SUPPLEMENTARY DATA

SUPPLEMENTARY MATERIALS AND METHODS

Co-culture experiments. Human SW620 cells ($5x10^5$ cells/well) were co-cultured with mouse macrophages ($5x10^5$ cells/well) in 6-well plates allowing direct cell-cell contact. Prior to siRNA treatment (100 nM) cells were allowed to adhere for 24 h. Co-cultures were treated with siRNA directed against human and mouse TNF-α. Three hours after transfection, co-cultures were fed with DMEM medium containing 1% FCS. Cells were incubated for 48 h at 37°C, followed by RNA isolation for real time RT-PCR. Experiments were performed in triplicate.

Western Blotting. Tissue lysates were prepared (1, 2) and 50 µg/lane were separated by 8%-12% SDS-PAGE prior to electrophoretic transfer onto Hybond C super (Amersham Pharmacia Biotech, Buckinghamshire, UK). The blots were probed with antibodies against MMP-2 (Chemicon) and VEGF-A (Neomarkers, Union City, CA) before incubation with horseradish peroxidase–conjugated secondary antibodies (Amersham Pharmacia Biotech). Proteins were immunodetected by chemiluminescence (Supersignal-West- Femto, Pierce, Rockford, IL), and quantified by Easy Win 32 software (Herolab, Wiesloch, Germany).

SUPPLEMENTARY REFERENCES

1. Aharinejad S, Abraham D, Paulus P, et al. Colony-stimulating factor-1 antisense treatment suppresses growth of human tumor xenografts in mice. Cancer Res 2002;62:5317-24.
2. Aharinejad S, Paulus P, Sioud M, et al. Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of human mammary tumor xenografts in mice. Cancer Res 2004;64:5378-84.

SUPPLEMENTARY LEGENDS

Supplementary Figure 1. Cancer cells upregulate macrophage gene expression. Co-cultured SW620 colon cancer cells and mouse CRL-2470 macrophages with cell-cell contact. Human TNF-α, human VEGF-A and mouse TNF-α, CSF-1, VEGF-A and MMP-2 mRNA expression were measured by real time RT-PCR in RNA from cultured SW620 cells, cultured CRL-2470 macrophages or from co-cultured human SW620 colon cancer cells and mouse CRL-2470 macrophages (Mph) following treatment with human TNF-α siRNA (huTNF-α-si) or mouse TNF-α siRNA (muTNF-α-si). Results were normalized to β-2 microglobulin mRNA levels and expressed as a percentage of the levels in RNA from the cultured SW620 cells (cancer cells) or CRL-2470 macrophages (macrophages). *, significantly different from SW620 cancer cells (human genes) ($P < 0.008$) and macrophages (mouse genes) ($P < 0.001$; except for mouse VEGF-A following huTNF-α-si treatment, where $P = 0.038$); †, significantly different from co-cultured SW620 cells and macrophages ($P < 0.001$; except for mouse VEGF-A following muTNF-α-si treatment, where $P = 0.046$).

Supplementary Figure 2. Effect of human TNF-α and mouse CSF-1 siRNA treatment on gene expression in SW620 colon cancer xenografts. **A,** Real time RT-PCR measurements of human and host (mouse) TNF-α and VEGF-A, and of mouse CSF-1 and MMP-2 mRNA levels in tumor lysates were normalized to the corresponding human and mouse β-2 microglobulin mRNA levels and expressed as a percentage of the mRNA levels in tumors receiving scrambled siRNA. Human mRNA levels: *, significantly different from scrambled siRNA (control) day 22 ($P < 0.01$); mouse mRNA levels: *, significantly different from scrambled siRNA (control) day 22 ($P < 0.04$). **B,** Representative Western blots and quantification of VEGF-A and MMP-2 protein expression levels in tumor lysates. *, significantly different from scrambled siRNA (control) day 22 ($P < 0.016$).