2.4. Combination Therapy

Since the pathogenesis of PAH involves multiple pathways, monotherapy may not completely control disease progression in the long term. Thus, the combined use of drugs with different mechanisms will result in an improved treatment of PAH. Combination therapy can be considered as an initial therapy or an add-on therapy in case of deterioration with monotherapy. As an initial combination therapy, a randomized placebo-controlled trial has compared the combined use of intravenous epoprostenol and oral bosentan with epoprostenol alone [33]. As add-on strategies, several non-randomized trials have demonstrated results from the combined use of sildenafil [34-36] or bosentan [37] with prostanoids. Interestingly, these studies exhibited greater improvements in the combination-therapy group without an increase in adverse events. However, well-designed randomized controlled trials are required before such approaches are accepted as standard practice because the efficacy, safety, and cost of these regimens are issues of high concern.

3. NOVEL AGENTS TARGETING PATHOGENESIS OF PAH

Numerous new compounds against PAH have been evaluated in either experimental studies or small case series. Evolving knowledge of these drugs has revealed novel molecular targets of PAH. Certain drugs may inhibit the inflammatory or proliferative process of PA remodeling as well as abnormal vasoconstriction. Others may be developed by formulating analogies to mechanisms observed in cancer progression. Here, we show the therapeutic effects of new molecules presented in the last five years, and briefly discuss the possibilities of their clinical application.

3.1. HMG-CoA Reductase Inhibitors

Inhibitors of 3-hydroxy-3-methylglutaryl (HMG)-Coenzyme A (CoA) reductase, i.e., statins, clinically used for the treatment of dyslipidemia, can also prevent several cardiovascular diseases through the inhibition of vascular inflammation and proliferation. Nishimura *et al.* [38] demonstrated marked inhibitory effects of simvastatin on neointimal VSMC proliferation in pneumonectomized rats with monocrotaline-induced pulmonary arterial hypertension (MCT-PAH). The simvastatin treatment prolonged the survival of these rats along with the downregulation of proinflammatory genes as well as upregulation of cell cycle inhibitors, endothelial NO synthase (eNOS), and bone morphogenetic protein receptor (BMPR) type 1α [39]. Simvastatin also induces EC apoptosis and blocks signals from the vascular endothelial growth factor (VEGF) to prevent PAH induced by chronic hypobaric hypoxia [40]. An open-label observational study on 16 patients with PAH has shown that the administration of simvastatin (20–80 mg/day) improves the 6-minute walk performance and hemodynamics without severe complications [41]. Since this is the only clinical study that used statins for PAH therapy, further studies are needed to confirm the efficacy and safety of this approach.

3.2. Rho-Kinase Inhibitors

RhoA guanosine triphosphate (GTPase) mediates contraction, growth, and gene expression of vascular cells. Acute hypoxia activates RhoA and its downstream effecter Rho-kinase. Rho/Rho-kinase inhibitors might modulate hypoxia-induced abnormal vascular responses in PAH. Intravenous administration of either of the two Rho-kinase inhibitors—fasudil or Y-27632—attenuated PAH and PA remodeling along with enhanced eNOS expression in MCT-induced or hypoxia-induced PAH in rodents [42, 43]. In addition, these drugs have been observed to act synergistically with sildenafil in the prevention of hypoxia-induced PAH [44]. Interestingly, inhaled fasudil selectively reduces PA pressure, while inhaled NO decreases both pulmonary and systemic arterial pressures [45]. Recently, intravenous fasudil (30 mg for 30 minutes) exhibited a significant acute reduction in the pulmonary vascular resistance of 9 patients with severe PH [46]. Larger controlled trials are needed to identify the long-term clinical benefits and anti-remodeling effects of Rho-kinase inhibitors in PAH therapy.

