

The Vascular Targeting Agent VEGF₁₂₁/rGel Inhibits Bone Remodeling and Skeletal Metastases through a Unique Mechanism

Khalid A. Mohamedali, Ann T. Poblentz, Chuck Sikes, Troy Luster, Nora Navone, Philip Thorpe, Bryant G. Darnay and Michael G. Rosenblum
University of Texas M.D. Anderson Cancer Center, Houston, TX and University of Texas Southwestern Medical Center, Dallas, TX.



Abstract

Cancer metastases to bone are associated with significant morbidity and mortality and patients with advanced cancer experience frequent bone metastasis. The pathophysiological processes leading to the development of skeletal metastases remains poorly understood. We developed a novel fusion construct designated VEGF₁₂₁/rGel, composed of VEGF₁₂₁ and the plant toxin gelonin (rGel), which targets the tumor neovasculature and exerts impressive cytotoxic effects by inhibiting cellular protein synthesis in target cells. We tested the ability of VEGF₁₂₁/rGel treatment to inhibit the growth of prostate cancer cells in a bone metastases model. VEGF₁₂₁/rGel inhibited tumor growth and enhanced survival of mice by targeting the tumor vasculature as well as normalizing the number of mature osteoclasts found in bone. Treatment of mice bearing PC-3 intrafemoral tumor xenografts with VEGF₁₂₁/rGel was shown to dramatically suppress PC-3 skeletal metastases. All control mice developed lytic lesions and were sacrificed by day 67. In contrast, 50% of the VEGF₁₂₁/rGel-treated mice survived past day 140 without any sign of skeletal tumor lesions. In vitro studies showed that VEGF₁₂₁/rGel but not rGel could dramatically suppress RANKL-induced osteoclast differentiation of RAW cells into TRAP⁺ multi-nucleated osteoclasts. The observed effect was not mediated by either VEGF₁₂₁ or gelonin alone but is a characteristic unique to the combined fusion protein. The IC₅₀ of VEGF₁₂₁/rGel on dividing RAW cells was 40 nM as compared with 900 nM for rGel itself, indicating the presence of a receptor for VEGF₁₂₁. Similar results were obtained for VEGF₁₂₁/rGel-treated bone marrow-derived monocytes. While immunofluorescence studies clearly show VEGF₁₂₁/rGel penetration into osteoclast precursor (RAW) cells, the receptor responsible for mediating the cellular entry of VEGF₁₂₁/rGel is unknown. RT-PCR analysis of RAW cells indicates the presence of only VEGFR-1 (Flt-1). In addition, Flt-1 levels are downregulated following stimulation of osteoclastogenesis by RANKL demonstrating that mature osteoclasts express comparatively low levels of Flt-1 and are insensitive to VEGF₁₂₁/rGel cytotoxic effects. These studies suggest an important role for VEGF and its receptors in tumor-mediated osteoclastogenesis and demonstrate that VEGF₁₂₁/rGel appears to suppress osteolytic lesions by acting directly on osteoclast precursor cells as well as on suppression of tumor vasculature. This suggests a previously unrecognized role for this unique agent in the treatment of skeletal metastases.

VEGF Receptors

- Flt-1 (Receptor 1) and KDR/Flk-1 (Receptor 2) have tyrosine kinase activity.
- Highly expressed on vascular endothelial cells.
- Receptors are over-expressed in tumor neovasculature of several cancers including lung, breast, prostate, skin and ovarian cancer.

Targeting Moiety: VEGF₁₂₁

- Endothelial cell-specific mitogen
- The shortest form of VEGF-A
- Does not bind to heparin or require heparin for binding to its receptors
- Functions as a homodimer

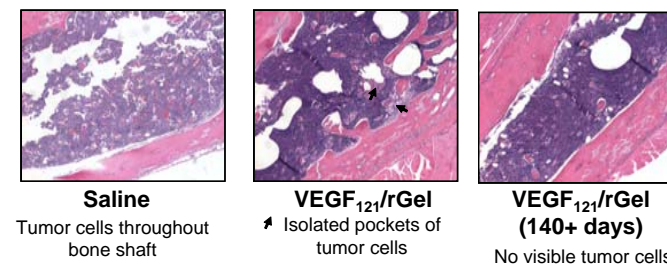
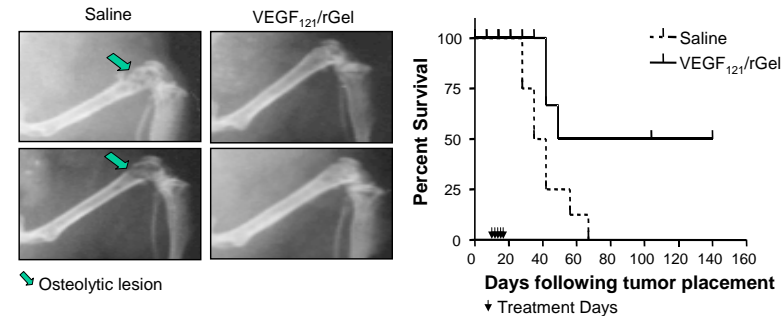
Effector Moiety: Gelonin Toxin

