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WNT receptor signalling in lung physiology and pathology☆

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ABSTRACT

The WNT signalling cascades have emerged as critical regulators of a wide variety of biological aspects involved in lung development as well as in physiological and pathophysiological processes in the adult lung. WNTs (secreted glycoproteins) interact with various transmembrane receptors and co-receptors to activate signalling pathways that regulate transcriptional as well as non-transcriptional responses within cells. In physiological conditions, the majority of WNT receptors and co-receptors can be detected in the adult lung. However, dysregulation of WNT signalling pathways contributes to the development and progression of chronic lung pathologies, including idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), asthma and lung cancer. The interaction between a WNT and the (co-)receptor(s) present at the cell surface is the initial step in transducing an extracellular signal into an intracellular response. This proximal event in WNT signal transduction with (cell-specific) ligand-receptor interactions is of great interest as a potential target for pharmacological intervention. In this review we highlight the diverse expression of various WNT receptors and co-receptors in the aforementioned chronic lung diseases and discuss the currently available biologicals and pharmacological tools to modify proximal WNT signalling.

Contents

Abbreviations: ABC, active β-catenin/non-phosphorylated β-catenin; APC, adenomatous polyposis coli; ATI, alveolar epithelial type I cell; ATII, alveolar epithelial type II cell; AXIN, axis inhibition protein; CBR2, carbonyl reductase 2; CK1, casein kinase 1; CELSR1, cadherin EGF LAG seven-pass G-type receptor 1; COPD, chronic obstructive pulmonary disease; CPI, composite physiologic index; CRD, cysteine-rich domain; CS, cigarette smoke; DKK, Dickkopf; DL_{CO}, diffusion capacity of the lung for carbon monoxide; ECM, extracellular matrix; FZD, Frizzled; GSK-3, glycogen synthase kinase-3; IL, interleukin; IPF, idiopathic pulmonary fibrosis; LGR, leucine-rich repeat-containing G-protein coupled receptor; LRP5/6, low density lipoprotein receptor-related protein 5/6; NFAT, nuclear factor of activated T-cells; NLK, nemo-like kinase; NSCLC, non-small cell lung cancer; PCP, planar cell polarity; PTK7, protein tyrosine kinase 7; RNF43, ring finger protein 43; ROR 1/2, receptor tyrosine kinase-like orphan receptor 1 and 2; RSPO, R-spondin; RYK, related to receptor tyrosine kinase; SCC, squamous cell carcinoma; sFRP, soluble frizzled-related protein; SNP, single nucleotide polymorphism; TAK1, TGF-β-activated kinase-1; TCF/LEF, T-cell factor/lymphoid enhancer-binding factor; VANGL, Van-Gogh-like protein; WNT, Wingless/integrase-1; ZNRF3, zinc and ring finger 3.

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1. Introduction

The Wingless/integrase-1 (WNT) family of secreted glycoproteins interacts with a plethora of transmembrane receptors and is involved in various aspects of mammalian development, physiology and pathophysiology. WNT proteins comprise an important class of glycoproteins (19 in human) critical for proper development and function of many organs, including the respiratory system. Each of the distinct WNT proteins conducts a signal from the cell surface through the cytosol to the nucleus, where it regulates the expression of coordinated sets of genes or via activation of kinases leads to cytoskeletal rearrangements regulating cell polarity (Macdonald, Semenov, & He, 2007; Semenov, Habas, Macdonald, & He, 2007). The WNT family of signalling proteins directs a complex network of downstream signalling events. Originally, WNT signalling is divided into two major signalling branches: (i) canonical WNT/β-catenin signalling, which relies on the activation of the pleiotropic transcriptional coactivator β-catenin and (ii) non-canonical WNT signalling, which is a collection of miscellaneous signalling cascades acting independently of β-catenin (Fig. 1). The nomenclature of canonical and non-canonical WNT signalling is outdated and therefore we ratherrefer to WNT signalling as being either β-catenin-dependent or –independent. The WNT/β-catenin pathway is the most investigated and best characterized WNT signalling pathway, in part due to the availability of several genetic and molecular tools. However, both the physiological and pathophysiological relevance of β-catenin-independent WNT signalling should not be underestimated and recent studies urge further investigations. Remarkably, single WNT proteins can activate multiple signalling pathways depending on the cell-surface receptors present at the cell. WNT-driven activation of β-catenin requires the binding of the ligand to a member of the Frizzled family of transmembrane receptors (FZD_1-FZD_{10}) and a concomitant interaction with the WNT co-receptor low-density lipoprotein-related protein

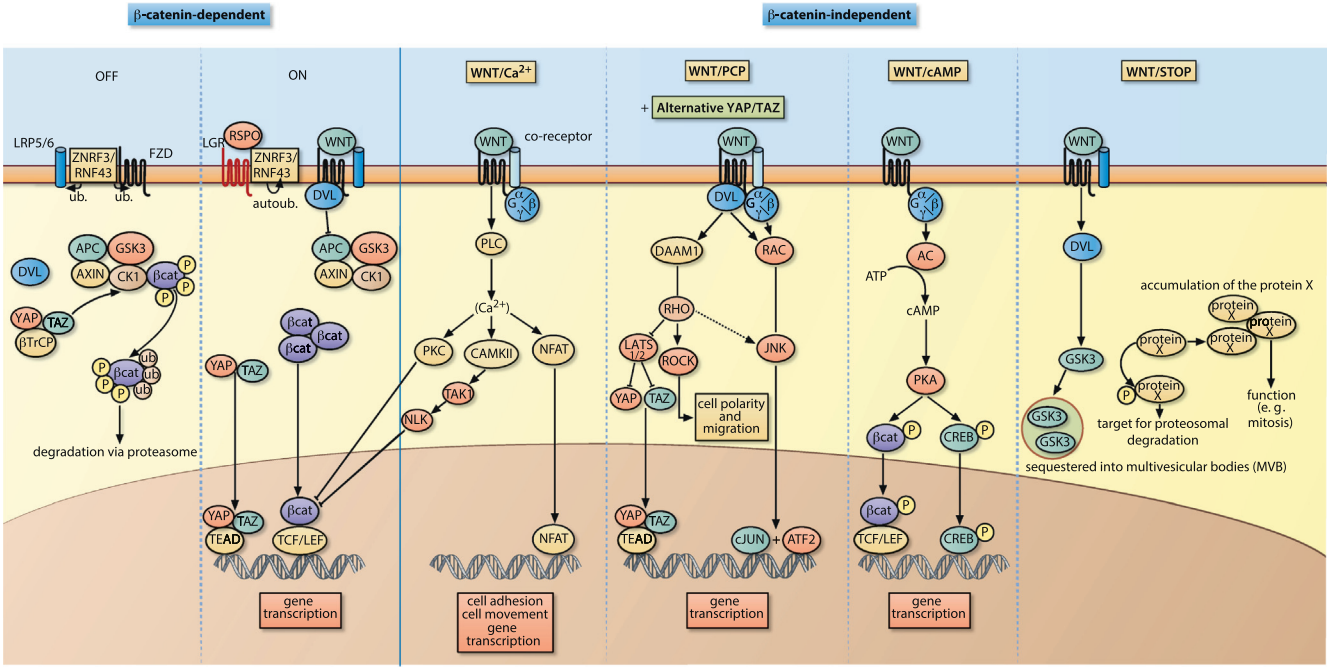

Fig. 1. Schematic representation of various WNT signalling cascades. β-Catenin-dependent WNT signalling pathway: *OFF* state (*left*; *first panel*): In the absence of WNTs, β-catenin is captured by the destruction complex, which is composed of AXIN, Adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK-3β) and Casein Kinase-1 (CK1). The YAP/TAZ/βTrCP complex can have dynamic interaction with the destruction complex. Subsequently, β-catenin is phosphorylated which targets the protein for proteasomal degradation. *Zinc and Ring Finger 3* (ZNRF3)/*Ring finger protein 43* (RNF43) bind the low-density lipoprotein receptor related proteins (LRP) 5/6 as well as Frizzleds (FZDs), which leads to ubiquitination and subsequent degradation of these receptors. *ON* state (*left*; *second panel*): WNT proteins bind to FZD and LRP5/6 which leads to activation of a signalling cascade, which results in the inhibition of the destruction complex. Cytosolic β-catenin can then accumulate and translocated to the nucleus, where it binds to T-cell factor/lymphoid enhancer factor-1 (TCF/LEF) and induces target gene expression. In addition, YAP/TAZ can translocate to the nucleus and contribute to WNT transcriptional responses. R-spondins (RSPOs) bind to leucine-rich repeat-containing G protein-coupled receptors (LGR) and ZNRF3/RNF43, which leads to autoubiquitination of ZNRF3/RNF43, thereby stabilizing FZD and LRP expression at the membrane. β-Catenin-independent WNT signalling pathways: (I) *WNT-Ca^{2+} pathway*: WNTs bind to receptors (i.e. FZD) and co-receptors (e.g. RYK) which in turn activates phospholipase C (PLC) and leads to the formation of inositol 1,4,5-triphosphate (IP3) and 1,2 diacylglycerol (DAG). This subsequently results in an increase of intracellular Ca^{2+}. The raise in Ca^{2+} activates calmodulin-dependent protein kinase II (CAMKII), protein kinase C (PKC) and the nuclear factor of activated T cells (NFAT) transcription factor, regulating transcription of the genes controlling cell fate and migration. This pathway via PKC and nemo-like kinase (NLK) can also negatively influence β-catenin dependent gene transcription. (II) *Planar cell polarity* (*PCP*) *pathway*: binding of WNT to FZDs and co-receptors (e.g. ROR1/2, RYK, MUSK or PTK7) transduces signal via small GTPases, such as RAC1 and RHOA, to activate RHO kinase (ROCK) and JUN-N-terminal kinase (JNK), which regulate cytoskeletal rearrangements and epithelial cell polarity. ROCK can also inhibit large tumour suppressor 1/2 (LATS1/2) resulting in alternative activation of YAP/TAZ signalling. (III) *WNT-cAMP pathway*: WNTs binding to a FZD results in the activation of heteromeric Gs protein, which subsequently leads to conversion of ATP to cAMP by adenylylcyclase (AC). In turn, PKA is activated by cAMP, which phosphorylates both β-catenin and cAMP response element binding protein (CREB) thereby influencing the transcriptional response of these proteins. (IV) *WNT/STOP pathway*: In response to activation of WNT signalling a large part of GSK-3 is sequestered into multivesicular bodies thereby preventing the interaction of GSK-3 with its substrates. Consequently, these GSK-3 substrates are not degraded, accumulate and are capable of inducing cellular responses. Figures modified from (Nakata, Phillips, & Goidts, 2014; Niehrs, 2012; Shi, Mao, Zheng, & Jiang, 2016) and see main text for further details.

