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Iron- and 2-oxoglutarate-dependent Dioxygenases: an emerging group of molecular targets for nickel toxicity and carcinogenicity

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Abstract

Nickel compounds are important occupational and environmental pollutants. Chronic exposure to these pollutants has been connected with increased risks of respiratory cancers and cardiovascular diseases. However, it is still not clear what are the specific molecular targets for nickel toxicity and carcinogenicity. Here, we propose that the iron- and 2-oxoglutarate-dependent dioxygenase family enzymes are important intracellular targets that mediate the toxicity and carcinogenicity of nickel. In support of this hypothesis, our data show that three different classes of enzymes in this iron- and 2-oxoglutarate-dependent dioxygenase family, including HIF-prolyl hydroxylase PHD2, histone demethylase JHDM2A/JMJD1A, and DNA repair enzyme ABH3, are all highly sensitive to nickel inhibition. Inactivation of these enzymes accounts for a number of deleterious effects caused by nickel in cells, namely hypoxia-mimic stress and aberrant epigenetic changes. Future studies on nickel's effects on these iron- and 2-oxoglutarate-dependent dioxygenases would deepen our understanding on nickel toxicity and carcinogenicity.

Keywords

Nickel; Dioxygenase; Iron; JHDM2A/JMJD1A; ABH3; HIF; Epigenetic; Histone methylation

Nickel (Ni) compounds are important occupational and environmental pollutants. Epidemiological studies have provided evidence showing a strong correlation between worksite exposure to Ni compounds and an increased incidence of nasal and lung cancers in nickel refinery workers (Polednak 1981; Roberts et al. 1984; Roberts et al. 1989). Recent studies have also indicated that nickel, a common component present in fine particulate matter of ambient air, plays an important role in development of cardiovascular diseases in susceptible human populations (Lippmann et al. 2006). Using in vitro cell models and animal models, nickel compounds have been found to generate various types of deleterious effects, including chromosomal aberrations, DNA strand breaks, excessive reactive oxygen species production, impaired DNA repair, hypoxia-mimic stress, aberrant epigenetic changes, and signaling cascade activation (reviewed by Lu et al. 2005). However, it is still not clear what are the specific molecular targets for nickel toxicity and carcinogenicity. Here, we propose that the iron- and 2-oxoglutarate-dependent dioxygenase family enzymes are important intracellular targets that mediate the toxicity and carcinogenicity of nickel. Preliminary data in support of this hypothesis are provided in this article.

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The first piece of supportive evidence came from our studies on nickel-induced hypoxia-mimic stress. One of the most prominent changes in cells following nickel exposure is an accumulation of hypoxia inducible factor 1 alpha (HIF1α), a transcriptional factor important for cell adaption to low oxygen tension environment (Costa et al. 2005). The intracellular protein levels of HIF1α are mainly regulated through protein stability. Under normoxia conditions, HIF1α is constantly synthesized but is rapidly degraded through proteasome pathway, while it becomes stabilized and trans-activated under hypoxic conditions. A number of iron-containing dioxgenases, such as prolyl hydroxylase domain proteins 1–3 (PHD1–3), have been found to actively hydroxylate several proline residues located in the oxygen-dependent degradation domain (ODDD) of HIF1α in the presence of oxygen, iron, 2-oxoglutarate, and ascorbic acid (Stolze et al. 2006). Hydroxylation of these proline residues leads to the association of HIF-1α with a Von-Hippel-Lindau (VHL) E3 ubiquitin ligase complex, and consequently causes an ubiquitin-dependent degradation of this protein (Stolze et al. 2006). Our previous studies have indicated that nickel replaces the iron in these HIF-prolyl hydroxylases, and causes inhibition of their enzymatic activity and stabilization of HIF1α (Davidson et al. 2005; Davidson et al. 2006). An inhibiting concentration 50 (IC_{50}) of nickel was found to be 22 μM for PHD2 at the presence of 100 μM Fe^{2+}, indicating this enzyme is highly sensitive to nickel inhibition (Davidson et al. 2006). These findings prompted us to ask the question whether other enzymes that belong to the same dioxygenase family as HIF-prolyl hydroxylases could have similar sensitivity to nickel inhibition.