3.3. Peroxisome Proliferator-Activated Receptors

Peroxisome proliferator-activated receptors (PPAR) is a nuclear receptor/ligand-dependent transcription factor that binds to hormone-response elements on target gene promoters. PPARs have three isoforms called α, β/δ, and γ. The PPARγ ligands can attenuate proliferative and inflammatory responses in vascular endothelial and smooth muscle cells. Recent studies may shed light on the role of PPARs in the pathogenesis of PAH. In the lungs of severe PAH patients, fluid shear stress decreases the PPARγ expression in ECs, producing an abnormal, proliferating, and apoptosis-resistant endothelial cell phenotype [47]. In contrast, pioglitazone and troglitazone, the synthetic ligands for PPARγ, can ameliorate rat MCT-PAH through the inhibition of proliferative vascular changes [48]. In addition, a selective PPARβ/δ ligand also inhibits the proliferation of human lung fibroblasts *in vitro*, suggesting a therapeutic role of PPARβ/δ activation [49]. Although pioglitazone is widely used in the treatment of insulin resistance, it may increase the risk of heart failure in patients with type 2 diabetes because of a propensity to cause fluid retention [50]. The underlying mechanisms of the fluid retention have yet to be fully elucidated, but appear to be a dose-related class effect. Thus, it is necessary to identify selective PPAR ligands in order to develop effective and safe therapy in human PAH.

3.4. Regulators of Pro-Inflammatory Cytokines

An inflammatory response is involved in the progression of human and animal PAH. Chronic hypoxia as well as MCT treatment produces PAH along with the inflammatory and proliferative changes in the PA. The transgenic mice that constitutively express heme oxygenase (HO)-1—an antiinflammatory mediator—are protected from the progression of hypoxic PAH and inflammatory responses [51]. The prostacyclin analogues, i.e., beraprost and treprostinil, can reduce nuclear factor kappa B (NF-κB) activation and decrease the levels of interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF) in alveolar macrophages from MCT-PAH rats [11, 52]. Chronic treatment with a recombinant human IL-1 receptor antagonist inhibited the development of MCT-PAH but not hypoxic PAH [53]. These observations suggest that the regulation of multiple proinflammatory signals would produce a more effective therapeutic strategy. Thus, we used IL-10—a pleiotropic antiinflammatory cytokine—in PAH therapy. We reported that adeno-associated virus (AAV) vector-mediated sustained IL-10 expression successfully

prevented the progression of MCT-PAH (abstract; Ito, T *et al. Circulation,* **2005**, 112 suppl II, 99). Since several clinical trials demonstrated a high tolerability to recombinant IL-10 in the treatment of inflammatory bowel disease, an adequate delivery system of IL-10 would help realize a better anti-inflammation therapy for human PAH.

3.5. Superoxide Dismutase

The reactive oxygen species (ROS) play an important role in the pathogenesis of PAH because they have been implicated as mediators of inflammation, proliferation, and gene expression of pulmonary vascular cells. Recent studies have shown that ROS, in particular, superoxide anion, are involved in hypoxia-induced vasoconstriction [54, 55]. Superoxide dismutase (SOD) is a key player of specific defense mechanisms that eliminates ROS in the lung. The serotonin-induced vasoconstriction was enhanced in the PA isolated from extracellular SOD knockout mice [55]. Interestingly, the treatment with polyethylene glycol-conjugated SOD or recombinant copper/zinc SOD enhanced the effects of inhaled NO in persistent pulmonary hypertension in lambs, since SOD protects NO from inactivation by superoxide anion [56, 57]. Although long-term effects of SOD remain unclear, SOD may be a promising candidate for PAH therapy.

3.6. Immunosuppressive Agents

Rapamycin is an immunosuppressive agent with antiproliferative properties not only against lymphocytes but also against vascular endothelial and smooth muscle cells. Rapamycin induces HO-1 expression in human pulmonary artery endothelial and smooth muscle cells [58]. HO-1, an inducible isoform of HO, is a regulator of vascular tone and cell proliferation through the production of endogenous carbon monoxide (CO). A recent study has shown that rapamycin-induced HO-1 expression can prevent PAH and PA remodeling in MCT-PAH [59]. In clinical settings, rapamycin-eluting stents exhibit a pronounced efficacy in the prevention of coronary artery remodeling. Therefore, an efficient delivery system to express the function of rapamycin would produce a novel treatment for human PAH.

Mycophenolate mofetil (MMF) is also a potent immunosuppressive agent with antiproliferative properties against VSMCs. MMF treatment have a great preventive potential in transplant arteriosclerosis through inhibition of VSMC proliferation [60]. Interestingly, MMF attenuated the development of MCT-PAH in rats through its antiinflammatory and antiproliferative properties [61]. These observations will provide new insights into the potential role of immunosuppressants in PAH therapy.

