Supplementary Materials and Methods

AAVrh.10 Vector Production and Characterization

AAVrh.10BevMab was produced by Polyfect-mediated (Qiagen) cotransfection into human embryonic kidney 293 cells (HEK 293; American Type Culture Collection) of two plasmids, pAAVαVEGF (500 μg), and pPAKMArh.10 (1.0 mg): (1) pAAVαVEGF is an expression plasmid containing (5′ to 3′) the AAV2 5′-inverted terminal repeat including packaging signal (ψ), the expression cassette of the humanized anti-VEGF monoclonal antibody, bevacizumab and the AAV2 3′-inverted terminal repeat; and (2) pPAKMArh.10 is a helper and packaging plasmid that provides the AAV Rep proteins derived from AAV2 needed for vector replication, the AAVrh.10 viral structural (Cap) proteins VP1, 2 and 3, which define the serotype of the produced AAV vector and provides Ad helper functions of E2, E4 and V_A RNA. At 72 hr after transfection, the cells were harvested; a crude viral lysate was prepared by four freeze/thaw cycles and clarified by centrifugation. AAVrh.10BevMab was purified by iodixanol gradient and QHP anion exchange chromatography, concentrated with an Amicon Ultra-15 100K centrifugal filter device (Millipore) and stored in PBS, pH 7.4, -80°C. The control vector was produced by the same method with pAAVanticoc, a plasmid coding for a monoclonal antibody against cocaine, substituted for pAAVαVEGF. Vector genome titers were determined by TaqMan quantitative PCR using a cytomegalovirus promoter-specific primer–probe set (Applied Biosystems, Grand Island, NJ). To verify AAVrh.10BevMab-directed expression of bevacizumab, HEK 293 cells were infected with AAVrh.10BevMab at 2 x 10^5 genome copies per cell (or mock infected), supernatant was harvested 72 hr later and immunoglobulin was purified with protein G sepharose. Bevacizumab expression was evaluated by coomassie blue

stain SDS-PAGE and Western analysis with a sheep anti-human IgG heavy chain and light chain secondary antibody (Sigma) and an enhanced chemiluminescence reagent (Amersham).

mRNA and Protein Expression of Bevacizumab

Blood was obtained from the tail vein, allowed to clot for 1 hr, 23°C, followed by 30 min, at 4°C, and then spun in microcentrifuge at 10,000 g for 20 min to collect serum. The levels of bevacizumab antibody were determined by ELISA. Wells of flat bottomed 96-well EIA/RIA plates (Corning, Corning, NY) were coated with 100 µl of 0.2 µg/ml VEGF165 (R&D Systems), in carbonate-buffer at pH 9.0 overnight at 4°C and then washed with 0.05% Tween 20 in PBS (PBS-Tween) and blocked with 5% dry milk in PBS for 30 min, 23°C. Serial dilutions of sera were added to the 96-wells and incubated for 90 min, 23°C. The plates were washed 4 times with PBS-Tween and 100 µl of 1:2000 diluted goat anti-human IgG conjugated to horseradish peroxidase (Sigma-Aldrich, St. Louis, MO) in 1% dry milk in PBS, incubated for 90 min, 23°C. After 4 wash steps, peroxidase substrate (100 µl/well; Bio-Rad, Hercules, CA) was added to each well, incubated for 15 min, 23°C and the reaction was stopped with addition of 2% oxalic acid (100 µl/well). Absorbance was measured at 415 nm. Bevacizumab antibody titers were calculated by interpolation of the log(OD)-log(dilution) with a cutoff value equal to twice the absorbance of background and converted to µg/ml based on standard curve with the bevacizumab protein. Total protein levels were quantified by the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL).

Immunohistochemical Analysis

AAVrh.10BevMab transduction of the mouse brain cells was assessed by immunohistochemical staining for bevacizumab. Mouse brains were perfused with 4% paraformaldehyde formulated in PBS were stored in 4% paraformaldehyde/PBS , equilibrated in

15% sucrose at 4°C for 24 to 48 hr, followed by an equilibration in 30% sucrose at 4°C for 48 to 72 hr. Serial 10 to 100 μm thick frozen coronal sections were produced with a microtome (Histoserv, Germantown, MD). For immunohistochemical analysis, bevacizumab was detected with anti-human kappa IgG antibody (1:100; overnight at 4°C; Sigma), neurons were detected with anti-neuronal nuclear antigen, anti-NeuN (Millipore), and astrocytes were detected with anti-glial fibrillary acidic protein, anti-GFAP (Millipore). Secondary antibodies conjugated to the fluorophores Alexa 488, Alex 555 or Alexa 647 (Invitrogen, Carlsbad, CA) were used to visualize the staining pattern. Fluorescence imaging was performed with an Olympus Fluoview confocal microscope (Olympus America, Center Valley, PA). For quantification of blood vessel density, frozen sections were stained with PECAM endothelial cell marker anti-CD31 (BD Pharmingen) and counterstained with anti-human specific antigen or anti-human mitochondrial antigen (millipore) and DAPI. Quantification of tumor blood vessel density was calculated separately in tumors and normal area of control-treated mice compared to AAVrh.10BevMab-treated mice. Activation of c-met pathway in tumor cell implantations was evaluated using anti-phosphorylated c-Met [pYpYpY1230/1234/1235] antibody (Invitrogen).