

Supplementary information

Materials and Methods

In situ hybridization In situ hybridization was carried out according to a standard method using radiolabeled oligonucleotide probes and high stringency conditions. Two probes complementary to PDGFR- α (nucleotides 423-470 and 3083-3130) and two probes complementary to PDGFR- β (nucleotides 946-996 and 2610-2657) were used. All probes were used separately and did not match any known sequence in Genbank except those of the intended genes. A control 50-mer random probe not complementary to any sequence deposited in Genbank, was also used. Following 3' end-labeling with [³³P]dATP (NEN Dupont) by terminal deoxynucleotidyl transferase (Amersham), probes were purified with the QIAquick™ Nucleotide Removal Kit Protocol (QIAGEN). Corneal histological slides at day 5 after implantation were incubated overnight (42°C) with 0.1 ml hybridization cocktail, containing 50% formamide, 4x SSC (0.15 M NaCl, 15 mM sodium citrate pH 7.0), 1x Denhardt's solution, 1% Sarcosyl, 0.02 M Na₃PO₄ pH 7.0, 10% dextran sulphate, 0.06 M DTT, 0.1 mg/ml sheared salmon sperm DNA and hot probe. Slides were then rinsed four times (45 min) in 1x SSC at 60°C and allowed to adjust to room temperature during a fifth rinse in 1x SSC. Further rinsing was carried out in distilled water and increasing concentrations of ethanol. Air-dried slides were then dipped in

emulsion (Kodak NTB2, diluted 1:1 with water). After five weeks of exposure, slides were developed, counterstained with cresyl violet and mounted in Entellan® (Merck). The control probe was hybridized and processed together with the other probes and gave rise to no specific pattern of hybridization signals in the mouse tissue. Specific labeling was confirmed by similar expression patterns revealed by two probes (complementary to different parts of the mRNA) for each PDGFR- α and PDGFR- β . Detection of positive autoradiographic signals was based on serial observations of adjacent sections from each tissue specimen, and accumulation of silver grains in the emulsion above specific cells and tissues identified by the staining procedures. Only cells over which silver grain accumulation was clearly above surrounding background levels and could be confirmed by both dark-field and high magnification bright-field, were regarded as positive. Positive signals (particles) in bright-field images were quantified from 7-12 random fields.