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EDITORIAL COMMENT

New Insights in the Pathology of Chronic Thromboembolic Pulmonary Hypertension*

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In this issue of *JACC: Basic to Translational Science*, Bochenek et al[1] present their recent findings describing a critical role of endothelial-derived mediators in thrombus remodeling in patients with chronic thromboembolic pulmonary hypertension (CTEPH).[1] CTEPH is a rare progressive vascular disease associated with pulmonary hypertension caused by prolonged thrombotic blockage of arteries in the lung and can lead to right heart failure and death. Patients with CTEPH can be treated by pulmonary thromboendarterectomy (PTE). This study is a continuation of work by the same group showing that increased transforming growth factor beta (TGF-β) signaling impairs thrombus resolution in mice and was associated with CTEPH.[2]

In their paper, Bochenek et al[1] indicate a possible mechanism for CTEPH development with regard to the role of TGF-β–induced (TGFBI), also called BIGH3, released from endothelial cells (ECs). The present study included patients with diagnosed CTEPH who underwent PTE after enrollment in the CTEPH Registry Bad Nauheim (CEPRA) in Germany. All CTEPH patients were receiving anticoagulation therapy, either with vitamin K antagonists (52%) or direct oral anticoagulants (48%). In addition, some CTEPH patients received antiplatelet drug treatment with aspirin (n = 4) or clopidogrel (n = 2).

First, the authors used CTEPH-derived ECs (a PTE outgrow model) and compared the gene expression pattern in CTEPH-derived ECs with the results from commercially obtained human pulmonary artery endothelial cells (HPAECs) or human umbilical vein endothelial cells (HUVECs).[1] Interestingly, the authors found a significant increase in expression of genes involved in matrix organization, hemostasis, signal transduction, immune system, metabolism, and protein life cycle. Moreover, while the CTEPH-derived ECs exhibited increased expression of mesenchymal cells such as actin alpha 2 compared with normal HPAECs, they kept their expression levels of the EC marker cadherin 5 and CD31.

Importantly, the new study[1] identified different candidate genes, including TGFBI, FSTL3 (follistatin like 3), STC2 (stanniocalcin 2), and TAGLN (transgelin); these are highly expressed in vessel-rich areas of PTE specimens from CTEPH patients and vessels of pulmonary arterial hypertension (PAH) and patients with idiopathic pulmonary fibrosis (IPF) compared with healthy control subjects. This suggests that these genes could be markers of pathologic pulmonary vessel remodeling and/or lung fibrosis. Interestingly, only TGFBI and STC2 protein expression were upregulated in CTEPH compared with PAH, IPF, and healthy control specimens. More importantly, TGFBI expression was limited to $CD31^+$ cells, whereas STC2 expression was not in the analyzed PTE specimens. The authors concluded that only TGFBI expression was EC dependent. These findings are in line with the group's previous findings that CTEPH was associated with increased TGF-β signaling in ECs.[2]

In confirming cell culture studies, Bochenek et al[1] found that TGFBI overexpression in HPAECs in vitro leads to a similar gene expression pattern compared with CTEPH-derived ECs.[1] More importantly, TGFBI-stimulated HPAECs or overexpression of TGFBI in

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HPAECs decreased fibrinolytic remodeling of whole blood clots in vitro. This observation confirms an earlier study showing that TGF-β stimulation of ECs leads to decreased thrombus resolution in vitro.[2] Moreover, recombinant TGBFBI, when administered exogenously in mice, delayed venous thrombus resolution (discussed later). Because TGFBI-exposed HPAECs exhibited a similar procoagulant phenotype compared with human pulmonary aortic fibroblasts, the authors concluded that during CTEPH, pulmonary ECs transition into a mesenchymal, smooth muscle actin expressing (SMA^+), fibroblast-like phenotype after TGFBI exposure.

In addition, the study showed that patients with CTEPH have higher TGFBI plasma levels compared with patients with PAH or healthy control subjects.[1] Moreover, TGFBI plasma levels decreased in CTEPH patients 12 months after successful PTE surgery. This observation suggests that the remodeled pulmonary thrombus is a source of the increased TGFBI levels, which can contribute to the severity of CTEPH.