Our studies on epigenetic effects of nickel led to the discovery of a new class of enzymes that belong to the same dioxygenase family as HIF-prolyl hydroxylases, histone H3 lysine 9 (H3K9) demethylases (Chen et al. 2006a). In our previous work, we studied the epigenetic effects of nickel on gene expression in detail by using G12 cells, which is a Chinese hamster V79-derived cell clone possessing a single copy of the bacterial *gpt* (xanthine guanine phosphoribosyltransferase) transgene near the telomere of chromosome 1. We found that exposure of G12 cells to nickel compounds silenced the *gpt* transgene via epigenetic mechanisms (reviewed by Costa et al. 2005). In the nickel-induced *gpt*-inactivated cell clones, the promoter of this transgene was associated with decreases in histone acetylation and H3K4 methylation as well as increases in dimethylated histone H3K9 (H3K9me2), DNA methylation, and chromatin condensation (Chen et al. 2006a; Lee et al. 1995; Yan et al. 2003). Since H3K9me2 is found to be a critical mark for establishment of DNA methylation and long-term gene silencing (Jackson et al. 2004), this modification is likely to play an important role in nickel-induced DNA methylation and gene silencing (Chen et al. 2006a). During our studies on the mechanism by which nickel increases the global level of H3K9me2 in cells, we found that hypoxia and several other hypoxic-mimic agents, such as iron-chelator (deferoxamine) and dimethyloxalylglycine (DMOG; an analog of 2-oxoglutarate), can also effectively increase this modification in cells by sharing a similar pattern as HIF-1α induction (Chen et al. 2006a). Using an in vitro histone demethylation assay, we demonstrated that histone H3K9me2 demethylase activity exists in cells and is dependent on iron and 2-oxoglutarate, and that nickel inhibits this demethylase activity (Chen et al. 2006a). It is now known that JMJD2A-D and JHDM2A/JMJD1A which all belong to the iron- and 2-oxoglutarate-dependent dioxygenase family, demethylate H3K9me2 by catalyzing the generation of highly reactive oxygen species in the presence of oxygen, iron, 2-oxoglutarate, and ascorbic acid. These resultant species attack the methyl groups on histone lysines and produce unstable oxidized intermediates that spontaneously release formaldehyde, resulting in the removal of methyl groups from histone lysines (Klose and Zhang 2007; Shi and Whetstine 2007).

To date, besides HIF-prolyl hydroxylases and histone demethylases, this family of iron- and 2-oxoglutarate-dependent dioxygenases also includes, but is not limited to, HIF-1α asparagine hydroxylases (FIH-1) and the human homologues of bacterial DNA repair enzyme Alkb (ABH1–9) (Ozer and Bruick 2007). FIH-1 hydroxylates the Asn(803) residue of HIF-1α and

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prevents the binding of histone acetyltransferase p300 to this site, which consequently inhibits the transcriptional activity of HIF-1α (Lando et al. 2002). Similar as Alkb, human homologues ABH2 and ABH3 catalyze oxidative demethylation reacation to repair alkylated lesions in DNA, such as 1-methyladenine or 3-methylcytosine, in an iron- and 2-oxoglutarate-dependent fashion (Aas et al. 2003; Duncan et al. 2002). After knowing what other members of this iron- and 2-oxoglutarate-dependent dioxygenase family are, we chose to test the sensitivity of JHDM2A and ABH3 to nickel inhibition.