5/6 (LRP5/6). This signal cascade results in the inactivation of the so-called β-catenin destruction complex, an assemblage of multiple proteins (including axis inhibition protein (AXIN), adenomatous polyposis C (APC), casein kinase-1 (CK1) and glycogen synthase kinase-3 (GSK-3)), which in the absence of WNTs targets cytosolic β-catenin for proteasomal degradation (Fig. 1) (Baarsma, Konigshoff, & Gosens, 2013; Clevers, 2006). Inactivation of the destruction complex allows β-catenin to accumulate and translocate to the nucleus, where it activates the T-cell factor/lymphoid enhancer factor-1 (TCF/LEF) transcription factors and alters expression of specific genes (Fig. 1). However, WNT-dependent β-catenin signalling is more complex than this straightforward representation suggests, as several other signalling pathways can interfere with the activation of β-catenin at various levels (Attisano & Wrana, 2013). For instance, the Hippo transducers YAP and TAZ can be both positive and negative regulators of WNT signalling (Azzolin et al., 2014). Azzolin and colleagues reported that, in the absence of WNTs, cytosolic YAP and TAZ can be integral components of the β-catenin destruction complex and have a negative effect on WNT signalling. Furthermore, they showed that when the WNT pathway is activated YAP, TAZ and β-catenin dissociate from the destruction complex, translocate to the nucleus, and activate gene transcription. The induced changes in gene transcription are conjointly called the WNT transcriptional response. Thus, when WNT/β-catenin signalling is activated nuclear YAP/TAZ may represent a branch of the WNT transcriptional effects, whereas cytosolic YAP/TAZ (i.e. when Hippo signalling is active and/or WNT signalling activity is low) facilitates proteasomal degradation of β-catenin (Azzolin et al., 2014; Bernascone & Martin-Belmonte, 2013). Consequently, YAP and TAZ likely have a defining role in WNT signalling activity in lung development and disease; but this is beyond the scope of this review. Furthermore, some WNTs, neurotransmitters and hormones can influence β-catenin mediated gene transcription via the activation of protein kinase A (PKA) (Fig. 1). These aforementioned ligands bind to G-protein coupled receptors (GPCR) that activate adenylyl cyclase (AC) via Gs proteins. AC in turn converts ATP into cAMP. The generated cAMP activates PKA, which can phosphorylate β-catenin thereby influencing the activity of the transcriptional co-activator (Fig. 1) (Hino, Tanji, Nakayama, & Kikuchi, 2005; Semenov et al., 2007).

