Histol Histopathol (2013) 28: 185-193
DOI: 10.14670/HH-28.185

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Crosstalk between endothelial cell and thrombus in chronic thromboembolic pulmonary hypertension: perspective

Seiichiro Sakao and Koichiro Tatsumi
Department of Respirology (B2), Graduate School of Medicine, Chiba University, Chuo-ku, Chiba, Japan

Summary. It is generally accepted that chronic thromboembolic pulmonary hypertension (CTEPH) results from pulmonary emboli originating from deep vein thrombosis. However, this consensus opinion has been challenged, and the concept that some aspects of CTEPH exacerbation might result from a small-vessel disease leading to secondary thrombosis has been suggested.

In addition to the effect of recurrent thromboembolism, a number of lines of clinical evidence indicate that progressive worsening is contributed to by remodeling in the small pulmonary arteries. Histopathological studies of the microvascular changes in CTEPH have identified vascular lesions similar to those seen in idiopathic pulmonary arterial hypertension (IPAH). Especially in *in vitro* and *ex vivo* experiments, pulmonary artery endothelial cells (ECs) in pulmonary hypertensive diseases are suggested to exhibit an unusual hyperproliferative potential with decreased susceptibility to apoptosis, indicating that dysfunctional ECs may contribute to the progression of the diseases. Although the degree and mechanisms of EC dysfunction as a contributor to CTEPH are unclear, EC dysfunction may occur in small arteries. Indeed, the cells stimulated by the microenvironment created by the unresolved clot may release substances that induce EC dysfunction. The EC dysfunctions in CTEPH may lead to disorders of the anti-coagulation properties in ECs and may result in additional clots *in situ*. Moreover, these may lead to the progression, not only of distal thrombus, but also of proximal clotting.

This article reviews the pathobiological concepts of CTEPH and explains a crosstalk between EC dysfunction and *in situ* thrombi which may contribute to the vascular lesions of CTEPH.

Key words: Endothelial cell, Thrombus, CTEPH

Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) has emerged as one of the leading causes of severe pulmonary hypertension. CTEPH is characterized by intraluminal thrombus formation and fibrous stenosis or complete obliteration of the pulmonary arteries (Klepetko et al., 2004). The consequence is increased pulmonary vascular resistance, resulting in pulmonary hypertension and progressive right heart failure. Pulmonary endarterectomy (PEA) is the current mainstay of therapy for CTEPH (Jamieson et al., 2003). Recently, there has been evidence suggesting that the existing consensus that the pathophysiology of CTEPH results from unresolved pulmonary emboli may have been too simplistic (Hoeper et al., 2006). Although acute pulmonary embolism is generally accepted as the main initiating event in CTEPH, small-vessel disease is believed to appear and worsen later during the course of disease, and to contribute to the progression of hemodynamic and symptomatic decline (Hoeper et al., 2006). Moreover, *in situ* thrombosis and pulmonary arteriopathy have been proposed as potential causes of CTEPH (Shure, 1996; Peacock et al., 2006).

This article reviews the pathobiological concepts of CTEPH, including pulmonary microvascular disease, the endothelial-mesenchymal transition (EnMT), EC dysfunction, and *in situ* thrombosis, which are important pathological features of pulmonary arterial hypertension (PAH) (Eisenberg et al., 1990; Welsh et al., 1996; Wolf et al., 2000; Bauer et al., 2002; Cool et al., 2004; Humbert et al., 2004; Reesink et al., 2004). Furthermore,

---

Offprint requests to: Seiichiro Sakao, MD, PhD, Department of Respirology (B2), Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. e-mail: sakaos@faculty.chiba-u.jp

it explains a crosstalk between EC dysfunction and *in situ* thrombi which may contribute to the vascular lesions of CTEPH.

Microvascular lesions

In addition to the effect of recurrent thromboembolism, a number of lines of clinical evidence indicate that progressive worsening is contributed to by remodeling in the small distal pulmonary arteries in the open vascular bed (Moser and Bloor, 1993; Azarian et al., 1997; Yi et al., 2000). Indeed, the PH and right ventricular dysfunction are progressive, even in the absence of recurrent thromboemboli (Azarian et al., 1997). Moreover, there is a low degree of correlation between the extent of vascular obstruction visible on pulmonary angiography and the severity of PH (Azarian et al., 1997). There is likely a vascular stealing phenomenon, which means that there is redistribution of the pulmonary blood flow from the nonoccluded to newly endarterectomized vasculature after PEA (Moser and Bloor, 1993). There is often no hemodynamic improvement and persistent PH despite successful PEA in approximately 35% of patients (Condliffe et al., 2008).

Pulmonary microvascular disease, which is an important pathological feature of PAH, leads to increased pulmonary vascular resistance and reduced compliance, with marked proliferation of pulmonary artery smooth muscle cells (SMCs) and endothelial cells (ECs), resulting in the obstruction of blood flow in pulmonary arteries (Humbert et al., 2004). Recently, we reviewed pathogenetic concepts of pulmonary arterial hypertension (PAH) and explained the vascular lesions with EC dysfunction, i.e., apoptosis and proliferation (Sakao et al., 2009, 2010). Taraseviciene-Stewart et al. showed that a vascular endothelial growth factor (VEGF) receptor blocker induced some of the "angioproliferative" features typical of advanced PAH in a rat model, i.e., worsening of the pathological vascular remodeling, and those features were reversed by inhibitors of apoptosis, suggesting that increased apoptosis of ECs in response to loss of survival signaling provided a selection pressure that induced the emergence of actively proliferating ECs without evidence of apoptosis (Taraseviciene-Stewart et al., 2001). Moreover, our *in vitro* experiments have demonstrated that the emergence of apoptosis-resistant proliferating ECs depended on initial EC apoptosis induced by blockade of VEGF receptors and these phenotypically altered ECs expressed the tumor marker survivin and the antiapoptotic protein Bcl-$_{XL}$ (Sakao et al., 2005). Consistent with our results, Masri et al. have shown that pulmonary artery ECs isolated from patients with idiopathic PAH (IPAH) were hyperproliferative and apoptosis-resistant (Masri et al., 2007). However, these results were from an animal model and tissue culture experiments, not from human. It remains unknown whether they actually contribute to pathobiology of human PAH.

