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Invited Perspective: New Insight into Cadmium-Related Osteoporosis Yields Hope for Prevention and Therapy

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In this issue of *Environmental Health Perspectives*, Liu et al. present a mechanistic mouse study on cadmium (Cd)-induced osteoporosis and propose a hypothesis that nuclear factor erythroid 2-related factor 2 (NRF2) and its Cap 'n' Collar and basic region Leucine Zipper (CNC-bZIP) family member nuclear factor erythroid 2-related factor 1 (NRF1) coordinate osteoclastogenesis during Cd exposure.[1] This study offered new insights into cadmium-related osteoporosis, providing hope for both prevention and therapy.

With an increasing life expectancy globally, we can expect an increased number of people with age-related bone loss and osteoporosis.[2] The causative or risk factors for osteoporosis include genetic factors, endocrine, gastrointestinal, rheumatic and autoimmune diseases, lifestyle change, and environmental factors.[3] Chronic exposure to Cd is associated with osteoporosis, even though there are sex and racial differences for the associated levels between Cd exposure and osteoporosis.[4–6] However, the specific mechanisms driving this association remain incompletely understood, and Liu et al. have provided an innovative and important insight.

Many metals induce an increase in reactive oxygen species (ROS) via mitochondria-dependent and -independent mechanisms, resulting in oxidative damage.[7,8] Nrf1 and Nrf2 are the two master transcription factors regulating antioxidant defense,[9–11] including its response to metal challenge. Emerging evidence indicates the protection of Nrf2-mediated antioxidant pathways against metal-induced oxidative damage. Beyond this protection against oxidative damage, as a nuclear transcriptional factor Nrf2 also acts directly and indirectly by modulating a wide range of cell fates, including cell metabolism, proteasome activity, and cell differentiation.[9–11] The insight gleaned by Liu et al. is their finding of the cross-regulation between Nrf1 and Nrf2 in osteoclastogenesis in response to Cd exposure,[1] as outlined in Figure 1.

Nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1 (NFATc1) signaling plays an essential role in osteoporosis by increasing a number of osteoclast-specific genes, such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, calcitonin receptor, and osteoclast-associated receptor.[12] Reportedly, human and mouse *Nrf1* (also called NFE2L1) genes can be transcribed into alternatively spliced forms, resulting in multiple protein isoforms.[13,14] For example, in mice, the *Nrf1* gene can be transcribed into multiple spliced forms, resulting in at least two long isoforms (L-Nrf1) containing 741 and 742 amino acids and two short isoforms (S-Nrf1) containing 453 and 583 amino acids.[13,14] In a previous study by Liu et al.,[15] Nrf1 regulated the expression of the *Nfatc1* gene in an isoform-specific manner: The long isoforms (L-Nrf1-742 or -741) promoted Nfatc1 expression, whereas the short isoform (S-Nrf1-453) inhibited Nfatc1 expression. Normally, cellular ROS activates both Nrf2 and L-Nrf1 to up-regulate multiple antioxidants to suppress ROS-induced effects.[13–16] However, only L-Nrf1 can activate Nfatc1 transcription of the downstream genes that stimulate osteoclast differentiation,[15] as shown in Figure 1. Therefore, in mice with *Nrf2* deficiency, Cd-induced ROS predominantly up-regulates L-Nrf1 (which in turn can up-regulate expression of both antioxidants) and Nfatc1 (which amplifies the osteoclast differentiation and osteoporosis), as reported by Liu et al.[1] and illustrated in Figure 1.

This finding may also explain several previous studies. With arsenite-treated HaCaT cells, Nrf1 protein was increased when *Nrf2* was silenced,[17] whereas *Nrf1*-knockout HepG2 cells increased Nrf2 protein accumulation and antioxidant response.[18] In addition, *Nrf2*-deficient mice did not show a significant wound-healing phenotype, partly because of the compensatory expression of other family members, such as Nrf1 and Nrf3.[19] These results highlight the role of Nrf1 and Nrf2 and their cross-regulation in response to metal toxicity. In addition, Cd-mediated oxidative stress not only stimulates L-Nrf1–mediated activation of NFATc1-mediated osteoclast differentiation but also causes osteoblast oxidative damage,[20,21] senescence,[22] autophagy,[23,24] and apoptosis.[21,25] Other studies have shown the critical role of Nrf2 in protection from Cd-induced oxidative stress and damage in osteoblasts.[26] Therefore, activation of Nrf2 function is an important approach to preventing Cd-mediated osteoporosis, as outlined in Figure 1; the Nrf2 activator sulforaphane (SFN) may be an attractive option for this role, given evidence it may prevent osteoporosis.[27,28]

The study by Liu et al. revealed the mechanisms underlying the cross-regulation of Nrf1 and Nrf2, which are complicated and apparently determined at least in part (but not solely) by ROS levels since reduction of ROS by *N*-acetyl-L-cysteine (NAC) and mitoquinone mesylate (MitoQ) inhibited this cross-regulation.[1] As detoxifiers of metals such as Cd, metallothioneins (MTs) may also be important molecules in the complicated mechanisms for the prevention of Cd-mediated osteoporosis, although the authors did not discuss this possibility. MTs contribute to several physiologic processes, including metal-ion homeostasis and detoxification, ROS scavenging, and apoptosis inhibition.[29] There are multiple elements in the promoter region of MT genes, including the antioxidant response element for Nrf2 binding, and the metal-responsive element for zinc-bound metal-regulatory transcription factor 1 (MTF-1) binding for its transcriptional activation.[29–31]

