workers from different work groups had different nickel concentrations in their lungs. The average concentration in nickel refineries was 150 ± 280 μg/g, where as the concentration in roasting and smelting groups was significantly higher than that in electrolysis groups (330 ± 380 μg/g–34 ± 48 μg/g). The reason for this difference is that workers in the former group are mainly exposed to insoluble nickel compounds, while the latter group is predominantly exposed to soluble compounds.

One obstacle in the evaluation of nickel content in lung tissue of nickel-exposed workers is the difficulty in setting the "normal" concentration in healthy lungs. Many factors may influence nickel content in lungs, such as age, sex, and smoking habits (*20,23,24,26*). Through the measurement of nickel content in 330 specimens of 15 random autopsies, Raithel et al. (*23*) showed substantial variation in nickel concentration among different sections within a single lung. The higher concentrations of nickel were found in the upper sections of the lung and in the right middle lobe because of the favorable ventilation conditions.

In addition, the environmental airborne nickel concentration and the geographical site where people live also affect lung nickel content (*27,28*). For example, Kollmeier et al. (*27*) found that the nickel lung content in 87 subjects from the Ruhr district in Germany, which was considered a particularly polluted area with locally high nickel emission, was 2.8 times higher than that in 23 cases from Munster with relatively less air pollution (0.65 ± 0.94 μg Ni/g dry lung weight to 0.17 ± 0.11 μg Ni/g dry lung weight).

Edelman et al. (*28*) established a model to estimate the effects of nickel-containing cigarette smoke and nickel in the ambient air on the amount of nickel accumulation in lungs and indicated the importance of these two factors in estimating lung nickel content in occupationally exposed workers. More research needs to be done to evaluate the effects of nickel in cigarettes on lung nickel content in occupationally exposed workers.

As for the relation between the lung nickel content and the incidence of occupational lung cancer, no consensus can be presented now. Akslen et al. (*29*) determined the nickel content in 20 cases of lung cancer and found a significant difference compared to 21 control individuals. Martin-Mateo et al. (*30*) also found similar results. On the other hand, Anderson et al. (*19*) suggested that the former nickel-refinery workers who had died of lung cancers displayed the same nickel content as the workers who had died of other diseases. Raithel et al. (*24*) showed that the nickel concentration in lung tissue was somewhat higher in the lung cancer patients than in noncancer cases, but a statistically significant difference could not be established. Recent investigations performed by Adachi et al. (*26*) on a relatively large population also revealed similar results (see Table 1).

According to the present studies, it may be concluded that the nickel content in pulmonary tissue may be regarded as an indicator of occupational nickel exposure. However, considering the relatively small number of test subjects and the considerable interindividual variation, evidence at present is not sufficient to reach a conclusion that the nickel detected in the lung tissue is a major contributory cause of the development of occupational lung cancer (*31*). Therefore, investigations using larger numbers of subjects are necessary to understand this relationship. Furthermore, the combination of nickel determination in lung tissue with epidemiological lung cancer investigations in the same population will be conducive to the assessment of nickel-induced lung cancer risk.

Immunotoxic Effects of Nickel

The effects of nickel on immunologic response have attracted much interest. Haley et al. (*32*) determined the immunotoxicity of three nickel compounds at various concentrations following 13 weeks of inhalation exposures in mice. It was found that the immunotoxic effects of nickel varied significantly depending on the dose and the physicochemical forms. Nickel subsulfide (1.8 mg Ni/m^3) could decrease the activity level of natural killer (NK) cells, while both nickel oxide (0.47, 2.0, 7.9 mg Ni/m^3) and nickel subsulfide (0.45, 1.8 mg Ni/m^3) inhibited the phagocytic ability of alveolar macrophages. Smialowicz et al. (*33,34,35*) carried out a series of experiments to investigate the immunological effects of nickel compounds (nickel chloride) in rats and mice. According to their results, the following conclusions could be reached: *a*) Nickel predominantly exerts its effects on the T-cell-mediated immune response; the humoral immune response was not affected significantly. *b*) The NK cell is the selective target cell of the immunotoxic effects of nickel. The activity level of NK cells was suppressed remarkably by nickel administration both *in vivo* and *in vitro*. *c*) Nickel injection might result in reduced clearance ability of syngeneic tumor cells from lungs and thus enhance the susceptibility to develop lung cancer. Judde et al. (*34*) further confirmed the correlation between the depression of NK cell activity and the development of tumors in nickel-injected rats. In contrast, the activation of NK cells may prevent nickel carcinogenicity. Recently, Kasprzak et al. (*37*) suggested that a single intramuscular injection of *Mycobacterium bovis* antigen (MB) significantly reduced the tumor incidence rate resulting from nickel subsulfide administration in F344/NCr rats. This might explain the numerous active NK cells and macrophages found at the injection site of nickel and MB.