The studies of the microvascular changes in CTEPH have identified histopathological characteristics similar to those seen in IPAH and Eisenmenger's syndrome (Moser and Bloor, 1993; Azarian et al., 1997; Yi et al., 2000; Piazza and Goldhaber, 2011). Therefore, dysfunctional ECs may contribute to the progression of the microvascular changes in CTEPH as shown in PAH. Although PEA is the current mainstay of therapy for CTEPH, a recent study showed that specific vasodilative compounds, e.g., prostanoids, endothelin receptor antagonists, phosphodiesterase type 5 inhibitors or a combination, as used for PAH therapy, improved cumulative survival in the patients with inoperable CTEPH, suggesting that there may be vasodilative reactivity in the vasculature of some populations of CTEPH patients as shown in the vasculature of PAH (Seyfarth et al., 2010). Indeed, there exists evidence that patients with CTEPH show similar acute vasoreactivity to inhaled nitric oxide and iloprost (Ulrich et al., 2006; Skoro-Sajer et al., 2009).

The similarities between the microvascular changes in CTEPH and those seen in IPAH suggest that specific vasodilative compounds as used for PAH therapy may be appropriate for some populations of CTEPH, as the patients with no hemodynamic improvement and persistent PH despite successful PEA.

Endothelial-mesenchymal transition (EnMT)

EnMT is a term which has been used to describe the process through which ECs lose their endothelial characteristics and gain the expression of other mesenchymal cell characteristics (Arciniegas et al., 2007). There is the intriguing possibility that intimal SMCs may arise from ECs (Majesky and Schwartz, 1997). In the systemic circulation, Arciniegas et al. demonstrated that mesenchymal cells that existed in the intimal thickening may arise from ECs (Arciniegas et al., 2000). Indeed, the existence of “transitional cells” demonstrating features of both ECs and vascular SMCs in the plexiform lesions in the lungs from patients with IPAH has been identified (Cool et al., 2004). Our *in vitro* studies of human pulmonary microvascular endothelial cells (HPMVECs) showed that blockade of VEGF receptors generated a selection pressure that killed some ECs and expanded resident progenitor-like cells to transdifferentiate into other mesenchymal phenotypes (Sakao et al., 2007). Although there is the limitation of this study based on *in vitro* experiment, this result may support the concept that transdifferentiation of pulmonary ECs to other mesenchymal cells may contribute to the muscularization of the pulmonary arteries. Because of histopathological similarity of the microvascular changes between CTEPH and IPAH (Moser and Bloor, 1993; Azarian et al., 1997; Yi et al., 2000, Piazza and Goldhaber, 2011), EnMT may contribute to the progression of the microvascular changes in CTEPH.

Recently, we have shown the existence of not only myofibroblast-like cells, but also endothelial-like cells in endarterectomized tissues from patients with CTEPH (Maruoka et al., 2012). Our experiments demonstrated that the endothelial-like cells included a few transitional cells (coexpressing both endothelial- and smooth muscle- cell markers). Moreover, experiments using commercially available HPMVECs and myofibroblast-like cells, which were isolated from the PEA tissues of CTEPH patients, demonstrated that substances associated with myofibroblast-like cells might induce the EnMT (Sakao et al., 2011). Indeed, transitional cells which co-expressed both endothelial- and smooth muscle- cell markers were identified in the PEA tissues of patients with CTEPH (Sakao et al., 2011). In support of our findings, Yao et al. showed the presence of CD34 (an endothelial marker) positive cells co-expressing α-smooth muscle actin (a smooth muscle- cell marker) in endarterectomized tissues from patients with CTEPH (Yao et al., 2009).

As shown in our experiment, Firth et al. demonstrated that a myofibroblast cell phenotype was predominant within endarterectomized tissues from patients with CTEPH, contributing extensively to the vascular lesion/clot (Firth et al., 2010). Moreover, the existence of putative endothelial progenitor cells in endarterectomized tissues of patients with CTEPH has been demonstrated (Yao et al., 2009). Firth et al. have reported the presence of multipotent mesenchymal progenitor cells within the tissues of patients with CTEPH (Firth et al., 2010). These studies suggested that the unique microenviroment created by the stabilized clot may promote these progenitor cells to differentiate into myofibloblast-like cells, and the misguided differentiation of these progenitor cells may enhance intimal remodeling (Yao et al., 2009; Firth et al., 2010). Therefore, myofibloblast-like cells may participate directly in vascular remodeling and they may induce EnMT to lead to EC dysfunction.

Indeed, it may be possible that the cells coexpressing both endothelial- and SM- cell markers in endarterectomized tissues are more likely progenitor cells rather than the cells which are differentiated by EnMT. However, in our *in vitro* experiments, there was no bone marrow-derived cell (defined as born marrow cell markers) in the cultured endothelial-like cells because *ex vivo* conditions may allow these cells to differentiate (Sakao et al., 2011).

EnMT may contribute to the development of vascular remodeling in the patients with CTEPH and interrupting this transition may provide a therapeutic target for CTEPH.

