Supplementary Information

MATERIALS AND METHODS

Cell culture and reagents

U87GM (U87, human glioblastoma) and HT1080 (human fibrosarcoma) cell lines were obtained from Cancer Research UK Cell Services. Generation of U87-EV and U87-DLL4 cells and growth conditions were described previously (26). Mycoplasma contamination in cultured cells was excluded by using Lonza Mycoplasma Detection Kit. Dibenzazepine (DBZ) was purchased from Syncom (The Netherlands) at a purity of 99.8% at 232nm. PD173074 was purchased from TOCRIS Bioscience or made by C.P.M. and A.J.R.. Sorafenib (BAY54-9085) was generously provided by Bayer HealthCare Pharmaceuticals. Recombinant FGF2 was purchased from R&D Systems Europe Ltd. Antibodies were purchased from the following companies: Notch1IC, ERK, phosphorylated ERK (Cell Signalling) and β-actin (Sigma). Rabbit anti-FGF2 antibody (sc-79), recognizing both human and mouse FGF2, was purchased from Santa Cruz. The anti-Hes1 antibody was a gift from Tetsuo Sudo, Toray Industries, Japan. The anti-VEGF antibody (VG1, 1/1) that detects both human and mouse VEGF (isoform 121, 165, and 189) was obtained from Dako, and the M75 mouse monoclonal antibody against CA9 was purchased from Novus Biologicals (Littleton, CO). Monoclonal rat anti-mouse CD34 (clone MEC14.7) was obtained form AbD Serotec, UK. Anti-mouse, anti-rat, and anti-rabbit HRP conjugated secondary antibodies were from DAKO and anti-goat HRP was from Perbio. Human anti-EphrinB2 monoclonal antibody and recombinant sEphB4 protein (huEphB4 WT delta4R-HAS-His) were kindly supplied by MedImmune, USA. This anti-EphrinB2 antibody specifically recognized both human and mouse EphrinB2 and effectively neutralized the EphB4-EphrinB2 interaction. It binds to EphrinB2 on cell surface, preventing internalization and phosphorylation of EphrinB2, with an affinity of 5-10nM. The sEphB4 protein could compete for the EphB4-EphrinB2 interaction both in solid phase and cell based assays and thus agonized EphrinB2 as well as all its other binding partners.

Quantification of immunohistochemical staining

Necrosis was identified histologically on haematoxylin-eosin counterstained sections as areas displaying cells with basophilic pyknotic nuclei undergoing karyorrhexis and/or karyolysis, and scored as the percentage of the necrotic area in the entire full-face section. Immunohistochemical stainings of CA9, VEGF, VEGFR3 and FGF2 were scored for the intensity and percentage area of positive staining in viable tumor areas. The intensity was graded 1, 2 or 3 (corresponding to low, medium and high) and multiplied by the percentage area to produce an intensity percentage system score.

Primers for qPCR

RNA extraction from tumours, qPCR protocol, *mHey1* and *β-actin* primers were previously described (26). Other primers were listed as follows: mouse *VEGFR1* (*Flt1*, forward 5'-GGCCCGGGATATTTATAAGAAC-3' and reverse 5'-CCATCCATTTTAGGGGAAGTC-3'), mouse *VEGFR2* (*Kdr*, forward 5'-CAGTGGTACTGGCAGCTAGAAG-3' and reverse 5'-ACAAGCATACGGGCTTGTTT-3'), mouse *VEGFR3* (*Flt4*, forward 5'-GAATGAGAGCCCCGGAAC-3' and reverse 5'-GGTCTCCAGACCAGCAACTC-3'), mouse *Efnb2* (forward 5'-GATCCTCATGAAAGTTGGACAAG-3' and reverse 5'-AGCTCTGGACGTCTTGTTGG-3'), mouse *EphB4* (forward 5'-ACTGGGACATGAGCAACCA-3' and reverse 5'-TCTGCCAACAGTCCAGCAT-3'), human *EFNB2* (forward 5'-TGTAATTCCATCATCATCGTTGT-3' and reverse 5'-AAGTGTCGTAAGGCTCAATCG-3'), and human *EPHB4* (forward 5'-CGGATCCTACCCGAGTGA-3' and reverse 5'- TGTGTTCAGCAGGGTCTCTTC-3').

Western blotting

HUVECS isolated from fresh human umbilical cords were cultured in M199 media supplemented with 10% fetal calf serum and EC growth supplements. Tissue culture dishes (10cm), coated with 0.2% gelatin (w/v) in PBS, were pre-warmed to 37^{o}C. After removed the coating solution, 10^6 HUVECs was added to each dish and incubated at 37°C in a CO_2 incubator for 48h such that cells were of sufficient confluency to allow endogenous Notch signalling. . Cells were treated with different concentrations of DBZ for 8h. Alternatively, cells were starved in M199 medium supplemented with 2% fetal calf serum for 24h and then pre-incubated with the PD173074 inhibitor at different concentrations for 1 hour before adding FGF-2 (15ng/ml) for 30min. Cells were lysed with urea lysis buffer. Protein extracts

(40μg) were separated by SDS-PAGE and Western blotting was performed using standard techniques (28).