

# On the Horizon From the ORS

## Wnt, Osteosarcoma, and Future Therapy

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Osteosarcoma is the most common type of primary bone cancer, with peak incidences in adolescence and at age >60 years. This tumor has a very high propensity for local invasion and distant metastasis. Pulmonary metastasis is the most common mode of spread, occurring in about 80% of patients. Aggressive treatment with high-dose chemotherapy and surgery leads to cure in 60% to 70% of patients. However, the long-term survival for patients with relapse is only 20%.<sup>1</sup>

The Wnt pathway is comprised of a family of highly conserved proteins that regulate cell-to-cell interactions during embryogenesis. In the absence of Wnt binding to cell-surface receptors called Frizzled and LRP-5/6, a cytoplasmic complex consisting of axin, adenomatous polyposis coli, and glycogen synthase kinase- $\beta$  phosphorylates  $\beta$ -catenin, thus promoting the ubiquitination and degradation of  $\beta$ -catenin. In addition to Wnt proteins and receptors, several negative regulators, called Wnt antagonists, exist.  $\beta$ -Catenin, together with LEF/TCF transcription factors, acts as a signal transducer for downstream genes involved in cell proliferation and growth.

Using clinical tissue samples, we examined the relationship between the Wnt pathway and clinical outcome of patients with osteosarcoma. In this study, RNA from fresh-frozen osteosarcoma specimens was isolated to characterize the expression of the Wnt receptor LRP-5 by polymerase chain reaction. We found a statistically significant correlation between LRP-5 expression and a worse event-free

survival in patients. More importantly, patients whose primary tumors expressed LRP-5 sustained a higher risk of developing metastasis.

Given the association between LRP-5 and osteosarcoma, we proceeded to examine whether blocking Wnt signaling by a soluble LRP-5 receptor affects tumor progression. Our results suggest that blocking Wnt/LRP-5 signaling halts tumor invasiveness by reversing the epithelial-to-mesenchymal transition, confirming the important role of Wnt in osteosarcoma progression. In contrast, downregulation of Wnt antagonists is a common event in osteosarcoma tumors and cell lines, suggesting that Wnt activation contributes to the tumorigenic phenotypes. Further analysis suggests that promoter hypermethylation is one of the key mechanisms by which osteosarcoma suppresses Wnt antagonist expression.<sup>2</sup> Consistent with this notion, re-expression of Wnt antagonists slows both tumor growth and the formation of lung metastasis in vivo. Therefore, drugs that target DNA methyltransferase may play an important role in preventing osteosarcoma progression.

Several strategies have been devised to exploit the Wnt pathway for therapeutic purpose. DeAlmeida et al<sup>3</sup> showed that a secreted Wnt antagonist, consisting of the ligand-binding domain of Frizzled-8 fused with human immunoglobulin G, had antitumor efficacy in an animal model. Monoclonal antibodies against Wnt-1 and -2 have been shown to induce apoptosis in several cancer cell

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lines, including sarcoma.<sup>4</sup> Another class of compound takes advantage of the PDZ-binding domain of Disheveled (Dvl), a Wnt-activating protein.<sup>5</sup> Dvl is involved in transducing the intracellular signal to inhibit  $\beta$ -catenin destruction complex. By targeting this PDZ domain, researchers hope to turn off Wnt signaling by preventing Dvl from keeping a high intracellular level of  $\beta$ -catenin. This class of drug will be able to address mutations leading to increased expression of Wnt ligand or decreased expression of negative regulators, or to mutation in extracellular receptors. However, it would be less effective against tumors harboring downstream mutations of the degradation complex or mutations of  $\beta$ -catenin itself.

Although strategies that directly target the Wnt pathway hold great promise, there may be significant side effects because this pathway is critical for tissue regeneration and for stem cells in the gut and bone marrow to self-renew. Another approach may involve drugs that specifically block downstream targets of

Wnt. One such target is the hepatocyte growth factor receptor c-Met, a receptor tyrosine kinase aberrantly expressed in most human osteosarcomas. It appears that c-Met not only plays a role in the progression of osteosarcoma but may also be essential to its initiation. Overexpression of c-Met can convert primary human osteoblasts into osteosarcoma cells, displaying the transformed phenotype both in vitro and in vivo.<sup>6</sup> Our data implicated c-Met as being tightly regulated by Wnt signaling and therefore susceptible to the negative effects of Wnt antagonists. Together, these findings suggest that drugs inhibiting Wnt signaling or Wnt target genes, such as c-Met, should exhibit anti-tumor effects against at least a subset of osteosarcoma.

It is clear that activation of the Wnt pathway plays an important role in the biology of osteosarcoma. Although treatment of osteosarcoma has improved, there is still much to gain, especially in the areas of recurrence and metastasis prevention. Strategies to target the Wnt pathway

have emerged as a viable alternative to current treatment paradigms.

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