CHAPTER 6

Reconciling paradigms of abnormal pulmonary blood flow and quasi-malignant cellular alterations in pulmonary arterial hypertension

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ABSTRACT

In pulmonary arterial hypertension (PAH) structural and functional abnormalities of the small lung vessels interact and lead to a progressive increase in pulmonary vascular resistance and eventually right heart failure. A current pathobiological concept characterizes PAH as a “quasi-malignant” disease focusing on cancer-like alterations in endothelial cells (EC) and the importance of their acquired apoptosis-resistant, hyper-proliferative phenotype in the process of vascular remodeling. While changes in pulmonary blood flow (PBF) have been long-since recognized and linked to the development of PAH, little is known about a possible relationship between an altered PBF and the quasi-malignant cell phenotype in the pulmonary vascular wall. This review summarizes recognized and hypothetical effects of an abnormal PBF on the pulmonary vascular bed and links these to quasi-malignant changes found in the pulmonary endothelium. Here we describe that abnormal PBF does not only trigger a pulmonary vascular cell growth program, but may also maintain the cancer-like phenotype of the endothelium. Consequently, normalization of PBF and EC response to abnormal PBF may represent a treatment strategy in patients with established PAH.
INTRODUCTION

In severe forms of pulmonary arterial hypertension (PAH), a progressive increase in the pulmonary vascular resistance (PVR) leads to right heart pressure overload and right heart failure (1). The increase in resistance is caused by vasoconstriction, narrowing, occlusion and possible rarefaction of microvessels (diameter < 80 μM) in the pulmonary circulation that culminates in the development of generic plexiform lesions (2). Some of these characteristic histological findings were already described in the very first report of “endarteritis pulmonalis deiformans” in 1865 by the Viennese physician Julius Klob (3). Initially, sustained vasoconstriction was seen as the root cause of a disease that was then called primary pulmonary hypertension and later termed idiopathic PAH (IPAH), to describe the condition of PAH with unknown cause (4,5). The typical histopathological changes in the vascular walls of pulmonary vessels in the hypertensive lungs, e.g. intimal thickening and fibrosis, media hyperplasia and the formation of plexiform lesions were thought to be secondary to the chronic and sustained pulmonary vasoconstriction and consequently increased pulmonary blood flow (PBF). Yet, the overall modest success of vasodilator treatment continues to motivate investigators to explore the details of the cellular and molecular aspects of the disease (6).

Over the past 20 years reports of phenotypic changes of lung vessel endothelial cells, exuberant monoclonal outgrowth and genetic abnormalities in pulmonary endothelial cells (ECs) have led to a paradigm shift where some investigators have applied concepts from cancer biology in order to explain the vasculopathy of PAH as a quasi-malignant disease (7–10). This “cancer-paradigm” of PAH has led to extensive research and preclinical testing of anti-angiogenic drugs with tyrosine kinase inhibitors (TKIs) leading the way. Several compounds were successfully tested with positive outcomes in animal models of pulmonary hypertension (PH), but the translation of these findings to the clinic continues to be slow and entails several risks. A particular concern pertaining to the use of TKIs in patients with PAH is that they may have serious side-effects including cardiotoxicity and, to some extent, even promote the development of PH (11–14).

High shear stress, which triggers cellular injury and cell death, can be the driving force for a phenotypic switch of the cells in the pulmonary vascular wall, including the emergence of apoptosis-resistance and exuberant proliferation resulting in vascular occlusions and increased PVR (15–19). Recent findings by our group, underline the importance of dysfunctional EC shear-responses in the disease progression, which are caused by increased pro-apoptotic signalling and consequent protein cleavage (20). Therefore angio-proliferative vascular remodeling and abnormal PBF are in all likelihood interrelated, whereby it is of interest to examine the mechanisms of such an interaction on the cellular level and attempt to reconcile the early pathophysiological concepts of vasoconstriction and increased shear stress with the current concept of exuberant cell growth. Further, it is desirable to examine, whether molecular evidence supporting one or more hallmarks of cancer is sufficient to imply a quasi-malignant transformation, or could also represent a normal or excessive repair response to sustained cellular stress.

Here we will review the effects of abnormal PBF on the pulmonary vascular bed and link...
these to quasi-malignant events by addressing several hallmarks of cancer that have been highlighted by Hanahan and Weinberg in their landmark papers (21,22).