Sunderman (*38*) speculated that the mechanism of nickel's inhibitory effect on NK cell activity is the substitution for Zn^{2+} at the active sites of an enzyme that is an important cytolytic factor in NK cells.

Interferon is another important immunologic factor inhibiting tumor development in organisms. It was found that pretreatment with carcinogenic nickel compounds (such as crystalline nickel sulfide) could reduce the induction of interferon, but amorphous nickel sulfide showed no effects (*39,40*). It appeared that this was consistent with the effect of nickel compounds on NK cells.

With the understanding of the immunologic effects of nickel, it may be possible to establish some immunologic methods or

Table 2. Chromosomal damage induced by nickel compounds *in vitro*.

Cell systems	Exposure conditions	Nickel compound	Chromosomal damage CA	Chromosomal damage SCE	References
Mouse FM3A carcinoma cell	0.6/0.8 mM × 48 hr	Nickel chloride	+	+	Nishimura et al. (*42*)
	0.6/0.8 mM × 48 hr	Nickel sulfide	+	+	
	0.6/0.8 mM × 48 hr	Nickel acetate	+	+	
Human lymphocytes	0.233–0.0233 mM × 72 hr	Nickel sulfate	/[a]	+	Wulf (*43*)
Human lymphocytes	1–100 μg/L × 48 hr	Nickel subsulfide	/	+	Saxholm (*44*)
Human lymphocytes	0.119–0.5 mM × 64 hr	Nickel chloride	/	×	Newman et al. (*45*)
C3H10T1/2 cells	0.1–1.0mM × 6/24 hr	Nickel chloride	+	/	Sen et al. (*46*)
	2.5–20 μg/mL × 6/24 hr	Nickel sulfide	+	/	
CHO cells	0.05–0.1 mM × 2hr	Nickel chloride	/	+	Sen et al. (*47*)
	1-10 μg/mL × 24/48 hr	Nickel sulfide	/	+	

Abbreviations: CA, chromosome aberrations; SCE, sister chromatid exchanges; CHO, Chinese hamster ovary.
[a]None reported.

tests to evaluate the risk of lung cancer in nickel-exposed workers. Kotlar et al. (*41*) adopted a leukocyte adherence inhibition test to investigate the serum from nickel-refinery workers for immune response to lung cancer antigens and suggested that this test might be helpful for screening the nickel workers with increased risk of lung cancer.

On the basis of above studies, we conclude that the inhibitory effects of nickel on the cellular immune response, especially on the bioactivity of NK cells and the production of interferon, may closely relate to the development of nickel-induced malignant tumors in animals, as well as to the high risk of lung cancer in nickel-exposed workers, although few studies on the effects of nickel on the human immunologic system in nickel-exposed workers have been performed directly.

Chromosomal Damage Induced by Nickel Compounds

Chromosomal damage, e.g., chromosome aberrations (CA) and sister chromatid exchanges (SCE), induced by nickel compounds have been investigated both *in vivo* and *in vitro*. The results of some *in vitro* tests are shown in Table 2.

The chromosomal damage caused by various nickel compounds seems to be consistent with their carcinogenicity (*38,48*) and is affected by many factors, including the exposure concentration, duration, and cell lines, as shown in Table 2. Sen et al. (*47,49*) addressed the concentration-dependent and time-dependent fashion of carcinogenic nickel compounds (crystalline nickel sulfide and nickel chloride) on the incidence of SCE in Chinese hamster ovary (CHO) cells. It was also found that the chromosomal damage induced by *in vitro* exposure of CHO cells to carcinogenic nickel compounds preferentially occurs in the heterochromatic regions, which contain more proteins and exist in a higher-condensed state (*46,47,49*). The high binding affinity of nickel ions for protein and the low affinity for DNA may explain these results (*47*). In addition, Conway et al. (*50*) stated that a large proportion of the nickel-transformed cell lines had complete or partial deletions of the heterochromatic long arm of the X-chromosome. This change appears to be important in nickel carcinogenesis, as a tumor-suppressor gene was located in the long arm of the X-chromosome (*51*).

To explore the question of whether nickel constitutes a genetic hazard for nickel-exposed workers, Waksivk et al. (*52,53*) determined the chromosomal damage in peripheral lymphocytes both in nickel refineries and in retired workers and demonstrated that the increased incidence of CA in peripheral lymphocytes might be a useful parameter in the risk assessment of nickel-exposed workers. Deng et al. (*54*) investigated chromosomal damage (CA and SCE) in peripheral blood lymphocytes in Chinese electroplating workers and revealed that the high frequency of CA and SCE was related to their high nickel-exposure level. A significantly elevated CA in cultured lymphocytes obtained from welders was observed by Elias et al. (*55*) and also confirmed the correlation between the nickel concentration in serum and the frequency of CA.

