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DPP-4 inhibition: A novel therapeutic approach to the treatment of pulmonary hypertension?

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ABSTRACT

Pulmonary hypertension (PH) is a progressive disorder characterized by alterations of the vascular structure and function in the lungs. Despite the success in its stabilisation by targeting pulmonary vascular tone and endothelial dysfunction, the prognosis remains poor and new therapeutic approaches via neglected macromolecular targets are needed. In the pathophysiology of PH the early stages of vascular remodelling are considered to be reversible, while endothelial to mesenchymal transition and proliferation/migration of fibroblasts play a critical role in staging the irreversible phase. Dipeptidyl peptidase-4 (DPP-4)/CD26 is present and active in the lungs and is expressed constitutively on lung fibroblasts, on which it exerts proliferative effects. Further, it is a marker of migrating fibroblasts and of their functional activation, including collagen synthesis and inflammatory cytokine secretion. Inhibiting DPP-4 improves the reversible phases of vascular dysfunction in PH, but is also highly likely to attenuate endothelial to mesenchymal transition and decrease the proliferation and migration of fibroblasts, preventing fibrosis and, consequently, should prolong or even inhibit entrance to the potentially irreversible phase of PH. Proposed mechanisms that support the multifaceted aspects of DPP-4 inhibition in terms of improving PH, involve pathways and mediators in pulmonary vascular and connective tissue remodelling. The latter are affected by the inhibition of this protease resulting in the synergistic beneficial antioxidative, anti-inflammatory and antifibrotic effects. We offer here an evidence-supported hypothesis that DPP-4 inhibitors are likely to be effective in the irreversible phase of remodelling in PH. Accordingly, we propose PH as a possible novel therapeutic indication for existing and new DPP-4 inhibitors.

Contents

Abbreviations: cAMP, Cyclic adenosine monophosphate; cGMP, Cyclic guanosine monophosphate; DPP-4, Dipeptidyl peptidase-4; DPP-4i, Dipeptidyl peptidase-4 inhibitor; ECM, Extracellular matrix; eNOS, Endothelial NO synthase; ERK, Extracellular signal-regulated kinases; ET-1, Endothelin-1; GLP-1, Glucagon-like peptide-1; GSHPx, Glutathione peroxidase; IL, Interleukin; LDH, Lactate dehydrogenase; MAPK, Mitogen-activated protein kinases; MCP-1, Monocyte chemoattractant protein-1; miR, microRNA; MMP, Matrix metalloproteinase; NADPH, Nicotinamide adenine dinucleotide phosphate; NF-κB, Nuclear factor kappa B; NO, Nitric oxide; PDGF, Platelet-derived growth factor; PGI, Prostacyclin; PH, Pulmonary hypertension; RAGE, Receptor for advanced glycation end products; ROS, Reactive oxygen species; SDF-1, stromal cell-derived factor-1; SMCs, Smooth muscle cells; SOD, Superoxide dismutase; STAT, Signal transducers and activators of transcription; TGF-β, Transforming growth factor-β; TNF-α, Tumor necrosis factor-α; VCAM-1, Vascular adhesion molecule-1; VIP, Vasoactive intestinal peptide; XO, Xanthine oxidase.

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1. Introduction

Pulmonary hypertension (PH) is defined by the existence of a mean pulmonary arterial pressure greater than 25 mm Hg (El Chami & Hassoun, 2012). Smooth muscle and endothelial cells are significant participants in the pathology of PH. Fibroblasts play a critical role by undergoing proliferation and inducing proliferation of smooth muscle cells (SMCs). Adventitial fibroblasts in the pulmonary vessels also produce cytokines and chemokines in PH driving the migration of the inflammatory cells to the area of vascular remodelling (Church et al., 2015). Fibroblasts may differentiate to myofibroblasts. This differentiation was initially thought to be irreversible, but recent results indicate that it can be reversed. In-depth knowledge of the signalling pathways and profibrotic mediators involved therein has provided the basis for discovering effective antifibrotic and anti-PH therapy (Yang, Chen, Liu, & Chen, 2014).