EC dysfunction

The degree and mechanisms of EC dysfunction as a contributor to CTEPH in small muscular arteries distal to nonobstructed pulmonary elastic vessels are unclear (Yi et al., 2000; Dartevelle et al., 2004; Hoeper et al., 2006). However, EC dysfunction may play a crucial role in these areas. Indeed, EC related humoral markers that have been linked to CTEPH include anticardiolipin antibodies, a known risk factor for venous thromboembolism (Torbicki et al., 2008), elevated endothelial factor VIII (Wolf et al., 2000; Bonderman et al., 2003), and monocyte chemoattractant protein 1 (Kimura et al., 2001). Moreover, markers of endothelial trauma or dysfunction, such as endothelins, regularly observed in IPAH, are also found in cases of pulmonary embolism (Sofia et al., 1997). In particular, the endothelin-1 levels in CTEPH closely correlated with the hemodynamic and clinical severity of the disease (Reesink et al., 2006). Endothelin-mediated vascular remodeling and impairment of nitric oxide function may play a crucial role in the development of vascular lesions distal to occluded vessels in CTEPH, as well as in severe PH (Bauer et al., 2002; Reesink et al., 2004). It has been observed that PH is more likely to occur following partial vascular occlusions of pulmonary artery segments than following complete occlusions (Robin et al., 1966), thus suggesting that vasoactive substances produced by the turbulent flow in CTEPH may be involved in EC dysfunction. However, it seems to be difficult to define EC dysfunction in patients with CTEPH.

Several lines of evidence indicate that autophagy has an important role in many different pathological conditions. Moreover, fewer mitochondria, the decreased expression of superoxide dismutase and normoxic decreases in reactive oxygen species have been shown to be the characteristics of mitochondrial abnormalities in PAH (Archer et al., 2008). Our recent findings demonstrated that endothelial-like cells lost their ability to form autophagosomes and had defective mitochondrial structure/function (Sakao et al., 2011), indicating that EC dysfunctions occur in the proximal lesions of patients with CTEPH. Moreover, experiments using commercially available HPMVECs and myofibroblast-like cells demonstrated that factors associated with myofibroblast-like cells might induce HPMVEC dysfunction through the inactivation of autophagy, the disruption of the mitochondrial reticulum, and the improper localization of superoxide dismutase-2 (Sakao et al., 2011). The PCR array data analysis showed that substances associated with myofibroblast-like cells induced the alterations in the endothelial cell biology of HPMVECs (Sakao et al., 2011). Although it is uncertain whether EC dysfunctions actually contribute to microvascular remodeling in patients with CTEPH, the myofibroblast-like cells in the proximal lesions may contribute to EC dysfunction in the vasculature of CTEPH. Indeed, it has been demonstrated that ECs in noninvolved pulmonary vascular beds are different from ECs in regions of organized thromboembolic material in patients with CTEPH (Lang et al., 1994a,b). In patients with CTEPH, primary ECs cultured from pulmonary arteries without thrombus had no abnormalities in the expression of fibrinolytic proteins or responses to thrombin stimulation (Lang et al., 1994a,b). However, ECs within yellowish-white thrombi, i.e., the highly organized tissues, showed elevated type 1 plasminogen activator inhibitor (PAI-1) mRNA levels (Lang et al., 1994a). Therefore, we have to separate them to consider EC dysfunction.

The correlation between endothelins and vascular remodeling in CTEPH seems to support the possibility that pharmacological therapy using endothelin receptor antagonists are effective treatment for the patients with CTEPH.

In situ thrombosis

ECs not only facilitate the thrombotic process, but also actively inhibit thrombosis and promote fibrinolysis. The production and release of nitric oxide and prostacyclin, two potent inhibitors of platelet aggregation, by ECs are important for the prevention of intravascular thrombosis (Moncada et al., 1991). In addition, the expression of thrombomodulin (TM), a high affinity receptor for thrombin, on the surface of ECs prevents the cleavage of fibrinogen to fibrin. ECs are also a source of tissue plasminogen activator (t-PA), a key activator of plasminogen in the fibrinolytic cascade. On the other hand, ECs also synthesize and release plasminogen activator inhibitor (PAI)-1, an inhibitor of t-PA, highlighting the role of the endothelium in regulating the fine balance between prothrombotic and antithrombotic processes.

Indeed, the plasma concentration of soluble TM in patients with CTEPH was found to be significantly lower than that in the control group, suggesting that a

Table 1. Clinical and pathobiological features of CTEPH.

Hallmarks	Features	Reference	Tissue Culture/ Clinical
Microvascular lesions	Progressive worsening by remodeling in the small distal pulmonary arteries	Azarian et al. (1997)	Clinical
		Moser and Bloor (1993)	Clinical
		Yi et al. (2000)	Clinical
	No hemodynamic improvement and persistent PH despite successful PEA	Condliffe et al. (2008)	Clinical
	microvascular changes in CTEPH similar to those seen in IPAH	Azarian et al. (1997)	Clinical
		Moser and Bloor (1993)	Clinical
		Yi et al. (2000)	Clinical
		Piazza and Goldhaber (2011)	Clinical
	Vasodilative reactivity in the vasculature of CTEPH	Seyfarth et al. (2010)	Clinical
EnMT	Transitional cells in endarterectomized tissues	Sakao et al. (2011)	Tissue Culture
		Yao et al. (2009)	Tissue Culture
	EnMT induced by substances associated with the cells in endarterectomized tissues	Sakao et al. (2011)	Tissue Culture
	The existence of endothelial and mesenchymal progenitor cells in endarterectomized tissues	Yao et al. (2009)	Tissue Culture
		Firth et al. (2010)	Tissue Culture
EC dysfunction	Humoral markers related with EC in CTEPH: Anticardiolipin antibodies Endothelial factor VIII Monocyte chemoattractant protein 1 Endothelins	Torbicki et al. (2008)	Clinical
		Bonderman et al. (2003)	Clinical
		Kimura et al. (2001)	Clinical
		Sofia et al. (1997)	Clinical
		Reesink et al. (2006)	Clinical
	Endothelin-mediated vascular remodeling	Reesink et al. (2006)	Clinical
	The loss of the ability to form autophagosomes	Sakao et al. (2011)	Tissue Culture
	Structure-function defects of mitochondria	Sakao et al. (2011)	Tissue Culture
	ECs with abnormalities in the expression of fibrinolytic proteins or responses to thrombin stimulation	Lang et al. (1994a,b)	Tissue Culture
		Lang et al. (1994a,b)	Tissue Culture
In situ thrombosis	A decreased plasma TM concentration	Sakamaki et al. (2003)	Clinical
	Elevated PAI-1 mRNA levels	Lang et al. (1994)	Tissue Culture
	The decreased expression of Annexin A and plasminogen activator genes in HPMVECs co-cultured with the cells from the PEA tissues	Sakao et al. (2011)	Tissue Culture