To this end, we expressed Flag-tagged JHDM2A recombinant protein in insect cells and purified it using anti-Flag antibody affinity chromatography (Fig. 1a). Consistent with a previous report (Yamane et al. 2006), JHDM2A specifically demethylates both H3K9me2 and me1, but not H3K9me3 (Fig. 1b). Similarly, 6×His-tagged ABH3 was expressed in bacterial cells and purified by Ni-NTA (nickel-nitrilotriacetic acid) chromatography (Fig. 1c). In vitro assay showed that the purified 6×His-tagged ABH3 actively demethylated 3-methylcytosines located in the synthesized oligonucleotides (Fig. 1d). We next studied the nickel inhibitory kinetics of purified Flag-JHDM2A and 6×His-ABH3 recombinant proteins. By incubating Flag-JHDM2A with different concentrations of nickel ions at the presence of 100 μM Fe(II), we found that nickel inhibited Flag-JHDM2A demethylase activity in a dose-dependent fashion with an IC_{50} = 25 μM (Fig. 2a–b). Similarly, nickel also inhibited the demethylase activity of 6×His-ABH3 in a dose-dependent manner with an IC_{50} = 75 μM (Fig. 2c–d). In contrast, purified aconitase, a Krebs cycle enzyme that binds iron in a form of iron-sulfur cluster, was not inhibited by up to 5 mM $NiCl_2$ (Fig. 2e). Taken together, these results provided direct evidence showing that the members of this iron- and 2-oxoglutarate-dependent dioxygenase family have similar sensitivity to nickel inhibition.

Why do these iron- and 2-oxoglutarate-dependent dioxygenases have similar sensitivity to nickel inhibition, even though they are involved in many different biological processes in cells? This is likely due to the fact that these enzymes utilize a similar mechanism to bind the cofactor iron. An analysis of the crystal structures of JMJD2A, ABH2–3, PHD2, and FIH-1 reveals that these enzymes all use a 2-histidines-1-carboxylate triad motif to bind iron (Chen et al. 2006b; Dann et al. 2002; McDonough et al. 2006; Sundheim et al. 2006; Yang et al. 2008). It is estimated that the affinity constant of Ni(II) binding to this iron-binding motif is at least three orders of magnitude greater than that of Fe(II) (Davidson et al. 2006). Based on this estimation, it can predict that nickel ions will effectively compete with iron ions for binding to this motif, and that iron ions will not be able to replace nickel ions once nickel ions bind to this motif first. This research is currently ongoing in our group. Different from our opinion, another group proposes that nickel exposure depletes intracellular ascorbate and consequently leads to inhibition of HIF-prolyl hydroxylases (Salnikow et al. 2004). This theory was based on the findings that nickel exposure decreased ascorbate uptake in an in vitro cell culture system, and that addition of ascorbate in the culture medium blocked nickel-induced HIF transactivation in cells. However, a different interpretation of these findings should be considered. First of all, it is commonly believed that an increase in ascorbate levels in culture medium promotes the availability of Fe(II) to the active site of HIF-prolyl hydroxylases (Knowles et al. 2003), which can effectively compete with Ni(II) for binding. Secondly, different from an in vitro tissue culture system that has only 1–5 μM ascorbate in medium and undetectable amount of this compound (<0.5 μM) in cells (Quievryn et al. 2002), it is estimated that the intracellular ascorbate level in humans is about 1 mM (Meister 1994). It is questionable how nickel exposure in humans can lower the intracellular ascorbate concentration to such a level that causes inhibition of these Fe- and 2-oxoglutarate-dependent dioxygenases. It is also difficult to reconcile this theory with our in vitro assay results that nickel inhibits JHDM2A and ABH3 demethylase activity at the presence of 2 mM ascorbic acid in each reaction mixture. In contrast with the estimated 1 mM intracellular ascorbate level in humans, the bio-available Fe level (also called the labile Fe pool) in cells is estimated to be just about 0.8 μM (Konijn et

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al. 1999) and is tightly controlled by iron responsive proteins (IRPs) (Muckenthaler et al. 2008). Because there is no known defensive mechanism against nickel ions in cells, nickel ions that enter cells would have unrestricted access to the iron-binding sites of these Fe- and 2-oxoglutarate-depenendent dioxygenases.

In summary, the iron- and 2-oxoglutarate-dependent dioxygenase family enzymes are emerging molecular targets for nickel toxicity and carcinogenicity. Because the members of this dioxygenase family are involved in many different biological processes in cells, their inhibition by nickel could have a broad and complex impact on cells. Future research should aim to elucidate the mechanism by which nickel inhibits these enzymes as well as to understand the biological consequences of these enzymes inhibition by nickel.

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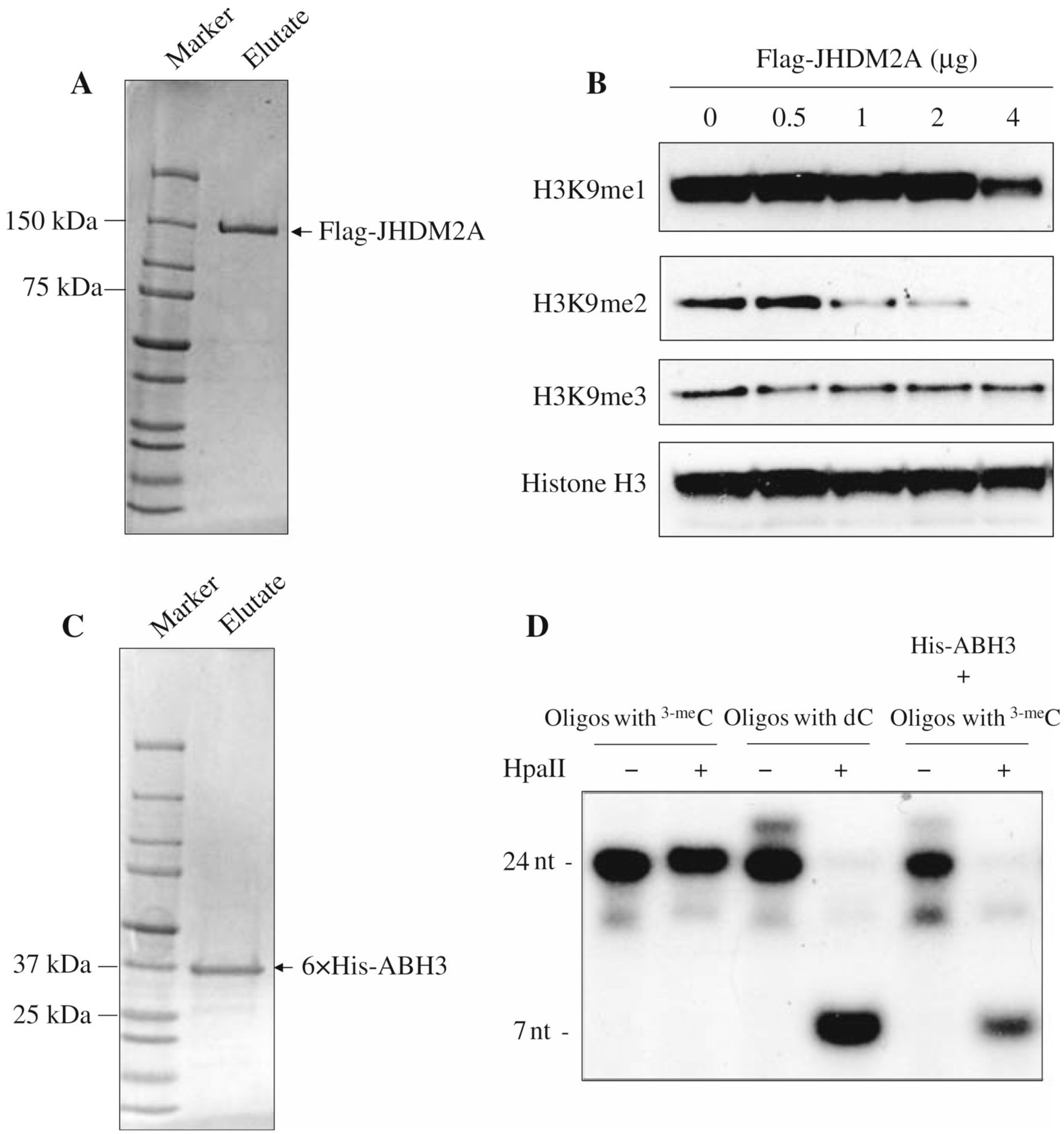