Dipeptidyl peptidase-4 (DPP-4, CD26) is serine protease that cleaves dipeptides with proline or alanine at the penultimate position from the N-terminus of substrates. Membrane bound and soluble circulating form of DPP-4 regulates biological activity of polypeptides such as chemokines, cytokines and vasoactive peptides. By cleaving incretins, this protease plays a significant role in glucose homeostasis with a wide spectrum of biological functions (Anderluh et al., 2016; Beckers, Gielis, Van Schil, & Adriaensen, 2017; Meyerholz, Lambertz, & McCray, 2016; Tomovic et al., 2019). DPP-4 additionally modulates some signalling pathways, which to date have not been fully elucidated (Shiobara et al., 2016). Pirozzi et al. published case reports on the use of DPP-4 inhibitor, vildagliptin, as a treatment option for diabetes mellitus type 2 in patients with PH (Pirozzi et al., 2015; Pirozzi et al., 2017). The aforementioned case reports indicate beneficial effects in cases of PH with the explanation that DPP-4 inhibition leads to increased levels of glucagon-like peptide-1 (GLP-1), promoting vasorelaxation in the pulmonary artery and exerting anti-inflammatory activity (Pirozzi et al., 2015; Pirozzi et al., 2017). Although GLP-1 most probably plays an important role in PH treatment, we believe it is just one of the major key players that arise from DPP-4 inhibition, leading to improvement of PH symptoms. Other pathways and mediators besides GLP-1 that might lead synergistically to the beneficial effects of DPP-4 inhibition in PH are, in our opinion, as important as GLP-1. Accordingly, in this work we focus on the involvement of DPP-4 in PH and describe the multifaceted aspects of DPP-4 inhibition as a therapeutic approach to vascular remodelling and to remodelling of connective tissue in PH. In particular, we present evidence for PH as possible novel indication for DPP-4 inhibitors that act in the irreversible phase of the pulmonary vascular remodelling initiated by endothelial to mesenchymal transition and proliferation/migration of fibroblasts.

2. Reversible phase of pulmonary vascular remodelling and significance of DPP-4 inhibition

Vascular remodelling in PH is characterized by the thickening of the media due to the proliferation of phenotypically altered SMCs, which may be reversible. Critical step for irreversible phase of PH might be associated with the impaired apoptotic regulation of endothelial cells, which contributes to intimal thickening, and distal pulmonary arteries obliteration. Further studies are necessary to determine the reversibility of vascular remodelling in PH (Ranchoux et al., 2018; Sakao, Tatsumi, & Voelkel, 2010).

The normal primarily paracrine communication between endothelial and smooth muscle cells is important for the maintenance of pulmonary circulation homeostasis, and is mediated primarily by nitric oxide (NO) and endothelin-1 (ET-1). NO can exert an antiproliferative effect on SMCs through activation of cyclic guanosine monophosphate (cGMP)-dependent protein kinase, followed by inhibition of mitogen-activated protein kinases (MAPK) (Barman, 2005; Gao, Chen, & Raj, 2016). Decreased generation of NO can be reversed by inhibition of p38 MAPK (Church et al., 2015; Weerackody, Welsh, Wadsworth, & Peacock, 2009). In contrast to NO, soluble DPP-4 activates MAPK and the nuclear factor kappa B (NF-κB) signalling cascade, resulting in induction of proliferation of human vascular SMCs and inflammation. This indicates that these processes could be successfully reduced by inhibiting DPP-4 (Shiobara et al., 2016). In cultured human pulmonary arterial SMCs, DPP-4 inhibitor sitagliptin suppressed their proliferation and migration (Xu et al., 2018).

Reactive oxygen species (ROS) are able to activate MAPK in endothelial and smooth muscle cells, stimulating their proliferation as well as that of pulmonary artery adventitial fibroblasts in PH (Freund-Michel et al., 2013). ET-1 elevates ROS generation in pulmonary arterial SMCs and decreases NO levels, thus promoting vasoconstriction (Gao et al., 2016). Furthermore, ET-1 participates in the proliferation of SMCs, fibrosis and inflammation (Freund-Michel et al., 2013). The release of ET-1 from endothelial cells can be induced by transforming growth factor-β (TGF-β) and angiotensin II, while the suppression of its release is mediated by prostacyclin (PGI2) and NO (Jeffery & Morrell, 2002). These facts imply that NO and ET-1 act as physiological antipodes in PH and that the action of one may be counterbalanced by that of the other. PGI2 acts as a further regulator of pulmonary circulation homeostasis, suppresses the proliferation of SMCs and exerts anti-inflammatory activity (Freund-Michel et al., 2013). ROS generation is elevated in PH, due to the increased activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that has been observed *in vitro* and *in vivo* in humans. In pulmonary arterial SMCs, TGF-β1 activates NADPH oxidase and increases ROS production. Xanthine oxidase (XO) is a further source of ROS in the pulmonary vasculature (Freund-Michel et al., 2013). Inflammatory cytokines and low levels of NO increase the expression of XO in the lungs and uric acid may serve as a marker of primary PH severity (Budhiraja, Tuder, & Hassoun, 2004). In PH, ROS take part in endothelial dysfunction, inflammation (partly through the activation of NF-κB signalling (Lee & Yang, 2012)), together with remodelling of extracellular matrix (ECM) mediated by the activation of matrix metalloproteinases (MMP)-2 and −9 (Freund-Michel et al., 2013). GLP-1 decreases the generation of free radicals by affecting NADPH oxidase activity through cyclic adenosine monophosphate (cAMP)/protein kinase A signalling. DPP-4 inhibition additionally protects against oxygen radicals by elevating levels of superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) expression (Beckers et al., 2017). Antioxidative effects, together with the regression of vascular SMCs, may contribute to the benefits of DPP-4 inhibition in PH.