CTEPH: Chronic thromboembolic pulmonary hypertension; PH: Pulmonary arterial hypertension; PEA: Pulmonary endarterectomy; IPAH: Idiopathic pulmonary arterial hypertension; EnMT: Endothelial-mesenchymal transition; EC: endothelial cell; TM: Thrombomodulin; PAI-1: type 1 plasminogen activator inhibitor; HPMVECs: Human pulmonary microvascular endothelial cells.

decreased plasma TM concentration might reflect pulmonary vascular EC dysfunction, leading to altered anticoagulant and fibrinolytic function in CTEPH (Sakamaki et al., 2003). ECs within the highly organized tissues in CTEPH exhibited elevated PAI-1 mRNA levels in comparison to patient pulmonary artery specimens that were free of thrombus, suggesting that the prevalence of PAI-1 expression within pulmonary thromboemboli may play a role in the stabilization of vascular thrombi (Lang et al., 1994a). Moreover, there were decreases in the expression of the Annexin A5 and plasminogen activator, urokinase genes in HPMVECs co-cultured with myofibroblast-like cells from the PEA tissues of CTEPH patients (Sakao et al., 2011). Annexin A5 plays an important role in anticoagulant function and is a protein that has a high affinity for negatively-charged phospholipids (Funakoshi et al., 1987; Tait et al., 1988), over which it forms trimers (Voges et al., 1994) that become an annexin A5 shield. The formation of this shield blocks the phospholipids from phospholipid-dependent coagulation enzyme reactions (Andree et al., 1992). Plasminogen activator, urokinase, is a thrombolytic agent. Its primary physiological substrate is plasminogen, which is an inactive zymogen form of the serine protease plasmin. The activation of plasmin triggers a proteolysis cascade that, depending on the physiological environment, participates in thrombolysis or extracellular matrix degradation (Collen and Lijnen, 2005). The decreased expression of Annexin A and plasminogen activator, urokinase, may contribute to the disorder of the anti-coagulation properties in CTEPH patients. However, there is no validation of these data in an *in vivo* experiment.

There are several lines of evidence indicating that EC dysfunction might interfere with the normal balance between the pro-thrombotic and anti-thrombotic mechanisms, resulting in local thrombosis, and may contribute to the pathophysiology of PAH (Eisenberg et al., 1990; Welsh et al., 1996; Wolf et al., 2000). The EC dysfunction in CTEPH may lead to disorder of the anti-coagulation properties in ECs, i.e., may inactivate a vascular fibrinolytic system, and result in the formation of additional clots *in situ*, like PAH, because the histopathological features in the CTEPH vasculature are similar to those seen in IPAH.

Crosstalk between the unresolved clot, EC dysfunction and *in situ* thrombi

Although the first pulmonary embolism is generally accepted as the main initiating event in CTEPH, we hypothesize that the emergence of the microenvironment created by the unresolved clot may result in the local induction of substances that circulate to cause a more widespread predisposition to vascular remodeling affecting the rest of the pulmonary vascular bed, i.e., beyond the site of initial thrombosis. Our recent study suggested that myofibroblast-like cells stimulated by the microenvironment created by the unresolved clot might release substances that promote ECs to transition to other mesenchymal phenotypes and/or induce EC dysfunction, contributing not only to the proximal vasculature, but also to the distal vasculature (Sakao et al., 2011). The precise reasons for the lung-specific action of these substances in CTEPH remain unknown. One explanation may be that the pulmonary vascular

