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COMMENTARIES

The Silent Wif of Death

Venkatesh Krishnan[1] and Ormond A. MacDougald[2]

[1]Musculoskeletal Research, Lilly Research Laboratories, Indianapolis, Indiana, USA
[2]Departments of Molecular and Integrative Physiology, and Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan, USA

Commentary on: Kansara M, Tsang M, Kodjabachian L, Sims NA, Trivett MK, Ehrich M, Dobrovic A, Slavin J, Choong PF, Simmons PJ, Dawid IB, Thomas DM. Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. *J Clin Invest*. 2009 Apr;119(4):837-51.

The Wnt signaling pathway regulates stem cell renewal, differentiation, and tissue/organ development and maintenance throughout the body (1). Activation of this pathway in bone offers promising therapeutic value for osteoporosis since Wnt signaling induces bone formation through stimulation of proliferation, differentiation, and mineralizing activity of osteoblasts (2). The extensive system of negative feedback mechanisms within the Wnt signaling cascade provides prime targets for pharmacological derepression of Wnt signaling; however, great care needs to be taken with this approach since dysregulation of this pathway is commonly associated with many types of cancer, including osteosarcoma. The recent article by Kansara and colleagues (3) identifies Wnt inhibitory factor 1 (WIF1) as one of the key checkpoints in controlling the switch between proliferation and differentiation of mesenchymal stem cells into mature osteoblasts, and the unbridled and deleterious growth of these cells observed in osteocarcinoma.

A screen for potential tumor suppressors epigenetically silenced in human osteocarcinoma cell lines was performed by demethylating DNA with 5-aza-2-deoxycytidine (dAC), profiling gene expression, and filtering out those genes involved in osteoblast differentiation. Potential caveats associated with this elegant approach include the very high concentration of demethylating agent (4), and the uncoupling of negative feedback by other inhibitors of Wnt signaling such as Dkk1, sFRPs, and WISP, which are normally up-regulated with Wnt/β-catenin signaling.

From the set of genes identified through this approach, the rationale to pursue WIF1 as a candidate tumor suppressor gene for osteosarcoma seems to derive mainly from independent observations that implicate WIF1 in other types of cancer (5). However, the selection of WIF1 is supported by the observation of increased spontaneous and induced osteosarcomas in WIF1 null mice, which strongly posits a causal role for WIF1 as an important osteosarcomagenesis checkpoint. One can anticipate that future studies will elucidate the precise mechanism(s) of deregulation of WIF1 silencing in osteosarcomas. For instance, which enzymes methylate WIF1 promoter/enhancer regions? In addition, are the enzymes associated with the silencing process regulated by Wnt activators? Such areas of future exploration may allow us to identify the mechanisms linking the epigenetic silencing of WIF1 to osteosarcomagenesis.

Although WIF1 is expressed in the skeleton of adult mice, and many components of the Wnt signaling pathway are known to influence bone mass, the lack of WIF1 in mice is not associated with significant alterations in cortical or trabecular bone mineral density through 52 weeks of age, nor are differences in osteoblast gene

expression observed. Importantly, these data suggest that WIF1 is not, at least in mice, a rational therapeutic target for stimulation of bone formation. In contrast, the high frequency of osteosarcomas in Ca^{45}-induced WIF1 null mice clearly demonstrates an antiproliferative and apparently unique role for WIF1, when compared to the established functions of Wnt inhibitors such as SOST, Dkk1, sFRPs, and WISP. While the latter cohort of Wnt repressors is now justifiably subject to additional scrutiny for risk of osteosarcomagenesis as a result of genetic or pharmacological ablation, the authors' extension of these findings to other inhibitors of Wnt signaling seems premature since mouse and human studies at this point demonstrate a stimulation in bone mass without an increased risk of osteosarcomagenesis (6). It is conceivable that the Wnt signaling pathway contains a set of negative regulators including WIF1, which regulates growth of pluripotent cells, and another set that controls subsequent steps in the differentiation and activity of osteoblasts. Only further investigation will determine whether cell fate-specific roles of WIF1, SOST, Dkk1 and sFRPs are temporally, spatially, and mechanistically distinct, such that bone formation can be stimulated through therapeutic intervention without increasing susceptibility to osteosarcomagenesis.

The authors also extrapolated their findings to raise concern about current therapeutics that increase bone formation, such as intermittent teriparatide. Assessment of osteosarcoma risk in humans treated with intermittent teriparatide is ongoing and the current teriparatide-induced osteosarcoma cases represent an incidence frequency below the normal public occurrences of osteosarcomas (7). While the mechanism of intermittent parathyroid hormone-mediated osteosarcomagenesis in Fisher rats is unknown, inhibition of SOST in mature osteocytes represents only a portion of the pleiotropic mechanism that mediates its anabolic activity. In addition, Wnt antagonists that favor bone formation (*e.g.* SOST, Dkk1 and sFRP1) may work within the boundaries of “rational osteogenesis” and could potentially avoid the increased osteosarcomagenesis seen with WIF1 silencing. The relative risk for induction and/or promotion of osteosarcomagenesis using pharmacological antagonists such as anti-SOST and anti-Dkk1 antibodies needs to be carefully examined using long-term observational safety studies, followed by watchful risk assessment during the clinical testing of these agents.

In summary, Kansara and colleagues have provided convincing evidence that WIF1 is a tumor suppressor silenced in osteosarcomas. This insight may allow us to pursue diagnostic methods to assess the relative silencing of molecules such as WIF1 and other tumor suppressors identified in their study, which could help identify individuals at risk for osteosarcomas. Hopefully these approaches will ultimately permit therapeutic activation of Wnt signaling to increase bone formation in osteoporotic patients without increasing the risk of osteosarcoma.

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