3. Irreversible phase of pulmonary vascular remodelling and significance of DPP-4 inhibition

Endothelial to mesenchymal transition, when endothelial cells lose junctions to the endothelium and gain migratory and proliferative capacities, might be considered as a critical player not only in the pathogenesis of tissue fibrosis but also in the pulmonary vascular remodelling in PH (Ranchoux et al., 2018). GLP-1 analogue could prevent TGF-β1/interleukin (IL)-1β-induced endothelial to mesenchymal transition, which was shown *in vitro* on human umbilical vein endothelial cells (Wang et al., 2019). It has been shown in mice that DPP-4

inhibitors, vildagliptin and linagliptin, significantly suppressed endothelial to mesenchymal transition in the absence of immune cells or GLP-1, by attenuating generation of ROS as inducers of transition in pulmonary vascular endothelial cells (Suzuki et al., 2017).

Endothelial dysfunction and imbalance of the vasoconstrictors/vasodilators ratio decrease endothelium dependent relaxation in PH (Freund-Michel et al., 2013). Therapies based on the endothelial dysfunction target the ET-1, NO/cGMP and PGI2 pathways (El Chami & Hassoun, 2012). In PH, endothelial NO synthase (eNOS) activity and NO levels in the pulmonary vasculature are both decreased (Chen, Watson, & Zhao, 2013; Giaid & Saleh, 1995). GLP-1 can induce vasodilation by indirect eNOS phosphorylation and elevation of NO and cAMP levels. GLP-1 receptors are expressed in lung tissue, including epithelium, alveoli and arterial smooth muscle cells (Nguyen, Linderholm, Haczku, & Kenyon, 2017). DPP-4 is widely expressed in the lung parenchyma, including pneumocytes, alveolar macrophages, bronchial epithelial and endothelial cells, with elevated expression in pathological states (Meyerholz et al., 2016). By catalysing GLP-1 cleavage DPP-4 reduces the beneficial effects of this incretin in lung homeostasis (Nguyen et al., 2017). Furthermore, in ischemia/reperfusion injury in mouse inhibition of DPP-4 improves lung function by increasing vasoactive intestinal peptide (VIP) levels in the alveolar macrophages (Jungraithmayr et al., 2010). VIP acts as a systemic and pulmonary vasodilator and suppresses the proliferation of vascular SMCs (Humbert et al., 2004). There are, therefore, other substrates, ligands, mediators and signalling pathways besides GLP-1 that can be directly or indirectly affected by DPP-4 inhibition, and all of them act synergistically to improve PH-related complications in the lung pathology (Fig. 1).

Impaired oxidant/antioxidant balance potentiates fibrogenesis, proliferation of fibroblasts and synthesis of ECM, and suppresses vasodilation (Behr & Ryu, 2008). Expression of ET-1, which promotes the growth of pulmonary arterial SMCs and stimulates proliferation of human lung fibroblasts, is elevated in pulmonary fibrosis (Jeffery & Morrell, 2002; Nathan, Noble, & Tuder, 2007). ET-1 acts as a growth factor for both endothelial cells and myofibroblasts. It induces fibrogenesis by interacting with MMPs, and initiates an epithelial to mesenchymal transition, inducing TGF-β1 activity (Behr & Ryu, 2008). Platelet-derived growth factor (PDGF), with notably elevated expression in PH, is another profibrotic cytokine (Nathan et al., 2007). ROS stimulate PDGF receptor activation (Lee & Yang, 2012), and potentiate its ability to induce the proliferation and migration of SMCs and fibroblasts (Humbert et al., 2008). TGF-β and PDGF participate significantly in the expansion of connective tissue in the lungs (Behr & Ryu, 2008). Fibroblasts produce and secrete TGF-β1 and IL-1β (Kendall & Feghali-Bostwick, 2014). TGF-β, whose effects are potentiated by tumor necrosis factor-α (TNF-α), takes part in the initiation of fibrosis in many tissues including the lungs. It elevates the activity of MMPs (Willis & Borok, 2007), NADPH oxidase (Kendall & Feghali-Bostwick, 2014), and induces the production of connective tissue growth factors in pulmonary fibroblasts. The mitogenic activity of angiotensin II, mediated by phosphorylation of MAPK, may be regulated by TGF-β in human lung fibroblasts (Jeffery & Morrell, 2002). Pulmonary fibrosis is characterized by uncontrolled myofibroblast proliferation (Hinz, 2012). The origin of myofibroblasts in lung fibrosis includes epithelial cells, bone marrow precursors and lung pericytes (Kramann, DiRocco, & Humphreys, 2013). TGF-β1 induces the epithelial to mesenchymal transition of

