

TUMOR NECROSIS FACTOR RELATED APOPTOSIS INDUCING LIGAND INHIBITS ANGIOGENESIS STIMULATED BY HUMAN GLIOBLASTOMA CELLS

Scuderi Mariagrazia Rita, Albini Adriana¹, Lempereur Laurence, Pennisi Giuseppa²,
Bernardini Renato, Giuseppina Cantarella

Department of Experimental and Clinical Pharmacology; and Department of Chemical Sciences;
University of Catania, 95100 Catania, Italy; Laboratory of Molecular Oncology, Istituto Tumori, 16100
Genova, Italy

Tumour growth is tightly related to new blood vessel formation, tissue remodelling and invasiveness capacity. A number of tissular factors fuel the growth of glioblastoma multiforme, the most aggressive brain neoplasm. In fact, gene array analyses demonstrated that the proapoptotic cytokine tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) inhibited mRNA expression of VEGF, along with those of matrix metalloproteinase-2 (MMP-2), its inhibitor tissue inhibitor of matrix metalloproteinases-2 (TIMP-2), as well as the tumour invasiveness-related gene secreted protein acid rich in cysteine (SPARC) in different human glioblastoma cell lines. Particularly, VEGF mRNA and protein expression and release from glioblastoma cells were also inhibited by TRAIL. To assess whether the antimitogenic effect of TRAIL on HUVEC encompasses actual blood vessel formation, we tested the effects of TRAIL in appropriate models, such as tube formation in matrigel *in vitro* (matrigel morphogenesis assay), as well as vessel formation in matrigel sponges containing a proangiogenic cocktail implanted subcutaneously *in vivo* in the mouse. In the matrigel morphogenesis assay, formation of the typical cellular network occurred 6 h after plating; TRAIL had concentration-dependent inhibitory effect on the morphogenesis of HUVEC cells and formation of capillary-like structures, which peaked at 200 ng/ml. The effects of TRAIL on angiogenesis-associated endothelial cell functions observed *in vitro* were confirmed *in vivo* in the matrigel angiogenesis assay. Matrigel suspensions containing a proangiogenic cocktail with VEGF, TNF- α and heparin (VHT) were injected subcutaneously into mice. The presence of VHT in the matrigel sponges promoted a haemorrhagic vascularisation within 4 days. Histology confirmed the absence of vascularisation in the samples treated with TRAIL alone, or reduced angiogenesis in the presence of VHT. Quantification of the extent of angiogenesis by haemoglobin content measurement showed that TRAIL (200 ng/ml) significantly ($P < 0.0089$; paired *t*-test) reduced the angiogenic response if compared to the positive control. Moreover, the expression of MMP-2, its inhibitor TIMP-2 and the tumour invasiveness-related protein SPARC were effectively inhibited by TRAIL in glioblastoma cell lines. In conclusion, our data indicate that TRAIL inhibits the orchestra of factors contributing to glioblastoma biological aggressiveness. Thus, the TRAIL system could be regarded as a molecular target to exploit for innovative therapy of this type of tumour.