The expression of proline-specific enzymes in the human lung

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Abstract: The pathophysiology of lung diseases is very complex and proteolytic enzymes may play a role or could be used as biomarkers. In this review, the literature was searched to make an overview of what is known on the expression of the proline-specific peptidases dipeptidyl peptidase (DPP) 4, 8, 9, prolyl oligopeptidase (PREP) and fibroblast activation protein α (FAP) in the healthy and diseased lung. Search terms included asthma, chronic obstructive pulmonary disease (COPD), lung cancer, fibrosis, ischemia reperfusion injury and pneumonia. Knowledge on the loss or gain of protein expression and activity during disease might tie these enzymes to certain cell types, substrates or interaction partners that are involved in the pathophysiology of the disease, ultimately leading to the elucidation of their functional roles and a potential therapeutic target. Most data could be found on DPP4, while the other enzymes are less explored. Published data however often appear to be conflicting, the applied methods divers and the specificity of the assays used questionable. In conclusion, information on the expression of the proline-specific peptidases in the healthy and diseased lung is lacking, begging for further well-designed research.

Keywords: Dipeptidyl peptidase 4 (DPP4); dipeptidyl peptidase 8 (DPP8); dipeptidyl peptidase 9 (DPP9); fibroblast activation protein α (FAP); lung; prolyl oligopeptidase (PREP)

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Introduction

Several respiratory diseases are leading causes of death and form a burden for many more (1). For example, around 235 million people suffer from asthma (2), and lung cancer is a top cause for cancer-related death (3).

The pathophysiology underlying lung diseases is complex and proteolytic enzymes may be involved or could serve as potential biomarkers (4,5). For this review, we focused on several members of the prolyl oligopeptidase (PREP) family (family S9), namely PREP (EC 3.4.21.26), dipeptidyl peptidase 4 (DPP4) (EC 3.4.14.5), dipeptidyl peptidase 8 (DPP8), dipeptidyl peptidase 9 (DPP9) and fibroblast activation protein α [(FAP) EC 3.4.21.B28] and their expression in the lung (6,7). All of these enzymes are serine proteases with the unique ability to cleave a peptide bond after a proline and classification in the S9 family highlights their structural conservation. Crystal structures are available for DPP4, FAP and PREP, while modeled structures for DPP8 and DPP9 have been published (8-12).

DPP4

DPP4 (also known as adenosine deaminase binding protein or CD26) was originally described in 1966 (13) and has DPP activity, meaning that it cleaves off dipeptides from the free amino-terminus preferentially when Pro or Ala are at the penultimate position (14). DPP4 is expressed as a type II transmembrane protein (15), but also a soluble form (sDPP4) can be detected in body fluids (16). The

sources of circulating DPP4 are not fully known, but bone marrow cells (17), adipocytes (18), skeletal muscle cells (19) and vascular smooth muscle cells (20) have been implicated. Several proteases, such as matrix metalloproteases, are able to induce shedding of sDPP4 from the plasma membrane (20). DPP4 has a widespread tissue distribution, being most abundant in the kidney and small intestine (21-23). Besides its expression in epithelial and endothelial cells, it can also be found in multiple cells of the immune system (24-28). Many substrates have already been identified for DPP4, amongst these are neuropeptides, chemokines and the incretin hormones glucagon-like peptide (GLP)-1 and gastric inhibitory polypeptide (GIP) (29-34). The latter are well-known, since DPP4-inhibitors are clinically used to treat type 2 diabetes by prolonging the biological activity of these incretins. Besides its enzymatic functions, DPP4 is able to interact with other molecules such as adenosine deaminase (35,36), caveolin-1 (37), mannose 6-phosphate/insulin-like growth factor II receptor (38,39), the sodium hydrogen exchanger type 3 (40), and with proteins of the extracellular matrix (41,42). However, the last interaction might be indirect (43,44).