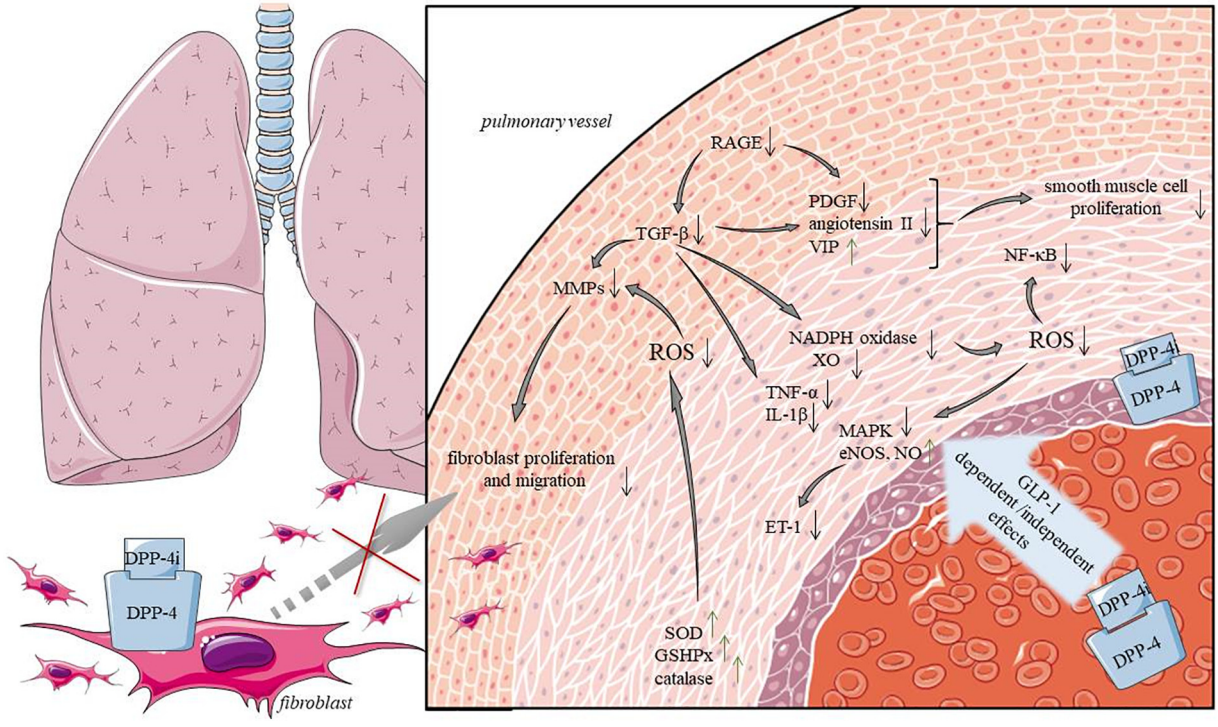

Fig. 1. Mechanisms by which DPP-4 inhibitors might exert beneficial effects in the vascular remodelling in pulmonary hypertension. In contrast to NO, soluble DPP-4 activates MAPK and NF-κB signalling, which could be reduced by inhibiting DPP-4 (Shiobara et al., 2016). GLP-1 can induce eNOS phosphorylation and elevate NO levels (Nguyen et al., 2017). The release of ET-1 from endothelial cells can be suppressed by NO (Jeffery & Morrell, 2002). ROS activate MAPK in endothelial and SMCs (Freund-Michel et al., 2013). In pulmonary arterial SMCs, TGF-β activates NADPH oxidase and increases ROS generation (Freund-Michel et al., 2013). Inflammatory cytokines and low levels of NO increase the expression of XO in the lungs (Budhiraja et al., 2004). In PH, ROS take part in the activation of NF-κB signalling and MMPs (Freund-Michel et al., 2013; Lee & Yang, 2012). GLP-1 decreases the generation of free radicals by affecting NADPH oxidase activity. DPP-4 inhibition elevates levels of SOD and GSHPx (Beckers et al., 2017). Inhibition of DPP-4 increases the levels of VIP, a vasodilator and suppressor of proliferation of vascular SMCs (Humbert et al., 2004; Jungraithmayr et al., 2010). ROS stimulate PDGF receptor activation (Lee & Yang, 2012), and potentiate its induction of the proliferation and migration of SMCs and fibroblasts (Humbert et al., 2008). TGF-β elevates the activity of MMPs (Willis & Borok, 2007). The mitogenic activity of angiotensin II may be regulated by TGF-β in human lung fibroblasts (Jeffery & Morrell, 2002). The deficiency of RAGE is accompanied by reduced pulmonary levels of TGF-β and PDGF (He et al., 2007). DPP-4 may increase expression of RAGE (Nguyen et al., 2017). TGF-β increases the expression of pro-inflammatory cytokines in lung fibroblasts. It was shown that DPP-4 inhibitor linagliptin and derivative of vitamin D calcipotriol suppress the activity of TGF-β, and NF-κB contents with elevation of GSHPx and catalase expression (Kabel et al., 2017).

alveolar epithelial cells to myofibroblasts *in vitro* and *in vivo*, reduces eNOS expression and activity in epithelial source cells and, in that way, suppresses NO mediated protective effects against epithelial to mesenchymal transition and fibrosis (Hinz, 2012; Vyas-Read, Shaul, Yuhanna, & Willis, 2007). The deficiency of the receptor for advanced glycation end products (RAGE), constitutively expressed in the lung, was accompanied by reduced pulmonary levels of profibrotic TGF-β and PDGF, suggesting involvement of RAGE in pulmonary fibrosis (He et al., 2007). DPP-4 may increase advanced glycation end products and RAGE expression (Nguyen et al., 2017). The inhibition of DPP-4 reduces RAGE expression and, in that way, among others, contributes to the lung homeostasis and the protection against fibrosis. Therefore, DPP-4 inhibition may result indirectly in reduced activity of profibrotic TGF-β and PDGF.

