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Molecular and Genomic Approaches on Nickel Toxicity and Carcinogenicity

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Abstract

Nickel is the one of potent environmental, the occupational pollutants and the classified human carcinogens. It is a serious hazard to human health, when the metal exposure. To prevent human diseases from the heavy metals, it is seemingly important that understanding of how nickel exerts their toxicity and carcinogenic effect at a molecular and a genomic level. The process of nickel absorption has been demonstrated as phagocytosis, iron channel and diffusion. Uptaked nickel has been suggested to induce carcinogenesis via two pathways, a direct DNA damaging pathway and an indirect DNA damaging pathway. The former was originated from the ability of metal to generate Reactive Oxygen Species (ROS) and the reactive intermediates to interact with DNA directly. Ni-generated ROS or Nickel itself, interacts with DNAs and histones to cause DNA damage and chromosomal abnormality. The latter was originated from an indirect DNA damage via inhibition of DNA repair, or condensation and methylation of DNA. Cells have ability to protect from the genotoxic stresses by changing gene expression. Microarray analysis of the cells treated with nickel or nickel compounds, show the specific altered gene expression profile. For example, HIF-I (Hypoxia-Inducible Factor I) and p53 were well known as transcription factors, which are upregulated in response to stress and activated by both soluble and insoluble nickel compounds. The induction of these important transcription factors exert potent selective pressure and leading to cell transformation. Genes of metallothionein and family of heat shock proteins which have been known to play role in protection and damage control, were also induced by nickel treatment. These gene expressions may give us a clue to understand of the carcinogenesis mechanism of nickel. Further discussions on molecular and genomic, are need in order to understand the specific mechanism of nickel toxicity and carcinogenicity.

Keywords: nickel, toxicity, carcinogenesis, microarray

As human society and science have been advanced, human health problems with the usage of heavy metals are remarkably increased. The Cancer is one of the well known health problem induced by toxic metals that we face. Epidemiological studies have shown that nickel compounds are associated with induction of human nasal and lung cancers[1,2,3]. The carcinogenic potential of water soluble and water insoluble nickel compounds were also confirmed *in vivo* and *in vitro*[4,5]. Previous studies have suggested that nickel was capable of silencing genes[6], inhibition of DNA repair[7], generating reactive oxygen species and other reactive intermediates[8]. These effects of nickel have been demonstrated to cause cell transformation. The effects would lead the cell cycle arrest, or recover the cell to normal condition and apoptosis as well. The genes, which has a role in this aspect may alter its own expression.

Past studies have been focused on molecular functions and regulatory mechanism of the genes. The techniques, such as the western and the northern blotting are based on hybridization technology, which have an advantage to determine the amount of gene product. However, these methods were able to analysis only a part of data. Recently, microarray analysis which deals with large scale gene expression pattern has been developed at once. This novel assay is a powerful tool to compare, or quantify the gene expression on large scale. In addition, differential gene expression pattern induced by external stimuli is sufficient to determine the function, the phenotype and the responses of the cells for environmental toxic chemicals. Therefore, the profiling of altered gene expression from microarray analysis would help us to un-

derstand at a toxicological mechanism of the specific carcinogenic nickel. In this review, the toxicity and the carcinogenicity of nickel (Ni) are discussed using molecular and genomic approaches.

Molecular Approaches on Nickel Carcinogenesis

Eighteen nickel compounds have examined for carcinogenecity in male Fischer rats within 2-year[8]. Many nickel compounds are highly carcinogenic (more than 50% of incidence rate) such as nickel subsulfide (Ni_3S_2), crystalline nickel monosulfide (NiS), nickel ferrosulfide (Ni_4FeS_4), nickel oxide (NiO), nickel subselenide (Ni_3Se_2), nickel sulfarsenide (NiAsS), nickel disulfide (NiS_2), nickel subarsenide, nickel dust, nickel antimonide (NiSb), nickel telluride (NiTe) and nickel monoselenide (NiSe). In contrast, other nickel compounds are not carcinogenic. One of the reasons among the different potentials of nickel compounds is on absorption rate that suggests carcinogenicity of nickel would be related to its transporting ability into cell. The mechanism of nickel admission into the cell is shown on Fig. 1. Insolubility, structure and size of nickel compounds have been identified to major factors to uptake Ni^{2+} and its derivatives. Nickel uptake was suggested to occur via three possible routes as phagocytosis, diffusion and iron channel. Phagocytosis, the most effective way of nickel transport, is dependent on both size and surface charge of the compounds[9,10] implicate that surface charge of nickel might affect its uptake into the cell[11]. Diffusion is another transport pathway of nickel compounds[12]. Basolateral membranes were suggested to allow passive transport of Ni^{2+} cation. The other possible transporting route depends on calcium channel[13,14] and iron channels such as divalent cation transporter (DMT-1; Nramp 2) [15,16]. Nickel compounds were transported to nucleus via vacuoles[17,18]. After the permeation into cell, the vacuoles released Ni^{2+} into the nucleus[19,20]. The ability to transfer Ni^{2+} is one if the significant factors in nickel carcinogenesis.