3.7. Imatinib Mesylate

Compelling evidence suggests that platelet-derived growth factor (PDGF) is involved in the proliferation of PASMC [62]. Further, PDGF receptors are upregulated in an animal model of chronic PAH [63]. These results suggest that imatinib mesylate (STI571)—a selective PDGF receptor antagonist—should prevent pulmonary arterial remodeling. This drug can reverse established severe PAH and prolong the survival MCT-PAH rats and hypoxia-induced PAH mice [64]. Since imatinib is tolerable and clinically used for the treatment of certain malignancies that present with c-kit-positive cells, the result of this drug might be easily transferable to PAH therapies. Interestingly, a case report by Ghofrani *et al.* [65] demonstrated that oral administration of imatinib improved exercise capacity, hemodynamics, and functional class in a patient who was refractory to the combination therapy: oral bosentan, inhaled iloprost, and sildenafil. These functional improvements lasted for at least 6 months from the initiation of imatinib treatment, without apparent adverse effects. Although single case reports pose certain limitations, this study provide new insights into targeted antiproliferative therapy using growth factor antagonists.

3.8. Serotonin Transporter Inhibitors

Serotonin (5-hydroxytryptamine; 5-HT) is a potent vasoactive substance released from activated platelets; its plasma levels are increased in patients with PAH [66]. 5-HT transporters (5-HTT) locally mediate the mitogenic effects of 5-HT on PASMCs by modulating cross talk between endothelial and smooth muscle cells in PAH [67]. Recent studies have reported that 5-HTTs play a crucial role in the pathogenesis of PAH as compared to the 5-HT receptors. Specific 5-HTT inhibitors but not the selective 5-$HT_{1B/1D}$ or 5-HT_{2A} receptor antagonists ameliorates PAH in chronic hypoxic mice and MCT-treated rats [68, 69]. The 5-HTT is significantly over-expressed in PASMCs from IPAH patients; however, this is not observed with regard to 5-$HT_{1B/1D}$, 5-HT_{2A}, or 5-HT_{2B} receptors [70]. Furthermore, the transgenic mice that overexpress the 5-HTT gene in PASMC can develop PAH [71]. These data support the possible therapeutic application of 5-HTT inhibitors such as fluoxetine in human PAH. However, this concept remains controversial because the maternal administration of fluoxetine, which is used as an anti-depressant, could lead to an increased risk of persistent pulmonary hypertension of the newborn (PPHN). A recent case-control study has demonstrated that the number of infants with PPHN is increased following maternal treatment with fluoxetine after the 20th week of gestation (odds ratio 6.1; 95% confidence interval, 2.2 to 16.8) [72]. In contrast, neither the use of the drug before the 20th week nor the use of other classes of antidepressants is associated with an increased risk of PPHN. These situations require further study in order to evaluate the clinical efficacy and safety of 5-HTT inhibitors in PAH therapy.

3.9. Elastase Inhibitors

In rats subjected to hypoxia or MCT, the levels of serine elastase increase in the PAs prior to the establishment of vascular remodeling. Elastase activates matrix metalloproteinases (MMPs) that amplify the proteolytic response in the vessel wall, resulting in the release of growth factors from the matrix in biologically active forms [73]. The mitogenic potential of the growth factors is enhanced by the elastase-MMP-mediated induction of the glycoprotein tenascin-C (TN-C) *via* $\alpha\upsilon\beta$-3 integrin signaling [74]. TN-C amplifies the response to growth factors such as the epidermal growth factor (EGF), by inducing phosphorylation of their receptors. Thus, the inhibition of elastase may attenuate PAH and vascular remodeling *via* the suppression of growth factor signaling. In a rat model of severe MCT-PAH, the oral administration of either of the serine elastase inhibitors i.e., M249314 or ZD0892, caused prolonged survival as well as a marked inhibition of vascular remodeling [75]. A selective blockade

of αvβ–3 integrin with SC-080 or that of EGF receptors with PKI166 also exhibited similar beneficial effects in MCT-PAH [76]. In addition, the overexpression of an endogenous serine elastase inhibitor, i.e., elafin, protected transgenic mice from hypoxic PAH [77]. These results support the promise of the therapeutic use of elastase inhibitors in PAH patients. However, no clinical study is currently underway to prove this hypothesis.