- Single-chain plant toxin (28 kDa)
- N-glycosidase specific for rRNA
- Mechanism of action identical to ricin A chain (protein synthesis inhibitor)
- Requires carrier to transit membrane
- Lethal to mammalian cells within 48 h

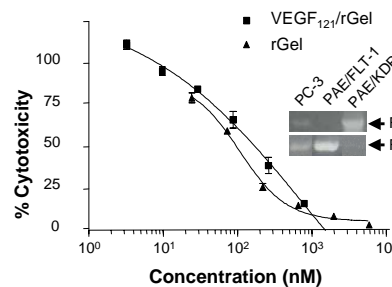
Characteristics of VEGF₁₂₁/rGel Fusion Toxin

- 84 kDa homodimer (disulfide linked)
- VEGF component binds to isolated Flk-1 (ELISA)
- Toxin component active with a specific activity similar to free gelonin
- The purified VEGF₁₂₁/rGel construct expressed and purified from soluble bacterial lysates

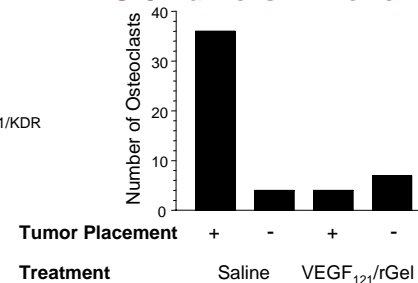
VEGF₁₂₁/rGel Inhibits Growth of Intrafemoral PC-3 Tumors and Increases the Survival of Nude Mice



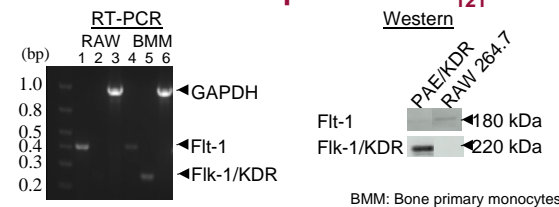
VEGF₁₂₁/rGel is Not Specifically Cytotoxic to PC-3 Cells



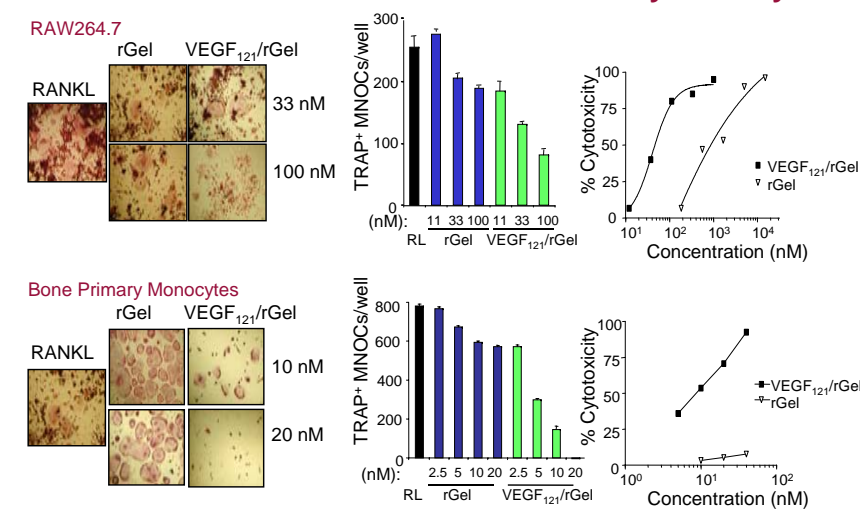
VEGF₁₂₁/rGel Reduces the Number of Osteoclasts in PC-3 Tumors in Bone



