Review
Gene therapies for pulmonary hypertension—from experimental trials to bedside aspects
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Summary
Accompanying the continuously deepening understanding of the mechanism of pulmonary hypertension that many genes are found to be aetiologically involved in its development, burgeoning literature manifest that gene therapies aimed at correcting these genetic defects have the ability to restore deficient pulmonary gene expression, over-express biologically active gene products, reverse established disease and regenerate pulmonary vasculature, and may constitute a promising therapeutic strategy for pulmonary hypertension. Therefore, to provide new information to basic scientists and clinical investigators, we present a review that attempts a clear description of the therapeutic potential of gene therapy in the treatment of pulmonary hypertension.

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Keywords: Pulmonary hypertension; Gene transfer; Gene therapy

1. Introduction
Pulmonary hypertension (PH), characterised by a marked and sustained elevation of pulmonary vascular resistance (PVR), is a fatal and refractory disease with consequent progressive right ventricular failure and death [1]. It has been recognised that abnormality of endothelial cells (ECs) function is an early feature of the disease [2,3] and endothelial dysfunction is characterised by an imbalance between vasodilator and vasoconstrictor factors, that is, a decrement in the production of vasodilators, such as prostacyclin and nitric oxide, and concomitantly an increment in that of vasoconstrictors, including thromboxane and endothelin-1 [4,5]. Thus, restoring the homeostasis between pulmonary endothelium-derived vasodilators and vasoconstrictors has been the basis of the rapid development of drugs including prostacyclin and endothelin receptor antagonists; however, a large number of patients are refractory to these agents and continue to be severely debilitated, with some ultimately progressing to heart—lung or lung transplantation [6,7]. Moreover, the use of vasodilator agents in the management of PH is hindered by either undesired systemic side effects or inconvenient drug administration routes [4,8—11]. These potential demerits have limited their widespread application in clinical settings. Therefore, developing a method for delivery of vasodilator agents directly to the pulmonary vascular bed is desirable for the treatment of PH.

While in its advanced stage, changes characterised by widespread arteriolar pruning and loss of pulmonary microcirculation [4,12] reduce the capacity of the peripheral pulmonary vascular beds. Unfortunately, most patients are not diagnosed until late in the course of the disease; for this reason, therapeutic approaches capable of rescuing vascular structural changes and regenerating pulmonary microvasculature are needed to restore pulmonary haemodynamics in advanced PH. Consequently, there is now a shift in the interest of the scientific community, focussing on therapies aiming to reverse the proliferative remodelling in PH [13].

A growing body of studies show that the pathogenesis of primary pulmonary hypertension (PPH) appears to involve a group of susceptible genes, including bone morphogenic protein receptor type II (BMPR-II) [14] and vasoactive intestinal peptide [15]. Furthermore, a signature set of 106 genes, which discriminated between patients with PH and normal individuals with high certainty, was identified [16], which makes the importance of genetic predisposition in the development of PH increasingly clear. Simultaneously, literature indicates that gene transfer into lungs, aiming at correcting the deficiency or mutation of these genes, opens a new window for the treatment of this devastating disease. So far, literatures documented great progress [11,17—23] mostly gene transfer into lungs has been made in this field, but problems still exist. Therefore, the various gene...
therapies as well as the problems encountered are described in this review.

1.1. eNOS gene therapy

Endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO), which could inhibit platelet aggregation and proliferation of vascular smooth muscle cells (VSMCs), is believed to play an important role in maintaining pulmonary arterial pressure (PAP) and PVR at normal physiological levels [18]. However, the role of eNOS in the pathogenesis of chronic PH remains controversial. In the lungs of patients with PH, eNOS mRNA and protein levels are decreased and correlated inversely with the severity of the pulmonic arteriopathy [24], while other studies contradictorily demonstrated that pulmonary vascular eNOS levels [25] and the expression of NOS3 in plexiform lesions [26] increased in patients with PPH, as well as in mice with severe hypoxia-induced PH [27]. In contrast, lungs from pre-term foetal eNOS/−/− pups revealed abnormalities in the microvascular structure, such as discontinuity of the distal arteriolar circulation, which are very similar to those of MCT-induced PH, suggesting that eNOS may play a critical role in pulmonary vascular development during lung morphogenesis [28]. Furthermore, in genetic models, it has been shown that targeted deletion of the eNOS gene results in increases in right ventricular pressure (RVP), PAP, right ventricular hypertrophy (RVH) and the proportion of muscularised small pulmonary vessels in mice after chronic hypoxic exposure [18,29], while in vivo gene transfer of eNOS to the lung in large part can correct the genetic deficiency resulting from eNOS deletion [17]. However, other investigators found a decrease in pulmonary arterial muscularisation early during the course of hypoxic exposure in eNOS-deficient mice [30].