3.10. Angiotensin II Type 1 Receptor Antagonist

Angiotensin II acts as a potent vasoconstrictor in the pulmonary circulation. The angiotensin II type 1 receptor (AT1R) blockade attenuates acute hypoxic pulmonary vasoconstriction in normal subjects. In patients with hypoxic cor pulmonale, the AT1R antagonist losartan (50 mg/day) exhibited acute improvements in the hemodynamics of PAH patients [78]. However, a recent randomized placebo-controlled trial on PAH with chronic obstructive lung diseases (n = 40) found no significant clinical benefits in patients treated with losartan (50 mg/day) in a 12-month follow-up period [79]. Since patients in the losartan group exhibited a trend of improvement, particularly in severe functional classes, the statistical significance is expected to be observed in a long-term study with higher doses and a larger sample size. No clinical study on the other types of PAH is currently available.

3.11. Natriuretic Peptides

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are cardiac hormones with potent diuretic, vascular relaxant, and antiproliferative properties. Both drugs act on a membrane-bound receptor that activates guanylate cyclase, leading to intracellular cGMP accumulation. In the pulmonary circulation, ANP physiologically modulates vascular resistance and remodeling. The mice with targeted disruption of the ANP gene exhibited moderate PAH. Under hypoxic condition, PAH of the ANP mutant mice was worse than that of heterozygous or wild-type controls [80, 81]. However, there are no positive data on the use of these natriuretic peptides in human PAH therapy. In a recent open-label study, a 3-hour infusion of BNP exhibited no significant improvement in the hemodynamics of PAH patients, although it showed high tolerability and augmented the acute vasodilating effects of sildenafil [81].

3.12. Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) is a neuropeptide with potent vasodilatory properties as well as inhibitory effects on vascular proliferation and inflammation [82]. VIP inhibits the proliferation of PASMCs in patients with IPAH [83]. VIP-containing nerves, normally plentiful in the wall of PAs, are completely absent from the PAs of IPAH patients [83]. In addition, VIP reduces the pulmonary vascular resistance of MCT-treated animals that have undergone cardiopulmonary bypass [84, 85]. These results suggest a possibility of VIP replacement therapy. A preliminary study demonstrated marked improvements in 6-minute walk performance and pulmonary hemodynamics in 8 PAH patients that received inhaled VIP [83]. Since VIP stimulates cAMP production, its combination with sildenafil—a promoter of cGMP production—may exert additive therapeutic effects on PAH. However, these results still await independent confirmation.

4. FUTURE APPROACHES

4.1. Therapeutic Approaches Based on the Genetics of PAH (Fig. 4)

Most cases of PAH appears to be sporadic, but approximately 6% of PAH demonstrate autosomal-dominant inheritance with reduced penetrance [86]. Although the clinical and pathological features of sporadic and familial PAH (FPAH) are similar, FPAH displays a genetic predisposition, suggesting a genetic basis of PAH. Since only 10–20% of the carriers of the relevant mutation described below exhibit PAH, the clinical manifestation of PAH may depend on the combined presence of genetic and environmental 'modifiers' that accentuate the risk for full expression of the disease [87]. Thus, many investigators support the "multiple-hit hypothesis" in the pathogenesis of PAH [88]. In order to develop more effective and specific therapies for PAH, the mechanistic relationships between gene mutations and exogenous stimuli should be widely explored.

4.1.1. Mutation of TGF-β Type II Receptors (BMPR2, ALK-1)

Transforming growth factor-β (TGF-β) signaling controls a diverse set of cellular processes including cell proliferation, differentiation, and apoptosis. The TGF-β ligand initiates signaling by binding to type I and type II receptor serine/threonine kinase on the cell surface, leading to the activation of Smad protein complexes that in tern regulate many transcription factors [89]. Mutation in two receptors of this group have been identified in the majority of FPAH patients: heterozygous mutation of bone morphogenetic protein (BMP) receptor type 2 (BMPR2) on chromosome 2q33 and activin-like kinase type 1 (ALK-1). Exonic mutations in *BMPR2* gene are present in approximately 50% of patients with FPAH and 10–20% of IPAH patients [90]. Additional intronic mutations have recently been found in a significant proportion of these patients [91]. The ALK-1 mutation is present in a minority of patients with PAH associated with hereditary hemorrhagic telangiectasia. These mutations may modulate the signaling activities in the proliferation and apoptosis of PASMCs and ECs. In the PASMCs of PAH patients, the antiproliferative and antiapoptotic effects of BMP signals are attenuated, resulting in the progression of PA remodeling. In the pulmonary ECs of these patients, the prosurvival and antiapoptotic effects of BMP signals are diminished, leading to vascular obstruction caused by monoclonal EC proliferation [92].