DPP8, DPP9

DPP8 and DPP9 were identified in 2000 and are similar to DPP4, but lack a transmembrane region and a secretion signal which led to the conclusion that they are located in the cytoplasm (45,46). Both DPP8 and DPP9 have multiple isoforms and for DPP9, it has been shown that the long isoform, which is longer at the N-terminus, contains a nuclear localization signal and localizes preferentially to the nucleus (47). The similarity between DPP8 and DPP9 is so high that, up to date, selective substrates or inhibitors that distinguish between both enzymes, have not been found, which hampers the search for their individual functions. DPP8 and DPP9 knock-out models have not been described. A DPP9 knock-in, replacing the active site serine for alanine, proved to be lethal, with neonates dying within 8–24 hours after birth (48). Analysis of gene expression patterns of these knock-in mice showed differential expression of genes involved in cell growth, innate immunity and metabolic pathways (49). DPP8 and DPP9 have DPP activity and are able to cleave DPP4 substrates *in vitro*, although at a lower rate than DPP4. The physiological relevance of cleavage of these extracellular peptides is not clear, considering the cytoplasmic location of DPP8 and DPP9 (50). DPP9 has been shown to be rate-limiting in the degradation of proline-containing peptides and to be involved in antigen presentation (51). A proteomics study using the terminal amine isotopic labelling of substrates approach identified several substrates, including calreticulin and adenylate kinase 2, providing possible leads into the functional role of DPP8 and DPP9. Involvement of DPP8 and DPP9 in adipogenesis has also been suggested, since the selective DPP8/9-inhibitor 1G244 resulted in impaired adipocyte differentiation (52). The same inhibitor exerted an anti-inflammatory effect in human as well as murine activated M1 macrophages (53,54). Other studies have implied that DPP9 is involved in cell migration, apoptosis and cell adhesion (53-56). SUMO1 has been identified as an interaction partner of DPP8 and DPP9 and for DPP9 it was demonstrated that SUMO1, or a sumoylated protein, is an allosteric regulator of its activity (57). Another, recently identified, interaction partner of DPP9 is filamin A, which recruits DPP9 to Syk, an important component in B-cell receptor signaling. Syk can be cleaved by DPP9 and hence influences the stability of Syk (58). DPP8 and 9 enzymes are widely expressed and can be found in cells of the immune system, epithelia, endothelia, brain, reproductive organs and others (24,53,59-61).

FAP

FAP, also known as seprase, is a type II membrane protein and was first described in 1986 (62). A soluble form of FAP can be found in the blood (63), but the sources of circulating FAP are currently not known. FAP has DPP activity, but in addition also possesses endopeptidase or gelatinase activity. FAP's endopeptidase activity is restricted because it can only cleave after an internal Pro when it is preceded by a Gly (64,65). The known substrate repertoire of FAP is limited and includes α2-antiplasmin and denatured collagen. *In vitro* work identified several DPP4 substrates such as neuropeptide Y, substance P and B-type natriuretic peptide also as FAP substrates (66-68). It is believed that FAP is largely absent from healthy adult tissues, but that it is expressed during embryogenesis, inflammation, wound healing, cancer and fibrosis (69-73). However, recent reports show FAP under normal conditions, with high expression in skin, pancreas and endometrium (74-76). In addition, FAP knock-out in a mouse model does not affect embryonic development or normal organ function (77). In epithelial cancers, FAP localizes to reactive stromal fibroblasts, and it can also be detected in certain sarcomas (69,76,78,79).

The absence of FAP from the majority of normal cells and tissues and its restricted expression in cancers make FAP an interesting target for the selective delivery of cytotoxic compounds to the tumor site (80-82). In addition, targeting FAP itself has also been the focus of anti-cancer research, since it has been implicated in extracellular matrix remodeling through its gelatinase activity, antitumor immunity and processing of substrates (83,84). Regardless, the exact role of FAP appears to be highly dependent of the tumor microenvironment and cancer type (80).