Fig. 1.

Purification of Flag-tagged JHDM2A and 6×His-tagged ABH3 recombinant proteins and characterization of their demethylase activity. **a** Purification of Flag-tagged JHDM2A recombinant protein from insect cells using Anti-Flag antibody affinity chromatography. The eluate was separated on a 4–20% SDS-polyacrylamide gradient gel. The Coomassie blue staining of the gel is shown here. **b** Detection of Flag-JHDM2A demethylase activity in vitro. Different amounts of purified Flag-JHDM2A recombinant protein were used in an in vitro histone demethylase assay, which contained 100 μM Fe(II), 1 mM 2-oxoglutarate, and 2 mM ascorbic acid in each reaction (Chen et al. 2006a). After the reactions, histones were separated by electrophoresis and transferred to PVDF membranes. Immunoblotting was then performed using specific antibodies to histone H3 and different methyl levels of histone H3K9. **c** Purification of 6×His-tagged ABH3 recombinant protein from bacterial cells using Ni-NTA

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affinity chromatography. The eluate was separated in a 4–20% SDS-polyacrylamide gradient gel. The Coomassie blue staining of the gel is shown here. **d** Detection of 6×His-tagged ABH3 demethylase activity in vitro. The assay was performed essentially as previously described by Lee et al. (Lee et al. 2005). In brief, purified 6×His-tagged ABH3 was incubated with a ^{32}P end-labeled single-stranded oligonucleotide (24 nucleotides in length) containing one 3-methylcytosine in its HpaII cutting sequence. After incubation in a reaction buffer containing 100 μM Fe(II), 1 mM 2-oxoglutarate, and 2 mM ascorbic acid, the single-stranded oligonucleotide was purified and annealed with its complementary sequence, which was then subject to digestion with methylation-sensitive restriction enzyme HpaII. The reaction products were separated on 20% denaturing polyacrylamide gels. The radioactivity on the gels was detected by autoradiography. The appearance of 7-oligonucleotide fragments indicates the removal of the methyl groups at the 3-N position of cytosines and subsequent cutting of 24-oligonucleotides by HpaII. Single-stranded oligonucleotides of the same sequence but without a 3-methylcytosine were used as a positive control, while the assay without addition of 6×His-tagged ABH3 was used as a negative control and performed in parallel