Recently, a large multi-center study has shown data regarding 144 distinct mutations of *BMPR2* gene of 210 independent PAH subjects [93]. It further demonstrated that heterozygous germline mutations in the *BMPR2* gene underlie the majority (>70%) of FPAH cases. Moreover, the study provided both disease-causing mutational hot spots within *BMPR2* and compelling genetic evidence that haploinsufficiency is the predominant mechanism underlying the predisposition for PAH. In addition, the recent animal models with *BMPR2* mutation partly explain the relationship between the basal mutation and additional modifiers. *BMPR2*-deficient mice that generally exhibit no change in hemodynamics and

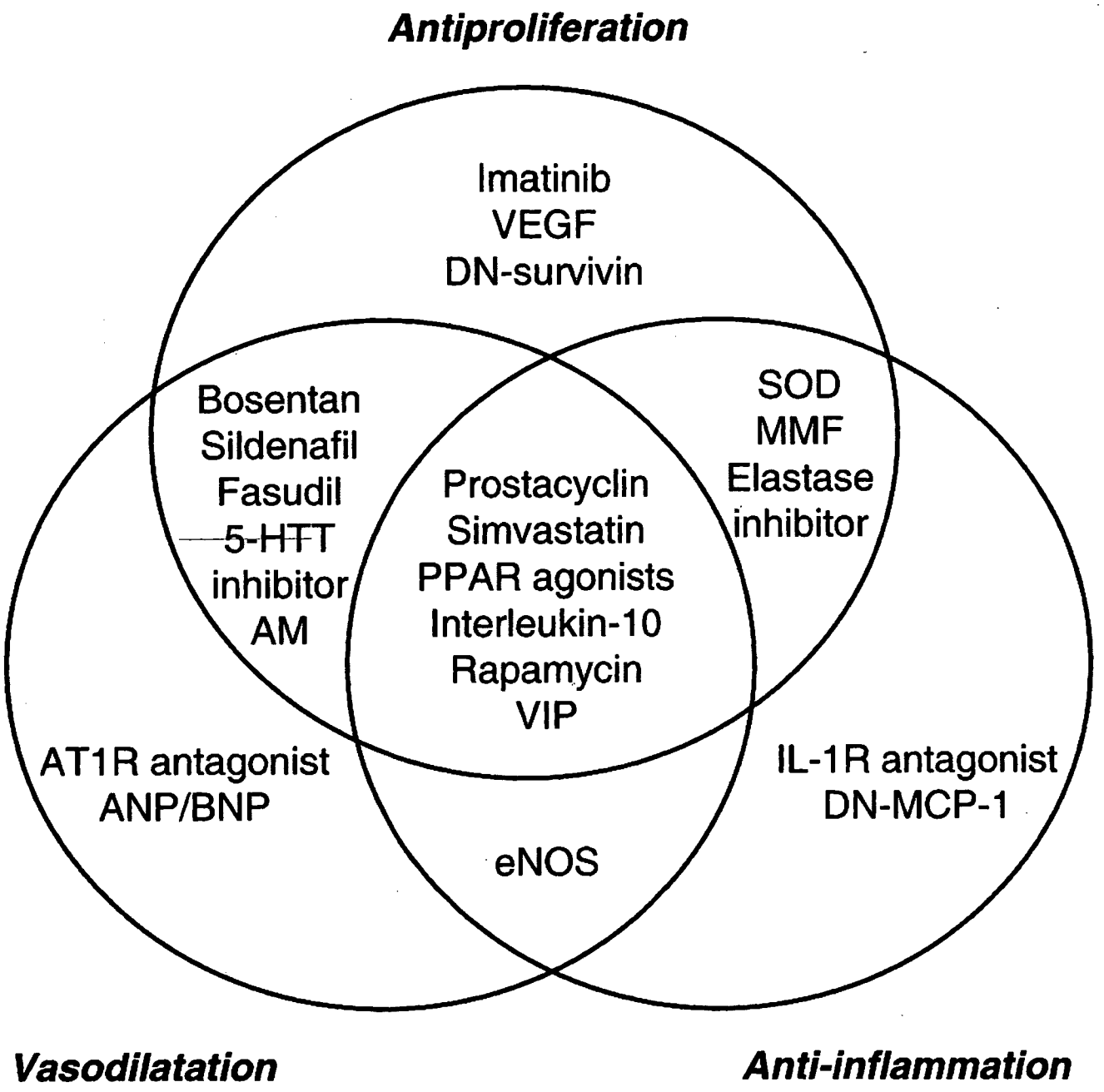

Fig. (4). Target pathological process and new therapeutic molecules of pulmonary arterial hypertension. DN-survivin: dominant-negative inhibitor of survivin, 5-HTT: serotonin transporter, AM: adrenomedullin, ANP/BNP: Artial/Brain natriuretic peptide, PPAR: peroxysome proliferator-activated receptor, VIP: vasoactive intestinal peptide, VEGF: vascular endothelial growth factor, eNOS: endothelial nitric oxide synthase, SOD: superoxide dismutase, MMF: Mycophenolate mofetil, IL-1R antagonist: interleukin-1 receptor antagonist, DN-MCP-1: dominant-negative inhibitor of monocyte chemoattractant protein-1.

vascular morphology even under hypoxic conditions developed marked PAH and vascular remodeling after chronic infusion of serotonin [94]. Inflammation may also promote the susceptibility to PAH. In heterozygous *BMPR2* mutant mice, the pulmonary overexpression of 5-lipoxygenase led to the development of PAH and PA remodeling along with inflammatory vascular changes [95].

4.1.2. Polymorphism of Serotonin Transporter

Allelic variations of the serotonin transporter (5-HTT) gene have been implicated in the pathogenesis of PAH. The 5-HTT gene is encoded by a single gene on chromosome 17q11.2. Compared with the short (S) allele, the long (L) allele is associated with an increased 5-HTT transcription and may be linked to an increased risk of developing PAH. Homozygosity for the L-allele (LL) may be associated with an early onset of IPAH during childhood [96]. In contrast, two recent reports have cast some doubt on the significance of 5-HTT polymorphism in the pathogenesis of human PAH [97, 98]. There are still conflicting data regarding the linkage of the LL-genotype to an earlier onset of FPAH.

4.2. Gene and Cell Therapies (Table 3)

