957 Treatment with a recombinant adeno-associated virus expressing the TFPI-2 gene simultaneously downregulates MMP-9 and VEGF in a human glioblastoma cell line

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Human tissue factor pathway inhibitor-2 (TFPI-2) is a Kunitz-type proteinase inhibitor that regulates a variety of serine proteinases. TFPI-2 is abundant in the extracellular matrix and highly expressed in non-invasive cells, but absent or undetectable in highly invasive human glioblastoma cells. Matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF) play major roles in tumor cell invasion and angiogenesis. Using a recombinant adeno-associated viral (rAAV) vector carrying the human TFPI-2 gene (rAAV-TFPI-2), we expressed TFPI-2 in the U251 cell line, a highly invasive human glioblastoma cell line. Results from the TUNEL assay, nuclear chromatin staining and FACS analysis revealed increased apoptosis in U251 cells after transfection with the construct. Results from an *in vitro* angiogenesis assay (co-culture of rAAV-TFPI-2-transfected U251 cells with human microvascular endothelial cells) demonstrated the inhibition of capillary-like structure formation in rAAV-TFPI-2-treated cells as compared with control and mock-transfected cells. *In vivo* angiogenesis studies in nude mice depicted decreased microcapillary formation in U251 cells infected with rAAV-TFPI-2. Zymography, western blot analysis and real time RT-PCR for MMP-9 demonstrated downregulation of MMP-9 expression in U251 cells after treatment with rAAV-TFPI-2. In addition, VEGF expression significantly decreased in rAAV-TFPI-2-transfected U251 cells as demonstrated by western blot and real time RT-PCR. The results of the present study indicate that TFPI-2 expression in highly invasive glioblastoma cells effectively prevents tumor invasion and angiogenesis through downregulation of MMP-9 and VEGF and that rAAV-TFPI-2-mediated gene expression offers a potential therapeutic application for cancer gene therapy.

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