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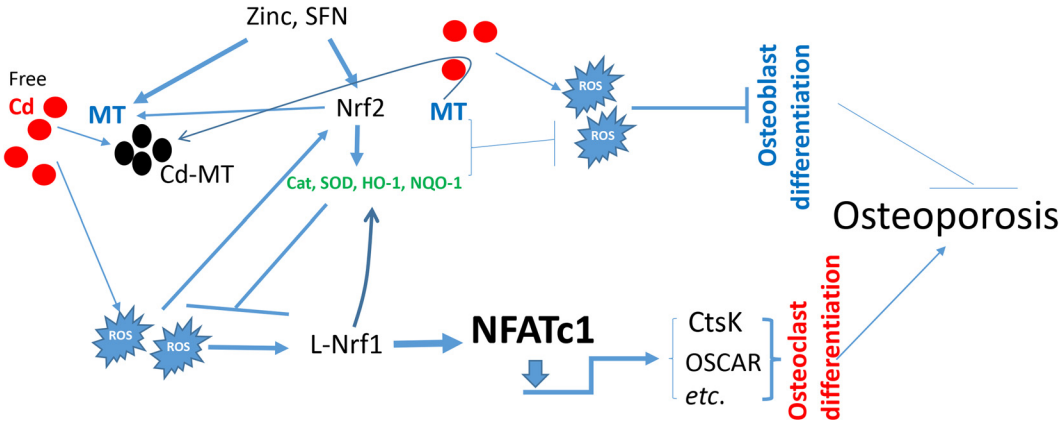

Figure 1. An illustration of the potential mechanism by which Nrf2 and Nrf1 coordinate to prevent cadmium (Cd)-mediated osteoporosis. Cd exposure may stimulate production of intracellular reactive oxygen species (ROS) via a complex mechanism. Through these ROSs, Cd directly damages osteoblast differentiation and indirectly induces osteoclast differentiation via ROS-mediated activation of L-Nrf1, which increases NFATc1 expression.[1,15] The latter is a key driver controlling the genes that induce osteoclast differentiation and associated osteoporosis, as one of the direct downstream transcriptional targets of L-Nrf1.[15] Nrf2, as a master transcription factor, regulates many antioxidant genes—including catalase (Cat), superoxide dismutase (SOD), NAD(P)H quinone dehydrogenase 1 (NQO-1), and metallothionein (MT)—to negatively control ROS levels.[9–11] Nrf2 activators, such as sulforaphane (SFN), and MT inducers, such as zinc, can synergistically or individually increase Nrf2 activation and MT expression.[34–39] Thus, both play important roles in preventing Cd-induced detrimental effects on osteoblasts and stimulation of osteoclasts via the L-Nrf1/NFATc1 pathway, leading to the final prevention of osteoporosis. The statements in this legend without citation are the author's opinion based on the discussion in the main body of this perspective. Note: CtsK, cathespin K; HO-1, heme oxygenase; L-Nrf1, long isoform of nuclear factor erythroid 2-related factor 1; NAD(P)H, nicotinamide adenine dinucleotide phosphate; NFATc1, nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1; Nrf1, nuclear factor erythroid 2-related factor 1; Nrf2, nuclear factor erythroid 2-related factor 2.

Up-regulated MTs can maintain cellular and mitochondrial redox balance to reduce oxidative stress, damage, and associated cell death.[32,33] Therefore, cells exposed to SFN exhibit elevated Nrf2 function to transcriptionally up-regulate multiple antioxidants including MTs,[34–36] and cells exposed to zinc show increased MT expression independently on Nrf2 function.[37–39] MTs can bind Cd to prevent it from undergoing the Fenton reaction to generate harmful ROS and can also directly scavenge ROS, thereby playing important roles in preventing Cd-induced osteoclast differentiation and osteoporosis. Meanwhile, zinc may also affect certain transcription factor pathways, including Nrf1/Nrf2, via MT-dependent or -independent means.[40,41]

I believe there is great potential of SFN and zinc for clinical use, given that the former can be extracted from broccoli and the latter is an essential nutrient; these agents have been clinically trialed or applied to (respectively) different chronic diseases. Furthermore, not only have several *in vitro* studies with osteoblastic cell lines[28,42–44] shown that SFN or zinc exhibited preventive effects on the cytotoxicity caused by different kinds of stresses, but numerous *in vivo* studies with animal models also showed the preventive effects of either zinc or Nrf2 activators, including SFN, on bone injury or loss caused by several pathologies.[44–46] We also know these agents may protect against the complications of both type 1 and type 2 diabetes—SFN via activation of Nrf2 function,[47–50] zinc via induction of MT expression,[37,51,52] and SFN and zinc synergistically.[53,54] Diabetes is a major risk factor for osteoporosis, most likely via oxidative stress.[55–57] Therefore, this study offers hope for osteoporosis prevention,[1] namely, the potential application of Nrf2 activators—including SFN alone or combined with zinc—to prevent and possibly treat osteoporosis caused by environmental exposures or chronic diseases, such as diabetes. Hopefully, more studies will be conducted to validate these promising findings and advance their clinical application in the near future.

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