Gene therapy was initially proposed in the context of inherited disorders, whereby, in theory, transfer of a normal copy of a single defective gene would revert the disease pathogenesis. At present, the concept of gene therapy encompasses the treatment of acquired disorders, including cardiovascular diseases, based on the transfer of genetic information. Recent advances in gene transfer technology have revealed that exogenous delivery of therapeutic molecule can reverse the development of PAH. Supplementation of therapeutic proteins, enhancement of enzyme induction, and delivery of dominant-negative oligonucleotides are the basic strategies for PAH gene therapy. However, optimal delivery system and a therapeutic gene yet remain unknown. Many hurdles still exist in the successful gene therapy of human PAH; these include site-specific gene delivery, sustained gene expression, and inflammatory and immune response to vectors. The AAV vector may be promising as a gene delivery system because it can achieve sustained transgene expression with minimal inflammatory responses and tissue-specific expression by selecting a suitable serotype *in vivo*. Hybrid therapy, which is a treatment using genetically modified cells or nanoparticles, is an important technology that can overcome these problems because some types of vectors can actively seek lesions and express therapeutic gene locally.

4.2.1. Supplementation of Therapeutic Proteins

Adrenomedullin

Adrenomedullin (AM) is a potent vasodilator peptide originally isolated from human pheochromocytoma. AM protects ECs from apoptosis and affect the vascular tone in the PA. Both AM and its receptor—calcitonin receptor-like receptor—are upregulated through a hypoxia-inducible factor 1-dependent pathway under hypoxic conditions [99]. Interestingly, intravenous and inhaled AM lower the pulmonary vascular resistance in patients with IPAH [100, 101]. Since AM is a nonselective vasodilator, the intravenous administration of AM will lead to an adverse drop in the systemic blood pressure. Thus, an efficient delivery system is required for successful AM-based technology. The intravenous administration of endothelial progenitor cells (EPCs) has been observed to restore endothelial function and improve the vascular remodeling of PAH. Nagaya *et al.* [102] reported that EPCs containing positively charged gelatin particles that hold negatively charged AM DNA in its lattice structure enhanced the therapeutic effects on rat MCT-PAH compared to EPC treatment alone. The AM DNA-gelatin complexes can release AM locally, and the increased levels of AM may prolong the survival of EPCs and enhance pulmonary endothelial function.