ABNORMAL PULMONARY BLOOD FLOW AND PULMONARY ARTERIAL HYPERTENSION

Fluid flow induced shear stress (τ), assuming an inelastic, cylindrical and straight vessel, can be calculated by the Hagen-Poiseuille equation (τ = (mean flow velocity (u) / vessel diameter (d)) × kinetic viscosity (μ)). A modification of this formula is used, because blood viscosity is not constant, but is affected by the vessel radius and flow velocity (Fåhræus-Lindqvist effect) (23). These formulas can be used to calculate shear stress, expressed in dynes per cm² (dyn/cm²). Additionally, flow is characterized as laminar, non-uniform, or turbulent by the determination of the Reynolds number (flow velocity × fluid density × vessel diameter/fluid viscosity). EC are directly exposed to blood flow and mechanical forces, which shape EC structure and affect their function via mechanisms such as altered expression of shear stress responsive elements (SSRE), structural and biochemical responses (24–27). The healthy lung microcirculation is a low-pressure, high volume system characterized by low blood flow velocity to ensure proper gas exchange (28). Abnormal alterations in this delicate equilibrium have been linked to the development of angio-proliferative remodeling and PH, wherefore we introduce abnormal PBF as a collective term describing a change in blood flow profile, pulsatility, velocity and blood distribution in the lung vasculature.

The link between increased PBF and PH was already established in the early nineteen fifties. Paul Wood and others published on the effects of "pulmonary hypertension with reversed central shunt" (Eisenmenger’s syndrome) and acknowledged the importance of an early normalization of the PBF before irreversible damage to the lung circulation would occur (29–31). To determine, whether shunt-reversal would be favourable, lung biopsies were deemed necessary to assess the severity of the vasculopathy. Examination of the lung vessel histology provided valuable insight into the vascular remodelling in the context of high PBF (32–34).

Today, several culture systems and animal models are available to study specific aspects of the disease, such as the role of abnormal PBF. Patient derived pulmonary microvascular endothelial cells (PMVECs), pulmonary artery endothelial cells (PAEC) and pulmonary artery smooth muscle cells (PASMCs) can be studied ex vivo under defined fluid flow/shear conditions (18). Thereby, valuable insight can be gained by properly designed flow experiments to determine effects of different types of shear stress (e.g. high/low, pulsatile, oscillatory shear stress) on cell monolayers. To investigate the flow dynamics in vivo multiple animal models are available. The chronic hypoxia and monocrotaline (MCT) models are well-described models of PH that are characterized by thickening of the media (smooth muscle cell layer) in the lung vasculature. These high flow models reveal that an increase in flow is not sufficient to induce the obliteratorative angiopathy observed in patients with PAH and in (rare cases of) congenital heart disease (CHD) with non-restrictive post-
tricuspid shunts (29,35–38). However, when the exposure to hypoxia or MCT is followed by a second stimulus (or “hit”), intimal obliteration develops in the lung vasculature and lesions that resemble the plexogenic alterations in the human PAH lungs. The “two-hit” hypothesis of tumorigenesis, originally formulated by Alfred Knudson, was proposed in the context of PAH in the late nineties, stating that both endothelial injury and changes in pulmonary artery hemodynamics must occur for neointimal lesions to arise (39–41). Known examples of such double-hit models are the MCT + pneumonectomy (PNx) model and the Sugen-Hypoxia model (SuHx), which combines the vascular endothelial growth factor receptor (VEGFR)-blocker SU5416 and chronic hypoxia (19,42). To date plexiform lesions have only been described in the SuHx model, thereby making this an unique model for PAH-like remodelling (43). Additionally, several variations to both models have been described (19,43–48).

**HALLMARKS OF CANCER IN PAH: SUSTAINED CELL GROWTH**

One of the central hallmarks of cancer as described by Hanahan and Weinberg is sustained cell growth. In order to proliferate all normal cells require growth signals transmitted into the cell via transmembrane receptors and phosphorylation of their tyrosine kinase domains. In cancer cells growth signals can be initiated via autocrine and paracrine stimulations from stromal cells (such as fibroblasts and pericytes) and their increased production of growth factors as well as their associated receptors (22). Caveolin-1 (CAV-1) a scaffolding protein that, among others, is one of the regulators of cell proliferation is of interest in PH and in mechanobiology (49).