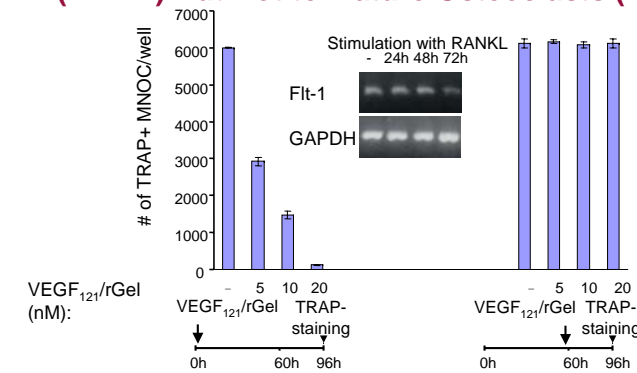
Osteoclast Pre-cursor Cells Express VEGF₁₂₁ Receptors



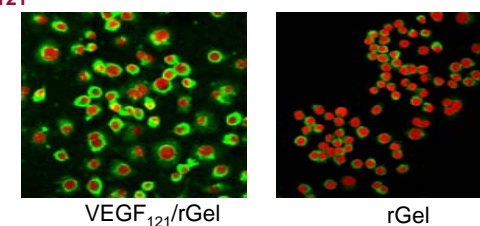
VEGF₁₂₁/rGel Inhibits RANKL-Mediated Osteoclastogenesis and Exhibits Targeted Cytotoxicity to RAW264.7 Cells and Mouse Primary Monocytes



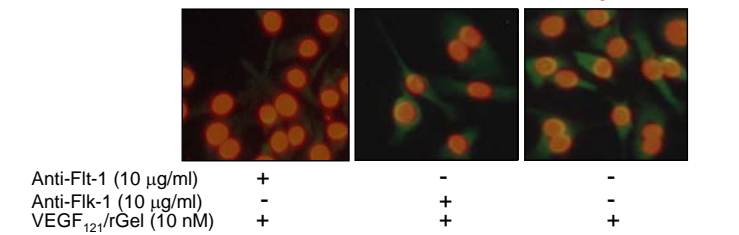
VEGF₁₂₁/rGel is Cytotoxic to Osteoclast Pre-cursor Cells (TRAP⁻) But Not to Mature Osteoclasts (TRAP⁺)



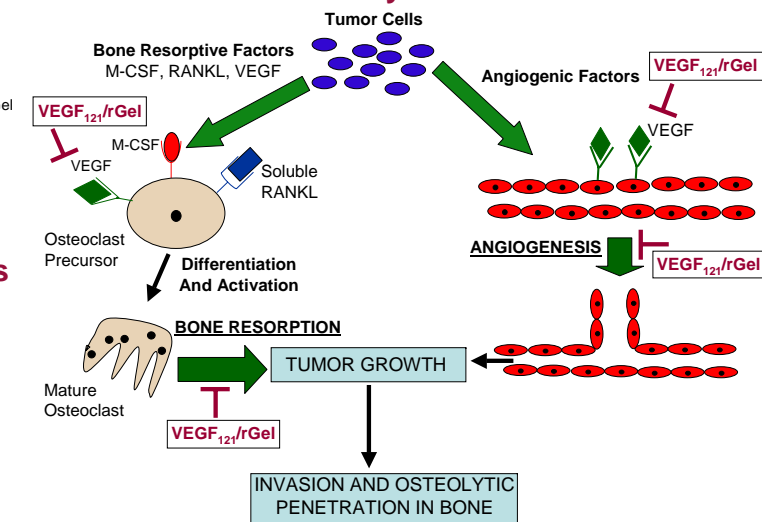
VEGF₁₂₁/rGel is Internalized into RAW264.7 Cells



Localization of VEGF₁₂₁/rGel into Osteoclast Pre-cursor Cells is Mediated by Flt-1



Proposed Dual Role for VEGF₁₂₁/rGel in Tumor Invasion and Osteolytic Penetration In Bone



Summary

- VEGF₁₂₁/rGel fusion protein inhibits the growth of orthotopic and subcutaneous tumors in vivo by targeting the tumor neovasculature.
- Cytotoxic effects of VEGF₁₂₁/rGel are due to effects on tumor vasculature, not directly on tumor cells themselves.
- Treatment of PC-3 tumor cells in a bone metastatic model results in 50% survival of mice treated with VEGF₁₂₁/rGel.
- VEGF₁₂₁/rGel inhibits growth of PC-3 tumor cells in bone by inhibition of angiogenesis as well as osteoclastogenesis.
- Internalization and cytotoxicity of VEGF₁₂₁/rGel into osteoclast precursor cells appears to be mediated by Flt-1 and not by Flk-1.
- VEGF₁₂₁/rGel is a useful probe to investigate the role of VEGF and its cognate receptors in skeletal metastasis.
- VEGF₁₂₁/rGel is a good candidate for further study alone or in combination with other therapies that target tumor cells.