SUPPLEMENTARY MATERIAL

Immunoblotting

Total protein from generated clones or parental cells treated with and without tiplaxtinin was extracted using a RIPA buffer with Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, Rockfold, IL). Twenty micrograms of total protein was electrophoresed using Mini-PROTEAN TGX gels (Bio-Rad Laboratories, Hercules, CA) and transferred onto nitrocellulose membranes. After blocking in Tris-buffered saline containing 5% skimmed milk or 5% bovine serum albumin, the membrane was incubated overnight with primary antibodies at 4^0C, followed by 1 hr incubation with horseradish peroxidase (HRP)-conjugated secondary antibody. The bound secondary antibody was detected using super signal West Dura Extended Duration Substrate (Thermo Scientific, Rockford, IL). PAI-1 antibody (612025, mouse monoclonal, dilution 1:1000) was purchased from BD Biosciences (San Jose, California). Cleaved Caspase-3 (Asp175) (5A1E) (#9664; rabbit polyclonal), Cleaved PARP (Asp214) (D64E10) (#5625; rabbit monoclonal), Fas (C18C12) (#4233; rabbit monoclonal), and FasL (#4273; rabbit polyclonal) antibodies all diluted at 1:1000 were purchased from Cell Signaling Technology (Danvers, MA). β-Actin (AC-15; mouse monoclonal, dilution 1:10,000) antibody (Sigma-Aldrich, St Louis, MO) was used as a loading control.

Immunohistochemical (IHC) analysis of xenograft tumors

Paraffin embedded specimens were deparaffinized followed by antigen retrieval using citric acid buffer (pH 6.0, 95^0C for 20 min). Slides were treated with 1% hydrogen peroxide in methanol to block endogenous peroxidase activity. After 20 min blocking in 5% horse serum, slides were incubated overnight at 4^0C with the following primary antibodies: anti-SERPINE1 (PAI-1)

(#HPA050039; rabbit monoclonal, dilution 1:100 in blocking buffer) from Sigma Aldrich (St. Louis, MO). PECAM-1 (CD31) (H3; mouse monoclonal, dilution 1:4,000) was from Santa Cruz Biotechnology (Santa Cruz, CA). Ki-67 (MIB-1; mouse monoclonal, dilution 1:100) was from Dako (Carpinteria, CA). Cleaved Caspase-3 (Asp175) (5A1E) (#9664; rabbit polyclonal) diluted at 1:1,500 was also from Cell Signaling Technology. Next, the slide was incubated with 2 μg/mL of biotinylated anti-mouse IgG secondary antibody (Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Subsequently, the sections were stained using Standard Ultra-Sensitive ABC Peroxidase Staining kit (Pierce/Thermo Fisher Scientific, San Jose, CA) and 3, 3'- diaminobenzidine (DAB; Vector Laboratories), counterstained by hematoxylin, dehydrated, and mounted with a cover slide. Slides were also counterstained with hematoxylin and eosin. Tumor angiogenesis was investigated on slides stained with anti-CD-31 monoclonal antibody and microvessel density (MVD, microvessel/mm^2) was assessed as previously described by (22). Single endothelial cells or clusters of endothelial cells positive for CD-31 were considered as a vessel. Four areas with the highest microvessel concentration from each specimen were identified at 4X magnification and images were taken for quantitation of MVD at 200X. Two independent observers counted the number of microvessels in each histological field. MVD of the specimen was estimated as a mean of MVD in at least 4 histological fields. Cell proliferation and apoptosis in tumors were determined by light microscopy of Ki-67 and cleaved caspase-3 staining, respectively. Ki-67 and cleaved caspase-3-positive cells were counted on a minimum of 4 randomly selected fields of each treatment group at 400X magnification. The density of proliferative cells (proliferative index, PI) and apoptotic cells (apoptotic index, AI) was expressed as the average of the five areas of highest intensity identified within a single 400X field (22). Cells with questionable nuclear staining were discounted. The numbers of positive

cells were reviewed by two independent observers. Results were scored by estimating the percentage of tumor cells showing positivity of Ki-67 (PI) and cleaved caspase-3 (AI).