PREP

PREP, also called prolyl endopeptidase, was discovered in 1971 as an oxytocin cleaving enzyme in the uterus (85). In contrast to the above described enzymes, PREP has endopeptidase activity only. High expression of PREP is found in the brain, which was the subject of several studies (86,87). PREP is a cytoplasmic enzyme, but its activity can also be measured in body fluids (88-90). All short peptides (up to 30 amino acids long) that contain a proline are possible substrates of PREP. Most research has been done *in vitro*, and examples of substrates are substance P, angiotensin II and bradykinin (91,92). PREP is involved in the generation of proline-glycine-proline (PGP) from collagen fragments, PGP is a neutrophil chemoattractant, and an important player in neutrophilic inflammation (93-95). Since PREP itself is also present in neutrophils, it also plays a role in sustaining neutrophilic inflammation (96), which links PREP to the pathology of many lung diseases. PREP is involved in the synthesis of the anti-fibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline (97). It has been shown that PREP interacts with α-synuclein and accelerates its aggregation (98,99). Additional interaction partners are glyceraldehyde-3-phosphate dehydrogenase (100), growth-associated protein 43 (101,102) and tubulin (90). Other suggested functions of PREP are a role in protein processing, secretion and axonal transport (90,103).

Possible problems when assaying DPPs, FAP and PREP

The discovery of DPP8 and DPP9 as proline specific DPPs uncovered a problem in DPP4 literature since, until then, the reported activity was often not supported by gene or protein expression analysis. When using antibodies for protein detection in immunoassays, one has to keep in mind that these enzymes share structural properties and that antibodies may cross-react. Likewise, overlapping substrate specificities complicate the interpretation of enzymatic activity assays *in situ* or *in vitro*. Consequently, the techniques, and often also the reagents, believed to be specific in the past, turned out to identify or measure multiple enzymes. This is certainly the case with the DPPs, and with FAP and PREP, when these enzymes are assayed with synthetic substrates. Examples are Gly-Pro-*para*nitroanilide (*p*NA) and Gly-Pro-4-methoxy-β-naphthylamide (Gly-Pro-4MeβNA), which both can be cleaved by DPP4, 8 and 9 or benzylcarboxy(Z)-Gly-Pro-*p*NA and Z-Gly-Pro-7-amido-4-methylcoumarin (Z-Gly-Pro-AMC), which are substrates of both FAP and PREP. In addition, the inhibitors used in the past and even the DPP4-inhibitors used clinically today differ in their selectivity towards the other family members (27). Therefore, researchers have to be careful when interpreting data from literature predating the discovery of the other enzymes. In addition, when designing new experiments, one should be aware of the specificity (or lack thereof) of the reagents and methods used.

With this warning in mind, the literature was searched for reports on the expression of each individual enzyme in the human lung in health and disease. Search terms included asthma, chronic obstructive pulmonary disease (COPD), lung cancer, fibrosis, ischemia reperfusion injury (IRI) and pneumonia. When relevant data appeared to be available for animal models only, these were incorporated as well. The information in this review is up-to-date until October 2016.

Expression of proline-specific enzymes in the lung in health and disease

Healthy lung

DPP4 has been identified as the functional receptor for the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (104). To better understand the pathogenesis of MERS-CoV, the distribution of DPP4 in the human respiratory tract was investigated using immunohistochemistry (IHC) (antibody clone 11D7) (105). In the epithelium of nasal mucosa and conducting airways, DPP4 was rarely observed, although it could be detected in small subsets of goblet cells, non-ciliated cells and ciliated cells in the airways, with a trend for higher DPP4 immunostaining in smaller airways. DPP4 was also present in mononuclear leukocytes, mainly T-lymphocytes, and in submucosal gland serous cells, but not in mucous cells. In

the parenchyma, DPP4 was detected in type I and II cells and alveolar macrophages. The endothelium of pulmonary vessels also had DPP4 staining, decreasing in staining intensity from lymphatics over venules to arterioles. These results are in line with an investigation that used enzyme histochemistry with Gly-Pro-4MeβNA and IHC (antibody Ta-1) to evaluate the distribution of DPP4 in the human bronchus, reporting staining in blood vessels, serosal glands and leukocytes, while bronchial epithelium, smooth muscle and connective tissue were negative (106). Another IHC study (antibody clone BA5) revealed similar results (107). A report on nasal mucosa described DPP4-expression (antibody clone BA5) in submucosal seromucous glands, leukocytes, endothelial cells of venules and capillaries, and in some epithelial cells (108).