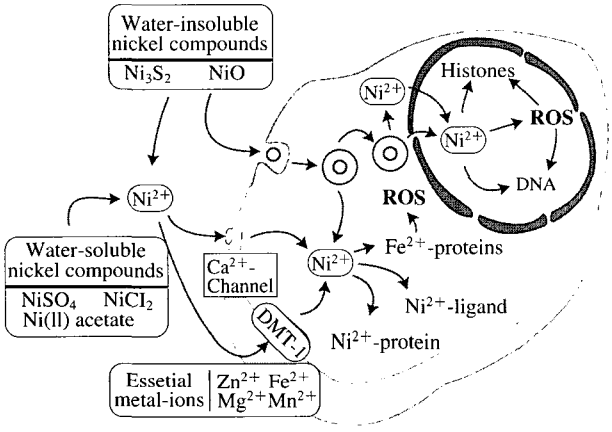

Fig. 1. Schematic view on nickel carcinogenic mechanism (adapted from Kasprzak *et al.*, 2003). Phagocytosis, diffusion and iron channel (such as calcium channel and DMT-1) are the route of nickel absorption into a cell. Absorbed nickel (form of Ni^{2+}) complexes with cellular ligands such as protein to lost its function and/or entered into nucleus by vacuole transportation. In the nucleus, nickel generate histone acetylation or methylation to suppress tumor suppresors or enhance DNA damage by direct or indirect manner via generation of reactive oxygen species.

Genotoxic effects of metal, which explained two possible ways though the mechanism, are not fully understood. One possible way is induction of DNA damage by the ability of nickel to generate reactive oxygen species and reactive intermediates[21], or react with DNA[21,22] in direct manner. Nickel sulfide has been reported to be entered into nuclei inducing DNA damage *in vivo*[23]. Nickel subsulfide also has known to induce transversion mutation from GGT to GTT in codon 12 of *K-ras*[24]. Ni^{2+} binds to histone to cause chromosomal abnormalities. In addition, treatment with Ni_3S_2 (10 μg/ml) in HeLa cells induced 1.5 fold increased in 8-OH-dG[25] which are biomarkers for oxidative DNA damage. Although NiO and $NiSO_4$

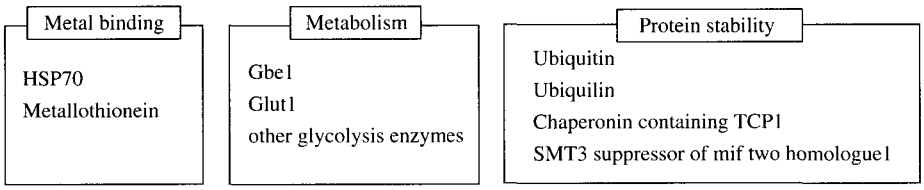

Fig. 2. Genes which are up-regulated by nickel treatment[33,37]. The genes may able to divided by their functions. Metallothionein and HSP70, which has metal-binding ability, was reported to up-regulate by nickel treatment. Nickel also induced the hypoxia mimicking states. In hypoxia states (low-oxygen), energy metabolism pathway such as glycolysis is changed. Glut1 and Gbe1 are included in this aspect. In addition, ubiquitin-like proteins are induced by nickel treatment and it may be the evidence that nickel enhance protein abnormality.

have been reportedly unable to enhance 8-OH-dG[21], these nickel compounds have been known as carcinogen[8,26,27]. On the other hand, previous reports have suggested that the silencing genes by chromatin condensation and DNA methylation nickel might be another mechanism of nickel toxicity and carcinogenicity[28,29,30]. Inhibition of DNA repair enzyme and/or gene silencing is considered to other route of carcinogenesis. Nickel is considered as the inhibitor of Nucleotide Excision Repair (NER) and Base Excision Repair (BER). For example, Ni (II) reduced the DNA binding ability of xeroderma pigmentosum group A complementing protein (XPA)[31] and the zinc finger domain of XPA and XPAzf, was lost in the Ni (II) substituted peptide[32].

