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Review

Nickel carcinogenesis

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Abstract

Human exposure to highly nickel-polluted environments, such as those associated with nickel refining, electroplating, and welding, has the potential to produce a variety of pathologic effects. Among them are skin allergies, lung fibrosis, and cancer of the respiratory tract. The exact mechanisms of nickel-induced carcinogenesis are not known and have been the subject of numerous epidemiologic and experimental investigations. These mechanisms are likely to involve genetic and epigenetic routes. The present review provides evidence for the genotoxic and mutagenic activity of Ni(II) particularly at high doses. Such doses are best delivered into the cells by phagocytosis of sparingly soluble nickel-containing dust particles. Ni(II) genotoxicity may be aggravated through the generation of DNA-damaging reactive oxygen species (ROS) and the inhibition of DNA repair by this metal. Broad spectrum of epigenetic effects of nickel includes alteration in gene expression resulting from DNA hypermethylation and histone hypoacetylation, as well as activation or silencing of certain genes and transcription factors, especially those involved in cellular response to hypoxia. The investigations of the pathogenic effects of nickel greatly benefit from the understanding of the chemical basis of Ni(II) interactions with intracellular targets/ligands and oxidants. Many pathogenic effects of nickel are due to the interference with the metabolism of essential metals such as Fe(II), Mn(II), Ca(II), Zn(II), or Mg(II). Research in this field allows for identification of putative Ni(II) targets relevant to carcinogenesis and prediction of pathogenic effects caused by exposure to nickel. Ultimately, the investigations of nickel carcinogenesis should be aimed at the development of treatments that would inhibit or prevent Ni(II) interactions with critical target molecules and ions, Fe(II) in particular, and thus avert the respiratory tract cancer and other adverse health effects in nickel workers.

Keywords: Nickel carcinogenesis; Epigenetic toxicity; Genotoxicity; Histones; Hypoxia; Oxidative damage

1. Introduction

Nickel,[1] discovered and named by Cronstedt in 1751, is the 24th element in order of natural abundance in the earth's crust. It is widely distributed in the environment. Natural sources of atmospheric nickel include dusts from volcanic emissions and the weathering of rocks and soils. Natural sources of aqueous nickel derive from biological cycles and solubilization of nickel compounds from soils. Global input of nickel into the human environment is approximately 150,000 metric tonnes per year from natural sources and 180,000 metric tonnes per year from anthropogenic sources, including emissions from fossil fuel consumption, and the industrial production, use, and disposal of nickel compounds and alloys [1,2].

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[1] The symbol Ni^{2+} is used to depict free nickel cations only; Ni(II) is used to depict divalent nickel in its compound, e.g., Ni(II) acetate; "nickel" is spelled out if the metal valency is unknown, or irrelevant. Other uncommon abbreviations are explained in the text.

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Major deposits of nickel ores, either oxidic or sulfidic are located in Australia, Canada, Cuba, Indonesia, New Caledonia, and Russia. Readers are referred to monographs and reviews for detailed discussions of the metallurgy, chemistry, environmental chemistry, biochemistry, toxicology, and biological monitoring of nickel [1–12].

The high consumption of nickel-containing products inevitably leads to environmental pollution by nickel and its derivatives at all stages of production, utilization, and disposal. Human exposure to nickel occurs primarily via inhalation and ingestion and is particularly high among nickel metallurgy workers [1]. In addition, implantation of nickel-containing endoprostheses and iatrogenic administration of nickel-contaminated medications (e.g., albumin, radiocontrast media, hemodialysis fluids) leads to significant parenteral exposures [13–17] and wearing or handling of jewelry, coins, or utensils that are fabricated from nickel alloys or that have nickel-plated coatings may result in cutaneous nickel absorption [18]. In industrialized regions and large cities, atmospheric nickel concentrations are related to fly-ash from burning fossil fuels in power plants and automobiles and may reach 120–170 ng/m^3 as compared to 6–17 ng/m^3 in suburban areas [19]. Cigarette smoking can further increase inhaled nickel [20]. Another source of human nickel exposure is dietary where some foods, especially plant foods, may contain well over 1 mg Ni/kg [2,5].

Occupational exposure to nickel occurs predominantly in mining, refining, alloy production, electroplating, and welding. In 1990, the International Committee on Nickel Carcinogenesis in Man suggested that respiratory cancer risks are primarily related to exposure to soluble nickel concentrations above 1 mg/m^3 and to exposure to less soluble forms at concentrations above 10 mg/m^3 [1]. The Committee was unable, however, to determine with confidence the level at which nickel exposure becomes a substantial hazard. Approximately 2% of the work force in nickel-related industries are exposed to airborne nickel-containing particles in concentrations ranging from 0.1 to 1 mg/m^3 [1,5].

Exposure to nickel compounds can produce a variety of adverse effects on human health. Nickel allergy in the form of contact dermatitis is the most common reaction. Although the accumulation of nickel in the body through chronic exposure can lead to lung fibrosis, cardiovascular and kidney diseases, the most serious concerns relate to nickel's carcinogenic activity which is reviewed below in more detail in regard to its human epidemiology, experimental animal models, and postulated molecular mechanisms.

2. Carcinogenic effects in humans

The propensity of nickel workers to develop cancers of the nasal cavities was first reported by Bridge in 1933. In 1937, Baader described 17 nasal and 19 lung cancer cases among workers of the same Welsh refinery. By 1949, these numbers increased to 47 nasal cancers and 82 lung cancers (diagnosed between 1923 and 1948), and cancers at both locations were proclaimed in Great Britain as industrial diseases among some classes of nickel refinery workers [2,21,22]. During the decades since these pioneering findings, the carcinogenicity of nickel compounds has been confirmed and corroborated by numerous epidemiological studies in humans and carcinogenesis bioassays in animals [1,2,5,22–28]. The epidemiological studies have demonstrated increased mortality from malignant tumors of the lung and nasal cavities in nickel refinery workers who were chronically exposed to inhalation of nickel-containing dusts and fumes from roasting and smelting. Welding of nickel alloys (e.g., stainless steel) also may be a source of such fumes [1]. For many years, it was believed that only water-insoluble nickel components of the dusts (e.g., Ni_3S_2, NiO) were carcinogenic. However, more recent epidemiological data clearly indicate that aerosols of water-soluble nickel compounds, generated in nickel electro-refining plants (e.g., from Ni(II) sulfate), are carcinogenic to the human respiratory tract as well, with a clear dose-related effect [23,24]. Tobacco smoking has been considered as a weak to moderate confounder [24].

Histopathology of the respiratory tract tumors in nickel refinery workers was compiled by Sunderman et al. [29]. Among the investigated 100 sinonasal cancers were squamous cell carcinomas (48%), anaplastic and undifferentiated carcinomas (39%), adenocarcinomas (6%), transitional cell carcinomas (3%), and other malignant tumors (4%). The 259 lung tumors examined were diagnosed as squamous cell carcinomas (67%), anaplastic, small cell, and oat

cell carcinomas (15%), adenocarcinomas (8%), large cell carcinomas (3%), other malignant tumors (1%), and unspecified cancers (6%). Thus, this study suggests some prevalence of squamous cell carcinomas induction by the occupational nickel inhalation.

There is no epidemiological evidence on possible cancer risk from general environmental and dietary nickel exposures. Nonetheless, based on available data on occupational exposure levels and health effects of inhaled metals, including nickel, the Canadian Environmental Health Directorate concluded that "the priority for analysis of options to reduce exposure to nickel in the general environment of Canada is considered to be moderate to high" [30]. Increased risks of other malignant tumors, such as carcinomas of the larynx, kidney, prostate, stomach, and soft-tissue sarcomas, have occasionally been noted, but the statistical significance of these findings is doubtful.

Besides occupational exposures, nickel released internally from endoprostheses, bone-fixing plates and screws, and other medical devices made of nickel-containing alloys, has been suspected, but not proven, to be the major cause of sporadic local tumors [31,32]. Overall, "implanted foreign bodies consisting of metallic cobalt, metallic nickel, and a particular alloy powder consisting of 66–67% nickel, 13–16% chromium and 7% iron" have been recently classified as "possibly carcinogenic to humans" (Group 2B) by the IARC Committee on Surgical Implants and other Foreign Bodies [33].

The carcinogenic effects of nickel and nickel compounds have been critically evaluated by the International Agency for Research on Cancer [1]. The evaluation was based on the combined results of epidemiological studies, carcinogenicity in experimental animals, and other relevant data, supported by the underlying concept that nickel compounds can deliver nickel ions to or generate such ions at critical sites in target cells. The IARC evaluation concluded: "There is sufficient evidence in humans for the carcinogenicity of nickel sulfate and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry. There is inadequate evidence in humans for the carcinogenicity of metallic nickel and nickel alloys [...]. Overall evaluation: Nickel compounds are carcinogenic to humans (Group 1). Metallic nickel is possibly carcinogenic to humans (Group 2B)"[1].

3. Carcinogenic effects in experimental animals

Following the findings of Baader of respiratory tract cancer in nickel workers, published in 1937, Campbell [34] reported that chronic inhalation of nickel dust caused a two-fold increase of lung tumor incidence in mice. Since that time, numerous bioassays in experimental animals have yielded positive results for nickel compounds with low or no aqueous solubility (e.g., $Ni(OH)_2$, Ni_3S_2, NiO) following inhalation or parenteral administration. Carcinogenesis of soluble nickel compounds (e.g., Ni(II) acetate) was studied less extensively, but also yielded positive results in rodents after parenteral injections [25,35–37], or intraperitoneal (i.p.)/transplacental administration [38]. The results were especially strong for bioassays under the initiation/promotion protocols in which Ni(II) acted as the initiating agent (see Section 3.2).

In experimental animals, nickel compounds induce tumors at virtually all sites of application (reviewed in refs. [1,2,4,5,28]). The carcinogenic activity depends strongly on the solubility of the nickel compounds in water and tissue fluids. As a rule, insoluble compounds, such as NiS, NiO, and Ni_3S_2, are better carcinogens than soluble compounds, Ni(II) acetate, chloride, or sulfate. The routes of administration that were shown to produce tumors include inhalation, intramuscular (i.m.), intrarenal (i.r.), intraperitoneal, intraocular (i.o.), subcutaneous (s.c.), and the intra-articular space (i.a.).