Therefore, it may be deduced that the genetic hazard of nickel, observed *in vivo* and *in vitro*, may constitute the occupational cancer risk in nickel-exposed workers. Research on nickel-induced DNA damage has further verified this hypothesis.

Effects of Nickel on DNA and Nuclear Proteins: Molecular Mechanisms of Nickel Carcinogenesis

The DNA damage caused by nickel may ultmately illustrate the mechanisms of nickel carcinogenesis, though many details need to be confirmed in future.

Binding of Nickel Ions to DNA and Nuclear Proteins

After endocytosis of nickel compound particulates, a portion of nickel ions enters the nucleus (*56,57*). Ono et al. (*58*) analyzed trace metal concentrations in nuclei and nucleoli of rat liver cells and found that the contents of nickel and chromium in nucleoli were significantly higher than those in nuclei (18 and 11 times, respectively). It has been confirmed that nickel ions exhibit lower binding affinity for DNA ($K = 7.3 \times 10^2$/M) compared to the higher binding affinity for animo acids [histidine $K = 1.9 \times 10^9$/M, cysteine $K = 4.37 \times 10^9$/M (*47,59*). Therefore, most nickel ions in the cell nucleus might interact with the histone, other than DNA, indicating the importance of nickel–protein interaction in the mechanism of nickel carcinogenesis. Ciccarelli et al. (*60,61*) demonstrated the presence of nickel-nucleic acid-histone complexes in nickel-treated rats and suggested that nickel may initiate DNA damage by forming this complex.

On the other hand, the direct effect of nickel ions on DNA recently attracted much interest. It was found that nickel ions could enhance the oxidation, hydroxylation, and deglycosylation of DNA bases (deoxynucleosides and deoxynucleotides) induced by active oxygen species (*62,63*). However, more details remain

Table 3. DNA strandbreaks and crosslinks induced by nickel compounds.

Cell system	Nickel compounds	Strandbreaks	Crosslinks	References
Rat renal cells *in vivo*	Nickel carbonate	+	+	Ciccarelli et al. (*65*)
CHO cells *in vitro*	Nickel chloride	+	/[c]	Robison et al. (*66,67*)
	Nickel subsulfide	+	/	
	Nickel sulfide[a]	+	/	
	Nickel sulfide[b]	−	−	
CHO cells *in vitro*	Nickel chloride	+	+	Patierno et al (*68,69*)
	Nickel sulfide[a]	+	+	
CHO cells *in vitro*	Nickel chloride	+	+	Conway et al. (*70*)
Rat liver cells *in vitro*	Nickel subsulfide	+	/	Swierenga et al. (*71*)
	Nickel chloride	+	/	

CHO, Chinese hamster ovary.
[a]Crystalline.
[b]Amorphous.
[c]None reported.

for further research. Sunderman (*38*) and Costa (*64*) proposed that the cellular bioavaibility of nickel, i.e., the ability of nickel compounds to enter target cells and to release nickel ions, appears to be a crucial factor of carcinogenic effects of various nickel compounds.

DNA Strand Breaks and Crosslinks

DNA strand breaks and DNA–protein, DNA–interstrand crosslinks are the most common kinds of DNA damage after the binding of nickel with DNA or protein. Some studies on nickel-induced DNA strand breaks and crosslinks are summarized in Table 3.

A concentration-dependent effect of nickel on the incidence of DNA damage has been established. Ciccarelli et al. (*65*) observed increased DNA strandbreaks and crosslinks in kidney nuclei from nickel carbonate-treated rats at doses of 15 or 20 mg/kg (ip), but no significant DNA damage was found in rats receiving 10-mg/kg nickel carbonate injection. Robison et al. (*67*) confirmed the results in cultured CHO cells exposed to nickel chloride (1 μ–10μg/mL) and crystalline nickel sulfide (1, 5, 20 μg/mL).

It was interesting to find that magnesium could prevent the effects of nickel ions on heterochromatin and then inhibit nickel-induced DNA damage significantly, while exhibiting no substantial effects on the DNA damage caused by nickel in euchromatin (*70*). As magnesium was found to be antagonistic to the nickel carcinogenicity tested in rats (*72*), it could be deduced that the DNA damage in heterochromatin might play an important role in nickel-induced carcinogenic processes.