Genomic Approaches on Nickel Carcinogenesis

If homeostasis of the cell is broken by environmental stresses, the cell expresses genes to protect itself via up regulation and down regulation of gene. When the modulation of gene expression is not able to overcome stress, diseases are occurred. Use of microarray has an advantage to find pathological mechanism and function of unknown gene. However, changes of gene expression tend to differ on each experiment due to different experimental condition and non established standards. Nevertheless, the tendency of gene expression pattern might provide evidence for a mechanism of harmful phenotypic outcome by nickel.

Metallothionein and heat shock protein families are well known as stress response genes. Metallothionein IE was markly up-regulated at both low and high nickel concentration. The up regulation of HSP families was also reported in dose response manner except at the exposure of toxic dose[33]. Upregulation of ubiquitin proteins such as ubiquitin, ubiquilin, chaperonin containing TCP1 and SMT3 suppressor of mif two homologue I has been reported[33]. The comparison study between fibroblast transforted by Nickl (II) and nickel (II)-resistant cell suggested that nickel (II) resistant cells markly up regulate antioxidant enzymes such as glutathione-s-transferase, Organic Cation Trasporter like protein 2 (Orctl2), Aldehyde Dehydrogenase (ALDH2), renal Na^+/Pi transporter, cytochrome p450, 4a14 Glutathione synthetase and glutathione-s-transferase α4 to survive in toxic condition[34]. These genes might play a role in reducing toxic stress to increase the rate of the cell survival in toxic condition. Thus, the altered genes, especially including metallothionein, were suggested as possible markers for nickel toxicity and carcinogenicity.

High glycolytic rates and accumulation of lactate and pyruvate, known as warburg effects[35], has been reported as nickel toxicity in glioma cell[36]. Triosephophate isomerase 1, Lactate dehydrogenase A and Endolase 3 (beta muscle) were reported to increase 2.5- to 3.5-fold in HPL1D cells treated with Ni^{2+}. These genes have been known to be involved in glycolysis[33]. Glucose transporter type 1 (*GLUT1*) was up regulated by nickel treatment[33]. 1, 4-α-Glucan Branching Enzyme 1 (GBE1) was up regulated via hypoxic signaling pathway[37]. These genes were known to play role in glycogen biosynthesis, generation of precursor metabolites and energy, carbohydrate metabolism[38,39]. The end product of glycolysis, lactate also might be able to a possible damage source to cell.

Recent study has suggested that pathological mechanism in response to nickel might involve the activation of hypoxia mediated primarily by the hypoxia inducible factor-1 (HIF-1)[40,41,42] inducing the cell growth and survival, tumor development and angiogenesis. For example, microarray data shows HIF dependent up regulation of oncogenes and down regulation of tumor suppressors. Many other genes including nip, EGLN1, hig, proyl 4-hydroxylase and focal adhesion kinase are HIF dependently activated. In contrast, genes such as gadd45, gadd153, p21, ATM and p53 are HIF independently activated[37,43]. These genes are involved in tumor suppressive responses such as apoptosis, cell cycle arrest and DNA repair[44,45]. Other genes such as Zinc Finger protein 3 (ZNF3) CCAAT/Enhancer Binding Protein Delta (CEBPD), General Transcription Factor IIA, 2 (GTF2A2), c-jun, and Ring Finger Protein (Rnf13) are increased in dose dependent manner but not in toxic dose[33].

From the genomic data previously described, the genes which were commonly modulated in response to nickel reportedly play a role in cell signaling, DNA damage induced response and metal metabolism, although there are cell type, exposure dose and time specific variations in alteration of gene expression. In dose dependent microarray analysis most alteration of gene expression has shown at the expose to low dose of nickel[33,42]. Since low dose of exposure occupationally and environmentally causes chronic phenotypes, gene expression at low dose exposure might be important to understand the mechanism of the cell level (or genomic level) defense.

In summary, molecular and genomic approach on nickel toxicity and carcinogenicity has been reviewed in this study. Several reports using microarray analysis have showed that the exposure to nickel induced the specific profiles of gene expression including metallothionein, family of heat shock protein, HIF-1 and its downstream genes. These data give a clue to

understand toxicological and carcinogenic mechanisms of heavy metal nickel. However, the possibility of argument still remains, due to experimental limitation and variable data. To find a specific biomarker for nickel toxicity and carcinogenicity, further investigation with more advanced tools of molecular biology and genomics should be are required.