A recent study reported on the changes in CAV-1 in lung vessels of children with CHD accompanied by an increased PBF. Increased flow caused a loss of CAV-1 expression in ECs, but concurrently, increased CAV-1 expression in pulmonary artery smooth muscle cells (PASMC) (33). The authors propose that the loss of CAV-1 in ECs stimulates the enhanced expression of CAV-1 in SMCs. In vitro, overexpression of CAV-1 has been linked to the proliferation of PASMCs most likely due to increased Ca\(^{2+}\)-influx, which subsequently initiates proliferation (50,51). In patients, a novel CAV-1 mutation has been associated with hereditary PAH (HPAH). Reduced levels of CAV-1 in EC have been reported in IPAH patients (52). Decreased levels of CAV-1 were reported to cause hyper-proliferation via eNOS hyper-activation and oxidative stress (53). In turn, sustained activation of the MAPK cascade, a hallmark of the EC changes in PAH, has been shown to be sufficient to reversibly down-regulate CAV-1 mRNA and protein expression (54). Shear also directly regulates eNOS via the endothelin-1 receptor mediated phosphorylation at Ser1177 in PAECs and lowers the bioavailability of NO, known to increase SMC proliferation (55). Reactive oxygen species (ROS), were shown increased in the lung endothelium from rats that underwent PNx combined with MCT (44). Resveratrol, an inhibitor of ROS, was shown to attenuate apoptosis in PMVECs stimulated by high shear stress and inhibits platelet derived growth factor (PDGF)-stimulated proliferation and cellular hypertrophy (56).
In HPMVECs shear stress resulted in a 10-fold upregulation of growth differentiation marker 15 (GDF-15) mRNA and increase in early growth response protein 1 (EGR-1) involved in both SMC and PMVEC proliferation (45,57). Additionally, EGR-1 is able to up-regulate different effector proteins that cause EC and SMC proliferation, inflammation and dysregulated apoptosis (58). Upregulation of fibroblast growth factor 2 (FGF2) induced by shear stress in a lamb model of chronic heart disease was linked to cell proliferation (59,60). Additionally, increased levels of FGF2 were shown in EC of IPAH patients contributing to medial remodeling and disease progression (61). Forkhead box transcription factor 1 (FoxO1) was recently shown to be partly inactivated in PH, thereby promoting pro-proliferative and inflammatory signaling (62). Unfortunately, no link to increased shear stress has been made in this report, but high shear stress has been shown to increase FoxO1 phosphorylation (thus suppressing function) in human umbilical vein endothelial cells (HUVECs). Additional factors such as angiopoietin-1 (Ang-1) and VEGF, both reported to be up regulated due to shear stress in PAH, are also involved in FoxO1 phosphorylation (63).

## EVASION OF TUMOR SUPPRESSOR FUNCTION, APOPTOSIS AND REPLICATIVE IMMORTALITY

Cancer cells exhibit powerful programs that inhibit cell proliferation (21). Many of these programs depend on the actions of tumor suppressor genes. The majority of anti-proliferative signals are funneled through the tumor suppressor retinoblastoma protein (Rb) and p53 (22). Inactivation of p53 promotes PH development and activation via Nutlin-3 can attenuate and reverse experimental PH (64–66). In bovine aortic ECs, p53 was shown to be regulated by shear stress in a time dependent manner and related to the magnitude of shear stress (67).

Rb is a known tumor suppressor involved in cell progression from the G1 to S state and is regulated by cyclin dependent kinases, and cyclin complexes (68). The Rb signaling circuit, guided by TGF-β, can be disrupted by the loss of responsiveness through the down-regulation or mutation of TGF-β receptors (15). Nishimura and colleagues found decreased protein expression of Rb in the MCT + PNx model of PH, which was restored by Simvastatin (69). Activation of Erk1/2 by shear stress has been shown to keep Rb in a non-active phosphorylated state thereby promoting the proliferation of osteoblasts. Erk1/2 phosphorylation is increased in PAH patients and PH animal models, implying that such a link could exist (70–72). NAPDH-oxidase 4 (NOX-4) can mediate TGF-β1 induced Rb phosphorylation, and Dorfmüller and colleagues found NOX-4 to be induced by high flow (44,73,74). In addition to Rb, other regulators may hamper tumor suppressor function in PAH. These include altered expression of peroxisome proliferator-activated receptor gamma (PPARγ), CAV-1 and superoxide dismutase 2 (SOD2). PPARγ levels are decreased in the angio-obliterated lesions in patients with PAH and it has been suggested that the loss of PPARγ can decrease tumor suppressor function in PAH ECs (75). Reduction of the expression of PPARγ has also been found in the SuHx model and
lamb-shunt models, whereas the treatment of mice with a PPARγ agonist prevented development of PH (76,77).