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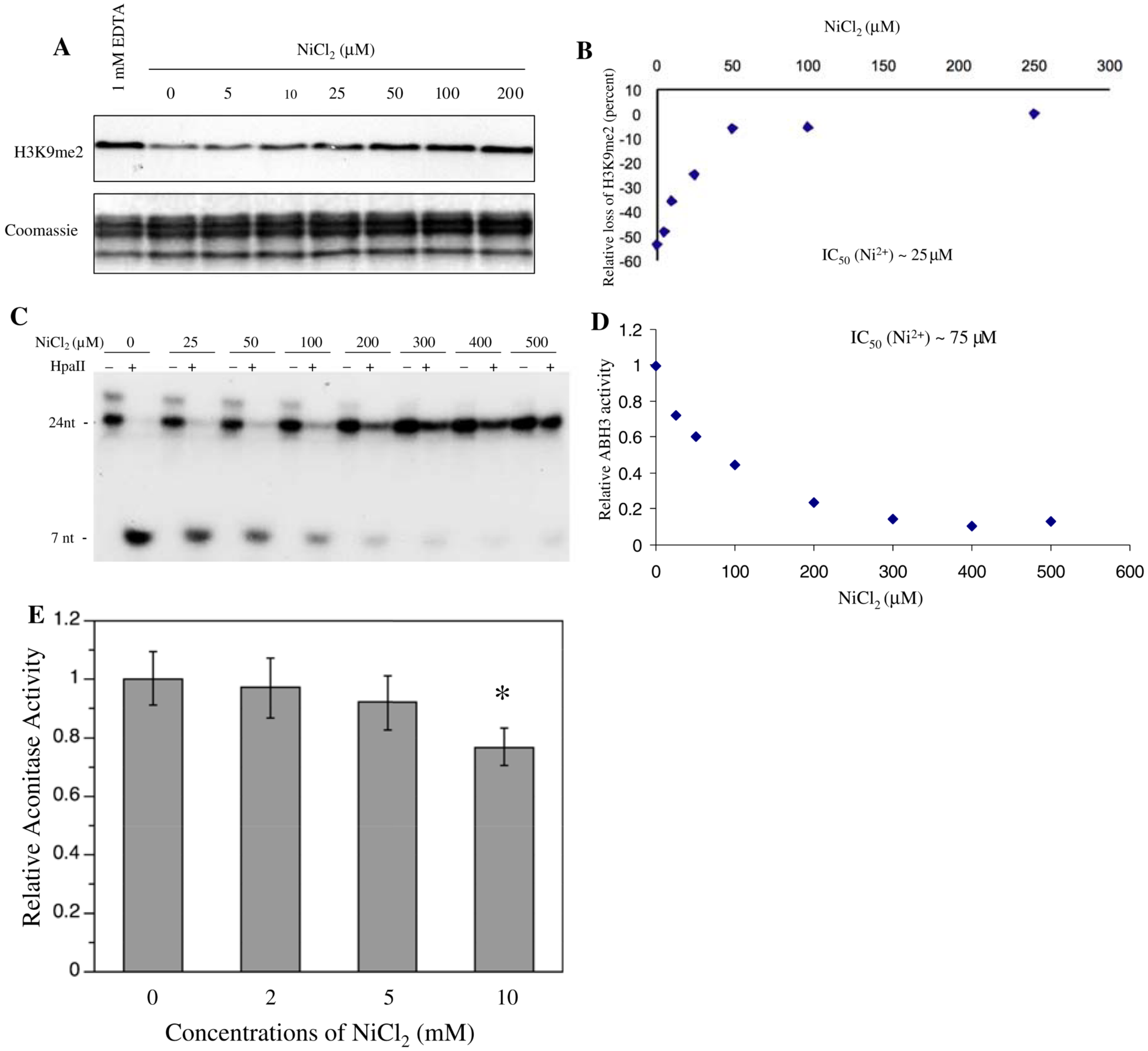

Fig. 2.

A kinetic study on Ni inhibition of JHDM2A and ABH3 demethylase activity. **a** Purified Flag-JHDM2A was assayed for its demethylase activity in the presence of different concentrations of Ni(II) ions as indicated. The assay with addition of EDTA, a chelator of divalent metals, was performed in parallel as a negative control. **b** Data quantification of Fig. 2a. **c**. Purified 6×His-tagged ABH3 was assayed for its demethylase activity in the presence of different concentrations of Ni(II) ions as indicated. **d** Data quantification of Fig. 2c. **e** Purified aconitase, an Fe–S cluster-containing enzyme, was incubated with different concentrations of Ni(II) ions for 4 h. After incubation, the aconitase activity was measured immediately as previously described (Chen et al. 2005). Aconitase activity is presented as that relative to levels in the control samples. Each bar represents the mean (±SD) from three samples per treatment. *Statistically significant change ($P < 0.05$) compared to control samples