DPP4 activity was shown to be measurable in bronchoalveolar lavage (BAL) fluid and to be increased in BAL fluid from patients (including tumors, sarcoidosis, infectious diseases and AIDS) (109). DPP4 activity was higher when the number of lymphocytes was increased, but there was no strict correlation (109). This might imply that sDPP4 in BAL fluid partially originates from other sources. DPP4 activity (measured with Gly-Pro-*p*NA) was lower in a bronchial epithelial cell line (BEAS-2B), than in an alveolar epithelial cell line (A549), which was in turn lower than in primary BAL macrophages (109). Schade *et al*. also measured DPP4-like activity with Gly-Pro-*p*NA in BAL fluids collected from DPP4-positive and DPP4-negative F344 rats. Low DPP4-like activity, most likely representing DPP8/9-activity, could be measured in the BAL fluid of DPP4-negative rats, accounting for approximately 20% of the total DPP4-like activity that was measured in DPP4-positive rats (110). In wild-type rats, DPP4 expression and activity was mainly found in lung parenchyma and less in the bronchi, while DPP8/9 (activity and expression) was located in bronchi and leukocytes, but weakly in the parenchyma. The expression of DPP10, an inactive member of the DPP family, was also detected in bronchi and leukocytes. Lavage of the lungs resulted in a lower expression of DPP8, 9 and 10 in the lung (110). Since DPP4 and DPP8/9 are differentially expressed in the lungs, the activity measured in the BEAS-2B cells might in fact represent DPP4, 8 and 9. For DPP4, also mRNA expression has been demonstrated in human bronchial epithelial cells, transformed bronchial epithelial cells, the Calu-3 cell line and A549 cells (111). However, as most cell lines originate from cancerous tissue these cells might not be representative for the corresponding primary airway epithelial cells *in vivo*.

Asthma

Since there are several reasons for a potential role of DPP4 in asthma, namely its function as a co-stimulatory molecule, its ability to process substrates involved in asthma and interactions with other proteins, DPP4 has already been extensively studied in various asthma models. For more information on DPP4 in asthma, the reader is referred to the recent review by Nieto-Fontarigo *et al*. (112).

Schade *et al*. induced asthma in DPP4-positive and DPP4-negative rats by sensitization and challenge with ovalbumin (110). This resulted in an increase of total DPP4-like and DPP8/9-activity in BAL fluid (DPP8/9-activity is responsible for approximately one fifth of the total activity). In addition, an increased expression and activity was detected for DPP4 in the parenchyma and for DPP8/9 in the bronchi. DPP10 expression was also upregulated after asthma induction (110) and the gene of DPP10 was previously identified as a susceptibility marker of asthma (113). Sensitization and challenge with different concentrations of ovalbumin in F344 rats resulted in a dose-dependent increase of $CD4^+/CD25^+/CD26^+$ T cells in the lung, but, in contrast to a subsequent study, no significant differences in the DPP4-activity (determined with Gly-Pro-*p*NA) could be found in BAL fluid (114). van der Velden *et al*. did not see any difference in the number of DPP4-positive cells in the bronchial epithelium between healthy controls and patients with asthma. These patients were treated with inhaled β-agonists and did not receive corticosteroids in the month prior to the study (106). The same authors could also not detect any differences in DPP4-activity, as determined with Gly-Pro-pNA, between BAL fluid from non-smokers, smokers and asthma patients (115). Treatment with inhaled fluticasone propionate for 12 weeks did not alter the activity of DPP4 in BAL fluid (115). Shiobara *et al*. demonstrated an increased DPP4 mRNA expression in bronchial epithelial cells of the distal airways from corticosteroid-naïve asthma patients (116). Using IHC on bronchial biopsies, DPP4 protein expression was shown in bronchial epithelial cells and in inflammatory cells (eosinophils, macrophages, lymphocytes) from corticosteroid-naïve patients, while in corticosteroid-treated patients the staining was reduced. IL-13 stimulation of bronchial epithelial cells resulted in an increased mRNA and protein expression of DPP4 (116).