Although the apparent discrepancy with regard to eNOS levels in some forms of pulmonary hypertension, it is generally believed that pathophysiological conditions of eNOS that reduce NO production have contributed to the course of PH and therapeutic strategies aimed at increasing NO availability in the lung may be beneficial in the management of PH. Over-production of eNOS-derived NO in eNOS-Tg mice can inhibit not only the increase in right ventricular systolic pressure (RVSP) associated with PH but also remodelling of the pulmonary vasculature and RVH induced by chronic hypoxia [18], while aerosolisation of AdCMVceNOS into rat lung reduced the rise in PAP, total pulmonary resistance (TPR) and hypoxic pulmonary vasoconstriction (HPV) during acute hypoxia [31]. Together, these literatures imply that up-regulation of eNOS or an increase of endogenous NO production can serve as useful therapeutic interventions for the treatment of hypoxia-induced pulmonary hypertensive disorders where eNOS activity reduced.

In MCT-treated mice, cell-based eNOS gene transfer significantly reduced PAP, inhibited RVSP progression, decreased the RV/body weight ratio and the RV/LV ratio and prolonged the survival time [20,32]. Moreover, fluorescent microangiography revealed that cell-based eNOS gene transfer reduced arteriolar muscularisation, regenerated pre-capillary arteriolar continuity, re-established the alveolar capillary perfusion, restored a more normal pattern of pulmonary microvasculature and alveolar capillary perfusion in reversal model [20,22]. Consequently, eNOS gene transfer into lungs aiming at restoring deficient pulmonary NOS gene expression or over-expressing the biologically endogenous NO may constitute a new approach to alleviate these pulmonary disorders.

1.2. CGRP gene therapy

Calcitonin-gene-related peptide (CGRP) is a 37-amino-acid endogenous peptide formed as an alternative splice product of the calcitonin gene [33], with many beneficial cardiovascular actions including potent vasodilator and anti-proliferative effects on VSMCs [34,35]. In the cardiovascular system, CGRP-containing nerves are located throughout the heart and lung and surround most arteries [36,37], while CGRP receptors are highly expressed on lung VSMCs [38]. In hypoxic rats, hypoxia-induced PH correlates well with declining blood CGRP levels and reduced bioavailability [39,40]; consistently, blockade of CGRP receptors [41], immunoneutralisation of CGRP receptors [41], depletion of CGRP from sensory nerves [42], targeted blocking of gene expressing for CGRP receptors [43] exacerbate the increase in PAP and right ventricular mass (RVM), whereas intravenous administration of exogenous CGRP has been shown to have a beneficial reversal effect in PH [41,44], suggesting that CGRP plays an important role in regulating pulmonary vascular tone under normal and pathophysiological conditions.

Studies showed that CGRP-over-expressing pulmonary artery smooth muscle cells (PASMCs) [35] inhibit proliferation in PASMCs, and CGRP-secreting modified mesenchymal stem cells (MSCs) inhibit VSMCs proliferation [45], which demonstrate the clinical potential of cell-based CGRP gene therapy for PH. Intratracheal injection of Adprepro-CGRP into the lungs of mice attenuates the increase in PVR and RVM, pulmonary vascular remodelling and pressor responses without altering systemic pressure in hypoxia-induced PH [11,46]. Similarly, intravenously administered CGRP-expressing endothelial progenitor cells (EPCs) effectively attenuate established PH and exert reversal effects on pulmonary vascular remodelling [22]. Interestingly, transfer of an adenoviral vector encoding prepro-CGRP into the lungs of CGRP knockout mice resulted into a similarly beneficial outcome [47]. These results suggest that, as the most potent endogenous vasodilatory peptide discovered so far, gene transfer of CGRP may represent a new strategy for PH.

1.3. VEGF gene therapy

Vascular endothelial growth factor (VEGF) is a peptide mitogen specific for ECs and fulfills its function by binding with high affinity to two highly specific tyrosine kinase receptors (flt-1 and KDR/flk-1) expressed almost exclusively on ECs [48]. VEGF acts as a potent survival factor for ECs by inducing proliferation, sprouting, migration and tube formation of ECs [49] and also plays a critical role in the regulation of vasculogenesis and angiogenesis [50], which could be verified by studies that homozygous VEGF knockout mice die at embryonic day 8–9 (E8–E9) from defects in blood island formation, ECs development and vascular formation [51]. Moreover, studies showed that increasing VEGF bioavail-
ability at the sites of endothelial injury accelerated endothelial repair, limited neointima formation [52,53] and also improved endothelium-dependent vasodilatation and blunted vasoreactivity in lungs from chronically hypoxic rats [54].