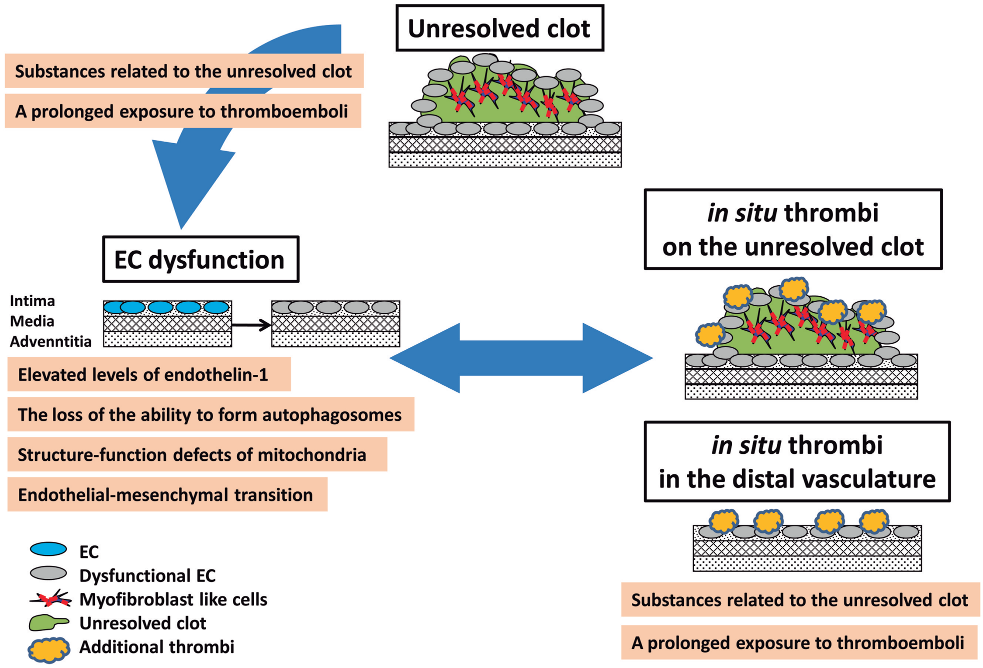

Fig. 1. Crosstalk between EC dysfunction and *in situ* thrombi in CTEPH (a hypothetical mechanism). The cells stimulated by the microenvironment created by an unresolved clot may release substances that induce EC dysfunction. The pulmonary vasculature in patients with CTEPH is subjected to prolonged exposure to thromboemboli. Indeed, thrombin is known to have potent effects on ECs, leading to endothelial barrier dysfunction due to mobilization of Ca^{2+} and rearrangement of the cytoskeleton. An impairment of the EC function in patients with CTEPH may lead to additional thrombi *in situ*, as is seen in patients with PAH, and these may lead to the progression of the proximal clot. A crosstalk between EC dysfunction and *in situ* thrombi may therefore contribute to the vascular lesions of CTEPH. CTEPH: chronic thrombo-embolic pulmonary hypertension, EC: endothelial cells.

beds, i.e., alveolar arteries, are exposed to the highest oxygen tensions in the body, which may induce the different response against substances related by an unresolved clot in comparison to systemic artery ECs. However, this explanation is not sufficient and further explanations are needed.

We fully recognize the limitation of our data interpretation which is based on *in vitro* studies of cultured cells and that this study does not confer any pathological evidence in CTEPH. Indeed, extensive small vessel disease may be a complication of a minority of CTEPH cases. Therefore, besides substances related to the microenviroment created by the stabilized clot, a second factor may be required to induce EC dysfunction which results in extensive disease. However, it remains unknown what is the second factor that is responsible for whether extensive small vessel disease occurs in a given patient.

In the pathogenesis of CTEPH, pulmonary microvascular lesions develop in the distal areas of unoccluded as well as occluded pulmonary arteries (Moser and Bloor, 1993; Azarian et al., 1997; Yi et al., 2000). The development of microvascular lesions distal to totally obstructed pulmonary arteries may be promoted by substances related to the microenvironment created by the unresolved clot. The development of the lesions distal to nonobstructed pulmonary arteries may be promoted not only by substances, but also by increased shear stress caused by hypoxic pulmonary vasoconstriction, because shear stress has been shown to inhibit apoptosis of ECs (Pi et al., 2004) and to stimulate EC growth (Ameshima et al., 2003; Sakao et al., 2005), contributing to vascular remodeling. However, unless the occlusion is enormous, it seems unlikely that vessel occlusion alone increases shear stress in unoccluded arteries because of the large reservoir capacity of the normal pulmonary vasculature. A more likely explanation for the lesions distal to nonobstructed pulmonary arteries may be that the pulmonary arteriopathy could be the initial pathology of the lesions in the patients with IPAH (Peacock et al., 2006). In any case, a persistent clot in the peripheral pulmonary arteries despite successful PEA may continue to create the microenvironment that induces microvascular changes. This may be the reason why there are patients who do not respond to PEA.

In the proximal lesions in patients with CTEPH, the pulmonary vasculature is subjected to a prolonged exposure to thromboemboli, i.e., components in the final common pathway of the coagulation cascade. Indeed, thrombin, a serine protease that catalyzes the conversion of fibrinogen to fibrin, is known to have potent effects on ECs, leading to endothelial barrier dysfunction due to the mobilization of Ca^{2+} and rearrangement of the cytoskeleton (Ellis et al., 1999). Moreover, chronic exposure to fibrinogen, fibrin, and thrombin caused changes in the cytosolic Ca^{2+} in pulmonary artery ECs, suggesting that such changes might contribute to EC dysfunction, thus leading to vascular changes in patients with CTEPH (Firth et al., 2009).

Based on these observations, it has been suggested that many kinds of insults to ECs of the pulmonary arteries may initiate a sequence of events which leads to the EC dysfunctions in CTEPH. Numerous factors such as hypoxia, endogenous vasoconstrictors, and inflammatory cytokines could help to sustain this process (Egermayer et al., 1999). An impairment of the EC function in patients with CTEPH may lead to additional thrombi *in situ* similar to that observed in patients with PAH, and these may also lead to the progression of the proximal clot.

It has been suggested that the core of the pathological process in CTEPH is not only related to thrombus formation, but it is also linked to disturbed thrombus resolution (Morris et al., 2006, 2007; Suntharalingam et al., 2008). An altered coagulation process may account for the pathological features of CTEPH (Wolf et al., 2000). Recently, the fibrinogen A Thr312Ala polymorphism was shown to correspond to significant differences in the genotype and allele frequencies between CTEPH and control subjects. The presence of these polymorphisms may confer resistance to fibrinolysis that subsequently contributes to the development of thrombus organization (Suntharalingam et al., 2008). The other mechanism may be the development of more fibrinolysis-resistant fibrin clots from patients with CTEPH, when compared with the fibrin clots from healthy control subjects (Morris et al., 2006). An abnormally elevated amount of disialylated fibrinogen γ-chain can render a clot resistant to plasmin, which could lead to the subsequent development of CTEPH (Morris et al., 2007). However, these explanations are not sufficient because there are many patients without known coagulation problems who have these factors, and because numerous genetic variants of human fibrinogen have been implicated in thrombotic diseases (Matsuda and Sugo, 2002). Therefore, the resistance could be ascribed to not only fibrinogen genetic polymorphisms, but also variations in the post-translational modifications.

Conclusion

Besides the altered coagulation process, a crosstalk between EC dysfunction and *in situ* thrombi may contribute to the vascular lesions of CTEPH (Fig. 1) (Table 1). Moreover, this may explain why pulmonary thromboemboli in CTEPH patients are stable. Indeed, pulmonary thromboendarterectomy may be the best way to break this crosstalk. Recently, we demonstrated that poor subpleural perfusion on pulmonary angiography might be related to distal vascular remodeling and an inadequate surgical outcome of CTEPH (Tanabe et al., 2012). No satisfactory hemodynamic improvement and persistent PH despite successful PEA in the patients with CTEPH (Condliffe et al., 2008) suggests the existence of distal vascular remodeling. Although it remains uncertain whether vascular remodeling is actually related to the crosstalk between EC dysfunction and *in situ* thrombi, the care for these patients should be directed toward pharmacologically reducing pulmonary vascular resistance with specific vasodilative compounds as used for PAH therapy. The next step in the future is to find out new ways to define EC dysfunction and vascular remodeling in CTEPH objectively.

