Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover

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**Introduction:**
Several growth factors are expressed in distinct temporal and spatial patterns during fracture repair. Of these, vascular endothelial growth factor, VEGF, is of particular interest because of its ability to induce neovascularization (angiogenesis). To determine whether VEGF is required for bone repair, we inhibited VEGF activity during secondary bone healing via a cartilage intermediate (endochondral ossification) and during direct bone repair (intramembranous ossification) in a novel mouse model.

**Methods:**

**Animals and reagents.**
C57 Bl6 mice and male New Zealand White (NZW) rabbits were used according to protocols approved by the Institutional Review Board or by Institutional Animal Care and Use Committee. Anesthetized mice were assayed for endogenous VEGF protein in vivo and ex vivo, using inhouse or commercial antibody reagents. A 1 cm segment of radius or femur was removed using a sterile saw blade with liberal saline irrigation to prevent overheating of bone margins. As assessed by radiographs, intramembranous ossification was evident in both bones within 6 weeks of injury.

**Creation of focal cortical defect in the tibia of mice.**
A full thickness unicortical defect was created on the tibia using a dental burr, with continuous saline irrigation to prevent thermal necrosis of margins. Mice were untreated (Control) or injected with intraperitoneal injections of a Control IgG or murine Flt-1 IgG on alternate days.

**Computed tomography (CT) analysis.**
X-ray micro-computed tomography (µCT) images were acquired using a µCT20/40 (SCANCO Medical, Bassersdorf, Switzerland). Axial images were obtained with an hydroxyapatite phantom for system calibration. Callus volume and mean voxel intensity were calculated for callus Volume of Interest (VOI_{callus}). A "calcification" threshold was applied to VOI_{callus} to determine volume and mean intensity of calcified callus. VOI_{callus} for mouse bones was determined manually using SCANCO image analysis software. VOI_{callus} for rabbit bones was determined with an in-house segmentation algorithm developed with Analyze software (AnalyzeDirect Inc., Lenexa, KS). Lower and upper thresholds, determined by histogram analysis of data from three rabbits, were applied to extract potential callus voxels. A series of morphological filtering operations (erode, open, conditional dilate, and close) were applied to extract the callus volume.

**Rabbit radius segmental gap model.**
The periosteum was excised from the radius along the mid-shaft of anesthetized, male NZW rabbits. A 1 cm of the radius was removed using a sterile saw blade with liberal saline irrigation to prevent overheating of bone margins. A local, subcutaneous osmotic pump was used to deliver VEGF. Analgesics were given before surgery and for 72 h post-surgery.

**In-vivo PECAM-1 labeling.**
Monoclonal antibodies (mAb) rat anti-mouse platelet-endothelial cell adhesion molecule, PECAM-1 labeled with 125I and a non-specific isotope control antibody, labeled with 131I were used. All antibodies were iodinated using the iodogen method. To measure PECAM-1 binding, a mixture of 125I PECAM-1 mAb and 131I non-specific mAb was used. Cold PECAM-1 mAb was added, and the mixture was injected through the jugular vein catheter and allowed to circulate.

**Discussion:**
Our results at specific time-points during the course of healing underscore the role of VEGF in endochondral vs. intramembranous ossification, as well as skeletal development vs. bone repair. The responses to exogenous VEGF observed in two different model systems and species indicate that a slow-release formulation of VEGF, applied locally at the site of bone damage, may prove to be an effective therapy to promote human bone repair. Furthermore, a patient with bone damage who has alterations in VEGF regulation and/or responsiveness may have a relatively poor prognosis. Given that significant cell death occurs in the first 24 hours after fracture and that shorter treatments with VEGF protein in vivo were less effective, a slow-release formulation may be necessary to promote optimal healing by VEGF. Such a VEGF formulation could also prove useful in additional indications such as spinal fusion, non-unions, and maxillo-facial surgeries.

**Essential Results:**
Treatment of mice with a soluble, neutralizing VEGF receptor decreased angiogenesis, bone formation, and callus mineralization in femoral fractures. Inhibition of VEGF also dramatically inhibited healing of a tibial cortical bone defect, consistent with our discovery of a direct autocrine role for VEGF in osteoblast differentiation. In separate experiments, exogenous VEGF enhanced blood vessel formation, ossification, and new bone (callus) maturation in mouse femur fractures, and promoted bony bridging of a rabbit radius segmental gap defect.