3.1. Intramuscular and subcutaneous administration

In the early 1960s, it was reported that the i.m. administration of insoluble nickel Ni_3S_2 or NiO resulted in the development of rhabdomyosarcomas in both mice and rats [39]. Since then, a number of studies have documented the induction of malignant tumors by i.m. administration of nickel compounds to various experimental animals [40–44]. In general, long-term persistence of a nickel compound within the target tissue was found to be critical for tumor development [43]; and most of the water-insoluble compounds meet this requirement. In contrast, water-soluble nickel salts are rapidly eliminated from the site of injection [45]. Therefore, an inverse relation between the yield of local tumors and rate of solubilization of the nickel compounds was found. The persistence of nickel at the site

of tumor formation was shown in experiments with ^{63}Ni- and ^{35}S-labeled Ni_3S_2 [46]. High incidence of local malignant tumors (rhabdo- and fibrosarcomas) was also observed in rats after s.c. injection of Ni_3S_2 [44,47].

3.2. Intraperitoneal injection

A significant increase in the lung tumor incidence was noted in strain A mice after multiple i.p. injections of 13 metal compounds, including Ni(II) acetate [48]. Local sarcomas also developed in rats following a single i.p. injection of feinstein dust (an intermediate in nickel ore processing; contains Ni_3S_2) [49]. Low incidence of renal cortical adenomas was observed in male F344 rats after a single i.p. injection of Ni(II) acetate followed by a prolonged dietary treatment with sodium barbital, a multitissue tumor promoter [36]. Intraperitoneal injection of Ni(II) acetate to pregnant F344 rats resulted in tumors in the offspring, both treated and untreated with sodium barbital after birth. Pituitary gland tumors developed without the barbital, and renal tumors occurred only when Ni(II) was followed by barbital administration. These results indicate that Ni(II) acetate is a complete carcinogen for fetal rat pituitary gland and a potent initiator of carcinogenesis in fetal rat kidney [38]. Interestingly, the pituitary gland is known to avidly accumulate Ni(II) [50].

3.3. Intrarenal injection

Renal tumors were found following i.r. injection of Ni_3S_2 in different strains of rats, with significant differences in tumor incidence among the strains, ranging from 64% in Wistar-Lewis rats, 50% in NIH Black rats, 28% of F344 rats, to none in Long-Evans rats [51–53]. However, wide differences in tumor incidence may also occur in the same strain, depending on experimental conditions [53,54]. No such cancers were observed in control animals or in animals injected with metallic nickel or NiS dust. Also, attempts to induce kidney tumors by this way in other animals were unsuccessful [52]. Histologically, most of the rat renal tumors resembled the sarcomatous variant of the classic renal mesenchymal tumor, while some were composed of bizarre undifferentiated cells [53,54].

It was also noted that i.r. injection of Ni_3S_2 elicited a strong increase in hemoglobin and erythrocyte levels [54,55]. The erythrocytosis that occurs in rats following i.r. injection of Ni_3S_2 is due to the induction of erythropoietin [56,57], a part of the hypoxia-mimicking response to Ni(II), reviewed in more detail in Section 5.3.1.1.

3.4. Intratesticular injection

Malignant testicular tumors developed in 16 of 19 rats within 20 months after a single injection of Ni_3S_2. These neoplasms were classified as fibrosarcomas, malignant fibrous histocytomas, and rhabdomyosarcomas. Since rhabdomyosarcomas normally do not occur in the testis, the authors suggested that Ni_3S_2 induces malignant transformation of undifferentiated, pluripotential mesenchymal cells [58].

3.5. Intraocular injection

Ni_3S_2 administration to Fischer rats by a single i.o. injection resulted in the development of local malignant tumors [59]. In some cases multiple tumors were induced. It is of interest that ocular tumors can also be produced by Ni_3S_2 in an evolutionary distant species like the Japanese common newt *Cynops pyrrhogaster* [60].

3.6. Inhalation

A comprehensive review covering nickel inhalation carcinogenesis has recently been published [26]. Here we provide details of some early experiments which showed that various nickel compounds and nickel-containing dusts are capable of causing lung tumors in experimental animals. The tumors were induced in rats following inhalation of $Ni(CO)_4$ vapor [61]. Pulmonary tumors were also found in rats after prolonged inhalation of Ni_3S_2 dust [62]. Rats inhaling feinstein dust for 5 h per day 5 days per week during a 6-month period developed squamous cell carcinomas [49]. Single intratracheal instillation of Ni_3S_2 resulted in the development of one tumor in 26 exposed rats; no tumors were observed in control animals [49]. Ni_3S_2 induced carcinomas in the epithelium of heterotopic tracheal transplants [63]. Ni_3S_2 particles persisted inside the implants for 7–9

months. The carcinoma incidence was 15% at 1 mg and approximately 70% at 3 mg Ni_3S_2 dose. The higher dose also produced fibro- and myosarcomas. Clear evidence for lung tumorigenicity of Ni_3S_2 dust in rats after exposure of more than one year was presented in another study [64]. In contrast, soluble Ni(II) was found to be non-carcinogenic in the large inhalation bioassay conducted by the National Toxicology Program; although there was evidence for tumorigenicity in rats using the less soluble form of nickel oxide [65]. Mice appeared to be more resistant to nickel inhalation carcinogenesis than rats [64].

3.7. Other routes of exposure

No tumors were found in animals that received Ni(II) compounds dissolved in drinking water [41]. In the same study, Ni_3S_2 did not induce any malignant tumors of the cheek pouch, oral cavity, or the gastrointestinal tract, despite multiple local applications to several groups of hamsters in total dosages as large as 1.1 g of Ni_3S_2. In addition, no malignant tumors developed in two groups of rats that received single injections into the submaxillary gland (2.5 mg of Ni_3S_2) or into the liver (5 mg of Ni_3S_2 via a portal vein).

3.8. Co-administration of nickel with other carcinogens

Co-administration of nickel compounds with classical mutagenic carcinogens produced a significant synergistic effect. Thus, after i.m. injection of Ni_3S_2 with 3,4-benzopyrene to rats, more sarcomas developed in a much shorter time than with Ni_3S_2 alone [66]. Likewise, intratracheal instillation of both carcinogens to rats resulted in more tumors and premalignant pulmonary lesions than produced by the individual carcinogens [67]. Most of the sarcomas induced by nickel alone or in combination with 3,4-benzopyrene, were classified as rhabdomyosarcomas (which is more typical for nickel carcinogenesis), whereas exposure to 3,4-benzopyrene alone produced fibrosarcomas [66].

3.9. Co-administration of nickel with essential metals

Compounds of the essential metals Mn(II) [68,69], Mg(II) [70,71], and Zn(II) [72], but not Ca(II) [70,71], co-administered i.m. to rats with Ni_3S_2, significantly reduced local tumor incidence in a dose dependent manner. Mg(II) was the strongest and Zn(II) was the weakest inhibitor. Separate administration of the essential metals through injection or in the diet did not produce this effect. Interestingly, Mg(II) carbonate co-administered with Ni_3S_2 was quickly absorbed from the muscle (hours) and had no effect on the gross retention of Ni_3S_2 particles at the injection site (weeks) [70]. Thus, Mg(II) could act only at the initiation step of the carcinogenic process. This finding provided an important mechanistic clue regarding multi-stage character of Ni_3S_2-induced carcinogenesis (compare Section 6.2). Mg(II) carbonate also strongly inhibited local renal carcinogenesis by Ni_3S_2 in the rat [73].

Co-injection of Ni_3S_2 with iron, as either metallic powder (Fe^0) or Fe(III) sulfate, resulted in strong inhibition of Ni_3S_2 carcinogenicity in the rat muscle [74]. In contrast, however, Fe^0 significantly shortened the latency of renal carcinogenesis by Ni_3S_2 in rats without, however, affecting the final yield of tumors as compared with those produced by Ni_3S_2 alone. Neither Fe^0 nor Fe(III) induced i.m. or i.r. tumors by themselves [73].

In strain A mice, multiple i.p. injections of Ni(II) acetate with Mg(II) or Ca(II) acetates resulted in lower incidence of pulmonary adenomas than that produced by Ni(II) alone [35].

3.10. Species and strain susceptibility to nickel carcinogenesis

Absolute species specificity has not been observed in nickel carcinogenesis, although rats are apparently more susceptible than mice, hamsters, or rabbits [75]. Also, significant variations in susceptibilities among rat and mouse strains have been reported [75,76]. Most rat organs have been found to be susceptible to nickel carcinogenesis following exposure via injection or inhalation. Intraocular and intramuscular administration has yielded the highest tumor incidences. The specific factors responsible for the differences in susceptibility are not clear. With i.m. exposure, mice appeared to be more resistant than rats [38]. The strain differences in rats have been suggested to depend on different abilities of phagocytes in various strains to ingest nickel particles. Therefore, the con-

centration of nickel available for carcinogenic action in other cells would be lower when phagocytes were more active [77]. In mice of different strains, the susceptibility to i.m. Ni_3S_2 carcinogenesis, ranked C3H > B6C3F$_1$ > C57BL, coincided with similar ranking of Ni(II)-induced lipid peroxidation levels in the muscles of the respective strains that seemed to reflect strain differences in tissue antioxidant capacity [76]. It is thus conceivable that genetic differences between animals resulting in the variable activity of antioxidant enzymes play a role in nickel carcinogenesis [76,78].

Differences in the carcinogenic activity of nickel compounds between rats and mice were also observed in inhalation or tracheal instillation experiments. Intratracheal Ni_3S_2 failed to induce tumors or preneoplastic lesions after 27 months of exposure in B6C3F1 mice [79], whereas the same compound induced adenomas and carcinomas in approximately 30% of the exposed F344 rats [65]. However, toxic effects of nickel such as inflammation and lung fibrosis were observed in both species.

4. In vitro transformation of cells

Ni compounds are not mutagenic in the *S. typhimurium* and *E. coli* test systems [80]. This may be due to efficient metal uptake/export control systems which protect microorganisms against Ni(II) overload [81]. Nonetheless, as shown by Pikalek and Necasek [82], Ni(II) chloride at higher, relatively toxic concentrations (36–50 mg/l), was markedly mutagenic in a strain of *Corynebacterium* sp. 887 (*hom*).

In contrast to its weak mutagenicity in microbial cells, nickel efficiently transforms human and rodent cells [83–86]. Fibroblastic and epithelial cells were transformed by soluble and insoluble nickel compounds. In rodent cells, in which transformation is achieved more easily than in human cells, the insoluble compounds acted like complete carcinogens. For example, exposure of Syrian hamster embryo (SHE) cells to Ni_3S_2 resulted in morphological transformation, soft agar growth, and the development of sarcomas upon injection to nude mice [87,88]. In the same cells, soluble Ni(II) was less potent and produced only fast growing immortalized colonies [89]. Several nickel compounds, including metal dust, Ni_3S_2, NiO, Ni_2O_3, and Ni(II) acetate, showed equal transformation potential in BHK-21 cells at equitoxic doses [90]. It should be noted, however, that many of these experiments did not confirm the malignant character of the morphologic cell transformation.