Increased VEGF expression has been found in association with plexiform lesions in pulmonary tissue from patients with PPH [55]; however, results obtained from the MCT [56,57] and hypoxia [58—60] models demonstrated controversial alterations of VEGF and VEGF receptor expression in lungs. Therefore, so far, there is no consensus on the significance of its changes of VEGF and VEGF receptor expression in PH lungs.

Nevertheless, recent data have shown that SU5416, a reportedly selective antagonist at VEGFR-2 receptors (which binds VEGF-A but not VEGF-B), exacerbates the pulmonary vascular remodelling and PH seen in hypoxic rats, and even causes the development of PH in normoxic rats [2]. A similar trend was attained by treatment with a monoclonal antibody to VEGF-A [61,62]. While adenovirus-mediated over-expression of VEGF-A in rat lungs attenuated the rise in PAP and the pulmonary vascular remodelling induced by chronic hypoxia [54], and in MCT-PH rats, cell-based gene transfer of VEGF-A prevented the development and progression of PH [21,63], but demonstrated no significant improvement in the muscularisation of small vessels [21]. Collectively, findings from these studies imply that VEGF-A may have a protective effect against pulmonary vascular remodelling induced by hypoxia or MCT except for its limitations on the reversal of established muscularisation of small vessels.

1.4. PGIS gene therapy

Prostacyclin, a major arachidonic acid metabolite in the vascular wall, has vasoprotective effects, including vasodilatation, anti-platelet aggregation [64] and inhibition of PASMCs proliferation in vitro [65,66]. The final committed step in the synthesis of prostacyclin, the conversion of prostaglandin H2 to prostacyclin, is catalysed by the membrane-bound enzyme prostacyclin synthase (PGIS), which is constitutively expressed in vascular endothelial and smooth muscle cells [67].

PGIS deficiency and impaired prostacyclin production in the lungs have been linked to the development of PH [68,69]. Studies showed significantly decreased production of prostacyclin relative to that of thromboxane [68] and also particularly reduced the expression of PGIS in remodelled pulmonary arteries containing plexiform lesions in patients with PPH [69]. Moreover, PGIS over-expression in transgenic mice protects against the development of hypoxic PH [70]. These findings raise the possibility that impaired prostacyclin synthesis resulting from decreased PGIS may be implicated in the pathogenesis of PH.

In fact, continuous intravenous infusion of prostacyclin has markedly improved the hemodynamics, exercise tolerance and survival span, beyond those attained with conventional therapy alone, in patients with PPH [71—73]. However, the short half-life of the drug, as well as the requirement for a continuous infusion system and catheter-related complications, has limited the use of this treatment fashion [74,75].

Considerable evidence indicates that PGIS gene transfer is a promising approach for the stable production of endogenous PG12, and has the potential to ameliorate progressive PH. Nagaya et al. showed that trans-tracheal gene transfection with PGIS augmented PGIS, attenuated the increase in mPAP, TPR and medial wall thickness of peripheral pulmonary arteries that resulted from MCT injection, and thereby improved survival in MCT rats [76]; while Suhara et al. documented that PGIS gene transfection to the liver could ameliorate MCT-induced PH [77], but the histological improvement of the medial wall thickness was minimum. Subsequently, they introduced a new strategy using co-transfection of HGF and PGIS [78], which showed significant improvement in medial hypertrophy of pulmonary arteries, indicating that co-transfection of HGF and PGIS may be a useful strategy for severe PH. Interestingly, PGIS gene transfer into skeletal muscle not only significantly lowered PVR and RVSP, notably suppressed medial thickening of pulmonary arteries and RVH, but also effectively improved the prognosis of MCT-induced PH rats [79,80] and hypoxia-induced PH rats [81]. Thus, PGIS gene therapy provides a better delivery system of endogenous prostacyclin for preventing PH.

1.5. AM gene therapy

Adrenomedullin (AM) is a potent, relatively long-lasting vasodilator peptide originally isolated from human pheochromocytoma [82] and has the ability to induce tube formation in HUVECs, drive sprouting in porcine pulmonary arterial endothelial cells and promote neovessel formation in a mouse Matrigel plug assay [83]. In knockout mice, AM is indispensable for vascular morphogenesis during embryonic development [84], while targeted null mutation of the AM gene is lethal in utero, with extreme hydrops foetalis and cardiovascular abnormalities [84—86]. These results imply that AM is a candidate for therapeutic angiogenesis or vasculogenesis.

In pulmonary vasculature, there are abundant binding sites for AM [87], and human AM messenger ribonucleic acid (mRNA) [88] as well as the mRNA for AM receptor [89] is also highly expressed in lung tissue. Moreover, the concentration of AM in the lung is several-fold higher than that in the cardiac ventricle and the kidney [90]. Studies also have shown that plasma AM level is elevated in humans with primary [91,92] and secondary PH [93], and the plasma AM level increases in proportion to the severity of PH [91,94].