COPD, cystic fibrosis and idiopathic pulmonary fibrosis (IPF)

Meyerholz *et al*. studied the influence of tissue remodeling on DPP4 distribution in lungs from patients with COPD or cystic fibrosis (105). A significant increase in DPP4 immunostaining was found in type I and II cells and in alveolar macrophages in the lungs of patients (105). Alveolar macrophages showed stronger staining when they were activated and also hypertrophy of mesothelial cells often corresponded with increased DPP4 staining. The DPP9 gene at chromosome 19p13 was identified as a novel risk locus for fibrotic idiopathic interstitial pneumonia and in IPF cases the expression of DPP9 mRNA in whole-lung samples was higher than in controls (117). Also FAP was shown to be upregulated in IPF patients (118). Using IHC for FAP (F19 antibody), no immunoreactivity could be detected in tissues (bronchiolar epithelium, type I and II pneumocytes, endothelium, smooth muscle, alveolar macrophages) of normal lung and centriacinar emphysema cases (118). This study could also not detect DPP4 (M-A261 antibody) in the same tissues except for a few positive cells in the alveolar epithelium, which is in contrast with the results of Meyerholz *et al*. (105,118). On the contrary, FAP expression was high in fibroblast foci, identifying more than what could be seen in H&E stained sections, and in fibrotic interstitium, suggesting a potential role for FAP in extracellular matrix modification. DPP4 showed a distinct expression pattern, staining the hyperplastic alveolar lining cells that overly fibroblast foci, but not the fibroblast foci themselves (118). Induction of pulmonary fibrosis in both bleomycin and thoracic irradiation murine models, resulted in an increased FAP mRNA expression, which was also confirmed by immunostaining of stromal cells rather than inflammatory cells or alveolar epithelial cells (119). Induction of pulmonary fibrosis in FAP-deficient mice resulted in a decreased survival and an increased lung collagen content in both models and this was not due to changes in the activity of matrix metalloproteinases (MMP), suggesting that FAP has an anti-fibrotic effect in the lung. FAP further processes MMP-degraded collagen, improving the internalization and clearance of collagen into macrophages and fibroblasts (119). As stated for PREP, PGP can be generated from collagen and has chemotactic effects on neutrophils. Based on the substrate specificity, FAP should also be able to produce PGP from MMP-processed collagen and hence might also exert its effects through this mechanism. However, this hypothesis needs to be further explored. In lung tissues of active smokers and COPD patients, PREP was highly expressed on neutrophils, macrophages and epithelial cells (89). The number of inflammatory cells and PREP expression was lower in lung tissues from ex-smokers (89). These data are in line with a study in mice (120). PREP activity, measured with Z-Gly-Pro-AMC in lung homogenates, was higher in cigarette smoke-exposed mice. Immunohistochemical analysis showed PREP in epithelial cells of control mice. In the smoke-exposed mice, the protein expression of PREP was increased due to the influx of inflammatory cells such as macrophages and neutrophils, which highly express PREP (120). PREP activity has been measured in sputum with Z-Gly-Pro-*p*NA and was 5 times higher in patients with cystic fibrosis compared to healthy controls (93). PREP mRNA, protein expression and enzymatic activity has been shown in multiple airway epithelial cells, both in cell lines and primary cells (121). PREP could be found in the medium of cystic fibrosis bronchial epithelial cells and was increased after stimulation with lipopolysaccharide (LPS), without an increase in PREP mRNA. Depletion of toll-like receptor 4 resulted in an attenuation of the LPS-induced release of PREP. It was reported that PREP is released from airway epithelial cells through exosomes. The sputum of patients with cystic fibrosis colonized with *Pseudomonas aeruginosa* showed more exosomes and increased PREP protein expression (121).