Infidelity of DNA Synthesis and Inhibition of DNA Repair

Many studies demonstrated that nickel ions may cause infidelity of DNA replication in various ways (*73,74*). The findings included altered substrate conformation at the substrate-binding site of DNA polymerase, altered enzyme conformation at the catalytic site of DNA polymerase, and changed template-base specificity (*73*). As the ability of various metals to induce infidelity of DNA replication and their carcinogenic activities (*75*) closely correspond, the tests for impaired fidelity of DNA replication may be used to screen the carcinogenicity of various nickel compounds (*38,75*).

The results of experiments on DNA repair after exposure to nickel compounds were not consistent. Some studies indicated that nickel-treated cells still possess the ability to repair the DNA damage caused by nickel exposure (*76,77*), whereas other experiments revealed that nickel inhibited the repair of DNA damage caused by ultraviolet light, X-ray, and other agents (*38,78*). Although the detailed effects of nickel on DNA repair are not clear yet, the inhibition of the DNA repair pathway may be involved in the mechanisms of nickel carcinogenesis.

Helical Transition of B-DNA to z-DNA

DNA normally exists as a right-handed double helix (B-DNA), but under the action of some nickel compounds, it may adopt a left-handed double helix form [z-DNA (*78,80*)]. With infrared spectroscopy, it was found that nickel ions induced poly-d(A-C), poly-d(G-T) to complete this transition (*80*). It was also suggested that the binding of nickel ions to the N7 site of deoxyadenosine might favor and stabilize the formation of z-DNA, which was related to nickel carcinogenesis as well (*38,79*).

Significance of Oncogene and Oncogene Protein in the Assessment of Nickel Carcinogenicity

The concept of "oncogene" was first put forth by Huebner and Todaro in 1969 (*81*). Since then, dozens of oncogenes in human cells have been identified. The studies on oncogenes promise both a challenge and an opportunity for the research on occupational lung cancer and may bring about the development of molecular occupational cancer epidemiology (*17,82,83*). As chemicals from smoking and occupational exposure contribute to as much as 40% of total cancers in human beings (*84*), to study the effects of occupational carcinogens on oncogenes is important for understanding the carcinogenic mechanisms and for monitoring occupational cancers further. Therefore, Hamm (*82*) stated that we now have stepped into the oncogene era in the field of occupational cancer research (*82*).

The development of cancer requires changes in two cellular genes: the activation of a proto-oncogene and the dysfunction of a tumor-suppressor gene. The proto-oncogenes, some normal DNA segments in cells, may be activated by carcinogens and become the active oncogenes through different mechanisms (*17,85,86*). For example, a single base change in the first exon in 12th coding triplet (GGC-GTC) can translate the proto-oncogene into *ras* oncogene, one of the best-studied oncogene systems at present (*86*).

Reynolds and Anderson (*87*) detected the activation of *ras*

genes by point mutations in many of the human lung cancers and virtually all of the mouse lung cancers. Hangen et al. (*88*) found that in transformed human kidney epithelial cells, nickel enhanced *ras* oncogene expression, suggesting synergistic action between nickel and the *ras* oncogene.

Another proto-oncogene activation route might be the inactivation of tumor-suppressor genes, caused by DNA damage and chromosomal aberrations (*82*). As mentioned above, the heterochromatic region is the sensitive site of nickel, and the deletion of the long arm of the X-chromosome in CHO cells occurred frequently with the treatment of nickel compounds (*46,47,49,50*), where a tumor-suppressor gene may correlate closely with the nickel carcinogenic process (*89*).

Oncogenes exert their effects through their protein products, i.e., oncoprotein. The *ras* oncogene encodes a protein of 189 amino acids that has a molecular mass of 21,000 daltons, and is hence designated p21 (*17,90*). The point mutations in *ras* oncogenes result in selected animo acid substitutions at various positions, and this mutated p21 may cause the malignant transformation of cells (*90,91,92*).

With monoclonal antibody technique, the existence of oncoprotein might be detected in the serum or in the tissue, and thus the risk of cancer in occupational carcinogen (such as nickel)-exposed workers could be identified. Brandt-Rauf et al. (*17*) demonstrated the presence of p21 in 14 of 15 lung cancer cases and in 1 of 16 workers, indicating that this p21-positive worker might be at high risk of lung cancer. Up to now, p21, as a molecular epidemiological biomarker, has shown good prospects in the risk assessment of occupational carcinogenesis and prevention of occupational lung cancer (*90,93,94*). Although similar investigations have not been performed in nickel-exposed workers directly, we can expect that with the advances in knowledge of oncogenes, a new approach for risk assessment of nickel and the biological monitoring of nickel-induced occupational lung cancer will be made in the near future.

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