Acknowledgments: All authors read and approved the final manuscript. This article shows a selective perusal of the relevant literature together with a discussion of our own findings, and not a comprehensive "balanced" review as outlined by a referee.

Conflict of interest: Dr. Tatsumi has received honoraria for lectures from Glaxo Smith Kline, and Actelion Pharmaceutical Ltd. The other authors report no conflicts of interest with regard to this manuscript.

Role of the funding source: This study was supported by Research Grants for the Respiratory Failure Research Group and the Cardiovascular Diseases (19-9, 22-33) from the Ministry of Health, Labor and Welfare, Japan, a Grant-in-Aid for Scientific Research (Category C 22590851) from the Japanese Ministry of Education and Science, and Takeda Science Foundation.

References

Ameshima S., Golpon H., Cool C.D., Chan D., Vandivier R.W., Gardai S.J., Wick M., Nemenoff R.A., Geraci M.W. and Voelkel N.F. (2003). Peroxisome proliferatoractivated receptor gamma (PPARgamma) expression is decreased in pulmonary hypertension and affects endothelial cell growth. Circ. Res. 92, 1162-1169.

Andree H.A., Stuart M.C., Hermens W.T., Reutelingsperger C.P., Hemker H.C., Frederik P.M. and Willems G.M. (1992). Clustering of lipid-bound annexin V may explain its anticoagulant effect. J. Biol. Chem. 267, 17907-17912.

Archer S.L., Gomberg-Maitland M., Maitland M.L., Rich S., Garcia J.G. and Weir E.K. (2008). Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1alpha-Kv1.5 O2-sensing pathway at the intersection of pulmonary hypertension and cancer. Am. J. Physiol. Heart. Circ. Physiol. 294, 570-578.

Arciniegas E., Ponce L., Hartt Y., Graterol A. and Carlini R.G. (2000). Intimal thickening involves transdifferentiation of embryonic endothelial cells. Anat. Rec. 258, 47-57.

Arciniegas E., Frid M.G., Douglas I.S. and Stenmark K.R. (2007). Perspectives on endothelial-to-mesenchymal transition: potential contribution to vascular remodeling in chronic pulmonary hypertension. Am. J. Physiol. Lung. Cell. Mol. Physiol. 293, L1-8.

Azarian R., Wartski M., Collignon M.A., Parent F., Herve P., Sors H. and Simonneau G. (1997). Lung perfusion scans and hemodynamics in acute and chronic pulmonary embolism. J. Nucl. Med. 38, 980-983.

Bauer M., Wilkens H., Langer F., Schneider S.O., Lausberg H. and Schafers H.J. (2002). Selective upregulation of endothelin B receptor gene expression in severe pulmonary hypertension. Circulation 105, 1034-1036.

Bonderman D., Turecek P.L., Jakowitsch J., Weltermann A., Adlbrecht C., Schneider B., Kneussl M., Rubin L.J., Kyrle P.A., Klepetko W., Maurer G. and Lang I.M. (2003). High prevalence of elevated clotting factor VIII in chronic thromboembolic pulmonary hypertension. Thromb. Haemost. 90, 372-376.

Condliffe R., Kiely D.G., Gibbs J.S, Corris P.A., Peacock A.J., Jenkins D.P., Hodgkins D., Goldsmith K., Hughes R.J., Sheares K., Tsui S.S., Armstrong I.J., Torpy C., Crackett R., Carlin C.M., Das C., Coghlan J.G. and Pepke-Zaba J. (2008). Improved outcomes in medically and surgically treated chronic thromboembolic pulmonary hypertension. Am. J. Respir. Crit. Care. Med. 177, 1122-1127.

Cool C.D., Wood K. and Voelkel N.F. (2004). Transdifferentiation of endothelial cells in primary pulmonary hypertension. Am. J. Resp. Crit. Care. Med. 167: A844.

Collen D. and Lijnen H.R. (2005). Thrombolytic agents. Thromb. Haemost. 93, 627-630.

Dartevelle P., Fadel E., Mussot S., Chapelier A., Herve P., de Perrot M., Cerrina J., Ladurie F.L., Lehouerou D., Humbert M., Sitbon O. and Simonneau G. (2004). Chronic thromboembolic pulmonary hypertension. Eur. Respir. J. 23, 637-648.

Egermayer P., Town G.I. and Peacock A.J. (1999). The role of serotonin in the pathophysiology of acute and chronic pulmonary hypertension. Thorax 54, 161-168.

Eisenberg P.R., Lucore C., Kaufman L., Sobel B.E., Jaffe A.S. and Rich S. (1990). Fibrinopeptide A levels indicative of pulmonary vascular thrombosis in patients with primary pulmonary hypertension. Circulation 82, 841-847.

Ellis C.A., Tiruppathi C., Sandoval R., Niles W.D. and Malik A.B. (1999). Time course of recovery of endothelial cell surface thrombin receptor (PAR-1) expression. Am. J. Physiol. Cell. Physiol. 276, C38-C45.

Firth A.L., Yau J., White A., Chiles P.G., Marsh J.J., Morris T.A. and Yuan J.X. (2009). Chronic exposure to fibrin and fibrinogen differentially regulates intracellular Ca2+ in human pulmonary arterial smooth muscle and endothelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 296, L979-986.

Firth A.L., Yao W., Ogawa A., Madani M.M., Lin G.Y. and Yuan J.X. (2010). Multipotent mesenchymal progenitor cells are present in endaretectomized tissues from patients with chronic thromboembolic pulmonary hypertension. Am. J. Physiol. Cell. Physiol. 298, C1217-C1225.