Additional supplementation of AM through chronic intravenous infusion [95,96] or inhalation [97—99] has beneficial effects on PH, such as decrement in PAP, reduction of RVH and inhibition of the increment in the medial wall thickness of peripheral pulmonary arteries. These results suggest that AM plays an important role in the regulation of pulmonary vascular tone, vascular remodelling and also implies that endogenous AM level in PH is insufficient to improve this deteriorated condition.

Transplantation of AM-expressing EPCs caused a marked decrease in mPAP, PVR, significantly greater improvement in pulmonary vascular remodelling and better survival in MCT
mice than transplantation of EPCs alone. Thus, the novel hybrid cell-gene therapy based on the properties to phagocytose DNA–gelatin complexes, the capability to sense injured tissues and the ability to migrate to sites of injured endothelium of EPCs may be a new therapeutic strategy for PH [100].

1.6. BMPR-II gene therapy

Heterozygous mutations in BMPR-II, a member of the transforming growth factor superfamily of receptors, have been identified to underlie many cases of familial and sporadic PPH [101—103]. In addition to these mutations, marked reduction of BMPR-II expression in the lung was also observed in patients with PPH in whom no mutation was identified in the BMPR-II gene and also among patients with secondary PH [104]. Furthermore, the expression of BMPR-1A was found to be reduced in pulmonary arteriolar ECs derived from non-familial PH [105]. These observations suggest that BMP signalling pathways may be implicated in the molecular pathogenesis of secondary PH as well as familial and sporadic PPH.

Homozygous BMPR-II mutant (BMPR-II+/−) mice have been shown to die in utero before mesoderm formation, while heterozygous BMPR-II mutant (BMPR-II+/−) mice, by contrast, are reported to be morphologically normal and fertile [106], but the pulmonary vascular remodelling response to prolonged hypoxic breathing was impaired in BMPR-II+/− mice [107]. Transgenic mice expressing a dominant-negative BMPR-II gene in smooth muscle have a significant increase of RVSP and relatively modest pulmonary vascular remodelling [108]. Although BMPR-II protein was abundantly expressed in the vascular wall of pulmonary arteries in the rats under normal conditions, a significant down-regulation of BMPR-II in the pulmonary vasculature was observed after prolonged exposure to hypoxia, so disruption of BMP signalling pathway through down-regulation of BMPR-II in chronic hypoxia may result in pulmonary vascular remodelling [109].

Targeted delivery of adenoviral vectors containing the BMPR-II gene to the pulmonary vascular endothelium of rats substantially reduced the pulmonary hypertensive response to chronic hypoxia, providing further evidence for a role for BMPR-II in PH and a rationale for the development of therapies aimed at improving BMPR-II signalling [110]. Nebulised intra-tracheal adenoviral gene therapy with hBMPR-II reliably distributed hBMPR-II to resistance pulmonary arterioles but did not ameliorate PH [111]. The failure of nebulised BMPR-II gene therapy in the MCT model suggests that depressed BMPR-II expression may be a marker of PH but is not central to the pathogenesis of this model of PH.

1.7. HGF gene therapy

Hepatocyte growth factor (HGF) was originally purified and cloned as a potent mitogen for hepatocytes [112]. Besides its mitogenic, motogenic and morphogenic activities in various cell types [113], accumulating data showed that HGF has a pulmotrophic effect on the regeneration and protection of the lungs [114], for instance, HGF plays a role in lung regeneration and protection from acute lung injuries [115,116]; while exogenous HGF administered in either a simultaneous or delayed fashion prevents the progression of bleomycin-induced chronic lung injury [117], suggesting that HGF may be a potent candidate to prevent or treat lung fibrosis in lung injuries.

HGF can not only induce VEGF production by a variety of cells and tissues [118—120], but also in vitro and in vivo act synergistically with VEGF to enhance VEGF-induced angiogenesis [121]. In contrast, HGF exclusively stimulates the growth of ECs without replication of VSMCs [122], but mediates angiotropoietin-induced smooth muscle cells recruitment in vascular maturation [123]. Moreover, HGF potently enhances EC barrier integrity in pulmonary vasculature, which is a critical feature of angiogenesis [124]. Local administration of HGF was shown to accelerate re-endothelialisation and attenuate neointimal proliferation [125]. In MCT rats, endogenous HGF production was dramatically down-regulated during the development of experimental PH, but c-met/HGF receptor was abundant in the medial layers of pulmonary arterioles, thus the deficiency of endogenous HGF may be a feature of the pathogenesis of PH [126], and studies showed that exogenous HGF supplementation may both minimise pathological lung conditions, even advanced PH [127,128] and notably reduce lung expression levels of endothelin-1 and transforming growth factor-β, which are critically involved in PH-linked fibrogenic events [126,129].