When the transforming potential of soluble Ni(II) was compared with such potential of other carcinogens, the efficiency of immortalization by Ni(II) was found to be higher than that by other carcinogens, including benzo[*a*]pyrene diol epoxide, *N*-methyl-*N*-nitrosourea or, γ- or X-rays [89]. The exposure of mouse C3H/10T1/2 cells in culture to Ni_3S_2 caused morphological transformation [91]. However, in cells of this line, Ni_3S_2 was a less efficient transforming agent than another carcinogen, methylcholanthrene. In primary human kidney epithelial cells, Ni(II) induced immortalization, soft agar growth, and abnormal karyotypes, but not tumorigenic growth in nude mice [92]. The latter could be achieved, however, through transfection of activated *Ha-ras* oncogene to these cells [93].

In addition to high cell transforming potential, typical for complete carcinogens, or tumor initiators [94], nickel also displays the property of a tumor promoter. Thus, exposure of NIH 3T3 cells to Ni(II) sulfate inhibited the intercellular communication [95]. The tumor promoter-like effect of Ni(II) was also observed in SHE cells initiated by benzo[*a*]pyrene [94].

5. Search for molecular mechanisms of nickel carcinogenesis

5.1. Uptake, distribution, and retention of nickel

The marked differences in the carcinogenic activities of various nickel compounds most likely reflect the differences in their uptake, transport, distribution and retention, and ultimately—the capacity to deliver Ni(II) ions to specific cells and target molecules. This, in turn, strongly depends on the physical and chemical properties of such molecules. Our knowledge of these factors is sketchy, but nonetheless, it allows for explaining at least some of the epidemiologic and experimental observations relative to the importance of solubility, particle structure and size, and redox activity of various nickel derivatives for the toxic and carcinogenic effects of this metal.

The pharmacokinetics and toxicokinetics of nickel at the whole-body and tissue levels have been studied experimentally in humans and animals and tested on mathematical models. Here, we only review certain aspects of studies conducted at the cellular and subcellular levels, which in our opinion are most relevant to the molecular mechanisms of nickel carcinogenesis.

As revealed by investigations of the gastrointestinal absorption of Ni^{2+} cations, nickel may be transported through the cell membranes by diffusion [96]. Another possibility is the transport of Ni^{2+} ions via calcium [97–99] and iron channels [100–102]. The latter is likely to involve a proton-coupled divalent cation transporter (DMT-1; Nramp 2). This may account for the observed mutual nickel/iron transport antagonism, since the transporter has a broad substrate range that includes Ni^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} [103–105] (Fig. 1). Ni^{2+} and other metal cations' interaction with iron transport and storage also seems possible at the transferrin/ferritin system [106]. Overall, these ways of nickel uptake by cells are relatively inefficient and concur with low carcinogenic activity of water-soluble Ni(II) compounds. The third, most effective, mechanism of cellular nickel uptake is phagocytosis of metallic nickel or nickel compound dusts, observed in cultured cells (Fig. 1); its efficiency depends on both the size and the surface electric charge of the particles [107–109].

The injection of experimental animals with crystalline Ni_3S_2 or crystalline NiS resulted in a high incidence of tumors at the injection site [41,43]. However, no tumors were found in animals which had been injected with soluble Ni(II) sulfate [43]. This striking difference is associated not only with the cellular uptake and clearance levels of nickel, but also with the delivery of Ni^{2+} cations to the nucleus. Thus, crystalline Ni_3S_2, NiS, and Ni_3Se_2 particles smaller than 5 μm, which are actively phagocytized by cultured cells, were found to form Ni^{2+}-generating intracellular vacuoles that might be localized close to the nuclei [110,111] (Fig. 1). Furthermore, a substantial portion of Ni(II) released from such vacuoles became available for interaction with nuclear components. These findings are consistent with results from other studies, which showed that Ni(II) released from Ni_3S_2 and NiO particles reached the nucleus in greater amounts than Ni(II) from water-soluble Ni(II) sulfate [112,113]. This may explain the higher cytotoxicity and genotoxicity of fine particles of water-insoluble nickel compounds. It is essential, however, that such particles are dissolvable inside the cells and thus able to deliver a high (but non-lethal) dose of Ni^{2+} to the nucleus. This, obviously, is the case of Ni_3S_2 and NiS whose dissolution mainly depends on their chemical reactivity with oxygen [46,114,115] (see Section 6.2). If the phagocytosed particles are resistant to the intracellular dissolution, the Ni^{2+} dose may not be sufficient for carcinogenic effect. This is the most likely reason for the low carcinogenicity of certain high temperature-modified nickel oxides [1,116]. Measurements of nickel concentration in subcellular fractions showed that exposure of cells to water-soluble salts resulted in high cytosolic, but very low nuclear nickel contents, whereas exposure to crystalline Ni_3S_2 resulted in high nickel contents in both the cytosolic and nuclear fraction [113]. Thus, efficient cellular uptake of a nickel compound and a high level of internal Ni^{2+} ions generation are essential for its carcinogenic activity [111].

5.2. Genotoxic effects

The ability of nickel to transform cells to neoplastic phenotype raises questions related to the molecular mechanisms of this effect. Nickel compounds generate specific morphologic chromosomal damage. This has been especially notable for the heterochromatic long arm of the Chinese hamster X chromosome which suffers regional decondensation, frequent deletions, and other aberrations following exposure of cultured cells to both insoluble Ni_3S_2 and soluble Ni(II) chloride [117]. Similar chromosomal abnormalities were also observed in nickel-transformed CHO cell cultures [118]. In these cells, nickel compounds were found to be weak inducers of sister chromatid exchanges (SCE), especially in heterochromatin [119]. In cultured human lymphocytes, a nearly two-fold increase in SCEs was detected for Ni(II) sulfate [120]. Also, exposure to Ni_3S_2 resulted in a statistically significant increase of micronuclei formation in human lymphocytes [121]. In addition to chromosomal damage, DNA–protein cross-links and oxidative DNA base damage were observed in Ni(II)-exposed cells [122,123]. Thus, in cultured human lung cancer cells, soluble Ni(II) induced microsatellite mutations consisting of both contraction and expansion of the

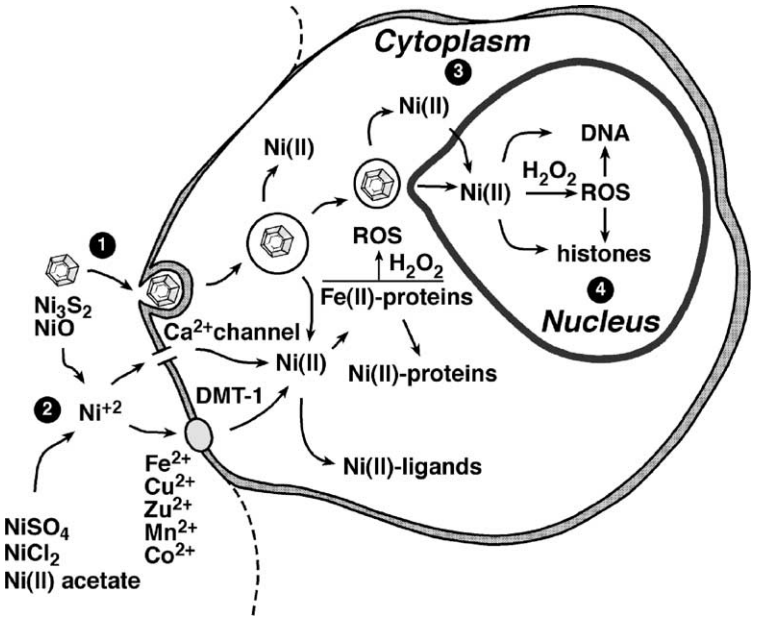

Fig. 1. Schematic representation of the uptake and major cellular interactions of Ni(II) derived from water-insoluble (e.g., Ni_3S_2 and NiO dust particles) and soluble (e.g., $NiSO_4$, $NiCl_2$, Ni(II) acetate) nickel compounds: (1) The insoluble particles enter the cell via phagocytosis; Ni(II) is released from the phagocytic vesicles into the cytoplasm and nucleus. (2) Soluble Ni^{2+} is transported into the cell via the Ca^{2+} channels, the divalent cation transporter system DMT-1 (Nramp 2), and by diffusion. (3) The cytoplasmic Ni(II) forms a variety of complexes with different ligands, such as amino acids, peptides, proteins, and glutathione, some of which are redox active and catalyze ROS production; the major effect is hypoxic stress due to Ni(II) interference with iron transport and iron-dependent hydroxylases. (4) The nuclear Ni(II) and Ni(II)-generated ROS interact with DNA and histones, causing promutagenic DNA damage (aggravated through inhibition by Ni(II) of DNA repair enzymes), and epigenetic alterations (stemming from Ni(II)-induced DNA hypermethylation, histone hypoacetylation and structural damage, and transcription factors activation).

cytosine-adenine repeat unit [124]. In rat kidney epithelial (NRK) cells infected with MuSVts110 retrovirus, Ni(II) induced insertion mutation of a 70 base pair-long stretch of DNA [125]. CHO cells cultured with soluble Ni(II) or Ni_3S_2 showed predominantly deletion mutations generated through various genotoxic mechanisms [126]. The G → T transversion mutation, typical for oxidative DNA damage at the G residues, was found in the *K-ras* gene (codon 12) in renal tumors induced by Ni_3S_2 alone or combined with iron powder [127]. The same type of point mutation in the *p53* gene was associated with nickel exposure-related human lung tumors [128].

Despite numerous reports of the DNA and chromatin damage observed in nickel-exposed cells and tissues, the mutagenic potential of this metal is generally considered to be low. This notion is based on the results of mutagenesis assays in bacteria, fruit fly, and mammalian cells [113,129–132]. However, some data suggest that nickel can be a potent co-mutagen with alkylating mutagens in some *E. coli* and *S. typhimurium* tester strains [133]. So, its effect depends on the model used. In one study, treatment of freshly isolated mouse nasal mucosa and lung cells with Ni_3S_2 resulted in DNA fragmentation in a dose-dependent manner. However, when similar treatment was applied to *lacZ* and *lacI* Big Blue rats and Muta Mouse mice, the mutation frequency of these genes in the respiratory tract tissues was not increased [134]. Also, no increase of ouabain-resistant or 6-thioguanine-resistant colonies formation has been found in human diploid fibroblasts even at concentrations of Ni_3S_2 that increased the frequency of anchorage-independence 200 times [84]. No 6-thioguanine-resistant colonies were observed in hamster V79 cells using two of the particulate nickel compounds NiS or black NiO [130]. Interestingly, in the same study, the G12 transgenic cell line was very responsive to four insoluble nickel

compounds producing 20–100-fold increase in the number of 6-thioguanine–resistant mutants. However, such mutants, as it was found later, appeared to be silenced variants in which the *gpt* gene expression was inactivated by DNA methylation [131].

