

## Essential roles of tumor necrosis factor receptor p55 in liver metastasis of intrasplenic administration of colon 26 cells

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Tumor necrosis factor (TNF)- $\alpha$  was originally identified as a cytokine responsible for endotoxin-induced tumor necrosis. Previous reports demonstrated that therapy with recombinant TNF- $\alpha$  was effective against several types of murine tumor models of hepatic and pulmonary metastasis, particularly when it was administered in combination with interferon- $\gamma$  or IL-2. Moreover, a Phase I clinical study of TNF- $\alpha$  treatment demonstrated partial efficacy against metastatic spread. However, in several models, the administration of TNF- $\alpha$  or the TNF- $\alpha$  gene transduction into tumor cells, enhanced the incidence of metastasis. These contradictory results may be explained by the differences in the cell types used in each experiment. Consequently, the roles of endogenous TNF- $\alpha$  in the metastatic process remains to be determined. Hence, in order to evaluate the roles of TNF- $\alpha$ , we injected a colon adenocarcinoma cell line into the spleen of wild-type (WT) and tumor necrosis factor receptor p55 (TNF-Rp55)-deficient (KO) mice.

WT mice exhibited enhanced TNF- $\alpha$  protein expression around the central and portal veins of the liver by 3 days after intrasplenic injection of a colon adenocarcinoma cell line, colon 26. Moreover, 90% of WT mice developed liver metastases by 24 days after the tumor injection. In contrast, liver metastasis developed in less than 50 % of TNF-Rp55 KO mice. Liver weights and the volumes of metastatic foci were significantly lower in TNF-Rp55 KO mice. These observations suggest the critical roles of TNF-Rp55-mediated signals in this liver metastasis model. The intrasplenic tumor injection induced mRNA expressions of vascular endothelial growth factor, heparin-binding epidermal growth factor, matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 at similar levels in the livers of both WT and TNF-Rp55 KO mice. Immunohistochemical analyses of the livers of WT mice after tumor injection demonstrated the enhanced expression of vascular cell adhesion molecule (VCAM)-1 and E-selectin on sinusoidal endothelial cells. Enhanced E-selectin expression was similarly observed in the liver of TNF-Rp55 KO mice after tumor injection. However, the enhancement in VCAM-1 mRNA expression and protein production was significantly attenuated in the liver of TNF-Rp55 KO mice, when compared with WT mice. Collectively, these observations suggest that TNF-Rp55-mediated signals can regulate both VCAM-1 expression in the liver and subsequent liver metastasis following intrasplenic tumor injection.