On the basis of above findings, potent angiogenic effect of HGF, as well as its anti-fibrotic function, EC barrier protection property, is considered likely to be useful in cases of decreased pulmonary vasculature such as PH, or hypoplastic pulmonary vasculature of congenital heart disease. Ono et al. showed the arterial transfection of HGF gene resulted in predominant expression in the pulmonary ECs, and the preferential expression of HGF gene in ECs of the pulmonary arterioles, thus the deficiency of endogenous HGF may be a feature of the pathogenesis of PH [126], and studies showed that exogenous HGF supplementation may both minimise pathological lung conditions, even advanced PH [127,128] and notably reduce lung expression levels of endothelin-1 and transforming growth factor-β, which are critically involved in PH-linked fibrogenic events [126,129]. Under normoxic condition, trans-pulmonary arterial transfer of the human HGF gene into the left lung increased capillary density and blood perfusion, and decreased PVR when blood flow increased, which suggested the therapeutic effect of HGF gene expression on treating subjects with decreased pulmonary vasculature or increased PVR [23]. Surprisingly in MCT-induced PH of rats, HGF gene transfection almost completely prevented medial wall thickening of pulmonary arteries and decreased the total collagen deposition in the lung of MCT rats [126], while similar results were obtained from trans-arterial HGF gene transfection to the lung of mouse using HVJ liposome method [78]. Furthermore, except for attenuating the medial hypertrophy of pulmonary arteries, in vivo gene transfection with HGF gene to the liver enhanced the ameliorating effects of gene transfection of PGIS in MCT-induced PH of rats [78]. Collectively, the potential therapeutic value of HGF gene therapy may be a promising strategy for the treatment of patients with severe PH.

1.8. Kv gene therapy

Resistance pulmonary arteries, preferentially expressing O2-sensitive Kv channels, have a virtual monopoly on the functional expression of O2-sensitive K channels, specifically Kv1.5 and, to a lesser extent, Kv2.1 [130], which account for
largely increases cytosolic Ca\(^{2+}\) levels ([Ca\(^{2+}\)\(_{\text{cyt}}\)]. Subsequently, the rise in [Ca\(^{2+}\)\(_{\text{cyt}}\)] triggers both the HPV [136] and PAMSC growth and proliferation [139], which are characteristics of hypoxic PH. Thus, decreased transcription and functional inhibition of K\(_{\text{v}}\) channels may play a pivotal role in the hypertrophy of small pulmonary arteries and muscularisation of pulmonary arterioles (PAs).

Michelakis et al. showed that restoration of K\(_{\text{v}}\) channel expression in PH by direct K\(_{\text{v}}\) gene transfer using an adenovirus (AdS-K\(_{2.1}\)) is feasible and might be beneficial [140]. While administration of K\(_{1.5}\) to the pulmonary circulation through an aerosol is also feasible and effective in eliciting transgene expression in resistance pulmonary artery smooth muscle cells, which not only restores HPV and O\(_2\)-sensitive K\(_{\text{v}}\) but also reduces PVR in rats with established CH-PHT [134]. Thus, the concept that a K\(_{\text{v}}\) channel-deficiency state is involved in the pathogenesis of PH [5,136] is now supported by the findings that augmentation of K\(_{\text{v}}\) channel expression by gene transfer [134] or dichloroacetate [138,140] reduces PH.

1.9 ANP gene therapy

Atrial natriuretic peptide (ANP) is stored within atrial secretory granules and is periodically released from these granules in response to the increase in atrial wall stretch or pressure [141]. Several evidences demonstrated ANP anti-proliferative and anti-hypertrophic effects on VSMCs [142], inhibition of the production of endothelin from cultured ECs [143] and anti-fibrogenic role in the pulmonary vascular adaptation to chronic hypoxia [144], implying the potential value of this peptide in the therapeutic application in PH; similarly, literature also show that ANP is a selective relaxant of the pulmonary arteries and counters the pathogenic mechanisms involved in pulmonary vasoconstriction and hypertension [145]. Clinical studies found that plasma ANP level was significantly higher in patients with PPH or chronic respiratory disease when PH was present, and ANP level correlated to mPAP, RVEDP and TPR [146,147]. Chronic hypoxia-exposure-induced PH, RVH, muscularisation of distal pulmonary arterioles, expression of extracellular matrix (ECM) and parenchymal remodelling are exacerbated in ANP null mice [144], suggesting that endogenous ANP plays an important role in regulating PAP, ECM production and pulmonary vascular remodelling in response to hypoxic stress.