Vascular Endothelial Growth Factor

VEGF, which is expressed at high levels in the lungs, is the principal growth factor for the survival of ECs as well as the maintenance of normal PA function. VEGF is strongly expressed in angioproliferative plexiform lesions in patients with severe PAH. VEGF binds to VEGF receptor-2 (VEGFR-2), resulting in increased expression of eNOS and PGI_2 [103]. A blockade of VEGFR-2 by the tyrosin kinase inhibitor SU-5416 can produce PAH in rats [40, 104]. These results suggests a crucial role of VEGF signaling in the prevention of PAH. Interestingly, the overexpression of VEGF may play a therapeutic role in PAH. Adenoviral vector-mediated VEGF expression attenuated hypoxia-induced PAH in rats [105]. Treatment with recombinant VEGF also ameliorated fatal PAH produced by the partial ligation of the ductus arteriosus [106]. In addition, intravenous VEGF gene transfer using cell-based vectors or DNA-liposomes attenuated MCT-induced or bleomycin-induced PAH, respectively [107, 108].

Table 3. Gene and Cell Therapy Strategies for the PAH Therapy

Therapeutic gene	Vector	Delivery	Model	Major Outcomes	Reference
Adrenomedullin	EPC	iv	MCT	remodeling↓, survival↑	[102]
VEGF	Adeno	it	hypoxia	remodeling ↓, RVH ↓	[105]
	VSMC	iv	MCT	remodeling ↓, survival ↑	[107]
	Lipo	it	hypoxia	remodeling↓, PAP↓	[108]
Angiopoitin-1	VSMC	iv	MCT	PAP↓, RVH↓, survival↑	[110]
soluble Tie-2	AAV	iv	MCT	PAP↓, remodeling↓	[113]
PGIS	HVJ-L	it	MCT	remodeling↓, survival↑	[115]
	HVJ-L	ih	MCT	PAP↓, survival↑	[116]
	Plasmid	im	MCT	remodeling↓, survival↑	[117]
eNOS	Adeno	it	hypoxia	PAP↓, PVR↓	[120]
	EPC	iv	MCT	PAP↓, survival↑	[121]
	FB	iv	MCT	PAP↓, remodeling↓	[122]
DN-MCP-1	Plasmid	im	MCT	PAP ↓, remodeling ↓	[124]
DN-survivin	Adeno	it	MCT	remodeling ↓, survival ↑	[125]

VEGF: vascular endothelial growth factor, PGIS: prostacyclin synthase, eNOS: endothelial nitric oxide synthase, DN-MCP-1: dominant-negative monocyte chemoattractant protein-1, EPC: endothelial progenitor cell, VSMC: vascular smooth muscle cell, Lipo: liposome, AAV: adeno-associated virus, HVJ-L: hemagglutinating virus of Japan-loposome, FB: fibroblast, iv: intravenous, it: intratracheal, ih: intrahepatic, im: intramuscular, MCT: monocrotaline, RVH: right ventricular hypertrophy, PAP: pulmonary arterial pressure, PVR: pulmonary vascular resistance.

Angiopoietin-1

Angiopoietin-1 (Ang-1) —a ligand of the endothelial-specific tyrosine kinase receptor Tie-2—has been found to promote cell survival and vascular maturation. Although Ang-1-based gene therapy holds promise for many vascular diseases, its role in PAH therapy is still controversial [109]. An intravenous injection of rat PASMCs that encoded the Ang-1 gene improved the survival and hemodynamics in MCT-PAH rats through the inhibition of vascular cell apoptosis [110]. In contrast, the rodents that constitutively expressed Ang-1 in the lung developed severe PAH through an Ang-1/TIE2/serotonin paracrine pathway [111]. Moreover, the long-term Ang-1 expression using an AAV vector (AAV-Ang-1) caused PAH in rats [112]. Interestingly, intravascular treatment with the AAV vector expressing soluble Tie-2 to block Ang-1 signals prevented the progression of both MCT-PAH and AAV-Ang-1-induced PAH in rats [113]. In addition, Ang-1 expression as well as the phosphorylation of Tie-2 was increased in the lungs of various forms of human PAH [112, 113].