Lung cancer

The two main groups of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts for approximately 85% of all cases. The most common types of NSCLC are adenocarcinoma, large cell carcinoma and squamous cell carcinoma (122,123).

Evaluation of the activity of DPP4, based on Gly-Pro-4MeβNA, in different lung carcinomas revealed positive staining in 93% of the cases of adenocarcinoma, while squamous cell carcinoma, small cell carcinoma, large cell carcinoma and carcinoid were negative (124). Study of different NSCLC cell lines in comparison with normal bronchial epithelial cells showed a lower enzymatic activity, protein and mRNA expression of DPP4 (125). Šedo *et al.* used Gly-Pro-AMC to study DPP4 and succinyl-Gly-Pro-AMC for PREP in different lung tumors and, in contrast to the previous reports, showed higher activities of both enzymes in squamous cell carcinomas and adenocarcinomas, as compared to matched lung parenchyma (126). Dimitrova *et al*. used a new fluorogenic substrate, namely 4-(Gly-Pro-hydrazido)-N-hexyl-1,8-naphthalimide, for the detection of DPP4 in their study with human fetal lung-derived, A549

and SK-MES-1 cells (127,128). Specificity of their substrate was validated with the inhibitor N-(H-Phe-Pro)-O-(4-nitrobenzoyl) hydroxylamine, which was reported to result in abolishment of the enzymatic activity (128). Problem is that this inhibitor was characterized before researchers were aware of the existence of DPP8 and 9, and might as well inhibit multiple family members (129,130). Taking this uncertainty into account, the fluorescence intensity was lower in tumor cell lines compared to the fetal cells and was confirmed in an enzymatic activity measurement with Gly-Pro-*p*NA. However, a comparison between fetal and cancerous tissue might not be optimal, since it was shown that DPP4-like activity was higher in human fetal lung compared to adult lungs (131). The relative surface activity (surface activity versus total activity) of the fetal and A549 cells was almost 100%, indicating that DPP4 was present on the membrane. Instead, the relative surface activity of SK-MES-1 cells was only 35 %, which means that two thirds of the DPP4-like activity is located intracellularly. The authors explain this by disturbances in the intracellular membrane transport system, but a possible contribution of the intracellular enzymes DPP8 and 9 cannot be neglected.

In silico analysis showed that FAP mRNA expression was upregulated in squamous cell carcinomas, large cell carcinomas and adenocarcinomas, as compared to normal lung tissue (76). IHC with antibody F19 confirmed that FAP is restricted to the stroma of epithelial cancers (76). FAP immunostaining (antibody ab53066, Abcam) in tumor tissue from patients with NSCLC demonstrated positive stromal staining in 76 % of all cases. Higher expression of FAP was associated with worse survival (132). Talabostat, also known as PT-100 or Val-boroPro, is an inhibitor of DPPs including FAP. A phase II trial of talabostat with docetaxel in advanced NSCLC, did not show enhanced clinical activity in these patients (133).

In malignant pleural mesothelioma (MPM), DPP4 protein was highly expressed, while weak expression was seen in adenomatoid tumor or reactive mesothelial cells (134). DPP4 activity and protein expression has also been detected in pleural fluid. In patients with an epithelioid subtype of MPM the sDPP4 levels and DPP4-activity (substrate Gly-Pro-*p*NA) were increased compared to patients with benign pleural diseases. In MPM patients with a sarcomatous subtype, sDPP4 was significantly increased in the pleural fluid, while DPP4-activity was higher, but not reaching statistical significance (135). DPP4 protein levels in pleural effusion (PE) were significantly higher in malignant NSCLC, than in paramalignant NSCLC or pneumonia patients, but not when compared to tuberculosis patients (136). Other studies have also shown that DPP4 protein levels and activity (measured with Gly-Pro-*p*NA) were higher in PE of patients with tuberculosis compared to other non-tuberculous PE, including malignant, heart failure and parapneumonic effusion (137,138).