Funakoshi T., Heimark R.L., Hendrickson L.E., McMullen B.A. and Fujikawa K. (1987). Human placental anticoagulant protein: isolation and characterization. Biochemistry 26, 5572-5578.

Hoeper M.M., Mayer E., Simonneau G. and Rubin L. (2006). Chronic thromboembolic pulmonary hypertension. Circulation 113, 2011-2020.

Humbert M., Morrell N.W., Archer S.L., Stenmark K.R., MacLean M.R., Lang I.M., Christman B.W., Weir E.K., Eickelberg O., Voelkel N.F. and Rabinovitch M. (2004). Cellular and molecular pathobiology of pulmonary arterial hypertension. J. Am. Coll. Cardiol. 43, 13S-24S.

Jamieson S.W., Kapelanski D.P., Sakakibara N., Manecke G.R., Thistlethwaite P.A., Kerr K.M., Channick R.N., Fedullo P.F. and Auger W.R. (2003). Pulmonary endarterectomy: experience and lessons learned in 1,500 cases. Ann. Thorac. Surg. 76, 1457-1462.

Kimura H., Okada O., Tanabe N., Tanaka Y., Terai M., Takiguchi Y., Masuda M., Nakajima N., Hiroshima K., Inadera H., Matsushima K. and Kuriyama T. (2001). Plasma monocyte chemoattractant protein-1 and pulmonary vascular resistance in chronic thromboembolic pulmonary hypertension. Am. J. Respir. Crit. Care. Med. 164, 319-324.

Klepetko W., Mayer E., Sandoval J., Trulock E.P., Vachiery J.L., Dartevelle P., Pepke-Zaba J., Jamieson S.W., Lang I. and Corris P. (2004). Interventional and surgical modalities of treatment for pulmonary arterial hypertension. J. Am. Coll. Cardiol. 43 (suppl S), 73S-80S.

Lang I.M., Marsh J.J., Olman M.A., Moser K.M., Loskutoff D.J. and

Schleef R.R. (1994a). Expression of type 1 plasminogen activator inhibitor in chronic pulmonary thromboemboli. Circulation 89, 2715-2721.

Lang I.M., Marsh J.J., Olman M.A., Moser K.M. and Schleef R.R. (1994b). Parallel analysis of tissue-type plasminogen activator and type-1 plasminogen activator inhibitor in plasma and endothelial cells derived from patients with chronic pulmonary thromboemboli. Circulation 90, 706-712.

Majesky M.W. and Schwartz S.M. (1997). An origin for smooth muscle cells from endothelium? Circ. Res. 80, 601-603.

Maruoka M., Sakao S., Kantake M., Tanabe N., Kasahara Y., Kurosu K., Takiguchi Y., Masuda M., Yoshino I., Voelkel N.F. and Tatsumi K. (2012). Characterization of myofibroblasts in chronic thromboembolic pulmonary hypertension. Int. J. Cardiol. 159, 119-127.

Masri F.A., Xu W., Comhair S.A., Asosingh K., Koo M., Vasanji A., Drazba J., Anand-Apte B. and Erzurum S.C. (2007). Hyperproliferative apoptosis-resistant endothelial cells in idiopathic pulmonary arterial hypertension. Am. J. Physiol. Lung. Cell. Mol. Physiol. 293, L548-L554.

Matsuda M. and Sugo T. (2002). Structure and function of human fibrinogen inferred from dysfibrinogens. Int. J. Hematol. 76, 352-360.

Moncada S., Palmer R.M. and Higgs E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109-142.

Morris T.A., Marsh J.J., Chiles P.G., Auger W.R., Fedullo P.F. and Woods V.L. Jr (2006). Fibrin derived from patients with chronic thromboembolic pulmonary hypertension is resistant to lysis. Am. J. Respir. Crit. Care. Med. 173, 1270-1275.

Morris T.A., Marsh J.J., Chiles P.G., Kim N.H., Noskovack K.J., Magana M.M., Gruppo R.A. and Woods V.L. Jr (2007). Abnormally sialylated fibrinogen gamma-chains in a patient with chronic thromboembolic pulmonary hypertension. Thromb. Res. 119, 257-259.

Moser K.M. and Bloor C.M. (1993). Pulmonary vascular lesions occurring in patients with chronic major vessel thromboembolic pulmonary hypertension. Chest 103, 685-692.

Peacock A., Simonneau G. and Rubin L. (2006). Controversies, uncertainties and future research on the treatment of chronic thromboembolic pulmonary hypertension. Proc. Am. Thorac. Soc. 3, 608-614.

Pi X., Yan C. and Berk B.C. (2004). Big mitogen-activated protein kinase (BMK1)/ERK5 protects endothelial cells from apoptosis. Circ. Res. 94, 362-9.

Piazza G. and Goldhaber S.Z. (2011). Chronic thromboembolic pulmonary hypertension. N. Engl. J. Med. 364, 351-360.

Reesink H.J., Lutter R., Kloek J.J., Jansen H.M. and Bresser P. (2004). Hemodynamic correlates of endothelin-1 in chronic thromboembolic pulmonary hypertension. Eur. Respir. J. 24, 110s.

Reesink H.J., Meijer R.C., Lutter R., Boomsma F., Jansen H.M., Kloek J.J. and Bresser P. (2006). Hemodynamic and clinical correlates of endothelin-1 in chronic thromboembolic pulmonary hypertension. Circ. J. 70, 1058-1063.

Robin E.D., Cross C.E., Kroetz F., Totten R.S. and Bron K. (1966). Pulmonary hypertension and unilateral pleural constriction with speculation on pulmonary vasoconstrictive substance. Arch. Intern. Med. 118, 391-400.

Sakamaki F., Kyotani S., Nagaya N., Sato N., Oya H. and Nakanishi N. (2003). Increase in thrombomodulin concentrations after pulmonary thromboendarterectomy in chronic thromboembolic pulmonary hypertension. Chest 124, 1305-1311.