It is important to notice that most of the evidence of nickel mutagenesis in mammalian cells was obtained using transgenic cell lines. Thus, Christie et al. have studied V79 cell transfected with pSV2gpt plasmid, G12 clone [135]. The transgenic clone G12, but not its parental cell line, was very responsive to nickel mutagenesis. Mutagenic effect of Ni(II) was also studied using rat kidney cells infected with murine sarcoma virus mutant "ts110" [136]. In these cells, Ni(II) chloride induced a seven-fold increase in the reversion of transformed phenotype as compared with the spontaneous reversion frequency. However, as in the G12 system, changes in the expression mutations leading to alternative viral RNA splicing were found in the transgene [125]. Other experiments, using the SHE cells, confirmed that cell immortalization could occur as an indirect result of carcinogen exposure following an induced high frequency change in the treated population, rather than a direct result of targeted mutagenesis [89].

5.3. Epigenetic effects

Further progress in understanding molecular mechanisms of nickel carcinogenicity has been achieved in a study showing that nickel compounds increase the extent of DNA methylation that leads to the inactivation of gene expression [131]. In that study, the position of the *gpt* transgene on the chromosome was found to be important, since exposure of cells to nickel compounds resulted in hypermethylation of the transgene when it was located near heterochromatin, but not when its location was distant from heterochromatin. The specificity and mechanisms of the induction of DNA hypermethylation by nickel are presently unknown [131]. It is likely that inactivation of a tumor suppressor gene by hypermethylation could assist in nickel-induced cell transformation. The search for such genes in nickel-exposed or transformed cells is in progress.

In addition to gene silencing by hypermethylation, a suppressive effect of nickel on histone H4 acetylation in vitro has been reported for both yeast and mammalian cells [137]. The acetylation at Lys-12 and Lys-16 in yeast was affected more than at Lys-5 and Lys-8; it was proposed that Ni(II) binding to His-18 in histone H4 might be responsible for this effect. These data suggest again that epigenetic toxicity resulting in aberrant gene expression may be involved in the mechanisms of nickel-induced cancer.

5.3.1. Alteration of cell signaling pathways

5.3.1.1. HIF-1 and the hypoxia mimicking effect. The intrarenal injection of carcinogenic nickel compounds has been known for many years to induce erythrocytosis through the increase of erythropoietin (Epo) levels [138,139]. Seventeen nickel compounds were administered to groups of rats by i.r. injection and renal cancers developed within 2 years post-injection in 9 of the 17 groups. Rank correlation of $P < 0.0001$ was observed between the incidences of erythrocytosis and renal cancers [139]. Under physiological conditions, Epo is induced by hypoxia; its expression is under the control of the hypoxia-inducible transcription factor (HIF-1) [140]. Like hypoxia, Ni(II) also induces expression of a glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase [141,142], the vascular endothelial growth factor VEGF [143], and the *Cap43/NDRG-1* gene product [144]. Strong induction of the *Cap43/NDRG-1* gene by various nickel compounds has been observed in all tested cell lines and rat tissues [144], and both nickel and hypoxia produce this effect through activation of the same transcription factor, HIF-1; therefore, this gene can be considered as a marker of hypoxia [142]. The induction of the HIF-1α protein and transcriptional activation of HIF-1 responsive reporter plasmids following nickel exposure was demonstrated in human and rodent cells [145].

HIF-1α is the hypoxia-inducible subunit of the HIF-1 transcription factor. It binds the HIF-1α subunit and transactivates target genes [146,147], including genes coding for glucose transporters 1 and 3 and eleven glycolytic enzymes [148]. During tumor development, HIF-1 facilitates angiogenesis which is essential for tumor growth [149,150]. In hypoxic cancer and stromal cells, HIF-1 transactivates autocrine and paracrine growth/survival factors including VEGF, FGF, PAI-1, adrenomedullin, nitric oxide synthase (NOS), and transferrin. These factors stimulate

endothelial cell proliferation, migration, invasion, and angiogenesis [151–153].

The GeneChip microarray technique revealed that genes coding for glycolytic enzymes, glucose transporters, and other hypoxia-inducible genes regulated by HIF-1, were induced by nickel in HIF-1α proficient, but not in HIF-1α-null mouse embryonic cells [154–156]. Like hypoxia itself, nickel causes transactivation of the hypoxia-inducible genes through the induction of HIF-1 transcription factor in a reactive oxygen species (ROS)-independent manner [157].

Under normoxic conditions, the HIF-1α protein is rapidly degraded by the proteasome, and therefore is maintained at low levels [158]. Under hypoxic conditions, the degradation stops and HIF-1α becomes quickly accumulated whereas reoxygenation results in a prompt resumption of its degradation [159]. Therefore, HIF-1 plays the role of an oxygen sensor. In the presence of O_2, proline hydroxylase, a Fe(II)-dependent enzyme, hydroxylates HIF-1α at Pro-564 [160–162]. The hydroxylated proline residue is recognized by the von Hippel–Lindau protein (VHL), a component of the E3 ubiquitin ligase complex [163]. The interaction with VHL leads to ubiquitination and subsequent degradation of HIF-1α. Under hypoxic conditions, Pro-564 is not hydroxylated, HIF-1α protein does not bind to VHL that leads to its accumulation. HIF-1α hydroxylation requires O_2 and the Fe(II)-dependent enzyme, proline hydroxylase. In addition, Asn-803 in the C-terminal transactivation domain of HIF-1α is hydroxylated by a factor inhibiting HIF-1 (FIH-1) under normoxic conditions, causing abrogation of the HIF-1α/p300 interaction [164,165]. This reaction also requires oxygen as a substrate and iron as a co-factor. Under hypoxic stress, i.e., in the absence of Asn-803 hydroxylation, p300 (an acetyltransferase) binds to HIF-1 and transcriptionally activates HIF-1-dependent genes. Thus, in order to activate the HIF-1-dependent pathways, Ni(II) should stabilize HIF-1α protein and facilitate HIF-1 interaction with p300. This is only possible when hydroxylation of the proline and asparagine residues in HIF-1α is prevented. The key factor for the hydroxylase activity is Fe(II). Therefore, Ni(II) is likely to induce hypoxia through the depletion of cellular iron, competitive inhibition of proline hydroxylase activity, or both. The first postulated effect may stem from the Ni(II)/Fe(II) antagonism at the DMT-1/Nramp 2 cation transporter, while the inhibition by Ni(II) of proline hydroxylase remains to be tested (Fig. 1).

5.3.1.2. ATF-1. Exposure of cells to nickel compounds induces changes in gene expression that leads to expression patterns characteristic for cancer cells. For example, acute treatment of rodent cells with nickel down-regulates the expression of thrombospondin I (TSP I), a potent suppressor of angiogenesis [166,167]. Loss of TSP I expression in tumors promotes angiogenesis and thus stimulates tumor growth. The down-regulation of TSP in nickel-transformed cells was not accompanied by any methylation changes in the promoter of the gene [167]. It was found, however, that the ATF-1 transcription factor was hyperactivated in these cells, acting as a negative regulator of TSP I [167]. ATF-1 belongs to the ATF/CREB family that was originally identified as a target of the cAMP signaling pathway (reviewed in ref. [168]). Elevation of intracellular calcium also activated a protein kinase cascade that mediated ATF/CREB phosphorylation. Thus, the available data indicate that one or both of these pathways may be modulated in nickel-exposed and transformed cells.

5.3.1.3. NF-κB. Intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1, E-selectin), are endothelial surface molecules that play a role in leukocyte recruitment to sites of inflammation during contact hypersensitivity. ICAM-1, VCAM-1, and ELAM-1 were found to be up-regulated by Ni(II) in cultured human umbilical vein endothelium cells (HUVEC) [169]. The induction of adhesion molecules by Ni(II) required de novo mRNA and protein synthesis. Up-regulation could be blocked by kinase inhibitor H-7 but not staurosporine, suggesting involvement of phosphorylation independent of protein kinase C. Moreover, pre-treatment for 24 h with Ni(II) produced hypo-responsiveness to IL-1 and TNF-α upon restimulation, suggesting that Ni(II) and these cytokines might partially share a common pathway of activation. When the transcriptional mechanisms underlying gene-inductive effects of nickel were studied, NF-κB transcription factor was found to be involved in the inducible expression of adhesion molecules. A strong increase of NF-κB binding with DNA was

found after stimulation of HUVEC with Ni(II) or Co(II) [170]. NF-κB is an important transcription factor in apoptosis and inflammation. It is clear that activation of NF-κB by nickel causes significant modulation of cellular and tissue responses. In addition, activation of NF-κB explains nickel-induced allergic effects and contact skin hypersensitivity in humans [169].

5.3.1.4. p53. This tumor suppressor gene and transcription factor is involved in the regulation of cell proliferation and apoptosis. Mutations in *p53* are the most common genetic alterations found in human cancer [171]. The *p53* gene was reported to be mutated in human kidney epithelial cells transformed by nickel [172]. However, no mutations in the *p53* gene were found in 10 analyzed nickel-induced rat renal tumors [173]. This raises the question as to whether *p53* mutations are essential for nickel-induced neoplastic transformation. The acute treatment of human cells with Ni(II) induced the expression of wild-type p53 protein, but not of mutant *p53* [145]. Another report suggested the induction of p53 protein by Ni(II) acetate in CHO cells [174]. It is not clear thus far whether the induction of p53 protein results from nickel-assisted DNA damage, or from stabilization of p53 by other factors as reported for HIF-1 [175]. Despite the initial induction of p53 in cells placed in Ni(II)-containing media, the functional activity of p53 decreased when the cell were transformed. Additionally, in human and rodent cells transformed by nickel, a shift in the balance of HIF-1-dependent transcription versus p53-dependent transcription was observed [145].

5.3.1.5. Retinoblastoma. This tumor suppressor protein was first reported to be lost or mutated in retinoblastomas [176]. The retinoblastoma (Rb) mutation may result in deregulation of the cell cycle. A substantial amount of the Rb-interacting proteins are transcription factors such as E2F, Elf-1, DRTF-1, and NF-IL6 [177]. In general, these transcription factors are inactive when they are bound to a hypo-phosphorylated form of Rb. Once Rb is phosphorylated, the transcription factors are released and thus activated. Rb itself also regulates the expression of c-fos, c-myc, Sp-1 and some other transcription factors by direct binding to specific sites on the promoters. These data suggest that Rb plays a fundamental role in the regulation of transcription and, most importantly, phosphorylation of Rb protein was found to be decreased in nickel-transformed cells [178].

