Modulation Of Bone Homeostasis And Suppression Of Adipogenic Differentiation By Sulforaphane, A Naturally Occurring Isothiocyanate

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Introduction: Nutritional drugs may have unanticipated anabolic effects on skeletal development and homeostasis. For example, sulforaphane (SFN) which is abundantly present in cruciferous vegetables like broccoli or cauliflower (1), is capable of ameliorating the pathology of different diseases. SFN can prevent, delay or even reverse carcinogenesis in vitro and in vivo (2), in rheumatoid arthritis SFN was found to inhibit synovial hyperplasia and T cell activation. Furthermore, SFN was demonstrated to inhibit osteoclastogenesis by inhibiting nuclear factor-kappaB (3). In this study, we addressed whether SFN has positive effects on skeletal development and homeostasis.

SFN is a member of a biochemical class of agents (e.g., DMSO) that may cause benign shifts in cellular phenotypes due to effects on active DNA-demethylation (5). For example, this mechanism has been invoked to clarify the osteogenic effects of DMSO and increased production of extra cellular matrix (ECM) mineralization in primary osteoblasts and osteoblast cell lines (i.e., MC3T3-E1 cells)(4). We evaluated the effect of SFN on bone cells and tissues, as well as on clinical grade adipose derived human mesenchymal stem cells.

Methods: We selected primary cells and cell lines representative for bone-forming osteoblasts (mouse MC3T3-E1) and osteocytes (mouse MLO-Y4 cells), bone-resorbing osteoclasts (mouse RAW-264.7 and primary pre-osteoclasts from bone marrow), as well as mouse bone-marrow derived mesenchymal stem cells (BMSCs) and human adipose-tissue derived mesenchymal stem cells (AMSCs) cultured in osteogenic or adipogenic medium. Experimental results were also validated in ex vivo calvarial explant cultures derived from either newborn or adult (7 weeks old) mice. All cell types and tissues were treated with increasing concentrations of SFN for up to 6 weeks.

Gene expression was measured by qRT-PCR, gene arrays and immunoblots. Alkaline phosphatase (ALP) activity was measured by pNitrophenyl-phosphate assay and ECM mineralization was quantified by alizarin red staining. Osteoclastic resorption on ivory was measured and caspases 3/7 and 8 activities were evaluated. DNA hydroxymethylation was visualized by immuno-fluorescence microscopy.

Results: Our results show that SFN has striking short-term and long-term effects on cell growth and differentiation, consistent with both pro-anabolic and anti-catabolic effects on bone.

For example, SNF administered at an optimal dose of 3 µM significantly increased the expression of BGLAP2, RUNX2, COLA1A1 and LOX in MC3T3-E1 osteoblasts and BMSCs and AMSCs after 14 to 20 days of treatment. In all cell culture models, as well as in our ex vivo newborn and adult calvarial explant cultures, SFN treatment significantly increases ECM mineralization.
Notably, exposure to SFN also significantly decreases expression of RANKL, a paracrine factor that promotes osteoclast differentiation, in MLO-Y4 osteocytes, as well as in newborn and adult calvaria. Consistent with this observation, SFN rapidly inhibits cell proliferation and activates caspases 3/7 and 8 in pre-osteoclastic RAW-264.7 cells (within 24 hr), compared to osteocyte-like MLO-Y4 and pre osteoblastic MC3T3-E1 cells. Furthermore, SFN treatment also has long term effects on in vitro resorption by osteoclasts on ivory (significantly inhibited by 40% at day 12 of treatment).

**Discussion:** From a bone-tissue engineering perspective, SFN is also capable of triggering osteoblastic differentiation of clinical-grade human AMSCs in part by suppressing adipogenic differentiation. SFN enhances ALP activity, ECM mineralization and expression of osteoblastic genes like COL1A1, RUNX2 or TNFRSF11B (OPG) in AMSCs cultured in osteogenic media. However, SFN dramatically decreased fat droplet formation and expression of the fat-related PPARY, PLIN1 or CEBPA genes with modestly increased expression of osteoblastic genes in AMSCs cultured in adipogenic media. Mechanistically, induction of differentiation by SFN triggers epigenetic reprogramming of the chromatin within a few hours, as well as induces >30 genes responsible for active DNA demethylation and epigenetic chromatin remodeling within 4 hours of treatment in AMSCs. Collectively, our results indicate that SFN preferentially stimulates osteoblastogenesis while suppressing adipogenesis in AMSCs by modulating key epigenetic events required for mesenchymal cell fate determination.

**Significance:** We propose that the natural food compound SFN may be an effective anabolic stimulator of bone homeostasis and suppressor of adipogenic differentiation.

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