Using a proof-of-concept inferior vena cava (IVC) stenosis (subtotal ligation) thrombosis model on male C57BL/6J mice, Bochenek et al[1] investigated the effects of TGFBI infusion on thrombus resolution in vivo. After confirmed in vivo thrombus formation 2 days after surgery, mice with IVC thrombus were subjected to continuous infusion of TGFBI or vehicle solution. As suggested by the group's other findings,[2] TGFBI infusion led to significantly increased thrombus sizes and delayed thrombus resolution.[1] The thrombi of TGFBI-infused mice also exhibited increased expression of EC (CD31) and mesenchymal cell (SMA) markers, suggesting more advanced thrombofibrosis. Importantly, the authors state that vessel and thrombus remodeling in IVC-stenosed and TGFBI-infused mice appeared similar to that of their CTEPH PTE specimens. The presented findings are in line with IVC stenosis results made in mice with reduced circulating TGFβ levels.[2]

From their combined in vitro and in vivo findings, Bochenek et al[1] concluded that in CTEPH, ECs express components and regulators of the TGF-β signaling pathway. Especially, TGFBI and STC2 were selectively expressed only in CTEPH and no other pulmonary vasculopathies such as PAH or IPF. TGFBI is the major TGF-β response gene and is highly expressed in the extracellular matrix, and mice lacking TGFBI exhibit defects in lung development and display bronchopulmonary dysplasia.[3] During lung development, TGFBI is dynamically expressed and its expression down-regulated at later stages of organ development. In the lung, TGFBI expression correlates with expression of myofibroblast markers such as SMA. Moreover, it was shown that TGFBI deficiency impairs lung fibroblast differentiation in mice.

Besides the data discussed by the authors, the study also measured the tissue factor (TF) expression in ECs. TF is the key initiator of the (extrinsic) coagulation cascade, and the role of TF expressed by ECs is still not fully resolved.[4] The supplementary data set presented by Bochenek et al[1] provides data about the specific phenotype of the CTEPH-derived ECs. The authors compared the expression of TF in CTEPH-derived ECs with commercially available ECs. Interestingly, HPAECs seem to have lower TF expression levels compared with HUVECs. Moreover, CTEPH-derived ECs exhibited increased TF expression compared with HPACs. However, the TF expression in CTEPH-derived ECs was similar to TF expression in HUVECs. This suggests that although CTEPH leads to TF induction in HPAEC-like cells, this induction was less than the baseline expression of cultured HUVECs. A major difference between arteries and veins is the vessel wall composition and the experienced pressure/sheer forces. This leads to the question of whether the TF expression in EC is dependent on surrounding smooth muscle cells or dependent on the pressure/shear forces applied to EC. Further studies could investigate if smooth muscle cells release an EC TF gene expressing limiting factor or if there are pressure/shear-dependent epigenetic changes in ECs regarding their procoagulant potential.

In a previous study, Bochenek et al[2] found that during thrombosis and in CTEPH, TGF-β released from platelets led to pathologic TGF-β receptor I (TGFBRI) signaling in ECs, causing increased thrombus formation and delayed thrombus resolution. Platelets are one of the major sources of circulating TGF-β. In addition, platelet TGF-β deficiency was associated with reduced IVC stenosis and thrombus resolution, suggesting that CTEPH is associated with platelet activation.[2] Here, the authors[1] report that only CTEPH samples exhibited significant increased STC2 expression levels compared with PAH or IPF patient samples. Interestingly, STC2 was shown to be involved in thrombin-mediated platelet activation.[5] In addition, we found that protease-activated receptor 4 (PAR4), which belong to the family of G-protein-coupled receptors is present on platelets, is responsible for platelet activation contributes to IVC stenosis in mice.[6] It could be argued that in CTEPH, ECs express TF that leads to thrombin-dependent platelet activation.

In conclusion, CTEPH is associated with increased TGF-β signaling and a dysregulated thrombus remodeling/ resolution.[1,2] The increased TGF-β:TGFBRI

signaling and TGFBI expression in CTEPH leads to changes in the EC phenotype[1,2] within the pulmonary vessels, transforming the ECs to a more prothrombogenic phenotype.[1] We hypothesize that TF-dependent thrombin generation enhances platelet activation, which leads to TGF-β release from platelets enhancing TGF-β-dependent pathologic pathways in ECs via the TGFBRI.[2] TGFBRI activation leads to increased expression of TGFBI in ECs,[1] which subsequently leads to EC transformation, delayed thrombus resolution, and increases in thrombus remodeling/fibrosis.[1,2]

Future studies could investigate the platelet-specific pathways leading to the TGF-β release and the role of TGFBRI in venous thrombosis or CTEPH. Investigating these pathways could lead to improved therapeutic approaches in the treatment and management of CTEPH.

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