Human osteosarcoma (HOS) cells do not grow in soft agar or form tumors in athymic mice. However, when these cells were treated with water-soluble Ni(II) sulfate, or water-insoluble NiS, an increase in anchorage-independent colony formation was observed [178–181]. As found, eight of nine examined clones of such transformed cells contained Rb protein in the hypophosphorylated form [178]; mutations in the Rb gene in some of them were also identified. In addition, some of the clones showed decreased expression of Rb (Salnikow, unpublished observation). When these cells were transfected with a plasmid containing wild-type Rb gene, normal phosphorylation pattern was restored and the cells lost their ability to grow in soft agar. Neither the mechanism for abnormal Rb phosphorylation nor its metal specificity are known. However, since lead acetate and uranyl chloride produced similar effects, one may suggest that Rb hypo-phosphorylation is not nickel-specific, but is rather a part of the transformed phenotype [181].

5.3.1.6. FHIT. The *FHIT* (for Fragile Histidine Triad) gene is a tumor suppressor gene located in a fragile chromosomal site sensitive to deletions. Therefore, its expression is frequently reduced or lost in tumors and pre-malignant lesions. Its product, Fhit protein (phosphohydrolase), induces apoptosis through a complex interaction with its substrate, diadenosine triphosphate (Ap_3A) [182]. Ni(II) was found to strongly inhibit the enzymatic activity of Fhit protein in vitro [183] and also suppress Fhit's expression in nickel-transformed BALB/c-3T3 cells. In these cells, Fhit protein levels were reduced by 50% versus those in the parental cells. A decrease in Fhit protein levels by up to 90% was also observed in 22 local sarcomas induced by i.m. injection of Ni_3S_2 in mice, as compared with normal mouse muscles [184]. Moreover, Fhit was absent in 4 of those sarcomas. The decrease in Fhit expression coincided with faster development of tumors. Overall, the decline of Fhit in cells or tissues malignantly transformed by nickel, along with inhibition by nickel of Fhit's enzymatic activity, may indicate possible contribu-

tion of these two effects to the mechanisms of nickel carcinogenesis [184].

5.3.2. Modulation of gene expression

In addition to the above, the advent of the differential display and microarray techniques allowed for identification of many other up- and down-regulated genes and gene products in nickel-exposed cells. Thus, exposure of CHO cells to Ni(II) acetate resulted in a dose-dependent down-regulation of the expression of vimentin and the hSNF2H chromatin remodeling factor, but up-regulation of H ferritin expression [185]. However, in many tumors, including nickel-induced muscle tumors, vimentin was found to be up-regulated [186], indicating a more complex response. Increased expression of vimentin is associated with invasion and metastasis of tumors [185]. The hSNF2H protein belongs to the family of chromatin remodeling factors including the SWI/SNF complex, which alter chromatin structure and facilitate gene transcription [187]. Inactivation of the SWI/SNF complex is known to block cell cycle at the G_2/M phase, an effect observed in Ni(II)-exposed CHO cells [174]. Increased expression of the H ferritin may be a part of cell response to the hypoxic effect of Ni(II) (see Section 5.3.1.1).

The analysis of gene expression in immortalized human lung epithelial cells, HPL1D, cultured for 24 h in media containing 0.05–1.6 mM Ni(II) acetate, revealed more than two-fold change in expression of over 800 known genes at any Ni(II) concentration. Of them, 113 genes responded to the non-toxic concentration range, up to 0.2 mM Ni(II), most relevant to in vivo nickel carcinogenesis. Among these genes were those coding for the metallothionein, ferritin, heat shock proteins, RhoA, dyskerin, interferon regulatory factor, RAD21 homologue, and tumor protein translationally controlled. Overall, most of the genes impacted by non-toxic Ni(II) concentrations were associated with gene transcription, protein synthesis and stability, cytoskeleton, cell signaling, metabolism, cell membrane, and extracellular matrix [188]. It remains to be explored how much the observed up- or down-regulation of these genes can contribute to the development of lung cancer.

Acute lung injury in mice inhaling soluble Ni(II) sulfate changed the expression pattern of nearly 9000 genes in a way characteristic for oxidative stress, hypoxia, cell proliferation, and extracellular matrix repair [189]. Both soluble Ni(II) sulfate and insoluble Ni_3S_2 induced similar signaling pathways in cultured mouse embryo fibroblasts following a 20 h exposure. They included up-regulation of HIF-1, p53, and Egr-1, and down-regulation of WT1 transcription factors which are similarly modulated by exposure to hypoxia. Modulation of these transcription factors by nickel is believed to exert a potent selection pressure that may lead to cell transformation [155].

5.3.3. Nickel effect on DNA repair

DNA damage by various insults may lead to cell transformation or death, but only if not repaired. Therefore, inhibition of any element of the complex DNA repair and/or pro-apoptotic systems bears the potential of assisting in carcinogenesis. In vitro, Ni(II) and some other heavy metals were found to impair the function of DNA polymerase and cause base misincorporation into newly synthesized oligonucleotides [190]. As discovered in a series of extensive investigations by Hartwig and co-workers, nickel along with several other carcinogenic metals is a potent inhibitor of base and nucleotide excision repair mechanisms. This enhances DNA damage by UV radiation, reactive oxygen species, benzo[*a*]pyrene-7,8-diol 9,10-epoxide, and methylating agents [191–195]. The proteins targeted by Ni(II) in the DNA repair systems include the Xeroderma pigmentosum group A complementing protein (XPA), a zinc-finger protein [195,196], and O^6-methylguanine-DNA methyltransferase [193], but not formamidopyrimidine-DNA glycosylase (Fpg) [197]. Effects of Ni(II), Cd(II), and other carcinogenic metals were also tested on another class of DNA repair enzymes, the bacterial and human 8-oxo-dGTPases, MutT and MTH1. These enzymes prevent utilization of 8-oxo-dGTP and other promutagenic nucleoside triphosphates in DNA synthesis. Ni(II) was found to be a weak, non-competitive inhibitor of these enzymes (reviewed in ref. [198]).

5.3.4. Nickel effect on calcium homeostasis

The role of changes in calcium homeostasis in cell transformation is not well understood. Ca^{2+} is recognized as one of the most important intracellular second messengers; its concentration is maintained at a very steep gradient between the outside and the inside of all mammalian cells [199,200]. Cytoplasmic Ca^{2+} pulses signal gene expression associated with

cell growth, differentiation, and apoptosis of many different types of cells in the body [200,201]. Although a "calcium theory of oncogenesis" had been formulated by Jaffe in 1982 (reviewed in ref. [202]), only a few studies have associated the carcinogenic and/or toxic effect of nickel with disturbances in calcium metabolism. One of the earliest observations that nickel-transformed cells could rapidly proliferate in a low-calcium media suggested alteration of intracellular calcium metabolism in nickel-transformed cells [203,204].

In vivo, parenterally administered Ca(II) acetate prevented the formation of lung adenomas caused by Ni(II) or Pb(II) acetates in mice [35], but Ca(II) carbonate had no significant effect on Ni_3S_2 carcinogenesis in the rat muscle [70]. When administered alone, Ca(II) acetate increased the incidence of mouse lung adenomas. In cultured cells, *Cap43* was similarly induced by Ni^{2+} and by the Ca^{2+} ionophore A23187. The induction of *Cap43* by these two metals was abolished when free intracellular Ca^{2+} was sequestered by a calcium chelator BAPTA-AM. These observations confirmed the notion that free intracellular Ca^{2+} was elevated in nickel-treated cells [205].

Soluble Ni^{2+} probably enters the cell via calcium channels since the Ca^{2+} ionophore ionomycin (3 μM) increases nickel uptake four- to five-fold [97,206]. Additionally, nickel uptake into IHKE cells was inhibited by calcium. Ni^{2+} has been found to block Ca^{2+} channels [207], and it is likely that an initial decrease in intracellular Ca^{2+} level in response to Ni^{2+} is followed by a compensatory release of Ca^{2+} from intracellular stores. In fact, nickel was found to have evoked the release of stored intracellular Ca^{2+} via a mechanism involving a cell surface receptor [208]. Another possibility is that Ni^{2+} ions interact with a Ca^{2+} sensor or receptor on the plasma membrane to activate intracellular Ca^{2+} release. However, modulation of extracellular Ca^{2+} levels from zero up to 7 mM neither affected the expression of the *Cap43*/*NDRG-1* gene, nor its induction by 1 mM of Ni(II) chloride [205]. This indicates that Ni^{2+} ions interact with a surface Ca^{2+} receptor in a non-competitive mode. Inhibition of Ca^{2+} channels by Ni^{2+} may also affect transport and homeostasis of another important physiologic metal, iron. For example, Ni^{2+} was found to drastically decrease the intracellular Ca^{2+}-dependent recycling rate of the transferrin receptor Tf-R [209].

6. Chemical basis of nickel carcinogenesis

6.1. Nickel-binding to amino acids, peptides, and proteins

6.1.1. Nickel carriers

At the physiological pH range, the strength of Ni^{2+} interactions with proteins depends on the type of amino acid residues, their positions relative to each other, and their accessibility in the protein molecule. Under certain conditions, deprotonated peptide nitrogen may also coordinate Ni^{2+} ions. In concordance with the highest relative affinity for Ni^{2+} of free histidine (thanks to imidazole nitrogen) and cysteine (the sulfhydryl group) and their small peptides (e.g., carnosine, anserine, glutathione), the greatest affinity for Ni^{2+} is shown by the histidyl and also cysteinyl residues in proteins, and especially by the Xaa-Yaa-His (or XYH in one-letter code; X and Y stand for any amino acid) motif at the N-terminus. This motif coordinates Ni^{2+} in a square planar ring that is created by (a) the terminal amino group; (b) deprotonated nitrogen atoms of the two subsequent peptide bonds; and (c) the imidazole nitrogen of the histidine residue at the third position [2,210–213]. The very strong affinity of Ni^{2+} for His is widely employed for purification of recombinant proteins having N-terminal hexa-histidyl tags, on agarose containing immobilized Ni^{2+} ions [214]. Interestingly enough, the ubiquitous metal carrier metallothionein appears not to be a major Ni^{2+}-binding ligand [215].

