

Supporting Information

Laklai et al. 10.1073/pnas.0908674106

SI Materials and Methods

Cell Culture, Treatments, and Transfection. Human pancreatic cancer BxPC-3 cells were transfected with the human 1.35-kbp sst2 cDNA or mock vector. Stable BxPC-3 transfectants were cultured in DMEM (LONZA). Human dermal microvascular endothelial HMEC cells were grown in MCB131 (GIBCO), and human pancreatic neuroendocrine tumor BON cells in DMEM/HAM F12 (50%–50%) (LONZA). All media were supplemented with 10% FCS (LONZA), 2.5 μ g/mL fungizone, 2 mM L-glutamine, and 5 U/mL streptomycin/penicillin (Invitrogen). When indicated, cells were treated with recombinant VEGF (BD Biosciences), or with the somatostatin analog RC-160 (provided by one of us, A.V. Schally), or with the PI3K inhibitor LY294002 (Calbiochem). For characterization of the TSP-1 IRES, BxPC-3 cells were transfected with 2 μ g of the pSL2-TSP-1S, pSL2-TSP-1AS, or pGL3-TSP-1 vector. Cells were harvested in Passive Lysis Buffer (Promega), and extracts were assayed for firefly and *Renilla* luciferase activities using the Dual Luciferase Reporter Assay System (Promega), detected with a Luminoscan Ascent Microplate Luminometer (Thermo Lab-systems).

Real-Time Quantitative RT-PCR. Total RNA (RNeasy Kit Qiagen) was reverse-transcribed (random hexamers) and resulting cDNAs were used in qRT-PCR using a GeneAmp 7000 Sequence Detection System and SYBR green (Applied Biosystems). Primer sequences (Invitrogen) were as follows: TSP-1-forward, 5'-GTAACCCCTGCACGGATGG-3'; reverse, 5'-TGCACTT-GCGTTCTTGTG-3'; VEGF-forward, 5'-CCAATC-

GAGACCCTGGTG-3'; reverse, 5'-CACACAGGATGGCTT-GAAGA-3'; and 18S-forward, 5'TGCATGGCCGTTCTT-AGTTG-3'; reverse, 5'-TGGCTGAACGCCACTTGTC-3'. PCR efficiencies were >90%. Target gene expression was normalized by using 18S expression as an internal control.

RNA Interference. One hundred nanomolar of TSP-1, SRIF, and CTR siRNAs were designed (SMARTpools, Dharmacon) and transfected using DharmaFECT1 reagent (Dharmacon).

Immunoprecipitation and Immunoblotting. CM was centrifuged to remove cell debris. Clarified supernatant was used for treatment of HUVEC cells, or concentrated by ultrafiltration (Amicon, Millipore) for immunoprecipitation and/or direct immunoblot analysis. HUVEC cells were treated with 30 ng/mL recombinant VEGF, or with CM for 5 min, and lysed in [50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM sodium orthovanadate, and protease inhibitor mixture (Roche Diagnostics)]. Protein immunoprecipitation from cell lysates or CM was performed using the appropriate antibody precoupled to protein A/G Sepharose beads. Immunocomplexes or CM were resolved on SDS/PAGE, transferred onto nitrocellulose membranes (Pall Life Sciences). Following probing with a specific primary antibody and a horseradish peroxidase-conjugated secondary antibody, the protein bands were detected by enhanced chemiluminescence (Pierce). Primary antibodies used were as follows: TSP-1, phosphotyrosine clone 4G10 and MMP-2 (Calbiochem), VEGFR2/Flk-1, and VEGF (Santa Cruz Biotechnologies).