中文題目：探討肺癌肋膜積液(LCPF)促血管新生的系列研究:從實驗室、臨床數據庫及未來治療著眼

英文題目：Serial studies of lung cancer associated pleural fluid on endothelial angiogenesis : from bench characterization, clinic database analysis to therapeutic investigation

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Background: Malignant pleural effusion (MPE) and paramalignant pleural effusion (PPE) remain debilitating complications in lung cancer patients. The role of vascular endothelial cells has not been explored in the pleural environment of lung adenocarcinoma. Combined anti-VEGFA antibody with EGFR target therapy could prolong patient survival, whereas the efficacy of concurrent blockade was not specified in patients with MPE. Furthermore, statin as cholesterol lowering drug seemed to suppress tumor angiogenesis, however, the effect of statin on pleural angiogenesis required more investigation.

Method: Pleural microvessels were examined by H&E and IHC staining in lung adenocarcinoma specimens. Our group integrated MPE and PPE as lung cancer-associated pleural fluid (LCPF). To recapitulate the impact of the pleural microenvironment on vascular endothelium, LCPF was adopted as conditioned medium in human umbilical vein endothelial cells (HUVEC) culture system. Immunofluorescence staining, MTT, transwell, tube formation and permeability assay were applied to evaluate LCPF-induced changes on HUVEC. In addition, the content of LCPF was investigated by exosome purification and bile acid ELISA assay. To correlate *in vitro* results with clinical response, our studies enrolled 883 lung adenocarcinoma patients complicated with MPE from 2010 to 2019 for database analysis.

Results: First, the subpleural layer of lung adenocarcinoma was characterized with increased capillaries. Positive staining of EGFR and FXR as bile acid receptor were observed on those pleural microvessels. Following that, LCPF ubiquitously promoted endothelial angiogenesis regardless of the driver gene mutation, prior treatments, and evidence of malignant cells in pleural fluid. Cultured with LCPF upregulated VEGF/VEGFR2, EGFR/p-EGFR and FXR/RXR/SHP expression on HUVEC.

The examination of LCPF unveiled EGFR-enriched exosomes that implied possible EGFR transfer to endothelial cells. Both sunitinib (VEGFR2 inhibitor) and bevacizumab (anti-VEGFA antibody) could reverse LCPF-induced HUVEC angiogenesis. The combination of gefitinib (EGFR target therapy) and bevacizumab revealed better inhibition on LCPF-induced endothelial angiogenesis and permeability. Kaplan-Meier analysis of MPE patients revealed the benefit of cotreatment with target therapy and bevacizumab.

Abundant bile acids were characterized in LCPF. Moreover, LCPF increased proteins of cholesterol metabolism and bile acid metabolism in HUVEC, including CYP7A1, StAR, HMGCR, and SREBP2. Statin treatment could alleviate pleural angiogenesis through FXR/RXR/SHP inhibition. The anti-angiogenesis effect of statin *in vitro* was extended to MPE patients exposed to stain revealed a survival benefit.

Conclusion:

Our data first exploited increased pleural angiogenesis in lung cancer and the angiogenic activities of LCPF on endothelial cells. Among multiple LCPF-induced angiogenesis pathway, endothelial EGFR and FXR were recognized as potent targets, respectively. Simultaneous targeting of VEGF and EGFR suppresses the LCPF-induced angiogenesis effects *in vitro* and in vivo. The usefulness of statin as an FXR antagonist were found to suppress pleural angiogenesis from bench to clinic. By applying LCPF, our serial studies provided insight of pleural endothelial angiogenesis that could serve as future therapeutic targets.