TGF-β1 increases the expression of pro-inflammatory cytokines (TNF-α, IL-1β), and elevates the activity of lactate dehydrogenase (LDH) via the hypoxia-inducible factor-1α in lung fibroblasts, promoting differentiation to myofibroblasts. It has been reported that the combination of one of DPP-4 inhibitors linagliptin and synthetic derivative of vitamin D calcipotriol significantly suppressed the activity of LDH, reduced lung malondialdehyde, TGF-β, TNF-α and NF-κB contents with elevation of GSHPx and catalase expression. This combination resulted in significant improvement of pulmonary fibrosis compared to that arising from the use of linagliptin alone (Kabel, Elmaaboud, Atef, & Baali, 2017). This constitutes evidence for the beneficial antioxidative, anti-inflammatory and antifibrotic effects of DPP-4 inhibition in the lungs as well as indicates to beneficial effects of combination of DPP-4 inhibitors with vitamin D analogues.

DPP-4 stimulates the production of fibronectin and proliferation of lung fibroblasts and bronchial SMCs (Shiobara et al., 2016). Lung and skin fibroblasts express CD26/DPP-4 constitutively (Nemoto, Sugawara, Takada, Shoji, & Horiuch, 1999). Rinkevich et al. identified the dermal fibroblast lineage responsible for the bulk of fibrosis, on the basis of the expression of the cell surface CD26/DPP-4 *in vivo,* which is important for the possibility to effectively influence and manipulate the fibrogenic response (Rinkevich et al., 2015). Soare et al. demonstrated that DPP-4 positive fibroblasts play an important role in the pathogenesis of fibrosis in systemic sclerosis (Soare et al., 2016). DPP-4 on the fibroblast surface can bind fibronectin and collagen, participating in ECM turnover (Ghersi et al., 2002). DPP-4/CD26 on fibroblasts also takes part in inflammatory processes regulating the expression and activity of biologically active peptides (Nemoto et al., 1999). Recently Bušek et al. reported that DPP-4 activity is actually a consequence of a family of proteins known as the "DPP-4 activity and/or structure homologues", capable of modulating the structure and activity of various peptides important among other activities for the regulation of fibroblasts migration (Bušek, Malík, & Šedo, 2004). The migration of fibroblasts through connective tissue is dependent on the activity of metallo- and serine- cell surface proteases. It has been shown that the complex of the membrane-bound seprase and DPP-4 on fibroblasts is a prerequisite for cell migration and invasion. The seprase/DPP-4 complex, as a marker of migrating fibroblasts, is partly responsible for the connective tissue invasiveness (Ghersi et al., 2002). After the activation of adventitial fibroblasts in the pulmonary vasculature, that express CD26/DPP-4, these cells undergo changes that promote their proliferation, migration, differentiation and inhibition of apoptosis. Proliferation is induced by activation of the MAPK/extracellular signal-regulated kinases (ERK) signalling, and is followed by the secretion of cytokines, chemokines, adhesion molecules and growth factors, as well as the production of ECM proteins, ROS and MMPs (Fig. 2) (Stenmark, Frid, & Yeager, 2010). The inhibition of DPP-4 might suppress the proliferation and migration of fibroblasts and, in that way, prevent fibrosis.