Transport of nickel in blood plasma is mediated by binding to albumin and ultrafiltrable ligands [216,217]. The primary Ni(II)-binding site of serum albumin has been identified and characterized as the N-terminal XYH motif: e.g., DAHKSEVA—in human, DTHKSEVA—in bovine, or EAHKSEIA—in rat albumin [218,219]. A secondary Ni(II)-binding site, likely to involve His-105, His-146, and/or His-247 in the folded molecule, has also been identified in human, bovine, and porcine albumins [220]. Small ultrafiltrable Ni(II)-binding ligands in blood plasma include amino acids (e.g., histidine), small peptides [217,219], and (provisionally) creatine phosphate [221].

A major fraction of plasma nickel is present in nickeloplasmin, which is a Ni(II)-containing α_2-macroglobulin [222,223]. The nickel content of

nickeloplasmin is not readily exchangeable with free Ni^{2+}, and nickeloplasmin seems not to be involved in the extracellular transport of nickel [224].

In the cytosol of rodent kidney, lung, and liver, parenterally administered Ni(II) is bound to several macromolecular and low molecular weight constituents [211,221,225–230]. Of particular interest is neuromedin C, a bombesin-like neuropeptide that avidly binds Ni^{2+} and Cu^{2+} cations [231].

The results from nickel equilibrium studies concluded that a Ni(II)–L-histidine complex is the major form of nickel transport across the cell membrane, and a Ni(II)–albumin complex is the form for systemic transport [210]. Using ^{63}Ni(II), it was shown that nickel was transported in vitro into mouse fibroblasts in both the form of a complex with serum protein and complexes with low molecular weight molecules, and subsequently incorporated into the nuclei [232].

6.1.2. Regulatory proteins

Recently, differential screening of genes that are suppressed in neuroblastoma cells resulted in the cloning of a new gene DAN, that encodes a protein which possesses Ni(II)-binding motifs (PHSHAH-PHP) in the C-terminal region [233]. This motif allows native DAN protein to be isolated on a Ni(II) affinity resin. The expression of DAN is significantly reduced in transformed cells and it has recently been demonstrated that DAN has tumor-suppressive activity [234]. Cell cycle analysis revealed that overexpression of the DAN gene product causes retardation of cellular growth by blocking entry into the S phase. Thus, possible interaction of DAN protein with Ni(II) may impair cell cycle regulation in nickel-treated cells.

Another Ni(II) binding protein is pNiXa, a serine protease inhibitor (serpin) abundant in *Xenopus* oocytes and embryos. It has similarity to tissue plasminogen inhibitor [235]. The serpin pNiXa has the histidine-rich domain –HRHRHEQQGHHDSAK-HGH– and forms six-coordinate octahedral Ni(II) complexes. As with the DAN gene product, binding of Ni(II) to pNiXa may lead to the loss of protein function and cause embryotoxicity and tissue injury. The importance of proteases like tissue plasminogen in carcinogenesis is well known [236]. *Xenopus laevis* oocytes and embryos also contain a 40 kDa protein that can be isolated on Ni(II)-agarose [237]. This nickel-binding protein shows similarity to eukaryotic aldolases and is 96% identical with human aldolase A. These data suggest that aldolase A may also be a target for nickel toxicity. Similarly, lipovitellin 2β appears to be a Ni(II)-binding protein [238].

Import of proteins from the cytoplasm into the nucleus is essential for signal transduction. One of the key components of nuclear transport, importin α also can be purified on a Ni(II)-agarose column [239]. This observation raises an interesting question if Ni(II) can impair transport of nuclear proteins and transcription factors. If so, this could cause a significant deregulation of gene expression.

In its substrate-binding groove designed to interact with Ap_3A, Fhit protein (Section 5.3.1.6) has several amino acid residues, including histidines His-35, His-96, His-98. Along with Cys-39 located nearby [240], this arrangement may provide a strong binding site for some transition metals, including Ni(II). The possible involvement of Cys-39 in metal coordination may increase Fhit's sensitivity to oxidation that blocks its enzymatic activity [183]. Therefore, Ni(II) binding has the potential to inhibit interaction of Fhit with its substrate Ap_3A and thus impair its tumor suppressive pro-apoptotic function. Likewise, nickel effects on the Cap43 protein, mentioned previously, are presumably mediated through Ni(II) coordination to His and other residues in the –TRSRSHTSEGTRSR– motif [241].

Ni^{2+}, like several other transition metal cations, can also bind to the iron regulatory protein-1 (IRP-1), a central regulator of iron homeostasis [242]. In this case, nickel modulates IRP-1 by replacing iron at the fourth labile position in the 4Fe-4S cluster. IRPs control RNA stability or the translation rate of several proteins, including transferrin receptor and ferritin. When cellular iron is scarce, IRP-1 loses one Fe with the formation of a 3Fe-4S cluster and in this state has little enzymatic (aconitase) activity. The latter effect is also produced by Ni(II) [242] that may falsely signal iron scarcity and thus contribute to the hypoxic response described in Section 5.3.1.1.

Generally, besides certain specific effects reviewed below, the ability of Ni^{2+} cation to react (form complex species) with a number of proteins raises the possibility that nickel may significantly alter their conformation, and thus change their functions and cellular homeostasis, producing a variety of pathogenic effects including stress similar to unfolded protein response.

6.1.3. Histones and protamines

Binding of Ni^{2+} to DNA is relatively weak, especially in the presence of the physiological DNA counter-ion, Mg^{2+}, and amino acid ligands [243]. Therefore, in nuclear chromatin, the major target for nickel binding appear to be the proteins, especially the histones and protamines. This subject has been recently reviewed by Bal et al. [244]. Briefly, at physiological pH, strong Ni(II)-binding motifs have been found in protamine P2 and in core histones H3 and H2A, and a weak one in histone H4. Protamine P2 contains the classic XXH N-terminal motif, –RTHGQSHYRR– [245]. The histone H3 motif –CAIH– is located in a hollow, metal-accessible structure of the core histone octamer [246,247], while the –TESHHKAKGK– motif of histone H2A is positioned near the end of its unstructured, 34-amino acid-long, C-terminal tail [247,248]. The weak Ni(II) coordination by histone H4 is offered by the –AKRHRK– motif, located around His-18 in the N-terminal tail of this histone [247,249]. Binding of Ni(II) by histone H3 in the histone tetramer was confirmed in vitro experimentally [250]. The sequestration of Ni^{2+} by histone tetramer $(H3/H4)_2$ and histone H2A has been evaluated using numerical models and found to be substantial even in the presence of maximal physiological concentrations of the major competing cellular ligands His and glutathione [244,251]. Very importantly, Ni(II) coordinated in the –CAIH–, –RTHGQSHYRR–, and –SHHKAKGK– complex systems appeared to be redox active, i.e., capable of mediating oxidative damage to other molecules (see below). The latter complex is derived from the original Ni(II)–TESHHKAKGK complex with histone H2A owing to a novel effect: nickel-facilitated hydrolysis of the E–S bond [252]. Since the C-terminal tail of H2A is involved in maintaining chromatin structures [247,253], its truncation in nickel-exposed cells may affect chromatin in a way disturbing orderly gene expression.

6.2. Nickel-induced oxidative damage

The possible involvement of reactive oxygen species in nickel carcinogenesis was reviewed previously [123,254,255]. As compared with copper, iron, cobalt and other redox-active metals, nickel produces relatively low, but measurable levels of ROS in cells as detected by the dichlorofluorescein (DCF) method [157,256,257] (Fig. 1). Both soluble Ni(II) chloride and insoluble Ni_3S_2 enhanced the formation of intracellular ROS after 6 h of exposure. After 18 h, more ROS were observed at the nucleus when cells were exposed to Ni_3S_2 [256]. In addition to the direct measurement of free radicals, depletion of the antioxidant glutathione represents another marker of oxidative stress. In vivo, hepatic glutathione levels diminished greatly after Ni(II) injection [78,258]. Likewise, glutathione was found to be depleted by nickel in cultured cells [257,259]. The 3T3 cells made resistant to high concentrations of Ni(II) chloride were found to exhibit cross-resistance to H_2O_2 and menadione. The nickel-resistant cells had their basal levels of glutathione nearly twice as high as the wild-type cells. These results suggest that the resistant cells acquire some defense mechanisms against oxidative stress.

The oxidative effects of nickel depend on its ability to form the Ni(III)/Ni(II) redox couple around pH 7.4. This is possible only when Ni(II) is complexed by some natural ligands, including peptides and proteins, especially these which form square planar nickel complexes, e.g., GGH, or GGGG [244,260]. A list of such ligands is provided by ref [261]. An important result of reactions of such Ni(II) complexes with oxygen species, e.g., endogeneous O_2 or H_2O_2, is generation of not only the hydroxy radical $OH^{\bullet}$ (or an oxo-cation NiO^{2+}), but also other oxygen-, carbon-, and sulfur-centered radicals originating from the ligands [261–263].

A variety of reactive intermediates can also be produced in the process of oxidative cellular solubilization of nickel sulfides Ni_3S_2 and NiS. Both are sensitive to oxidation by ambient oxygen which facilitates their dissolution in biological fluids, though with different kinetics [28]. The simplified summary oxidation reaction of Ni_3S_2 is stepwise and eventually leads to intracelluar formation of soluble Ni(II) complexes with natural ligands (L), e.g., amino acids and proteins [76,114]. The first step requires less oxygen:

$$Ni_3S_2 + \tfrac{1}{2}O_2 + 2HL \rightarrow 2NiS + Ni(II)L_2 + H_2O \qquad (1)$$

Oxidation at this step is initially rapid but slows in time because of the formation of a thickening layer of crystalline NiS and other insoluble products (e.g., with sulfhydryl ligands) on the surface of Ni_3S_2 particles.

Further release of Ni(II) requires oxidation of NiS's sulfur that consumes much more oxygen and is slower:

$$NiS + 2O_2 + 2HL + 2OH^- \rightarrow Ni(II)L_2 + SO_4{}^{2-} + 2H_2O \quad (2)$$

Therefore, just after application, Ni_3S_2 particles are able to deliver a short burst (hours) of soluble Ni(II) to target cells that is followed by a slow, but prolonged (weeks) release of Ni(II). The first phase may just facilitate tumor initiation (high, but short-lasting Ni(II) dose) while the second phase would allow Ni(II) to act in a tumor promoter mode [76]. The oxidation reactions are, in fact, more complex and generate reactive intermediates. It was found, that O_2 reacting with Ni_3S_2 can be reduced to H_2O_2 [264] and the sulfur oxidation goes through reactive sulfur species, including the sulfite anion [265]; such intermediates are capable of inflicting promutagenic DNA damage through base oxidation or deamination [123,254,261]. This makes the nickel sulfides able to generate greater and more diverse oxidative damage than that produced by other compounds, and this is likely to underlay their high carcinogenic activity. However, one has to remember that if the damage is too extensive, as in the case of the highly redox-active metals copper, iron, or cobalt, the result may be "overkill" rather than survivable damage of cells [254,261]. The "overkill" effect may be a major reason for weaker carcinogenic activity of these metals, as compared with nickel. For example, Tkeshelashvili et al. [266] noticed that the ratio of increased mutagenesis to loss of survival of cells transfected with DNA damaged by metal-generated ROS was greater for Ni(II) than for Fe(II) or Cu(II). It is also important mechanistically that in the redox reactions of nickel complexes, oxidative damage is inflicted not only upon the bystander molecules, like DNA or non-binding proteins, but also on the complexing ligand itself [244,252,260,267,268].