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References

1. International Agency for Research on Cancer, IARC Monographs on the evaluation of Carcinogenic Risks to Humans, vol. 49, chromium, Nickel and Welding, IARC Scientific Publications, Lyon, 257-445 (1990).
2. Haber, L.T. *et al.* Hazard identification and dose response of inhaled nickel-soluble salts. *Regul. Toxicol. Pharmacol.* 210-230 (2000).
3. Doll, R., Mathews, J.D. & Morgan, L.G. Cancers of the lung and nasal sinuses in nickel workers: a reassessment of the period of risk. *Br. J. Ind. Med.* **34**, 102-105 (1977).
4. Conway, K. & Costa, M. Nonrandom chromosomal alterations in nickel-transformed Chinese hamster embryo cells. *Cancer Res.* **49**, 6032-6038 (1989).
5. Oller, A.R., Costa, M. & Oberdorster, G. Carcinogenicity assessment of selected nickel compounds. *Toxicol. Appl. Pharmacol.* **143**, 152-166 (1997).
6. Klein, C.B. *et al.* Senescence of nickel-transformed cells by an X chromosome: possible epigenetic control. *Science.* **251**, 796-799 (1991).
7. Chen, C.Y., Wang, Y.F., Huang, W.R. & Huang, Y.T. Nickel induces oxidative stress and genotoxicity in human lymphocytes. *Toxicol. Appl. Pharmacol.* **189**, 153-159 (2003).
8. Sunderman, F.W. Jr. Carcinogenicity of nickel compounds in animals. *IARC. Sci. Publ.* **53**, 127-142 (1984).
9. Costa, M., Simmons-Hansen, J., Bedrossian, C.W., Bonura, J. & Caprioli, R.M. Phagocytosis, cellular distribution, and carcinogenic activity of particulate nickel compounds in tissue culture. *Cancer Res.* **41**, 2868-2876 (1981).
10. Kuehn, K., Fraser, C.B. & Sunderman, F.W. Jr. Phagocytosis of particulate nickel compounds by rat peritoneal macrophages *in vitro*. *Carcinogenesis.* **3**, 321-326 (1982).
11. Costa, M. & Mollenhauer, H.H. Phagocytosis of nickel subsulfide particles during the early stages of neoplastic transformation in tissue culture. *Cancer Res.* **40**, 2688-2694 (1980).
12. Foulkes, E.C. & McMullen, D.M. On the mechanism of nickel absorption in the rat jejunum. *Toxicology.* **38**, 35-42 (1986).
13. Refsvik, T. & Andreassen, T. Surface binding and uptake of nickel (II) in human epithelial kidney cells: modulation by ionomycin, nicardipine and metals. *Carcinogenesis.* **16**, 1107-1112 (1995).
14. Azula, F.J., Alonso, R., Marino, A., Trueba, M. & Macarulla, J.M. Ni^{2+} impairs thrombin-induced signal transduction by acting on the agonist and/or receptor in human platelets. *Am. J. Physiol.* **265**, 1681-1688 (1993).
15. Gunshin, H. *et al.* Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature.* **388**, 482-488 (1997).
16. Tallkvist, J. & Tjalve, H. Transport of nickel across monolayers of human intestinal Caco-2 cells. *Toxicol Appl. Pharmacol.* **151**, 117-122 (1998).
17. Costa, M. & Mollenhauer, H.H. Carcinogenic activity of particulate nickel compounds is proportional to their cellular uptake. *Science.* **209**, 515-517 (1980).
18. Kasprzak, K.S., Sunderman, F.W. Jr. & Salnikow, K. Nickel carcinogenesis. *Mutat. Res.* **533**, 67-97 (2003).
19. Evans, R.M., Davies, P.J. & Costa, M. Video time-lapse microscopy of phagocytosis and intracellular fate of crystalline nickel sulfide particles in cultured mammalian cells. *Cancer Res.* **42**, 2729-2735 (1982).
20. Fletcher, G.G., Rossetto, F.E., Turnbull, J.D. & Nieboer, E. Toxicity, uptake, and mutagenicity of particulate and soluble nickel compounds. *Environ. Health Perspect.* **102**, 69-79 (1994).
21. Kasprzak, K.S. Oxidative DNA and protein damage in metal-induced toxicity and carcinogenesis. *Free Radic. Biol. Med.* **32**, 958-967 (2002).
22. O'Brien, T.J., Ceryak, S. & Patierno, S.R. Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms. *Mutat Res.* **533**, 3-36 (2003).
23. Costa, M. & Heck, J.D. Perspectives on the mechanism of nickel carcinogenesis. *Adv Inorg Biochem.* **6**, 285-309 (1984).
24. Higinbotham, K.G. *et al.* GGT to GTT transversions in codon 12 of the K-ras oncogene in rat renal sarcomas induced with nickel subsulfide or nickel subsulfide/iron are consistent with oxidative damage to DNA. *Cancer Res.* **52**, 4747-4751 (1992).
25. Kawanishi, S. *et al.* Oxidative DNA damage in cultured cells and rat lungs by carcinogenic nickel compounds. *Free Radic. Biol. Med.* **31**, 108-116 (2001).
26. Kasprzak, K.S., Gabryel, P. & Jarczewska, K. Carcinogenicity of nickel (II) hydroxides and nickel (II) sulfate in Wistar rats and its relation to the in vitro dissolution rates. *Carcinogenesis.* **4**, 275-279 (1983).
27. Lechner, J.F., Tokiwa, T., McClendon, I.A. & Haugen, A. Effects of nickel sulfate on growth and differentiation of normal human bronchial epithelial cells. *Carcinogenesis.* **5**, 1697-1703 (1984).

