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

+*Ellen H. Filvaroff, **John Street, *Min Bao, *Leo deGuzman, *Stuart Bunting, *Frank Peale, *Napoleone Ferrara, *Hope Steinmetz, *John Hoeffel, *Jeffrey L. Cleland, *Ann Daugherty, *Nicholas van Bruggen, **H. Paul Redmond, *Richard A.D. Carano

*Genentech, Inc., 1 DNA Way , South San Francisco, CA **Department of Academic Surgery, National University of Ireland, University College Cork, Cork, Ireland.

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 conforming to national laws and regulations.

Murine femur fracture.

A midshaft, fixed femur fracture was created in anesthetized mice. VEGF was applied at the fracture site. Any animals in which the pin came out, the fracture was grossly displaced, or the fracture was not midshaft (as assessed by radiographs) were not analyzed. Using inclusion criteria, experiments used a minimum of 7 animals/group.

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 were given 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 a 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. 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 prior to surgery and for 72h post-surgery.

*In-vivo PECAM-1 labeling***.**

Monoclonal antibodies (mAb) rat anti-mouse platelet-endothelial cell adhesion molecule, PECAM-1 labeled with ^{125}I and a non-specific isotype control antibody, labeled with ^{131}I were used. All antibodies were iodinated using the iodogen method. To measure PECAM-1 binding, a mixture of ^{125}I PECAM-1 mAb and ^{131}I 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. Blood samples were obtained from carotid catheters to measure circulating radiolabeled antibody levels. Organs and muscles were collected, weighed, and radioactivity was counted.

Primary Human Osteoblast Cultures.

After extensive washes of trabecular bone explants from consenting young adults with no evidence of metabolic bone disease, small bone chips were placed in flasks with and cultured at 37°C and 5% CO_2. Experiments were performed on osteoblasts subcultured to passage 3 - 6.

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.

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 distinct 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.

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