Intravenous infusion of ANP blunts acute HPV [148] and decreases PAP and PVR during acute hypoxia without affecting systemic circulation in conscious animals [149], while continuous infusion of exogenous ANP mitigates the development of PH, RVH and pulmonary vascular remodelling during chronic hypoxia [150]. In contrast, transgenic mice that over-express ANP in heart develop less RVH and pulmonary vascular remodelling than non-transgenic control mice to chronic hypoxic exposure [151]. Consistent with these studies, disruption of ANP-NPR-A signalling by neutralisation of endogenous ANP with monoclonal antibodies [148,152] or targeted disruption of the genes for ANP [153,154] or NPR-A [155—157] develop more severe PH and RVH. Together, these findings strongly provide further evidences that ANP/NPR-A signalling plays an important role in modulating pulmonary vascular tone, pulmonary vascular remodelling and protecting against the development of PH in response to hypoxia.

A growing number of investigations on NPR-C in the pulmonary vascular bed, which plays an important role in ANP clearance, provide further evidence that ANP plays a role in modulating the pulmonary vascular response to hypoxia. The half-life of ANP in the circulation is two-thirds longer in mice with homologous deletion of the NPR-C gene compared with that in wild-type mice [158], while administration of ANP(4-23), a selective NPR-C ligand, delays the metabolism of ANP and attenuates hypoxia-induced PH in the rat [159]. Furthermore, down-regulation of NPR-C likely represents an adaptation aimed at retarding ANP clearance from the circulation, thus enhancing the biological effects of ANP and mitigating the severity of hypoxia-induced PH [154].

Interestingly, Louzier et al. demonstrated that adenovirus-mediated ANP over-expression in lungs protects rats against the chronic hypoxia-induced PH, distal pulmonary arteriole muscularisation and RVH compared with control Ad.Gal-treated rats [160]. These results suggest that induction of ANP expression in the lung may hold promise in the treatment of PH.

1.10 Ang-1, Tie-2 gene therapy

Angiopoietin 1 (Ang-1) is a muscle-secreted ligand that plays a critical role in vasculogenesis and is an important modulator of both physiological and pathological angiogenesis [161]. Ang-1 activity is modulated by the endothelial-specific receptor Tie-2 [162]. Ang-1 regulates endothelial-cell recruitment of muscle cells to encase and stabilise primitive endothelial tubes [163], thus mice lacking Ang-1 or Tie-2 die in utero from defects in vascular development, including severe vascular abnormality of the lungs: [164] ECs were arrayed into tubes, however these vessels lacked muscular investment, had few branches and displayed minimal graduation in size. In addition, observations in humans have revealed that venous malformations are associated with mutations in Tie-2 [165], which implies that
Ang-1 stabilises vessel development by stimulating muscle cells to proliferate around nascent endothelial tubes and plays critical roles in creating mature arterial structures.

Sullivan et al. found that Ang-1 is constitutively expressed in the lungs of patients with different aetiologies of PH and is absent in normal adult lung tissue [105], indicating that Ang-1 correlated with the pulmonary hypertensive phenotype. Later, their studies show that Ang-1 plays a causal role in the disease process: rats engineered to express angiopoietin 1 constitutively in the lung develop severe PH and that Ang-1/Tie-2 serotonin cross-talk between vascular ECs and VSMCs is the molecular mechanism responsible for the generation of PH [166].

Gene transfer of a Tie-2 receptor antagonist mediated by adeno-associated virus containing an extracellular fragment of the Tie-2 receptor (AAV-sTie-2) into the pulmonary artery blocks the interaction between Ang-1 and its endothelial receptor (Tie-2) in the lung, which in turn prevents PH in MCT and Ang-1 groups [167]. However, another report suggests that Ang-1 may play a protective role in the pathogenesis of experimental PH. Cell-based gene transfer of Ang-1 to the pulmonary microvasculature effectively inhibits MCT-induced PH, simultaneously reduces the down-regulation of Tie-2 expression and MCT-induced ECs apoptosis, suggesting that the Ang-1/Tie-2 system is important in the protection of the pulmonary endothelium against MCT-mediated injury and that Ang-1 gene therapy may be useful in the treatment of PH [168]. So the different results increase the controversy regarding the role of Ang-1 in PH. In order to better interpret the observational data derived from animal experiments, it is now necessary to evaluate the in vivo effects of the angiopoietin pathway in this disease, and then further understanding of the role of Ang-1 in the pulmonary artery tree may reveal clues for treating PH and modulating vascular wall biology in this organ system.

