

The major themes of our research projects are the roles of growth factors and proteolytic enzymes in tissue remodeling, in particular tumor invasion and blood vessel formation (angiogenesis).

Growth Factor Interactions in Angiogenesis

Angiogenesis, the formation of capillaries from preexisting blood vessels, is required for the growth and metastasis of solid tumors. A number of cytokines and growth factors induce angiogenesis. Among them vascular endothelial growth factor (VEGF) has been implicated as the major tumor angiogenesis inducer. VEGF overexpression in most tumors correlates with high tumor vascularity, metastasis and a poor prognosis, and inhibition of VEGF in tumor-bearing animals results in decreased tumor vascularity and arrest of tumor growth. Pharmacological agents targeting VEGF are currently used or in advanced clinical trials for the therapy of several malignancies. However, these anti-angiogenesis therapies have limited effect and only if combined with chemotherapy. Therefore, developing novel anti-angiogenic agents would be most beneficial to cancer patients.

VEGF controls a number of vascular endothelial cell functions. Notably, VEGF protects endothelial cells from programmed cell death (apoptosis). We found that the prosurvival activity of VEGF on vascular endothelial cells is converted into an apoptotic activity by transforming growth factor beta 1 (TGF- β 1) (*Ferrari et al., Proc Natl Acad Sci USA 2006, 103: 17260–17265*). Our work has shown that TGF- β 1 induces VEGF expression in endothelial cells, and that VEGF-VEGF receptor-2 (flk-1)-mediated activation of p38^{MAPK} is necessary for TGF- β 1 induction of apoptosis. VEGF activates p38 β , which relays survival signaling; however, in context with TGF- β 1 VEGF activates the proapoptotic p38 α .

Our finding that VEGF - the major tumor angiogenesis inducer - can be converted into an inducer of endothelial cell apoptosis indicates a novel approach to block tumor angiogenesis. Pharmacological treatments that mimic the effect of TGF- β 1 on VEGF signaling could take advantage of high local levels of tumor-derived VEGF to induce uncontrolled endothelial cell apoptosis and efficiently block angiogenesis.

Our current work aims to understand the mechanism that mediates the crosstalk between the TGF- β 1 and VEGF signaling pathways. For this purpose we use molecular and cellular biology methods to dissect the signaling pathways upstream of p38^{MAPK} including protein kinases, small GTPases, phosphatases and scaffolding proteins. We also plan to use a cell-based assay to screen a library of synthetic compounds to identify molecules that convert VEGF into an apoptosis inducer for endothelial cells. We will then use affinity chromatography and molecular biology methods to identify the cellular target(s) of the active compounds, and characterize their role in p38^{MAPK} activation and induction of endothelial cell apoptosis. The ultimate goal of our work is to develop a novel anti-angiogenesis agent based on a revolutionary mechanism of action: converting VEGF, the major tumor angiogenesis factor, into an inducer of apoptosis in the endothelial cells of newly forming vessels.

Non-proteolytic signaling via Membrane-Type 1 Matrix Metalloprotease

Matrix metalloproteinases (MMPs) are family of proteolytic enzymes that degrade a broad spectrum of extracellular substrates. Some MMPs have been shown to contribute to tumor invasion and metastasis through the proteolytic degradation of cell membrane and/or extracellular matrix components. Experimental and clinical data have implicated membrane-type 1 MMP (MT1-MMP) as the most ubiquitous and important MMP. MT1-MMP, a transmembrane MMP with a short cytoplasmic domain and an extracellular proteolytic domain, acts as an oncogene, stimulates tumor cell invasion and metastasis, and controls tumor cell growth. High levels of MT1-MMP are associated with a variety of aggressive malignancies. In human breast carcinoma MT1-MMP expression levels correlate significantly with lymph node and distant metastases,

clinical stage and tumor size. Based on these observations, inhibitors designed to efficiently block the proteolytic activity of MMPs were expected to arrest tumor progression. However, these inhibitors failed all clinical trials. A number of reasons can explain this failure, the main of which is certainly our incomplete understanding of the roles of MMPs in tumor biology.

Our work has shown a novel, non-proteolytic function of MT1-MMP. MT1-MMP activates intracellular signaling that upregulates cell proliferation and migration, as well as tumor growth *in vivo*, by a mechanism independent of its proteolytic activity. This mechanism is triggered by MT1-MMP binding of its physiological inhibitor, tissue inhibitor of metalloproteinase-2 (TIMP-2) (D'Alessio *et al.*, *J Biol Chem* 2008, 283: 87-99). Our current work aims to identify the molecular determinants of TIMP-2 – MT1-MMP binding required for activation of intracellular signaling in tumor cells, and to study the effect of MT1-MMP – TIMP-2 interaction on tumor growth and invasion *in vivo*. The ultimate goal of this project is to develop inhibitors of TIMP-2 – MT1-MMP binding that can effectively block tumor progression by inhibiting the non-proteolytic activation of intracellular signaling by MT1-MMP.

Our work also aims to study the physiological role of MT1-MMP-mediated intracellular signaling *in vivo*. The analysis of the phenotype of MT1-MMP^{-/-} mice has shown multiple roles of this proteinase in skeletal development and angiogenesis. MT1-MMP^{-/-} mice show marked deceleration of postnatal growth, with severe defects in skeletal and alveolar development, impaired angiogenesis, and death by 3-5 weeks of age. It has been proposed that this phenotype results from the lack of MT1-MMP proteolytic activity. Based on our studies, we hypothesize that the phenotype of MT1-MMP^{-/-} mice results at least in part from the lack of signaling capacity. For this purpose we are generating a strain of mice that expresses a mutation in the cytoplasmic tail that abolishes the signaling capacity of MT1-MMP, and compare their phenotype to those of wt and MT1-MMP^{-/-} mice. We expect that expression of the MT1-MMP mutant devoid of signaling capacity will reproduce, at least partially, the phenotype of MT1-MMP^{-/-}. Therefore, the analysis of the phenotype of our mutant mice will afford understanding the role of MT1-MMP-mediated signaling in normal physiology and in pathology.