4. Epigenetic targets of DPP-4 inhibitors related to pulmonary fibrosis

PH is a disease with multifactorial etiologies including epigenetic influence. Epigenetic factors influence fibroblasts genetic rearrangement that contributes to pulmonary fibrosis and might serve as potential targets for treatment options. Epigenetic regulation may refer to nucleosomal histone modification, methylation of CpG-DNA islands and non-coding RNA (miR) metabolism, all reported to occur in PH (Ranchoux et al., 2018; Yu, Ibarra, & Kaminski, 2018). Functional studies indicate that miRs participate in the regulation of multiple biological processes and changes in their expression are observed in human pathologies. Most miRs derive from longer primary miRs (produced by polymerase II from independent transcription units or introns of protein-coding genes) that are cleaved into shorter precursor miRs by ribonuclease III enzymes. As a short polynucleotide transcripts of about 22 nucleotides, small regulatory miRs, after complete or partial complementary binding to specific nucleotide mRNA sequence (3′UTR, 5′UTR or coding regions), can silence translation and synthesis of target proteins (Ameres & Zamore, 2013; Cushing et al., 2011; Filipowicz, Bhattacharyya, & Sonenberg, 2008). Accumulating evidence suggests that miRs might be involved in the endothelial to mesenchymal transition process as well as in the regulation of disease progression (Kim, 2018). Among a number of miRs, the deregulation of miR-29 was emphasized in cardiometabolic disorders, chronic diabetic complications and pulmonary fibrosis (Slusarz & Pulakat, 2015; Widlansky et al., 2018). MiR-29 levels are inversely correlated with the expression of profibrotic genes and the severity of the fibrosis. This miR is suppressed by TGF-β in human fetal lung fibroblasts, and many fibrosis-associated miR-29 target genes such as collagens, ECM-associated and remodelling genes are derepressed by miR-29 knockdown (Cushing et al., 2011).

How is this related to DPP-4 and its inhibition? The possible epigenetic influences of DPP-4 inhibitors may be related to the regulation of expression of specific genes or the influence on the silencing of protein synthesis on specific mRNA-templates. The hypothesis about the possible role of DPP-4 inhibitors as epigenetic modulators came from the experimental data where suppressed DPP-4 mRNA expression in peripheral blood mononuclear cells by DPP-4 inhibitor was reported (Makdissi et al., 2012). In rats with metabolic syndrome sitagliptin induced histon posttranslational methylation modifications with beneficial effects on hyperglycemia-induced vascular dysfunction (Amber et al., 2014). It has been shown that the treatment with DPP-4 inhibitor linagliptin reduced inflammation and DPP-4 expression in kidney endothelial cells in streptozocin-induced diabetic mice. The hypothesis that DPP-4 up-regulation may be associated with the miR-29 family down-regulation comes after *in vitro* insertion of miR-29 antagonists in culture of endothelial cells, which counteracted linagliptin-induced DPP-4 inhibition. From the other side, the insertion of miR-29 mimetics down-regulated DPP-4 expression. It was found that the 3′UTR region can serve as a complementary silencing target for the DPP-4 mRNA. Kanasaki et al. reported that linagliptin inhibited TGF-β2-induced endothelial to mesenchymal transition, DPP-4 level and endothelial cell migration, *in vitro*, and such effects was mediated via miR-29 induction. TGF-β2-suppressed miR-29 s were restored by linagliptin in *in vitro* analysis. DPP-4 inhibition might be involved in the antifibrotic effects of miR-29 s (Kanasaki et al., 2014). The mRNAs transcripts, which code for proteins that may orchestrate tissue fibrosis (in the heart, kidney, liver and lung) can be the complementary templates for miR-29. Furthermore, it may explain decreased level of miR-29 in pulmonary fibrosis. In human fetal lung fibroblasts miR-29 is suppressed by TGF-β1, and miR-29 suppresses proliferation of pulmonary artery SMCs (Chen et al., 2018; Cushing et al., 2011). Albeit more experimental evidence is needed for final conclusions, we believe that the epigenetic cross-talk of DPP-4 inhibitors with miR-29 may additionally justify their therapeutic usefulness in the treatment of pulmonary fibrosis (Fig. 3). Not only lung fibroblasts are the cells which exert miR-29 specific response. MiR-29 expression patterns were documented in the lung endothelial cells and vascular SMCs of distal small arteries, acting as regulator of their differentiation (Cushing et al., 2015). Additionally, potential regulatory role of miR-21 in PH development was declared (Parikh et al., 2012). TGF-β acts as an inducer of miR-21 expression and maturation