1.11. Survivin gene therapy

Recently, several abnormalities that have been described in PH contribute to a resistance to apoptosis and a proliferation/apoptosis imbalance within the vascular wall and might explain the PA remodelling, for instance, bcl-2, a mediator of apoptosis in the PA wall that favours suppression of apoptosis, is up-regulated in PH [169], while activation of the BMPRII pathway leads to suppression of proliferation and activation of apoptosis in normal PASMCS [170], but not in PASMCS from patients with PH [171].

Survivin, a member of inhibitor of apoptosis protein family [172], is virtually undetectable in normal adult differentiated tissues but is expressed in most human cancers [173] and promotes cancer by suppressing the body’s ability to limit excessive cell growth. Studies prompt that dysregulated survivin expression is considered to be a major pathologic mechanism of apoptosis inhibition. Vascular injury increases survivin expression in vessel wall, concomitantly with neointima formation [174]. Similarly, McMurtry et al. demonstrated that survivin is expressed in remodelled resistance PAs, but not in normal PAs, from PH patients and rats with MCT-induced PH. Furthermore, the expression of survivin is positively associated with the progression and severity of PAs remodelling. These findings make the proliferation of lung blood vessels in PH ‘a form of cancer or a form of neoplasia’ to be more precise [19].

In addition, adenovirus-mediated over-expression of survivin induces PH in rats, whereas inhalation of an adenovirus vector encoding a mutant survivin gene with dominant-negative properties reverses established MCT-induced PH [19]. Their findings demonstrate for the first time that, as in cancer, apoptosis is suppressed and excessive cell growth is accelerated in the PH lung vessels, thus raising important issues regarding the role of survivin in the pathogenesis of PH, its value as a prognostic indicator and its use as a target for new therapeutic strategies [174].

2. Problems and prospects

The fact that pulmonary circulation is selectively diseased in human PH proposes a major therapeutic challenge in the management of PH, and the present clinical agents have some drawbacks as follows:

(1) Besides being expensive, recently approved therapies lack efficacy and are often associated with toxicity [4].

(2) The majority of currently approved drugs for PH targeting the vasculature will, if given systemically, affect the healthy normal circulation and often result in significant side effects, such as systemic hypotension [175].

(3) The half-life of vasodilator agents, such as PGI₂, CGRP, HGF and ANP, is only a few minutes, even that of AM, the most long-lasting peptide, is merely about 20 min, so the key point for these vasoactive agents in the treatment of PH is how to maintain a high-level blood concentration in the lung.

(4) Currently, systemic delivery as well as intra-tracheal inhalation is the usual administration routine to surmount these obstacles. However systemic delivery of recombinant protein has limitations that include the necessity of repeated injections of a large amount of peptide with possible side effects in other organs. Although the use of implantable pumps for delivery of vasodilator agents to sustain an effective level in the lung has been useful, agents escape to the systemic circulation is still a major limiting factor [11,176]. Moreover, continuous intravenous infusion of prostacyclin derivatives [71,177—179] is associated with significant morbidity [71,180,181]. Similarly, although the administration of NO has met with some clinical success, difficulties associated with long-term inhalational delivery and concerns over safety and efficacy have limited its widespread application [10].

(5) Clinical experience shows that optimal medical treatment could stabilise many patients for some time but cannot reverse the established disease, which eventually leaves patients to lung transplantation; however, this is not a realistic or satisfactory option, because there are too few donors and the long-term outlook is not good for most patients.

Therefore, new, efficacious, selective and non-toxic therapies that could deliver vasodilator agents directly to the pulmonary vascular bed are urgently required. Because
most PH patients do not present until their late phase of the disease, at which time advanced arterial narrowing and loss of the pulmonary microcirculation predominates [4], reversal approaches are much more clinically relevant than prevention. As an alternative to conventional therapies simply promoting vasodilatation, an ideal strategy would be to combat the pathological processes that drive the increased PVR and loss of pulmonary microvasculature. Fortunately, gene therapies aiming at elevating the blood concentration of vasoactive substances in the lung seem to be a much better choice.

Several evidences show that many genes [14,19,24,91] are aetiologically associated with the development of PH, and gene therapy aiming at correcting the deficiency or mutation of these genes opens a new window for the treatment of this devastating disease. Thus far, literature have documented that mostly gene transfer into lungs can (a) restore deficient pulmonary gene expression [17], (b) over-express biologically active gene product [18], (c) reverse the established disease [19,20], (d) regenerate pulmonary vasculature [21—23], (e) protect endothelial structural and functional integrity [182] and (f) modify responses to vasodilators [183] without systemic hypotensive side effects often associated with pharmacological vasodilator therapies, which suggest that gene transfection could represent a novel treatment paradigm for this progressive and lethal disorder (Table 1).