Further evidence for the loss of tumor suppression function is the downregulation of CAV-1 in ECs under high shear conditions. A decrease in CAV-1 and 2 expressions was also found in the plexiform lesions from PAH patients (18). In addition to its role in tumor suppression, caveolin has also been implicated in the regulation of proliferation and apoptosis by regulating Survivin (78,79). Upregulation of Survivin (Birc5) was shown in sheared ovine PAECs after 3 days (59). Among the functions of Survivin are the inhibition of caspase-dependent apoptosis and caspase-independent cell death. Loss of CAV-1 has been reported to be associated with the activation of signal transducer and activator of transcription 3 (STAT3), which is persistently activated in ECs from PAH patients (80). Besides Survivin, BCL-2 and BCL-XL (classified as oncogenes) are downstream effectors of STAT3 (81). Administration of cell-permeable CAV-1 prevented the development of PH in the MCT rat model and SOD2 (possibly a tumor suppressor), was found to be lowered in PAECs from lambs with increased PBF (82–84). Archer et al. described a similar result in PASMC from fawn hooded rats with the addition that correction of SOD2 levels had a therapeutic effect (85).

**SHEAR STIMULATED ANGIOGENESIS AND GROWTH FACTOR SIGNALING IN PH**

In cancers, neovascularization is driven by angiogenesis due to activation of the “angiogenic switch” (22). Disordered or misguided pro-angiogenic signaling is a key process in severe PAH and potent growth factors, such as VEGF, FGF, platelet derived growth factor (PDGF), TGF-β, angiopoietins and BMPs, contribute (59,60,86–89).

Increased levels of VEGF and VEGFR-2 are found in the plexiform lesions of PAH patients or patients with congenital heart disease (90,91). Li et al. showed increased VEGF expression in PAEC under various shear stress conditions, which was mentioned as being associated with angiogenesis (92). Paradoxically, blocking VEGFR (combined with a second hit) causes PAH in the SuHx model, which has been described as the “angiogenesis paradox” (93). FGF2 increases due to shear and FGF2 levels are increased in the lungs from PAH patients contributing to the progression of PAH (60). PDGFR-B transcription in PAEC is increased due to mechanical stretch, which was confirmed in the MCT model (94). Additionally, Perros et al. showed that PDGF and PDGFR mRNA is over-expressed in the pulmonary arteries of PAH patients (95). A SSRE for PDGF-B has been identified in BAECs, but to date this has not been confirmed for the pulmonary endothelium (24). Increased mRNA and protein levels of angiopoietin-2 (Ang-2) in the serum of PAH patients are linked to disease progression, EC damage and SMC proliferation (96). Blocking of the angiotensin II receptor I (ATR1) via Losartan was shown to be beneficial in experimental PH, due to the inhibition of the renin-angiotensin-aldosterone (RAAS) system (97). Using systemic ECs, ATR1 was also shown to be a shear stress dependent activator of ERK1/2 in ECs (98).
INVASION, MIGRATION AND METASTASIS

A critical hallmark of cancer is the formation of new tumors in other organs by primary cells via invasion, which is termed metastasis. The newly formed metastases arise as a mixture of cancer cells and benign cells of the host tissues (21,22). Actual metastasis and/or invasion of cells have not been reported in the context of PAH.

Endothelial progenitor cells (EPC) are specialized cells that are implied in vascular repair, specifically re-establishing the ECs monolayer after damage. EPC differentiation into mature ECs can be achieved by laminar shear stress, and EPCs are linked to cancer metastasis (99,100). The involvement of EPCs in PAH has been proposed in the early 1990s and evidence is accumulating on the participation of EPCs in PH and Eisenmenger patients (101–105). While discussed here in the context of invasion, migration and metastasis, EPCs are also postulated to drive angio-proliferative PAH (106). Reports have been made on the effects of shear stress on EPC proliferation and differentiation, speculating that links may exist but to date none have been investigated or reported (107,108). Besides EPCs, other cell types involved in metastasis and invasion such as pericytes and fibrocytes have been linked to PAH, but not to shear stress (109–112). Endothelial-to-mesenchymal transition (EndoMT), likely a key process leading to pulmonary vascular remodeling, is stimulated by TGF-β signaling but inhibited by BMPR2 signaling. Recent studies have shown data in support of EndoMT in PAH and the inhibition of EndoMT appears to be beneficial in animal models (113–115). EndoMT is modulated by shear stress, in the systemic circulation in vitro, in an ERK5 dependent manner (116). In this context pericytes are of interest as they are located specifically around capillaries, where their coverage increases in human PAH and give rise to SMC-like cells and directly contribute to the vascular remodeling of distal pulmonary arteries (109)

REPROGRAMMING OF ENERGY METABOLISM

In cancer cells the reprogramming of energy metabolism is mainly due to the upregulation of GLUT1, the receptor responsible for the uptake of glucose into the cytoplasm (117,118). Otto Warburg observed that neoplastic cells change their energy metabolism under aerobic conditions from a mitochondria-dependent metabolism to glycolysis even when there is sufficient oxygen. This phenomenon is known as the Warburg effect and coincides with mitochondrial fragmentation, which are observed in cancer (119–122). Evidence for deregulated mitochondrial metabolism in PAH has been found in both the lung and right ventricle (RV) (123–125). PET scans using the glucose analog tracer (18F)fluoro-deoxy-D-glucose showed a higher lung standardized uptake of IPAH patients and the MCT rat model compared to controls, although results in PAH patients are mixed (126).

Although, direct effects of shear stress effects on cell metabolism have been reported, few direct links have been established between metabolism and PAH (127,128). Mitochondria and carnitine homeostasis were found to be deregulated in a lamb model for increased pulmonary blood and associated with decreased mitochondrial function and disrupted bioenergetics (129,130). This deregulation was associated with asymmetrical
dimethylarginine mediated redistribution of eNOS to the mitochondria. Dimethylarginine deregulation has been observed in hypoxia-induced PH and patients with high PBF due to left-to-right shunt (131,132). A link between CAV-1 and metabolic switching has been described in a CAV-1 null mouse model, where CAV-1 was suggested to directly affect mitochondrial oxidative metabolism (133). An additional role for abnormal shear stress affecting EC metabolism via Krüppel-like-factor 2 (KLF2) has also been suggested (134,135). Although the role of KLF2 in PAH is unclear, it has been shown to be affected in several animal models of PH (136). Also, the recent “metabolic theory” of PAH defines the metabolic alterations as a consequence of the phenotypic, cancer-like cellular changes defining mitochondria as common integrators and thereby interesting treatment targets in the pathogenesis of PAH (137).

**TUMOR-PROMOTING INFLAMMATION**

Besides the established role of the immune system to permanently recognize and eliminate the vast majority of primary cancer cells that form tumors, new evidence shows a contributing role of the immune system to the development as well as the maintenance of cancer (138,139). The role of inflammation in PAH, both in vascular remodeling and disease susceptibility in the context of PAH is well acknowledged but not well understood (2,140).

While direct links between shear stress and inflammation are few in the context of PH, it is well accepted that endothelial damage, due to altered shear stress, initiates an inflammatory response within the pulmonary vasculature (141). Laminar shear stress is protective in the systemic circulation as lesions are predominantly found at areas where PBF is abnormal, such as curvatures and branch points. Shear directly activates shear-responsive genes, among which are nuclear factor-kappa B (NFκB), an important inflammation related transcription factor (24,142). Further, it is known that PBF can influence leukocyte adhesion in the pulmonary vasculature (143). Additionally, the occurrence of non-uniform and turbulent flow is suggested to be involved in the formation of characteristic plexiform lesions found distally of bifurcations and branching points (7, 144). PBF affects polymorphonuclear leukocytes accumulation in dog lungs (145). With regard to the systemic circulation the link between shear stress, increased flow and inflammation is a well-researched topic, whereby data may be translatable to the lung vasculature (142,146,147). As anti-inflammatory treatments have yielded some success, this perspective might give additional opportunities to target disease development (2)

**DISCUSSION AND PERSPECTIVES**

In this review we discussed that an altered PBF can account or synergize with the majority of cancer-like features observed in PAH (Figure 1). Current established therapies for PAH consist of vasodilatory treatment, but cannot reverse the vascular changes. While more specific therapies aimed at targeting other aspects of the disease appear to be
Figure 1: Processes that link quasi-malignant events in PAH to an abnormal pulmonary blood flow.

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<th>Hallmark</th>
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<td>NO bioavailability</td>
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<td>Reactive oxygen species</td>
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<td>Growth differentiation marker 15</td>
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<td>Early growth response protein 1</td>
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<td><strong>Invasion, migration and metastasis</strong></td>
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<td><strong>Reprogramming of energy metabolism</strong></td>
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<td><strong>Tumor-Promoting Inflammation</strong></td>
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beneficial in animal models, the translation to the clinic has been rather unsuccessful so far (14,148). The most promising treatment option Imatinib, successful in the treatment of acute myeloid leukemia, failed in PAH treatment trials (11). While the therapeutic potential of TKIs is recognized, the possible side-effects are considerable (14,149–151).