Others

PREP activity has been measured with Z-Gly-Pro-sulfamethoxazole in healthy lung tissues from patients with lung carcinoma and in the BAL fluid of patients with various lung diseases (139). PREP could be detected both in the supernatant of the BAL fluid, as well as in the cell pellet after centrifugation. Higher enzymatic activities in the pellet were seen with higher cell counts and percentage of macrophages (139).

PREP was determined in BAL fluid from lung transplant patients using Z-Gly-Pro-*p*NA as a substrate and an antibody targeted against a synthetic peptide representing residues 190–219 of murine PREP. Both protein expression and enzymatic activity were higher in patients with bronchiolitis obliterans syndrome at time of diagnosis, compared to acute rejection, no rejection and matched samples from 3 months before the diagnosis (140).

The role of DPP4 in IRI has also been extensively studied in multiple models, including the lung, and has been reviewed by Matheeussen *et al.* (141). However, in these studies the main focus lies on the effect of pharmacological inhibition of DPP4 and less on the expression pattern of DPP4. For the other enzymes covered in this review, to the best of our knowledge, no information on their expression in lung IRI models is available.

Pneumonia was another search term that was employed for this review. Unfortunately we weren't able to retrieve information on the expression of any of the enzymes in infectious pneumonia. One abstract shows a possible role of FAP in the host response to bacterial infection (142), and the clinically used DPP4-inhibitors were theoretically suspected to increase the risk of pneumonia based on DPP4's involvement in the immune system. However, several recent studies have not been able to identify an association (143,144).

Conclusions

From this literature review it is clear that DPP4 is by far the best characterized member of the PREP family in healthy

and diseased lungs, while less data was found on the other enzymes.

In healthy lungs, DPP4 was reported in lung parenchyma, immune cells, endothelial cells and submucosal glands. In certain cases DPP4 was also detected in bronchial epithelial cells. DPP4 has been implicated in asthma, but the different studies do not corroborate each other in terms of a possible differential expression of DPP4 in asthma. The available data on DPP4 in lung cancer (and cancer in general) are often conflicting. Certain studies showed decreased expression or activity, while others reported the opposite. Comparison between studies, however, is hindered by the use of a wide variation of methods, tissue types and cell lines. The expression and activity of DPP4 in pleural fluid has been investigated in multiple studies as a possible biomarker. Unfortunately, the results are also not unambiguous and DPP4 is probably not the best marker in pleural fluid to differentiate between different lung diseases.

On the expression of DPP8, 9 and 10 in the lung, only data from a rat asthma model is currently available, showing activity and expression in the bronchi and leukocytes, which increases after asthma induction.

Also in COPD, cystic fibrosis and IPF some of the enzymes were studied and shown to be increased. PREP was detected in epithelial cells in normal lungs and was highly expressed on inflammatory cells in different lung pathologies. The limited studies on FAP show no expression in normal lung, but FAP was increased in several lung cancers and was shown to be expressed in the stroma.

In conclusion, there is still a lot of missing information on the expression of proline-specific peptidases in the healthy and diseased lung. Published data often appear to be conflicting, the applied methods divers, and the specificity of the used assays questionable, begging for further well-designed research. Knowledge on the loss or gain of protein expression and activity during disease might tie these enzymes to certain cell types, substrates or interaction partners that are involved in the pathophysiology of the disease, ultimately leading to the elucidation of their functional role. The apparently differential expression of the enzymes in lung diseases makes them interesting candidates for validation as biomarkers.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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