Given the advances of gene therapy over conventional treatment in experiment animals, we can definitely conclude that gene transfer has the potential to ameliorate and reverse progressive PH; however, although therapeutic gene transfer for PH, especially adenoviral-based vectors have major advantages over other vectors such as high-level transgene expression, a broad host range, the ability to infect quiescent cells and ease of preparation of high-titre viral stock [184], successful application of gene-transfer technology to pulmonary circulation requires some daunting challenges be met.

One obstacle to extension of such studies to the clinic is the potential for inactivation of vectors, which may lead to great reduced in vivo half-lives. Many viral vectors are derived from non-human packaging cell lines expressing the terminal alpha-Gal glycosylation epitopes, while complement and a-galactosyl natural antibody in human serum can lyse and inactivate the non-human packaging cell lines, as

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ELPCs: endothelial-like progenitor cells; FB: fibroblasts; CH-PH: chronic hypoxia-pulmonary hypertension; HVJ: haemagglutination virus of Japan; RV/L V + S: right ventricular weight over left ventricular plus septum weight.
well as derived virus vectors, by complement and/or antibody-mediated mechanisms [185]. For these reasons, that low stability and infection efficiency result into reduction of circulating vectors activity of gene transfer remains a major barrier for the horizontal transmission of virus vectors to humans from non-primate species.

An attempt to avoid immunity to vectors has been explored: (a) use of human packaging cell lines which are alpha-Gal negative can produce vectors with different levels of resistance to complement [186, 187] and low sensitivity to the abundant anti-alpha-Gal antibody [188], and such virus vectors derived from human packaging cell lines exhibit longer half-lives and more efficacious transduction in vivo gene therapy [189]. (b) Adenovirus neutralising antibodies were found to be virus-specific and the immune response against the vector could prevent the transgene expression following subsequent inoculation of the same vector, so human and non-human adenovirus-based vectors could be enforced sequentially for human gene therapy as a means to avoid immunity to the vector [190]. Interestingly, the humoral response to AAV may be T-cell dependent; the inhibition of T-cell function using anti-CD4 antibodies prevents neutralising antibodies formation and allows vector re-administration [191, 192].

Induction of inflammatory reactions and cellular toxicity is another impediment that can be circumvented [193, 194]. In the case of PH, the deleterious effects of an inflammatory immune reaction by the viral vector especially need to be avoided due to the potential of accelerating the progression of PH. Moreover, inflammation that it provoked, together with the lack of viral genome integration, has limited the duration of gene expression in animal experiments. Similarly, clinical sequelae to immune response have also limited their utility in human trials.

In order to minimise the host immune and inflammatory responses and finally implement the clinical application, a new, sophisticated method of gene transfection, which could enable long duration of gene expression and maintain effective plasma concentration of agents without any side effects, is required. Therefore, it is necessary to develop improved viral vectors or non-viral vectors for prolonged transgene expression. To circumvent these problems, helper-dependent Ads that lack viral sequences in their genome have been developed [195]. It has recently been shown that these agents achieve longer transgene expression with less inflammatory response after vascular gene delivery [196]. Furthermore, non-viral gene transfer approaches, such as cell-based [21, 61, 168] and plasmid-based [79, 197] therapies, have attracted increasing attention because of their reduced toxicity, simplicity of use and lack of specific immune responses [198]. Cell-based methods of gene transfer rely upon the ex vivo transfection of a specific cell or cell line and the re-introduction of that transfected cell population into either the original host or an immunologically identical or immunosuppressed individual. The low-pressure characteristic and natural filtering function of the pulmonary microvasculature could provide ideal conditions for this method of delivery. In addition, the delivery of genetically engineered cells into the pulmonary circulation offers the promise of selective pulmonary vascular gene transfer, thus avoiding unwanted systemic effects. Especially, gene-engineered ex vivo-expanded adult stem cells, such as EPCs and MSCs, are more attractive than other cells, because MSCs are non-haematopoietic adult stem cells from bone marrow, are relatively easy to isolate and expand ex vivo and have multipotential differentiation capability [199, 200], while EPCs could sense the injury, incorporate into pulmonary arterioles and capillaries, replenish endothelial cells, enhance endothelial repair [100] and also are involved in vascular regeneration in experimental PH [20]. However, even EPCs can be problematic, as EPCs-based therapies require significant ex vivo manipulation of syngeneic or autologous cells. Although plasmid-based [79, 197] method is a non-viral gene transfer and the immune complications related to viral protein expression can be completely avoided, due to the lack of long-term transgene expression, the therapeutic efficacy of plasmid-based therapy is limited. Perhaps the use of integrating transposons may overcome this obstacle of plasmid-based therapies [201].

In summary, great progress in the recognition of the pathogenesis and the methodology of PH has been made recent years, considerable further refinement of the vector approach, which is the critical part of the gene transfer methods, would be required to expedite translation of this new technology to clinical care for patients with PH.

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