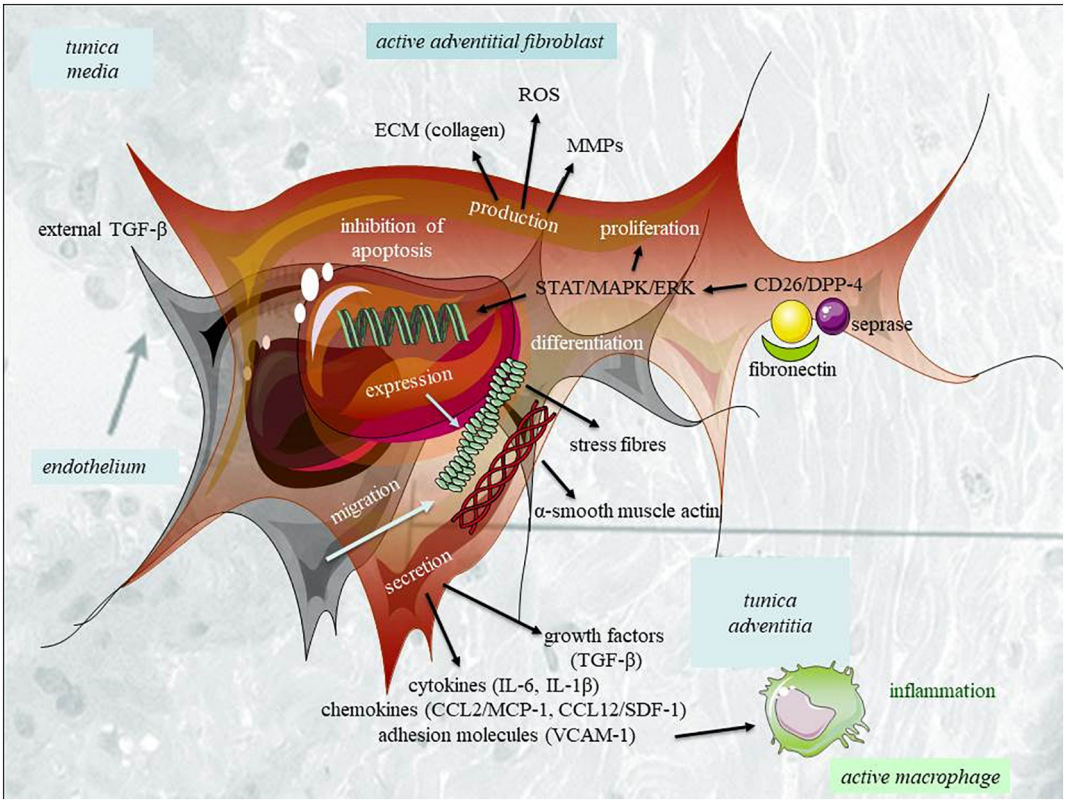

Fig. 2. An active adventitial fibroblast bearing CD26/DPP-4 and its possible role in vascular and connective tissue remodelling in pulmonary hypertension (partly adapted from Stenmark et al., 2010).