28. Costa, M. *et al.* The role of oxidative stress in nickel and chromate genotoxicity. *Mol. Cell Biochem.* 234-235(1-2), 265-275 (2002).
29. Salnikow, K. & Costa, M. Epigenetic mechanisms of nickel carcinogenesis. *J. Environ. Pathol. Toxicol. Oncol.* **19**, 307-318 (2000).
30. Lee, Y.W. *et al.* Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. *Mol. Cell. Biol.* **15**, 2547-2557 (1995).
31. Asmuss, M., Mullenders, L.H., Eker, A. & Hartwig, A. Differential effects of toxic metal compounds on the activities of Fpg and XPA, two zinc finger proteins involved in DNA repair. *Carcinogenesis.* **21**, 2097-2104 (2000).
32. Bal, W., Schwerdtle, T. & Hartwig, A. Mechanism of nickel assault on the zinc finger of DNA repair protein XPA. *Chem. Res. Toxicol.* **16**, 242-248 (2003).
33. Cheng R.Y. *et al.* Gene expression dose-response changes in microarrays after exposure of human peripheral lung epithelial cells to nickel (II). *Toxicol. Appl. Pharmacol.* **191**, 22-39 (2003).
34. Su, A.I. *et al.* Large-Scale analysis of the human and mouse transcriptomes. Large-scale analysis of the human and mouse transcriptomes. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4465-4470 (2002).
35. Warburg, O. On respiratory impairment in cancer cells. *Science.* **124**, 269-270 (1956).
36. Galarraga, J. *et al.* Glucose metabolism in human gliomas: correspondence of in situ and *in vitro* metabolic rates and altered energy metabolism. *Metab. Brain. Dis.* **1**, 279-291 (1986).
37. Zhao, J. *et al.* Nickel-induced 1, 4-alpha-glucan branching enzyme 1 up-regulation via the hypoxic signaling pathway. *Toxicol. Appl. Pharmacol.* **196**, 404-409 (2004).
38. Ward, T.L. *et al.* Glycogen branching enzyme (GBE1) mutation causing equine glycogen storage disease IV. *Mamm. Genome.* **15**, 570-577 (2004).
39. Bao, Y., Kishnani, P., Wu, J.Y. & Chen, Y.T. Hepatic and neuromuscular forms of glycogen storage disease type IV caused by mutations in the same glycogen-branching enzyme gene. *J. Clin. Invest.* **97**, 941-948 (1996).
40. Salnikow *et al.* Nickel induced transfiormation shifts the balance between HIF-1 and p53 transcription factors. *Carcinogenesis.* **20**, 1819-1823 (1999).
41. Salnikow *et al.* Carcinogenic metals induce hypoxia-inducible factor-stimulated transcription by reactive oxygen species-independent mechanism. *Cancer Res.* **60**, 3375-3378 (2000).
42. Andrew *et al.* Nickel requires hypoxia-inducible factor-1α, not redox signaling to induce plasminogen activator inhibitor-1. Am. J. Physiol. *Lung Cell Mol. Physiol.* **281**, 607-615 (2001).
43. Salnikow *et al.* The Involvement of Hypoxia-inducible transcription factor-1-dependent pathway in Nickel carcinogenesis. *Cancer Reserch.* **63**, 3524-3530 (2003).
44. Seo, Y.R. & Jung, H.J. The potential roles of p53 tumor suppressor in nucleotide excision repair (NER) and base excision repair (BER). *Exp. Mol. Med.* **36**, 505-509 (2004).
45. Smith, M.L. *et al.* p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol. Cell Biol. May.* **20**(10), 3705-3714 (2000).