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Tumor-associated fibroblasts enhance angiogenesis in lung adenocarcinoma through TIMP-1: potential implications in early dissemination and immunosuppression

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Background: Lung cancer is the leading cause of cancer-related deaths worldwide, with adenocarcinoma (ADC) and squamous cell carcinoma (SCC) being the most common histologic subtypes. Despite both being epithelial in origin, the fibrotic tumor microenvironment, enriched with tumor-associated fibroblasts (TAFs), plays a crucial role in various aspects of tumor progression, including migration, invasion, angiogenesis and metastasis. Our previous research revealed that TAFs in ADC exhibit enhanced fibrosis compared to SCC due to the epigenetic repression of SMAD3 in SCC-TAFs. Moreover, they respond differently to Nintedanib and other antiangiogenic drugs, suggesting a possible dependency on the histologic subtype for angiogenesis. Since abnormal tumor vasculature can trigger immunosuppression, there is a renewed interest in using antiangiogenic therapies to enhance the response to immune checkpoint inhibitors. Nevertheless, the specific role of lung TAFs in the promotion of angiogenesis and the resistance to anti-angiogenic therapies remains poorly defined.

Methods: a panel of angiogenesis markers was assessed in patient samples using publicly available databases. The pro-angiogenic function of the conditioned medium of TAFs from ADC and SCC patients pre-activated with TGF-?1 was analyzed *in vitro* using migration and Matrigel network formation assays of endothelial cells. The secretion of pro-angiogenic factors in TGF-?1-activated TAFs was analyzed using an angiogenesis antibody blot array. Selected factors were functionally validated *in vitro* and *in vivo* using genetic models.

Results: all angiogenesis markers were consistently upregulated in ADC compared to SCC concomitantly with a lower necrosis in ADC, which correlates with the clinical observation that ADC patients exhibit metastasis earlier than SCC patients. The conditioned medium of TGF-?1-activated TAFs elicited a larger endothelial cell network formation in ADC than SCC, revealing that ADC-TAFs exhibit enhanced angiogenesis. Our immunoblot array analysis identified a subset of pro-angiogenic factors that were selectively overexpressed in ADC-TAFs compared to SCC-TAFs, including TIMP-1 and VEGF-A. Notably, TIMP-1 overexpression in ADC-TAFs was SMAD3-dependent, and knocking down TIMP-1 by siRNA in ADC-TAFs impaired angiogenesis *in vitro* and in tumor xenografts *in vivo*.

Conclusions: our results reveal a larger angiogenesis in ADC compared to SCC, and implicate the TGF-?1/SMAD3/TIMP-1 pathway in the enhanced angiogenesis of ADC-TAFs. Our findings identify a biological process underlying the poor response of SCC to antiangiogenic therapies, and provide a rationale for the earlier metastasis of ADC patients based on the enhanced pro-angiogenic role of ADC-TAFs.