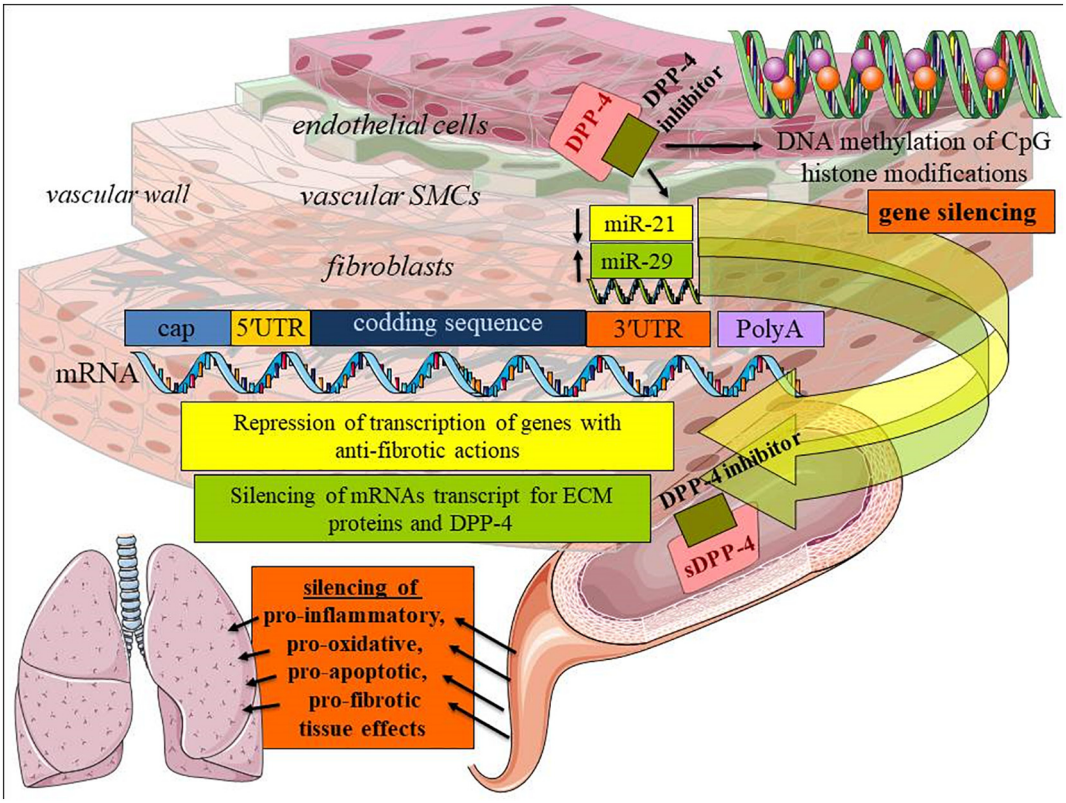

Fig. 3. Experimental epigenetic targets of DPP-4 inhibitors on mRNA transcript silencing.

in vascular SMCs and endothelial cells (Davis, Hilyard, Lagna, & Hata, 2008; Krichevsky & Gabriely, 2009; Ranchoux et al., 2018). miR-21 might up-regulate proliferation of vascular SMCs (Ji et al., 2007). Its expression in endothelial cells and action on Akt kinase downstream signalling was considered as an important step in endothelial to mesenchymal transition (Guo et al., 2015; Ranchoux et al., 2018). MiR-21 was identified as a long-term memory keeper of the fibrogenic program in mesenchymal stem cells. It has been considered as a profibrotic regulator in a variety of different cell types and organs (Li et al., 2017). Significant upregulation of miR-21 was confirmed in renal cortex in rats with experimental type 2 diabetes, with downregulation after the treatment with sitagliptin. Obseved miRs profile was closely related to the improvement of type 2 diabetes, followed by the attenuation of inflammation and oxidative stress and increased antioxidative response (Civantos et al., 2017). Potential effect of DPP-4 inhibition on miR-21 in pulmonary fibrosis remains to be elucidated, but hypothetically, based on the above mentioned results, inhibitors of DPP-4 might decrease miR-21 in the lung. These preliminary relations of DPP-4 inhibitor/miR pathway may help in possible justification of innovative therapeutic strategy for prevention and treatment of PH even in irreversible phase through their influence on endothelial cells and fibroblasts.

5. Conclusion

As the obligatory step, the irreversible phase of PH includes functional activation, proliferation, migration and differentiation of lung fibroblasts, which leads to fibrosis in PH. Their further production of ECM proteins, cytokines and growth factors contributes to remodelling of the pulmonary vasculature. An emerging novel therapeutic approach for DPP-4 inhibitors has here been elucidated by explaining the critical role of CD26/DPP-4 positive fibroblasts in staging from the reversible to the irreversible phase of PH.

The mechanisms proposed here support DPP-4 inhibition as a novel therapeutic approach in PH and involve pathways and mediators in the pulmonary vascular and connective tissue remodelling that are affected directly or indirectly by the inhibition of this protease. This result in antioxidative, anti-inflammatory and antifibrotic effects with consequent improvement of PH. DPP-4 inhibition reduces the activity of profibrotic mediators, suppresses endothelial to mesenchymal transition, proliferation and migration of fibroblasts. This offers the possibility that DPP-4 inhibitors might prevent fibrosis and delay or even fully suppress the entry to the irreversible phase of vascular remodelling in PH.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

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