Sakao S., Taraseviciene-Stewart L., Cool C.D., Tada Y., Kasahara Y., Kurosu K., Tanabe N., Takiguchi Y., Tatsumi K., Kuriyama T. and Voelkel N.F. (2007). VEGF-R blockade causes endothelial cell apoptosis, expansion of surviving CD34+ precursor cells and transdifferentiation to smooth muscle-like and neuronal-like cells. FASEB J. 21, 3640-3652.

Sakao S., Taraseviciene-Stewart L., Lee J.D., Wood K., Cool C.D. and Voelkel N.F. (2005). Initial apoptosis is followed by increased proliferation of apoptosis-resistant endothelial cells. FASEB J. 19, 1178-1180.

Sakao S., Tatsumi K. and Voelkel N.F. (2009). Endothelial cells and pulmonary arterial hypertension: apoptosis, proliferation, interaction and transdifferentiation. Respir. Res. 10, 95.

Sakao S., Tatsumi K. and Voelkel N.F. (2010). Reversible or irreversible remodeling in pulmonary arterial hypertension. Am. J. Respir. Cell. Mol. Biol. 43, 629-634.

Sakao S., Hao H., Tanabe N., Kasahara Y., Kurosu K. and Tatsumi K. (2011). Endothelial-like cells in chronic thromboembolic pulmonary hypertension: crosstalk with myofibroblast-like cells. Respir. Res. 12, 109.

Seyfarth H.J., Halank M., Wilkens H., Schäfers H.J., Ewert R., Riedel M., Schuster E., Pankau H., Hammerschmidt S. and Wirtz H. (2010). Standard PAH therapy improves long term survival in CTEPH patients. Clin. Res. Cardiol. 99, 553-556.

Shure D. (1996). Thrombendartectomy: some unanswered questions. Ann. Thorac. Surg. 62, 1253-1254.

Skoro-Sajer N., Hack N., Sadushi-Koliçi R., Bonderman D., Jakowitsch J., Klepetko W., Hoda M.A., Kneussl M..P, Fedullo P. and Lang I.M. (2009). Pulmonary vascular reactivity and prognosis in patients with chronic thromboembolic pulmonary hypertension: a pilot study. Circulation 119, 298-305.

Sofia M., Faraone S., Alifano M., Micco A., Albisinni R., Maniscalco M. and Di Minno G. (1997). Endothelin abnormalities in patients with pulmonary embolism. Chest 111, 544-549.

Suntharalingam J., Goldsmith K., van Marion V., Long L., Treacy C.M., Dudbridge F., Toshner M.R., Pepke-Zaba J., Eikenboom J.C. and Morrell N.W. (2008). Fibrinogen AThr312Ala polymorphism is associated with chronic thromboembolic pulmonary hypertension. Eur. Respir. J. 31, 736-741.

Tait J.F., Sakata M., McMullen B.A., Miao C.H., Funakoshi T., Hendrickson L.E. and Fujikawa K. (1988). Placental anticoagulant proteins: isolation and comparative characterization four members of the lipocortin family. Biochemistry 27, 6268-6276.

Tanabe N., Sugiura T., Jujo T., Sakao S., Kasahara Y., Kato H., Masuda M. and Tatsumi K. (2012). Subpleural perfusion as a predictor for a poor surgical outcome in chronic thromboembolic pulmonary hypertension. Chest 14, 929-934.

Taraseviciene-Stewart L., Kasahara Y., Alger L., Hirth P., Mc Mahon G., Waltenberger J., Voelkel N.F. and Tuder R.M. (2001). Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. FASEB J. 15, 427-438.

Torbicki A., Perrier A., Konstantinides S., Agnelli G., Galiè N., Pruszczyk P., Bengel F., Brady A.J., Ferreira D., Janssens U., Klepetko W., Mayer E., Remy-Jardin M. and Bassand J.P. (2008). ESC Committee for Practice Guidelines (CPG). Guidelines on the diagnosis and management of acute pulmonary embolism: the Task

Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). Eur. Heart J. 29, 2276-2315.

Ulrich S., Fischler M., Speich R., Popov V. and Maggiorini M. (2006). Chronic thromboembolic and pulmonary arterial hypertension share acute vasoreactivity properties. Chest 130, 841-846.

Voges D., Berendes R., Burger A., Demange P., Baumeister W. and Huber R. (1994). Three-dimensional structure of membrane-bound annexin V. A correlative electron microscopy-X-ray crystallography study. J. Mol. Biol. 238, 199-213.

Welsh C.H., Hassell K.L., Badesch D.B., Kressin D.C. and Marlar R.A. (1996). Coagulation and fibrinolytic profiles in patients with severe pulmonary hypertension. Chest 110, 710-717.

Wolf M., Boyer-Neumann C., Parent F., Eschwege V., Jaillet H., Meyer D. and Simonneau G. (2000). Thrombotic risk factors in pulmonary hypertension. Eur. Respir. J. 15, 395-399.

Yao W., Firth A.L., Sacks R.S., Ogawa A., Auger W.R., Fedullo P.F., Madani M.M., Lin G.Y., Sakakibara N., Thistlethwaite P.A., Jamieson S.W., Rubin L.J. and Yuan J.X. (2009). Identification of putative endothelial progenitor cells (CD34+CD133+Flk-1+) in endarterectomized tissue of patients with chronic thromboembolic pulmonary hypertension. Am. J. Physiol. Lung. Cell. Mol. Physiol. 296, L870-878.

Yi E.S., Kim H., Ahn H., Strother J., Morris T., Masliah E., Hansen L.A., Park K. and Friedman P.J. (2000). Distribution of obstructive intimal lesions and their cellular phenotypes in chronic pulmonary hypertension: a morphometric and immunohistochemical study. Am. J. Respir. Crit. Care. Med. 162, 1577-1586.

Accepted September 26, 2012