6.2.1. Protein damage

The oxidation of amino acids and proteins by ROS and the roles of toxic metals in this process have been reviewed before [269–271]. Along with the other types of oxidative damage, protein oxidation is believed to be mechanistically involved in a wide variety of adverse effects, including cancer [261–263,269–271]. Nickel, like many of the other transition metals, may promote oxidative modification of both free amino acids [269] and the amino acid residues in proteins. In the latter, major targets are the side chains of Cys, His, Arg, Lys, and Pro, residues. The sulfhydryls are commonly oxidized to disulfides, but they may be turned into sulfino-, sulfeno-, and solfono-derivatives as well; the latter is also true for the Met residue. The His imidazole may be oxidized to aspartic acid, asparagine, or 2′-OH-His. Arg is converted to γ-glutamic-semialdehyde; Lys, to 2-amino-adipic-semialdehyde; and Pro is turned into glutamic acid, pyroglutamic acid, γ-aminobutyric acid, and γ-glutamic-semialdehyde [4–6,8]. According to most recent results from Stadtman's laboratory, glutamic and aminoadipic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins [270].

The formation and rearrangements of radical intermediates arising in the oxidation process of protein molecules also result in protein fragmentation and intra- and inter-protein cross-linking. Because of that, proteins also may become cross-linked with DNA [261–263]. It seems obvious that such a variety of oxidative effects on proteins must be widely pathogenic. The site-specificity of Ni(II)- and other metals-mediated protein damage depends on the coordination of transition metal ions by proteins and peptides predominantly through the imidazole, sulfhydryl, and the deprotonated peptide bond and side chain nitrogens, followed by generation of ROS (e.g., from metabolic H_2O_2) at these particular sites. Therefore, Trp, Tyr, Phe, and Met, which are also sensitive to oxygen radicals, but do not bind Ni^{2+} under physiological conditions, are less likely to be targeted by metal-catalyzed oxidation [269]. They may, nonetheless, be damaged if they are located close to the metal binding site.

A good example of both oxidative and conformational effects of Ni(II) on polypeptides is that observed in experiments with a 15-mer peptide, RTHGQSHYRRRHCSR-amide ($HP2_{1-15}$), modeling the N-terminal sequence of human protamine P2 [245,267,268]. When bound to the RTH-end of this peptide, Ni(II) catalyzes oxidation by H_2O_2 of not only Arg-1 and His-3, but also of Tyr-8. The reason for this is a strong structuring effect of Ni(II) on the peptide ligand [268]. This effect brings Tyr-8 close to the metal center. It also shifts all the positive Arg side

chains to one side of the molecule. Thus, by imposing conformational changes on its ligand, the bound Ni(II) can focus oxidative damage on a particular target, and also modulate the function of the ligand, e.g., increase the DNA–peptide binding [245,267,268].

Ni(II) coordination mode was the most likely cause of a profound difference in redox activity between two Ni(II) complexes originating from the C-terminal "tail" of histone H2A. Ni(II) is bound by this peptide through the –TESHHK– motif and forms a non-redox-active octahedral complex [248,252]. However, Ni(II) binding causes hydrolysis of the ES peptide bond with liberation of the SHHKAKGK peptide ($H2A_8$). The latter binds Ni(II) through the –SHH– motif, yielding a square planar complex. The latter, like many other square planar Ni(II) complexes, is redox-active. Reaction with H_2O_2 results in degradation of its Ser and His residues and collateral oxidative damage to DNA [252].

6.2.2. DNA damage

In nuclear chromatin, the DNA molecule, having an abundance of phosphate anions and nitrogen and oxygen donor groups, is an ideal binding partner for metal cations, including Ni^{2+}. As reviewed above, the chromatin proteins can bind Ni^{2+} even stronger [245,248,261–263,272]. This helps to explain why, following in vivo exposure, heavy metals, including nickel, are found in cell nuclei [273–277]. The generation of $O_2^{\bullet -}$ and H_2O_2, was also detected in cell nuclei [277]. Hence, the bound metal can catalyze ROS generation in the cell nucleus and thus facilitate oxidative damage to DNA and other nuclear components, as observed experimentally [4,261–263]. Important targets for metals are also mitochondria and mitochondrial DNA [261–263,276].

The major oxidative effects in DNA associated with exposure of experimental animals and cultured cells to nickel and other transition metals include strand scission, depurination, cross-linking, and base modifications [4,261–263].

6.2.2.1. DNA base damage. The spectrum of chemical changes produced by hydroxyl radical, $OH^{\bullet}$, in the base moiety of DNA is well established [278]. Many modified DNA bases from that spectrum have been found in isolated chromatin or DNA exposed to H_2O_2 plus Ni(II), Co(II), Cu(II), or Fe(III). The most abundant is usually 8-oxoguanine, most often measured as 8-oxo-2′-deoxuguanosine (8-oxo-dG). Under ambient O_2, Ni(II) generated such bases in chromatin, but not pure DNA, indicating possible facilitation of its redox activity by chromatin proteins [279]. Indeed, as described above, amino acid binding domains for Ni(II) and redox activity of the bound Ni(II) were identified in core histones. Some other nuclear proteins are also able to produce this effect. For example, heterochromatic proteins isolated from CHO cells enhanced the formation of 8-oxo-dG by Ni(II) plus H_2O_2, in vitro, whereas euchromatic proteins inhibited this reaction [280]. It is thus possible that nickel may inflict DNA damage predominantly in genetically inactive heterochromatin that would be consistent with its low mutagenic potential. Besides attacking DNA bases directly in DNA strands, nickel-generated ROS can also damage the bases in the triphospho-nucleoside pools (e.g., generate 8-oxo-dGTP) from which the bases may be misincorporated into genomic DNA, or RNA. To prevent this, cells are equipped with "sanitizing" enzymes like MutT or MTH1 (8-oxo-dGTPases) [281].

Following in vivo exposure to nickel compounds, elevated amounts of at least one damaged DNA base were found in organs of F344 [282–284] and Wistar rats [285] and in BALB/c mice [261,286]. The distribution of the damaged DNA bases in the rat kidney, which is a target organ for nickel carcinogenesis, differed significantly from that in liver, a non-target organ [282]. In the lungs of rats, both insoluble Ni_3S_2 and NiO (black and green types) and soluble Ni(II) sulfate, instilled intratracheally, increased pulmonary 8-oxo-dG levels, but in cultured HeLa cells only Ni_3S_2 was active. This difference seems to indicate different mechanisms of the damage in vitro and in vivo by the same compounds, most likely involving contribution of ROS generated by inflammatory cells in the rat lung [285]. In Ni(II)-treated mice, renal 8-oxo-dG levels were increased only in the BALB/c strain, which had low glutathione and glutathione-peroxidase levels compared to two other strains, B6C3F1 and C3H [261,286].

6.2.2.2. Cross-linking. The most common effect of toxic metals in chromatin observed in vitro and in vivo is DNA–protein cross-linking (reviewed in refs. [261–263,287,288]). Generally, metal ions can generate DNA–protein cross-links in two ways: by

bringing both partners together in mixed-ligand complexes, or by inducing the formation of strong covalent bonds directly between DNA and the proteins. In the case of nickel, the formation of cross-links of both types has been found in vitro to be facilitated by microsomal proteins [265] and tetraglycine in the presence of ambient oxygen [289]. The redox active Ni(II)–tetraglycine complex enhanced oxidative DNA–protein cross-linking in isolated nucleohistone and protein–protein cross-linking among free histones [289]. Histidine promoted DNA–protein cross-linking in the rat kidney following an in vivo treatment with Ni(II) [283].

Intra- and inter-strand cross-linking between neighboring DNA nucleotides is also possible, but the effect of nickel and other metals on its formation has not been fully explored. Formation of cross-links between two cytosines or two thymines in the same strand is typical of DNA damage produced by free radicals generated in water by γ- and UV-radiation. Such cross-links are believed to result in tandem double CC → TT mutations. Interestingly enough, such mutations followed exposure of template DNA to Ni(II), or other metals, plus oxidant [266].

The formation of cross-links in chromatin may manifest itself in morphologic aberrations of chromosomes. Such aberrations were observed, for example, in lymphocytes of workers exposed to nickel and chromium compounds [261–263]. In cultured CHO cells, chromosomal alterations caused by Ni(II) were predominantly localized in the protein-rich, heterochromatic region of the X chromosome [280].

6.2.2.3. Strand scission. DNA single-strand scission was found in kidneys and lungs, but not in livers of rats after parenteral administration of Ni(II) chloride [290]. In vitro, single and double strand breaks were observed in Ni(II)-treated blood lymphocytes [291] and in HeLa cells cultured with Ni_3S_2, but not with different preparations of NiO [285]. Single-strand breaks also were produced in isolated DNA by Ni(II) plus H_2O_2 [261–263,287,288]. This effect was enhanced by the peptides forming square planar Ni(II) complexes [245,267,292].

Kawanishi et al. [293] have found that Ni(II) promotes in vitro DNA cleavage by H_2O_2 in a site-specific way characteristic for action of a reactive nickel–oxygen complex rather than free $OH^{\bullet}$ or singlet oxygen. The most sensitive sites appeared to be at the cytosine, thymine, and guanine residues. DNA cleavage mediation by Ni(II), Cu(II) and Fe(III) complexes and ligand effects on the selectivity of DNA oxidation with various oxidants were studied in detail by several laboratories [261–263].

6.2.2.4. Depurination. The spectrum of DNA damage resulting from exposure to toxic and carcinogenic metals also includes depurination. Thus, Ni(II) produced apurinic sites in the DNA molecule, and released guanine from 2′-deoxyguanosine in vitro [294,295]. The underlying mechanisms are thought to involve oxygen radicals. Indeed, the depurination occurs concurrently with DNA strand scission and both effects can be the result of $OH^{\bullet}$ attack on the DNA sugar moiety; modified sugars constitute alkali-labile sites that are frequently found in DNA from Ni(II) and other metal-treated cells [261–263,278,294,295].