β-Catenin-independent WNT signalling (classically defined as non-canonical WNT signalling) is also initiated by the binding of a WNT protein to one of the FZDs on the cell membrane. However, in β-catenin-independent WNT signalling, the FZDs as well as several non-Frizzled WNT receptors, including receptor tyrosine kinases (e.g. ROR2 and RYK), act independently of LRP5 or LRP6 to transduce the extracellular signal into a cellular response. The WNT/planar cell polarity (PCP) pathway, which regulates the coordinate alignment of cell polarity across tissue, and the WNT/Ca^{2+} pathway, regulating gene transcription and cell migration, are the most commonly studied β-catenin-independent WNT signalling pathways. These WNT signalling cascades can regulate both transcriptional and non-transcriptional responses in cells by either activating transcription factors, such as nuclear factor of activated T-cells (NFAT), or small GTPases, including RAC1 and RHOA (Fig. 1) (Komiya & Habas, 2008; Li, Bellusci, Borok, & Minoo, 2015; Semenov et al., 2007). In addition to WNT/PCP and WNT/Ca^{2+} several other β-catenin-independent WNT signalling pathways are described, including alternative WNT/YAP/TAZ signalling and WNT/STOP signalling (Acebron, Karaulanov, Berger, Huang, & Niehrs, 2014).

Besides regulating β-catenin signalling, YAP and TAZ can also be effector molecules of alternative WNT signalling cascades (Hot et al., 2017; Park et al., 2015). In this scenario, a WNT-FZD interaction results in the downstream activation of a heteromeric G protein (e.g. $G\alpha_{12/13}$) which subsequently leads to the inactivation of the Hippo pathway kinases large tumour suppressor 1/2 (LATS1/2). Consequently, YAP and TAZ are activated and translocate to the nucleus, where they bind to the TEAD transcription factor family and induce expression of a wide range of genes (Park et al., 2015).

WNT/STOP signalling (the abbreviation of *WNT-dependent stabilization of proteins*) refers to the stabilization of GSK-3 substrates in response to WNT signalling activation (Fig. 1). GSK-3 is a constitutively active kinase and has a wide variety of putative substrates (Jope & Johnson, 2004). In many cases, phosphorylation of substrates by GSK-3 results in the generation of phospho-degrons that are recognized by E3-ubiquitin ligases, which further target these proteins for proteasomal degradation (Fig. 1). In response to activation of WNT signalling, a large part of GSK-3 is sequestered into multivesicular bodies thereby preventing the interaction of GSK-3 with its substrates. Consequently, these GSK-3 substrates are not degraded, accumulate and are capable of inducing cellular responses (Fig. 1) (Acebron et al., 2014; Acebron & Niehrs, 2016).

Selectivity in ligand-receptor interaction is critical for the direction of downstream signalling initiated by specific WNTs (Dijksterhuis et al., 2015). Moreover, due to the involvement of the WNT signalling pathways in a wide range of cellular functions, the signalling potential of WNT proteins is tightly regulated by endogenously expressed extracellular modulators. These extracellular WNT modulators can act as scaffold proteins binding to WNT proteins in the extracellular space thereby limiting the bioavailability of the WNTs (e.g. secreted Frizzled related proteins: sFRPs). In addition, some of the extracellular WNT modulating proteins (e.g. Dickkopf proteins: DKKs) inhibit signal transduction by binding to WNT co-receptors (i.e. LRP6) (see Fig. 1) (Baarsma et al., 2013; Clevers, 2006).

Over the last decades WNT signalling and the complex regulation of these pathways have been extensively investigated. