Zinc ions have a potential to attenuate both Ni ion uptake and Ni ion-induced inflammation

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Nickel ions (Ni²⁺) are eluted from various metallic materials, such as medical devices implanted in human tissues. Previous studies have shown that Ni²⁺ enters inflammatory cells inducing inflammation. However, the regulation of Ni²⁺ uptake in cells has not yet been reported in detail. In the present study, we investigated the effects of various divalent cations on Ni²⁺ uptake and Ni²⁺-induced interleukin (IL)-8 production in the human monocytic cell line, THP-1. We demonstrated that ZnCl₂, MnCl₂, and CoCl₂ inhibited the Ni²⁺ uptake, while CuCl₂, FeCl₂, MgCl₂, and divalent metal transporter (DMT)-1 inhibitor, Chlorozol Black, did not. Furthermore, ZnCl₂ inhibited Ni²⁺-induced IL-8 production, correlating with the inhibition of Ni²⁺ uptake. These results suggested that Ni²⁺ uptake occurred through Zn²⁺, Mn²⁺, and Co²⁺-sensitive transporters and that the inhibition of Ni²⁺ uptake resulted in the inhibition of IL-8 production. Furthermore, using an Ni wire-implanted mouse model, we found that Ni wire-induced expression of mouse macrophage inflammatory protein-2 (MIP-2) and cyclooxygenase-2 (COX-2) mRNA in the skin tissue surrounding the wire were enhanced by low Zn conditions. These results suggested that the physiological concentration of Zn²⁺ modulates Ni²⁺ uptake by inflammatory cells, and a Zn deficient state might increase sensitivity to Ni.

Nickel (Ni) is included in several medical devices, including prostheses, pace makers, stents, and dental implants, owing to its beneficial properties such as resistance to corrosion and durability. However, Ni ion elutes from Ni-containing materials possibly causing inflammation. Actually, the prevention of neointima formation by Ni-free stainless stent was demonstrated. We also reported that the implantation of an Ni wire subcutaneously into the back of mice induced the elution of Ni²⁺, the expression of several inflammatory proteins such as cyclooxygenase-2 (COX-2) and neutrophil chemokine macrophage inflammatory protein-2 (MIP-2, CXCL2), and leukocyte infiltration as the initial responses. Importantly, infiltration and activation of neutrophils enhanced further elution of Ni²⁺. Thus, inhibition of Ni²⁺-induced inflammatory cell activation would be one of the strategies to prevent Ni²⁺ elution.

It was generally accepted that Ni²⁺ binds to various extracellular proteins to form a novel antigen causing delayed-type hypersensitivity. For example, Ni²⁺ binds to human serum albumin inducing activation of human T cells. Furthermore, Ni²⁺ forms different Ni epitopes leading to polyclonal Ni-specific T cell activation. However, Ni²⁺ directly activates various inflammatory cells and induces death of monocytes. For example, Ni²⁺ binds to Toll-like receptor 4 (TLR4) on the cell surface, activating the NF-κB pathway. In addition to cell surface proteins, Ni²⁺ binds to and modulates intracellular proteins; these ions enter the cells and inhibit prolyl hydroxylases (PHDs), resulting in the activation of a transcription factor called the hypoxia-inducing factor-1α (HIF-1α) 

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ions. Each of these members exhibits specificity toward a specific metal. However, the metal specificity of the transporter involved in Ni\(^{2+}\) uptake remains unclear.

Ni\(^{2+}\) uptake in cells and nuclei in the human monocytic cell line, THP-1, has already been reported\(^{18}\). THP-1 cells also have the ability to produce IL-8 by treatment with Ni compounds\(^{19,20}\). Therefore, using THP-1 cells, we examined whether the competition between Ni\(^{2+}\) and other ions affected IL-8 production. Especially, to assess the accumulation of metals in the cells and Ni\(^{2+}\) elution in the tissues precisely, we used inductively coupled plasma mass spectrometry (ICP-MS), a highly sensitive and efficient analysis technique for detecting various metal ions. In this study, we found that the physiological concentration of Zn\(^{2+}\) affected the uptake of Ni\(^{2+}\) by THP-1 cells and the sensitivity of mouse to Ni\(^{2+}\).

**Results**

**NiCl\(_2\)**-stimulated increase in Ni\(^{2+}\) content and IL-8 production in THP-1 cells. THP-1 cells were treated with various concentrations of NiCl\(_2\) for 24 h and the Ni\(^{2+}\) content in the cells and IL-8 level in the medium were determined. Both Ni\(^{2+}\) content and IL-8 production increased in a NiCl\(_2\) concentration-dependent manner (Fig. 1a and b). As IL-8 production was significantly induced by NiCl\(_2\) at the concentration of \(\geq 0.2\) mM (Fig. 1b), 0.2 mM NiCl\(_2\) was used in all the experiments. Ni\(^{2+}\) content in the cells increased in a time-dependent manner (Fig. 1c), consistent with the concentration-dependent increase in the cells, and IL-8 level in the medium increased significantly from the 4-h mark (Fig. 1d). The incubation of THP-1 cells in 0.2 mM NiCl\(_2\) for 24 h did not affect the viability as determined by the MTT assay (data not shown).

**Effects of metal ions on the uptake of Ni ions.** THP-1 cells were treated with 0.2 mM NiCl\(_2\) in the presence of various divalent cations (0.03 mM), including Zn\(^{2+}\), Mg\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\), Cu\(^{2+}\), or Mn\(^{2+}\), added as dichloride.

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*Figure 1.* Ni\(^{2+}\) uptake and IL-8 production in THP-1 cells. THP-1 cells were treated with various concentrations of NiCl\(_2\) for 24 h (a and b) and 0.2 mM NiCl\(_2\) for the indicated times (c and d). The amount of Ni\(^{2+}\) in the cells (a and c) and IL-8 in the supernatant (b and d) were determined using ICP-MS and ELISA, respectively. The vertical lines represent the S.E.M. of 3 samples. \(*p < 0.01\) vs. 0 mM (a and b) or 0 h (c and d).
salts. The Ni\(^{2+}\) content in the cells after 24 h of incubation was determined by ICP-MS. The increase in the intracellular Ni\(^{2+}\) content was inhibited by ZnCl\(_2\), CoCl\(_2\), and MnCl\(_2\) (Fig. 2a). In contrast, the increase in Ni\(^{2+}\) content was not inhibited by the divalent metal transporter 1 (DMT1) inhibitor, Chlorazol Black (Fig. 2b). Because Ni\(^{2+}\) activates Toll-like receptor 4 (TLR4), the effects of the TLR4 inhibitor, TAK-242, on Ni\(^{2+}\) uptake were determined. The results suggested that TAK-242 did not affect Ni\(^{2+}\) uptake (Fig. 2c), suggesting that TLR4 activation was not involved in Ni\(^{2+}\) uptake. To confirm whether ZnCl\(_2\) also inhibits Ni\(^{2+}\) uptake in the other cell lines, a human monocytic cell line, U937 (Fig. 2d), and a human embryonic kidney cell line, HEK293 (Fig. 2e) were treated with 0.2 mM NiCl\(_2\) in the presence of 0.03 mM ZnCl\(_2\), Ni\(^{2+}\) content in these cells was increased by NiCl\(_2\) treatment, and this increase was reduced by ZnCl\(_2\). These findings suggested that Ni\(^{2+}\) uptake occurred generally via a Zn\(^{2+}\)-sensitive transporter.

Figure 2. Effects of divalent cations and inhibitors on Ni\(^{2+}\) uptake. (a, b, and c): THP-1 cells were treated with 0.2 mM NiCl\(_2\), in the presence or absence of 0.03 mM metal chlorides (a), 0.1 mM Chlorazol B (b), or 0.01 mM TAK-242 (c) for 24 h. (d and e): U937 (d) and HEK293 (e) cells were treated with NiCl\(_2\) in the presence or absence of 0.03 mM ZnCl\(_2\) for 24 h. The Ni\(^{2+}\) uptake of the cells was determined using ICP-MS. The vertical lines represent the S.E.M. of 3 samples. \(*p < 0.01\) vs. Control, \(**p < 0.01\) vs. 0.2 mM NiCl\(_2\).
Cellular compartmentalization of Ni ions and the effects of ZnCl₂. To confirm whether Ni²⁺ entered the cells or was bound to the cell membrane, the cellular compartmentalization of Ni²⁺ was determined by the fluorescence indicator, Newport Green. This compound was used to detect Ni²⁺ in the immune cells in a previous study. Although Newport Green could bind to both Zn²⁺ and Ni²⁺, the concentration of ZnCl₂ used in this experiment, 0.01 mM, did not apparently increase the fluorescence. In contrast, treatment with 0.2 mM NiCl₂ increased the fluorescence in the cells, indicating that Ni²⁺ entered the cells. Consistent with the data of ICP-MS, treatment with ZnCl₂ inhibited the NiCl₂-induced increase in fluorescence (Fig. 3), indicating that even at a low concentration, Zn²⁺ inhibited Ni²⁺ uptake.

Effects of ZnCl₂ and MnCl₂ on Ni²⁺-induced IL-8 production. To clarify whether the inhibition of Ni²⁺ uptake resulted in the inhibition of IL-8 production, the cells were treated with 0.2 mM NiCl₂ in the presence of 0.01 and 0.03 mM ZnCl₂ and MnCl₂. The increase in the Ni²⁺ content was reduced by ZnCl₂ and MnCl₂ in a concentration-dependent manner (Fig. 4a and d). Treatment with ZnCl₂ did not affect the Zn²⁺ content in the cells, but that with MnCl₂ increased the Mn²⁺ content. In these conditions, IL-8 production was also inhibited by these cations (Fig. 4c and f). MnCl₂ at 0.03 mM concentration slightly induced IL-8 production by itself, both in the presence and absence of NiCl₂ (Fig. 4f), indicating that Mn²⁺ has a weak ability to induce IL-8 production by itself.

Effects of ZnCl₂ on CoCl₂- and LPS-induced IL-8 production. To confirm the selectivity of the action of ZnCl₂, the effects of ZnCl₂ on CoCl₂- and LPS-induced IL-8 production were examined. Treatment with 0.2 mM CoCl₂ increased Co²⁺ content in the cells and IL-8 production. ZnCl₂ (0.01 and 0.03 mM) inhibited this increase in a dose-dependent manner (Fig. 5a and b). In contrast, the same concentrations of ZnCl₂ and MnCl₂ did not inhibit LPS-induced IL-8 production (Fig. 5c and d), indicating that Zn²⁺ did not affect the signaling pathway inducing IL-8 expression in this case.

Enhancement of Ni wire-induced inflammation in a Zn-deficient state. Finally, we examined whether the physiological concentration of Zn²⁺ affects Ni²⁺-induced inflammation in low Zn diet-fed mice. Consumption of the low-Zn diet for two weeks reduced Zn²⁺ levels in the serum to one third of the normal levels (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b). As previously reported, implantation of the Ni wire on the back of mice induced inflammation, visible as vasodilation/erythema (Fig. 6a), but the level in the skin tissues was unchanged (Fig. 6b).

Discussion

In this study, we found that Ni²⁺ entered the THP-1 cells in a Zn²⁺, Mn²⁺, and Co²⁺-sensitive manner, and that Zn²⁺ inhibited Ni²⁺ uptake, resulting in reduced IL-8 production. More importantly, we showed that Ni²⁺-induced inflammation was enhanced in a systemic low-Zn state. Our findings suggest that maintaining a normal level of Zn²⁺ is important to reduce the incidence of Ni-induced inflammation and allergy.

As expected, the incubation of THP-1 in the presence of NiCl₂ elicited an increase in intracellular Ni²⁺ level and IL-8 production. The accumulation of Ni²⁺ in THP-1 cells was induced rapidly until 4 h and then it accumulated gradually. The findings, consistent with those in the previous report, suggested that the increase was regulated by Ni²⁺ influx and efflux balance. The increase in Ni²⁺ level in the cells was antagonized by Zn²⁺, Mn²⁺, and Co²⁺, indicating the involvement of transporter(s) sensitive to these divalent cations. The antagonizing effects of ZnCl₂ and MnCl₂ were observed at concentrations lower than those of NiCl₂, indicating that the affinity of Zn²⁺ and Mn²⁺ was much higher than that of Ni²⁺ to the transporter. The putative transporters were DMT1 and ZIPs. Although DMT1 has an affinity to Ni²⁺, it was likely to contribute minimally to Ni²⁺ uptake in THP-1 cells, because the DMT1 inhibitor, Chlorazol Black, did not decrease Ni²⁺ uptake. The ZIP family consists of several members and some of them have an affinity to Ni²⁺. All ZIPs except for ZIP12 were expressed in THP-1 cells, and ZIP2, ZIP3, ZIP8, and ZIP14 have been shown to have an affinity to Zn²⁺, Mn²⁺, and Co²⁺. In addition, ZIPs are known to be induced by the stimulation of TLR4. However, the possibility that Ni²⁺ induced Zn transporters via the stimulation of TLR4 was rejected, because TAK-242 did not affect the increase in Ni²⁺ uptake in THP-1 cells, but that with MnCl₂ increased the Mn²⁺ content. In these conditions, IL-8 production was also inhibited by these cations (Fig. 4c and f), indicating that Mn²⁺ has a weak ability to induce IL-8 production by itself.

The protective effects of Zn²⁺ at physiological concentrations were also observed in an in vivo model. We had reported that Ni²⁺ elution from the Ni wire induced inflammatory events, such as neutrophil infiltration and prostaglandin and histamine production, and that the initial inflammatory responses induced further elution of Ni²⁺. Using the Ni wire-implanted mouse model, we showed that Ni²⁺-induced inflammation was enhanced in a Zn-deficient state. Additionally, the mice fed with Zn-deficient diet for 2 weeks showed an enhanced Ni...
wire-induced expression of MIP-2, a neutrophil chemokine, and COX-2. The elution of Ni^{2+} was also enhanced, probably via augmentation of the inflammation, as consistent with the previous study. The severe Zn deficiency causes various defects in the function of the skin, such as barrier function. However, in our condition, although Zn^{2+} concentration in the serum was apparently decreased, that in the skin was unchanged, indicating that functions of the skin were not impaired. Even though the Ni^{2+} elution and Ni^{2+}-induced cytokine expression were enhanced, this suggested that the concentration of Zn^{2+} in the serum and/or in the intercellular fluids affected the Ni^{2+} uptake of leukocytes infiltrated from the blood stream. These results suggested that Ni^{2+}-induced

**Figure 3.** Detection of Ni^{2+} in the cells by Newport Green. THP-1 cells were treated with 0.2 mM NiCl₂ in the presence or absence of 0.01 and 0.03 mM ZnCl₂ for 24 h. Intracellular Ni^{2+} content was detected with Newport Green. The white scale bar indicates 10 μm.
inflammatory cell responses were enhanced in the Zn-deficient state, resulting in the increase in Ni\(^{2+}\) elution. As we focused on the initial responses induced by the uptake of Ni\(^{2+}\), whether the changes in these responses affect the induction of Ni allergy remain to be elucidated. The effects of Zn-deficient condition on Ni allergy are under investigation.

Figure 4. Effect of ZnCl\(_2\) or MnCl\(_2\) on Ni\(^{2+}\) uptake and IL-8 production in THP-1 cells. THP-1 cells were treated with NiCl\(_2\) in the presence of 0.01 and 0.03 mM ZnCl\(_2\) (a–c) or MnCl\(_2\) (d–f) for 24 h and then the amounts of Ni\(^{2+}\) (a and d), Zn\(^{2+}\) (b), and Mn\(^{2+}\) (e) in the cells, and IL-8 in the supernatant (c and f) were determined using ICP-MS and ELISA, respectively. The vertical lines represent the S.E.M. of 3 samples. 

**p < 0.01 vs. Control, **p < 0.01 vs. 0.2 mM NiCl\(_2\), ††p < 0.01 vs. 0.03 mM MnCl\(_2\).
The present in vitro and in vivo findings suggested that Zn$^{2+}$ modulated Ni$^{2+}$ uptake and the activation of the inflammatory cells. Our findings also suggested the need to issue a warning that a Zn-deficient state may exacerbate medical device-induced inflammation. A recent report indicated that the prevalence of Zn deficiency in Japanese adult males and females increased with increasing age, and that infants were also susceptible to Zn deficiency. Therefore, it is important to ascertain whether people with Zn-deficiency are susceptible to Ni allergy, and to determine Zn$^{2+}$ levels to avoid the induction of Ni-induced inflammation in people implanted with medical devices.

Figure 5. Effects of ZnCl$_2$ on Co$^{2+}$ uptake and IL-8 production induced by CoCl$_2$ and LPS. THP-1 cells were treated with CoCl$_2$ (a and b) and 0.2 μg/ml LPS (c and d) in the presence of 0.01 and 0.03 mM ZnCl$_2$ for 24 h. The amounts of Co$^{2+}$ (a) in the cells, and IL-8 in the supernatant (b, c, and d) were then determined using ICP-MS and ELISA, respectively. The vertical lines represent the S.E.M. of 3 samples. **$p < 0.01$ vs. Control, ***$p < 0.01$ vs. 0.2 mM CoCl$_2$. 
Figure 6. Enhancement of Ni\textsuperscript{2+}-induced inflammation in a Zn-deficient state in mice. Mice were fed a low-Zn diet or normal diet for 2 weeks and then an Ni wire was implanted subcutaneously in their dorsa. The mice were sacrificed 0, 8, or 24 h after the implantation. The amounts of Zn\textsuperscript{2+} in the serum (a) and skin (b) of mice before the implantation were determined using ICP-MS. The skin around the wire was photographed (c) and weighed (d). Ni\textsuperscript{2+} in the serum (g) and skin (h) were determined using ICP-MS. The expression of MIP-2 (e) and COX-2 (f) was measured by qRT-PCR for the respective times. Values are normalized to those of GAPDH. The vertical lines represent the S.E.M. of the respective values for 3–4 mice. \textsuperscript{**}p < 0.01 vs. 0 h control diet group, \textsuperscript{#}p < 0.05, \textsuperscript{##}p < 0.01, \textsuperscript{###}p < 0.001 vs. the corresponding control diet group, \textsuperscript{††}p < 0.01 vs. 0 h low-Zn diet group.
Methods
Nickel chloride (NiCl₂), zinc chloride (ZnCl₂), cobalt chloride (CoCl₂), copper (II) chloride dihydrate (CuCl₂·2H₂O), iron (II) chloride tetrahydrate (FeCl₂·4H₂O), magnesium chloride hexahydrate (MgCl₂·6H₂O), manganese (II) chloride tetrahydrate (MnCl₂·4H₂O), lipopolysaccharides (LPS) from *Escherichia coli* O111, and 30% (w/v) H₂O₂ were purchased from Wako Pure Chemical Industries (Osaka, Japan). Chlorazol Black and TAK-242 were purchased from Sigma-Aldrich Co. (St. Louis, MO) and Calbiochem-Merck Millipore (Darmstadt, Germany), respectively. Newport Green TM DCF diacetate was purchased from Invitrogen (Carlsbad, CA) and the Ni wire (purity 99.98%, diameter 0.8 mm) from Nilako (Tokyo, Japan). HNO₃ (69% (w/w)) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

Cell culture. The human monocytic cell line, THP-1 (Cell Resource Center, Tohoku University) and U937 (JCRB Cell Bank, National Institute of Biomedical Innovation, Health and Nutrition, Japan), and the human epithelial cell line, HEK293 (ATCC, Manassas, VA) were used. Cells were cultured in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Biowest, Miami, FL), penicillin G potassium (18 μg/ml), streptomycin sulfate (50 μg/ml), L-glutamine (0.3 mg/ml), and NaHCO₃ (1.8 mg/ml), and incubated at 37 °C under a humidified atmosphere containing 5% CO₂.

Mice. Four-week-old male ICR mice were purchased from SLC (Shizuoka, Japan). They were fed a standard diet (CE-2, CLEA, Tokyo, Japan) (control diet group, n = 12) or a Zn-deficient diet (CLEA, Tokyo, Japan) (low-Zn diet group, n = 12) for two weeks under a 12-h light/dark cycle in a specific, pathogen-free barrier facility. All animal experiments were approved by the Institutional Animal Care and Use Committee of Tohoku University, and performed in accordance with the Regulations for Animal Experiments and Related Activities at Tohoku University and Guidelines for Proper Conduct of Animal Experiments by the Ministry of Education, Culture, Sports, Sciences, and Technology of Japan.

Treatment of cells with stimulants and inhibitors. NiCl₂, ZnCl₂, CoCl₂, CuCl₂, FeCl₂, MgCl₂, MnCl₂, and LPS were dissolved in water. Chlorazol Black and TAK-242 were dissolved in dimethyl sulfoxide. THP-1 cells (5.0 × 10⁵ cells/ml) were seeded into 24-well plates, and stimulated with various concentrations of these reagents. The inhibitors were added with NiCl₂.

Implantation of the Ni wire. The Ni wire was cut into 5-mm length, sterilized by ultraviolet irradiation, and then washed with ethanol. Mice were anesthetized using isoflurane (Wako, Osaka, Japan) and then sterilized Ni wires were implanted subcutaneously in their dorsa using a 13 G implant needle (Natsume, Tokyo, Japan). In the control group, mice underwent a similar surgical procedure, but without the implantation of the Ni wire.

ELISA. After incubation of each of the sample, IL-8 in the supernatants was assayed using an ELISA kit (eBioscience, San Diego, CA) according to the manufacturer’s protocol.

Real-time PCR. Total RNA was extracted from the mouse skin tissue surrounding the Ni wire using RNAiso Plus (Takara, Shiga, Japan) according to the manufacturer’s protocol. The total RNA was reverse-transcribed into complementary DNA (cDNA) using the PrimeScript RT reagent kit (Takara, Shiga, Japan). Subsequently, real-time PCR was performed using an SYBR® Premix Ex Taq™ II (Takara, Shiga, Japan) and the Takara PCR Thermal Cycler Dice® real time system (TP800, Takara, Shiga, Japan). The oligonucleotides used for RT-PCR were the following: Mouse GAPDH: (forward) 5′-TGT GTC CGT CGT GGA TCT GA-3′ and (reverse) 5′-TGG CTG TTG AAG TCG CAG GAG-3′, mouse MIP-2: (forward) 5′-AGG CTC CTC CTT TCC AGG TCA GTT AGC-3′ and (reverse) 5′-GAA GTC TTT GTG CTG GTG CCT G-3′ and (reverse) 5′-TGTC TGG TGTT GTG AAA GTTA GTC G-3′. The normalization and fold changes were calculated using the ∆∆Ct method.

Determination of Ni²⁺, Zn²⁺, Mn²⁺, Co²⁺ concentrations with ICP-MS. THP-1 cells were stimulated by NiCl₂ for 24 h in Fig. 1a and b, or for the indicated time in Fig. 1c and d. The cells were stimulated by NiCl₂ and/or other metal chlorides for 24 h in Figs. 2, 4 and 5. After the incubation, they were collected and washed five times with PBS (phosphate-buffered saline), and then suspended in 150 μl PBS. The cell suspension was sonicated for 30 s and the aliquot was diluted 10-fold with 5% (w/w) HNO₃. The concentration of Ni²⁺ and other metal ions in each sample was determined by Agilent 7500 Series ICP-MS (Agilent Technology, Santa Clara, CA).

To determine the metal concentrations in the mouse skin and serum, circular skin tissue sections (1 cm in diameter) from the region surrounding the Ni wire were excised and the wet weight of skin was measured. The skin tissue sample, approximately 80 mg, was boiled in 3 ml 69% (w/w) HNO₃ for 30 min, and then, 300 μl 30% (w/v) H₂O₂ was added to the samples, on ice. The skin samples were then boiled again for approximately 30 min, and pure water was added to attain a total weight of 10 g. Mouse blood was incubated for 12 h at 4 °C and then centrifuged at 1,200 × g, 4 °C for 30 min. The supernatant was diluted 10-fold with 5% (w/w) HNO₃, and centrifuged at 500 × g, 4 °C for 5 min. The supernatant was collected. The Ni²⁺, Zn²⁺ concentration of each sample was also determined by ICP-MS.

Bradford determination of protein concentration. The protein contents in the sonicates of cells were determined using the Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, Tokyo, Japan), according to the manufacturer’s protocol.
Newport green fluorescence staining of intracellular Ni ions. THP-1 cells were stimulated by NiCl₂ and/or ZnCl₂ for 24 h. After the incubation, the cells were collected and washed five times with 1 × PBS, and then treated for 30 min with 5 μM Newport Green-DCF diacetate (Invitrogen, Carlsbad, CA) dissolved in dimethyl sulfoxide. After this treatment, the cells were washed once with 1 × PBS and placed on a Micro Slide Glass (76 × 26 mm, 0.9–1.2 mm thickness, Matsunami-glass, Osaka, Japan), cover-slipped with Fluoromount (DBS, Diagnostic BioSystems, CA). Fluorescence images (excitation at 505 nm and emission at 535 nm) were acquired using a laser scanning confocal microscope LSM 800 (Carl Zeiss, Germany).

Statistical analysis. The statistical significance of the results was analyzed using the unpaired two-tailed Student’s t-test, and the Bonferroni multiple comparison test or Student-Newman-Keuls test for multiple comparisons. For some experiments, a statistical outlier removal was performed using the Smirnov-Grubbs’ rejection test and the Thompson test.

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Acknowledgements

We thank Dr. T. Narushima and K. Ueda for helping us in the metal ion measurements by ICP-MS. This work was partly supported by the Cooperative Research Project Program of Joint Usage/Research Center at the Institute of Development, Aging, and Cancer, Tohoku University.

Author Contributions

R.O., S.A., and N.H. designed the experiments. R.O. conducted the experiments, analyzed the data, and wrote the manuscript. S.A., R.S., N.M., K.O., and M.H. contributed to materials and analysis tools. Animal experiments
were conducted by R.O. and S.A. The manuscript was edited by N.H. The authors declare that they have no competing interests.

**Additional Information**

**Competing Interests:** The authors declare no competing interests.

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