6.2.3. Molecular pathogenicity of the damage

Thus far, the strongest association of oxidative damage with carcinogenesis comes from the promutagenic nature of many DNA base products resulting from the attack of Ni(II)-generated ROS on DNA. Strand scission and depurination are thought to induce mutations as well [261–263,294,295]. Hence, emergence of these lesions in DNA may be considered as a genotoxic, tumor initiating event. Most importantly, Ni(II) can also inhibit DNA repair and thus augment such lesions [194,296,297]. However, mutations resulting from the mispairing properties of the damaged bases [296] are not the only result of their presence in DNA. As found, 8-oxoguanine may also misdirect DNA methylation [298] and disturb orderly binding of transcription factors to DNA [299]. These effects have the potential to disturb chromatin compaction and gene expression and thus constitute epigenetic events consistent with tumor promotion and progression.

Reactive oxygen species serve as physiological signal transduction messengers in controlling expression of genes, including oncogenes, tumor suppressor genes, and many others [263,300]. It seems likely, therefore, that the redox reactions driven by adventitious cellular metals like Ni(II) may disturb the timely and orderly generation of these messengers and affect the oxidation status of redox-dependent regulatory proteins, such as NF-κB, AP-1, p53, K-ras,

Bcl-2, HIF-1 [301,302], and others; the result must be detrimental to proper progression of the cell cycle and/or apoptosis [300]. The reduced level of binding of NF-κB and AP-1 transcription factors to their DNA consensus sequences in Ni(II)-transformed cells compared to wild-type cells, and their more reactive response following treatment of resistant cells with H_2O_2 or buthionine sulfoximine, clearly indicates that nickel resistance is closely allied to oxidative stress responses [257].

Nickel-mediated oxidation of regulatory proteins not belonging to the redox signaling network may affect their structure and function, as exemplified by the Fhit protein inhibition [183] (Section 5.3.1.6). Also, the binding of Ni(II) to histones that leads to their damage, reviewed here, may be mechanistically responsible for the effects of Ni(II) on heterochromatin morphology and gene expression regulation, reported by Costa et al. [303]. Unfortunately, the wide diversity of the protein oxidation products and the enormous complexity of the protein- and other molecule-dependent redox signaling network make it impossible at this moment to understand fully the mechanistic role of the oxidative protein damage in the lengthy multi-stage process of tumor induction and growth.

7. Conclusion

7.1. Mechanistic considerations

Since there is no convincing evidence of a direct mutagenic Ni(II)–DNA "adduct" formation in cells exposed to nickel compounds, current hypotheses on the mechanisms of nickel carcinogenesis consider the reported genetic and epigenetic effects of Ni(II) as indirect results of Ni(II) binding to various other molecular components of the cell, including chromatin proteins. Owing to that, the competition of Ni(II) with essential divalent metal cations for common cellular ligands and binding sites may underlie the observed inhibition of the experimental nickel carcinogenesis by Mg(II), Mn(II), Zn(II), and in some cases also Fe(II) and Ca(II) [35,54,68–74,127,202]. The binding, involving rigid Ni(II) coordination by several donor atoms in a protein molecule, must produce conformational change in its physiological structure (folding) and may thus derange its function. This type of effect is most likely responsible, at least in part, for Ni(II) inhibition of certain enzymes, e.g., the DNA excision repair enzymes, MTH1, Fhit, and inhibition of calcium transport channels and calcium-activated signaling molecules [97,199–209].

Examples of the most profound conformational alterations resulting from Ni(II) binding to macromolecules would include the B → Z transition of DNA [304,305] and the strong structuring effect of Ni(II) on a protamine P2 model peptide that increases its affinity for DNA and directs oxidative damage to the Tyr-8 residue [268]. The observed Ni(II)-facilitated hydrolysis of the C-terminal tail of histone H2A also is a likely result of a conformational change favoring the formation of reactive intermediate structures within the otherwise unstructured peptide chain. These effects would be consistent with the epigenetic/tumor promotional activity of Ni(II), reviewed in the preceding sections.

However, the widest possible spectrum of effects relevant to carcinogenesis may result from redox activity of Ni(II) complexes with certain cellular ligands, including amino acids, peptides, proteins, and other molecules, but not DNA [261–263,279,306,307]. The reactive oxygen species emerging from reactions of such complexes with ambient oxygen and its metabolic derivatives (O_2, $O_2^{\bullet -}$, H_2O_2, lipid peroxides) are capable of inflicting both site-specific and collateral damage to the ligands themselves and to other molecules. Thus, if the Ni(II) complex is located in the chromatin, as is the case of histones H3 and H2A, ROS may be generated very close to the DNA molecule and produce all the observed types of oxidative DNA damage. The close delivery of Ni(II) to the nucleus is best assured by phagocytosis of Ni_3S_2 followed by the obligatory intracellular generation of Ni(II). Therefore, oxidative DNA damage in cultured cells has been reported for this particular compound, but not for NiO [285], which is also phagocytosed, but practically not solubilized by a cytosol [28]. In addition, Ni(II) capacity to inhibit DNA repair [191–195] may contribute to the persistence of oxidative DNA damage caused by Ni(II) or other insults, such as UV, ionizing radiation, and endogenous metabolic oxidants.

As described in Section 6.2, the exceptionally high carcinogenic potential of Ni_3S_2 among nickel compounds may stem from redox reactions as well.

Oxidation is likewise involved in the mechanisms of metabolism and carcinogenesis by another potent nickel carcinogen, nickel carbonyl, $Ni(CO)_4$. Delivery of nickel to the cell by this volatile liquid compound is assured by its lipid solubility and high penetrance through cell membranes. However, to acquire biologic activity, the zero-valent nickel of this compound must be oxidized by cellular oxidants to Ni(II) [308,309].

The oxidative DNA damage has the capacity to produce both mutagenic and epigenetic effects. The former may be due to mispairing properties of the modified DNA bases and/or erratic repair of the other types of DNA damage in the presence of Ni(II). The results of both, namely the point mutations typical for base mispairing [127,266], and insertions typical for strand breaks [125] have been reported to occur on Ni(II)/oxidant-treated DNA templates as well as in nickel-exposed cells and nickel-induced animal tumors. The epigenetic effects of Ni(II)-mediated ROS attack on DNA would result from the derangement of gene expression caused by alterations in DNA methylation patterns [298] or in transcription factor binding at sites of the oxidatively damaged DNA bases [299].

The data reviewed in this paper clearly indicate that Ni(II), like many other transition metals, has an enormous potential to affect cellular functions through direct (e.g., conformational), or indirect (e.g., ROS-mediated) insults on proteins and nucleic acids [261–263,310,311]. The quality and extent of the damage strongly depend on intracellular Ni(II) dose and time of action. In this respect, high Ni(II) doses are more effective in producing genotoxic effects. Such doses are best delivered into the cells by phagocytosis of sparingly soluble nickel-containing dust particles. Lower doses presumably delivered by soluble nickel compounds may trigger only epigenetic toxicity [297,312] in cells targeted for neoplastic transformation. In humans and animals, Ni(II) target may also include the immune system, e.g., through activation of the NF-κB. The observed activation of the inflammatory response of this system to nickel may increase the oxidative stress and damage [285], whereas inhibition of the natural killer cells by Ni(II) [71] may suppress recognition and elimination of mutated cells. Thus, both effects have the potential to assist in tumor induction and growth.

Thus far, the strongest epigenetic effects of Ni(II) have been associated with the hypoxic response associated with Ni(II)/Fe(II) antagonism at the transport and "oxygen sensor" levels leading to the prevention of HIF-1α degradation. HIF-1 is involved in the coordinated up-regulation of numerous genes involved in glucose transport and glycolysis [313]. The exposure of animals to Ni(II) chloride or $Ni(CO)_4$ causes hyperglycemia, hyperglucagonemia and hyperinsulinemia [314]. Thus, the induction of HIF-1 by nickel is responsible for the up-regulation of glucose metabolism and glycolysis even in the presence of oxygen [142,315]. Prolonged nickel exposure is likely to promote selection of cells that maintain a high glycolytic rate and thereby acquire a phenotype similar to cancer cells. Such phenotype was first described by Warburg [316]. Glycolysis is an inefficient way of obtaining energy because the net ATP yield is much lower than that produced by the oxidation of glucose in the mitochondria. However, it leads to the accumulation in cells of the phosphorybosyl pyrophosphate needed for nucleotide synthesis and DNA replication of proliferating tumor cells [317]. Thus, exposure of cells to nickel would induce the "Warburg effect" and provide selective advantage to cells with a higher glucose metabolism and proliferation rate.

7.2. Practical implications

Nickel is ubiquitously present in the environment and the exposure to low doses of its compounds is unavoidable. It may not be harmful to the general population. However, in some nickel-rich regions, environmental nickel has already raised health concern stemming from the knowledge of its toxicokinetic and pathogenic properties derived from occupational epidemiology and animal studies [30]. Human exposure to highly nickel-polluted environments, such as those encountered in nickel refining, electroplating, and welding, has the potential to poison organism with nickel doses that are high enough to disturb cellular homeostasis and lead to a variety of pathologic effects, including neoplastic transformation via both genetic and epigenetic routes. The breadth and magnitude of the latter led to formulation of an "epigenetic hypothesis of nickel carcinogenesis" [131,303], stating that neoplastic transformation may result solely from gene silencing, especially of tumor suppressor and senescence genes, even in the absence of mutations. This interesting hypothesis, consistent

with tumor-promoting activity of low Ni(II) doses, typical for soluble, sparingly absorbed nickel compounds, deserves further attention. However, the present review also clearly demonstrates the genotoxic and mutagenic capacity of Ni(II), typical for DNA damage by high intracellular Ni(II) doses, best delivered by phagocytized particulate compounds. Therefore, the mechanistic importance of mutations in nickel-induced carcinogenesis deserves proper attention, as well. The investigations of the genotoxic and epigenetic effects of nickel can greatly benefit from the growing understanding of the chemical basis of Ni(II) interactions with cellular and tissue ligands and interference with the metabolism of essential metals. Research in this field is therefore equally, if not more, important. It will allow for more precise identification of putative Ni(II) binding sites in the growing number of bio-molecules relevant to carcinogenesis and predict other possible pathogenic effects of the binding. Ultimately, the mechanistic investigations of nickel-induced carcinogenesis should be aimed at the development of treatments that would inhibit Ni(II) interactions with critical target molecules and ions, Fe(II) in particular, and thus prevent the respiratory tract cancer and other adverse health effects in workers of nickel-related industries.

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