4.2.2. Enzyme Induction

Prostacyclin Synthase

Prostacyclin synthase (PGIS) is the final committed enzyme in the metabolic pathway leading to PGI_2 production. Patients with severe PAH have a PGIS deficiency in their precapillary vessels. Selective pulmonary overexpression of PGIS in transgenic mice can protect against the development of hypoxic PAH [114]. Thus, PGIS-based gene therapy for replacement of PGI_2 would be effective in PAH therapy. Previous PGIS gene therapies successfully prevented MCT-PAH by delivering viral vectors through the airway, liver, and skeletal muscles [115-117]. The intramuscular approach may be efficient because of fewer complications even in severe PAH patients. However, the previous plasmid-based method requires frequent vector injection to maintain sufficient PGI_2 levels [117]. To address this issue, we recently employed an AAV typel vector expressing PGIS (AAV1-PGIS). A single intra-muscular injection of AAV1-PGIS achieved long-term PGI_2 production, resulting in successful prevention of MCT-PAH (abstract; Ito, T *et al. Circulation*, **2006**, 114 suppl II, 82). For successful translation into clinical settings, this system requires a machinery to regulate adequate levels of gene expression and a strategy against the formation of anti-vector antibodies.

Endothelial Nitric Oxide Synthase

NO is responsible for resting pulmonary vasorelaxation. Endothelial NO synthase (eNOS) effects the conversion of L-arginine to citrulline, thereby producing NO. In addition to the baseline constitutive expression, eNOS can be modified by diverse stimuli such as shear stress and increased pulmonary blood flow. The overexpression of eNOS in transgenic mice prevents hypoxic PAH [118]. Conversely, eNOS-deficient mice can develop severe PAH after exposure to mild hypoxia [119]. These results suggest a promising role of eNOS gene transfer in PAH therapy. Intratracheal transduction of the eNOS gene by using the adenoviral vector successfully ameliorated hypoxia-induced PAH in mice [120]. Interestingly, an eNOS-transduced bone marrow-derived EPC (eNOS-EPC) reversed the establishment of MCT-PAH [121]. EPCs, which normally function to repair and regenerate blood vessels, restored the pulmonary hemodynamics and increased microvascular perfusion. Autologous transplantation of eNOS-EPC could allow homing to the pulmonary vascular bed without provoking a severe immune response. Interestingly, eNOS may be more effective than VEGF as a therapeutic transgene in fibroblast-based gene transfer against PAH [122]. Thus, the optimal combination of cell vectors and therapeutic transgenes should be widely explored in the future.

4.2.3. Dominant-negative oligonucleotide

Monocyte Chemoattractant Protein-1

The inflammatory process is involved in the pathogenesis of PAH. Monocyte chemoattractant protein-1 (MCP-1) is recognized as a potent chemotactic factor for monocytes. Plasma MCP-1 levels are increased in patients with PAH and reflect the severity of the disease [12, 123]. Thus, a blockade of systemic MCP-1 signaling may prevent PAH progression. An intramuscular injection of a plasmid expressing dominant-negative inhibitor of MCP-1 retarded the progression of MCT-PAH with a decrease in the macrophage infiltration in the lung [124].

Survivin

Survivin—an "inhibitor of apoptosis" protein—has been thought to be expressed primarily in cancer cells. Recent studies revealed a possible link between the abnormal proliferation of vascular cells and the blunted apoptosis signaling in the PAs. McMurtry *et al.* [125] observed an increased expression of survivin in the PAs of humans and rats with PAH, but not in those without PAH. They also demonstrated that an adenovirus-mediated overexpression of survivin induced PAH in rats, whereas the inhalation of an adenovirus vector expressing dominant-negative inhibitor of survivin reversed the development of MCT-PAH. These findings raise important issues regarding the role of survivin in the pathogenesis of PAH, its value as a prognostic indicator, and its therapeutic applications [126].

CONCLUSION

Great strides have been taken in the last couple of years toward a better understanding of the pathogenesis and more effective treatment of PAH. Insights from genetic studies and experimental models may open new perspectives for molecular targeting of pathological pulmonary vascular remodeling. For improved PAH therapy and future exploration of its pathobiology, the following unanswered questions should be addressed in the coming years: What are the full genetic determinants and crucial modifiers of PAH? What is the therapeutic implication of BMPR2/Smad signaling? Are attractive results from the various animal experiments transferable to clinical practice? Can therapeutic gene transfer be a more rational therapy? How beneficial is combined therapy likely to be in the long term? These problems can be solved by a collaboration of many study groups implementing comprehensive research from bench to bedside in this field. More integrated translational research will generate greater clinical benefits for patients with PAH.

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