

Thrombin activatable fibrinolysis inhibitor promotes development of chronic thromboembolic pulmonary hypertension -A possible novel therapeutic target-

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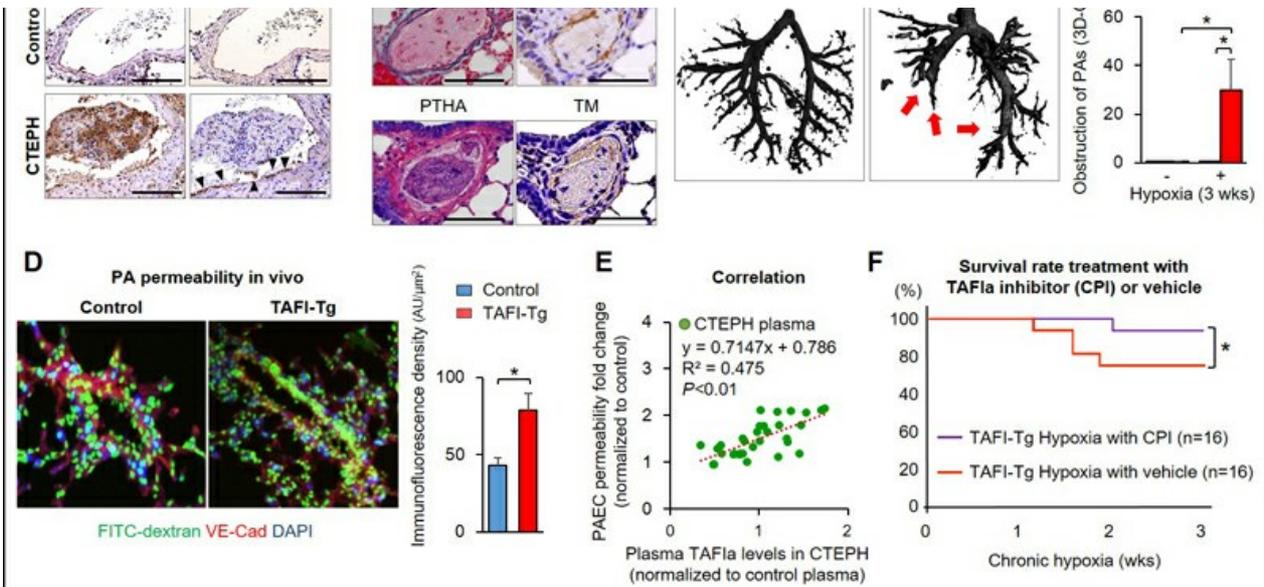
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Background: Chronic thromboembolic pulmonary hypertension (CTEPH) remains a serious disorder. Thrombin-activatable fibrinolysis inhibitor (TAFI, encoded by CPB2) is synthesized by the liver and inhibits fibrinolysis. The purpose of the present study was to determine the role of activated TAFI (TAFIa) in the pathogenesis of CTEPH.

Methods and results: CTEPH patients had higher prevalence of single nucleotide polymorphisms of CPB2 compared with controls. Moreover, plasma TAFIa levels were significantly increased in CTEPH patients (n=32) compared with controls (n=13) (8.7-fold, P<0.01). Immunostainings showed that TAFI and its linker protein thrombomodulin (TM) were highly expressed in the distal pulmonary arteries (PA) of CTEPH patients (Fig. A). In mice, chronic hypoxia caused significant increases in plasma levels of TAFIa, thrombin, and TM, resulting in PA thrombus formation, vascular remodeling, and PH (all P<0.05, n=6 each) (Fig. B). Cpb2^{-/-} mice showed significantly reduced right ventricular systolic pressure (RVSP), RV hypertrophy, and PA remodeling compared with Cpb2^{+/+} mice after 3 weeks of hypoxia (all P<0.05, n=14 each). In contrast, TAFI-overexpressing mice (TAFI-Tg) exhibited increased RVSP, RVH, and PA thrombus formation compared with controls (all P<0.01, n=19 each). Three-dimensional computed tomography showed that TAFI-Tg mice had multiple PA obstructions after the hypoxia, which were not observed in controls (P<0.01, n=10 each) (Fig. C). Moreover, TAFI-Tg mice showed increased PA permeability (Fig. D) and adventitial accumulation of monocytes/macrophages compared with controls (all P<0.01, n=8 each). Bone marrow transplantation showed that circulating plasma TAFI from the liver, but not that from the bone marrow, was activated locally in PA endothelial cells (PAECs) through interactions with thrombin and TM. Moreover, liver-specific TAFI overexpression increased RVSP, RVH, and PA thrombus formation compared with controls (all P<0.01, n=12 each). Mechanistic experiments demonstrated that treatment with human TAFI reduced VE-cadherin expression, increased PAEC permeability, and dysregulated the metabolic homeostasis of PA (all P<0.01, n=8 each). Consistently, treatment with CTEPH plasma (n=32) promoted PAEC permeability compared with control plasma (n=13) (P<0.05). Importantly, there was a significant correlation between PAEC permeability and plasma TAFIa levels (R²=0.475, P<0.01) (Fig. E). Finally, to evaluate the effect of TAFIa inhibition in hypoxia-induced PH in mice, we performed in silico screening and found several TAFIa inhibitors. Among them, we used carboxypeptidase inhibitor (5 mg/kg/day), with which we found significant amelioration of hypoxia-induced PH and improved prognosis (-76%) in TAFI-Tg mice (all P<0.01, n=16 each) (Fig. F).

Conclusions: TAFI is a crucial molecule in the development of CTEPH and PA thrombus formation, and thus could be a novel biomarker and a therapeutic target of CTEPH.





(A) Representative immunostaining for TAFI and thrombomodulin (TM) of the pulmonary arteries (PA) in CTEPH patients and controls. Scale bars, 50 μm .
 (B) Representative Elastica Masson (EM), phosphotungstic acid-hematoxylin (PTHA), and immunostaining for TAFI and TM of the PA thrombus in wild type mice exposed to hypoxia (10% O_2) for 3 weeks. Scale bars, 50 μm .
 (C) Representative images of 3D-CT of PAs in TAFI-Tg and control mice exposed to hypoxia (10% O_2) for 3 weeks. Quantification of frequency of obstruction in large PAs detected by 3D-CT ($n=12$ each).
 (D) Representative confocal images of immunostaining for FITC-dextran (green), VE-cadherin (red), and DAPI (blue) and quantification of PA permeability in TAFI-Tg and control mice exposed to hypoxia for 3 weeks ($n=8$ each).
 (E) Correlation between PAECs permeability and plasma levels of activated TAFI (TAFIa) in CTEPH patients ($n=32$).
 (F) Survival rate of TAFI-Tg exposed to hypoxia (10% O_2) treatment with a TAFIa inhibitor (CPI, potato carboxypeptidase inhibitor) (5 mg/kg/day) or vehicle for 3 weeks. Results are expressed as long-rank test.
 Results are expressed as mean \pm SEM. * $P < 0.05$.

TAFI in CTEPH