Here we summarize the reported effects of an abnormal PBF flow and describe its role as an initiating and maintaining factor in pulmonary hypertension. Many different triggers can lead to an abnormal flow thereby causing vascular remodeling. Within a “two-hit hypothesis”, this can imply that one hit (e.g. high PBF/high shear) needs to coincide with an additional hit (e.g. a genetic predisposition, environmental factors) for PAH and characteristic lesions to develop (152). Recently, shear by itself has been postulated to cause endothelial cell mutation, thereby introducing a second genetic hit (8,153). Consequently, interventions aimed at restoring normal flow and appropriate vascular response to increased flow should be considered. Although not formally proven, PH can be alleviated after shunt repair surgery (154,155). Also, two case reports described the reversal of PAH in the native lung after single lung transplantation (156,157). In the near future, it will be of importance to investigate the role of flow as a maintenance factor in PAH.

A subgroup of patients characterized by the occlusion of pulmonary vessels by large or several smaller thrombi termed chronic-thromboembolic-pulmonary hypertension (CTEPH) might be able to provide valuable insights in the role of shear stress. CTEPH is characterized by the occlusion of vessels by large or several smaller thrombi. Due to the reduction in number of patent vessels, flow is increased in the remaining non-occluded vessels, which causes the remodeling of those vessels. In some cases CTEPH can be surgically alleviated via pulmonary endarterectomy (PEA) or less invasively via balloon pulmonary angioplasty (BPA) thus normalizing the blood flow (158,159). It will be of interest to assess flow and shear dynamics after PEA over time. If increased flow is responsible for the maintenance of vascular abnormalities, a reversal of remodeling should be observable after PEA or BPA.

Another interesting perspective is the recent discovery of the involvement of microRNAs (miRs) in flow-dependent vascular remodeling and apoptosis (160). Currently, several miRs have been linked to PH but direct links with shear stress are few. The miR-21 is flow regulated and has been linked to PAH (161). White et al. reported that the programmed cell death 4/saspase-3 axis was shown to be miR-21 responsive, and of particular interest in disease onset (162). Also, interestingly, BMPR-II has been linked to miR-21 in various forms of cancer and miR-21 is down-regulated in IPAH patient lung tissue and serum (163,164). Recently, miR-27b has also been linked to PAH secondary to CHD. Ma and colleagues linked the upregulation of miR-27b with decreased NOTCH-1 signaling (165). The miR-17-92 is known to be involved in BMPR-II expression and regulated via STAT3 and interleukin-6, this cluster has been shown to be shear stress inducible (160).
SUMMARY AND CONCLUSION

For decades the concepts of the pathogenesis of severe PAH were solely based on vasoconstriction, the consequent increase in mechanical forces and their impact on pulmonary resistance vessels. However, increased blood pressures and flow alone left many pulmonary vascular diseases mechanistically unexplained. In particular the flow only models of chronic hypoxic vasoconstriction and pulmonary shunt could not satisfactorily explain the phenomenon of pulmonary angio-obliterative disease, but only resemble parts of the vasculopathy.

Once the number of pulmonary arterioles has been reduced and the resistance to blood flow proximal to vascular obstructions has increased it is probable that shear stress contributes to the maintenance and progression of the lung vascular structural abnormalities. Thereby emphasizing that shear stress is one of the triggers leading to the evolution of a quasi-malignant vascular pathobiology.

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REFERENCES


20. R. Szulcek, C.M. Happe, N. Rol, R.D. Fontijn, C. Dickhoff, K.J. Hartemink, et al., Delayed microvascular shear-adaptation in pulmonary arterial hypertension: Role


42. L. Taraseviciene-Stewart, Y. Kasahara, L. Alger, P. Hirth, G.M. Mahon, J. Waltenberger, et al., Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell


Receptor gamma (PPARγ) expression is decreased in pulmonary hypertension and affects endothelial cell growth, Circ. Res. 92 (2003) 1162–1169.


98. B. Ramkhelawon, D. Rivas, S. Lehoux, Shear stress activates extracellular signal-regulated kinase 1/2 via the angiotensin II type 1 receptor, FASEB J. 27 (2013) 3008–3016.


