Targeting Neuropilin, A VEGF Receptor, Reduces Angiogenesis and Tumor Growth in Osteosarcoma

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INTRODUCTION:
Osteosarcoma (OS) hypervascularity makes targeting tumor angiogenesis a promising new strategy for this cancer. We have previously shown that inhibition of Wnt signaling leads to decreased OS angiogenesis and tumor progression; However, the mechanism by which this occurs is largely unknown. Neuropilin-2 (NRP-2) is a co-receptor for vascular endothelial growth factor (VEGF). Recent studies indicate that disrupting NRP-2 and Notch signaling may inhibit tumor angiogenesis. Here, we investigated whether NRP-2 is regulated by Wnt and whether disrupting the function of NRP exerts a negative effect on OS angiogenesis and tumor growth.

METHODS:
NRP-2 mRNA and protein were measured in several cell lines. OS cell lines were stably transfected with the Wnt inhibitors and dominant-negative Wnt transcription factors. Analysis of gene expression after sLRP5 transfection was performed using Genechip™ microarray, qRT-PCR, and western blot. Depletion of NRP-2 in 143B OS cells was achieved by stable shRNA techniques and knockdown was confirmed by real-time PCR and western blot. Chromatin immunoprecipitation (IP) was used to confirm regulation of NRP-2 by Wnt. Tumorigenicity was assessed by agar colony formation assays. Control and NRP-2 depleted 143B cells were injected into nude mice and in vivo analysis was performed.

RESULTS SECTION:
Microarray analysis demonstrated that inhibiting the Wnt pathway lead to decreased expression of NRP-2. Chromatin IP assays demonstrated TCF transcription factor binds to the NRP-2 promoter, suggesting NRP-2 is regulated by Wnt. OS cell lines transfected with Wnt inhibitors consistently showed decreased expression of NRP-2 and Notch ligand Jag-1 by Western Blot and qRT-PCR. Inhibition of nuclear Wnt signaling also led to down-regulation of NRP-2 and Jag-1. Knockdown of NRP-2 by shRNA dramatically reduced the expression of Jag-1, suggesting control of Jag-1 expression by NRP-2 in OS. NRP-2 knockdown showed significant decrease in colony formation as well as in vivo tumor growth in xenograft mouse models. Analysis of mouse tumors showed reduced microvessel formation, suggesting that NRP-2 depletion leads to decreased angiogenesis.

DISCUSSION:
Tumor growth and metastasis depend on vascularization and a chemical signal from tumor cells can shift resting endothelial cells into a phase of rapid growth. The high metastatic potential and high recurrence rate of osteosarcomas are considered to be associated with a positive angiogenic phenotypes and rapid growth. A large number of angiogenic associated factors have been cloned.

Vascular endothelial growth factor plays a major role in the induction of endothelial cell proliferation and increase of the vascular endothelium permeability. NRP-2, a mediator of neuronal guidance, is a specific VEGF receptor.

Our data demonstrates a novel Wnt-related mechanism to control tumor angiogenesis via NRP-2 and Jag-1 in OS. NRP-2 expression affected the increased vascularization and major prognostic factor in osteosarcoma. NRP-2 suppression resulted in decreased proliferation, angiogenesis, and tumor formation. Our understanding of this mechanism opens new avenues for developing therapeutics for this malignancy.

SIGNIFICANCE:
Our data demonstrates a novel mechanism to control tumor angiogenesis via NRP-2 and Jag-1 in OS. Our understanding of this mechanism opens new avenues for developing therapeutics for this malignancy.

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REFERENCES: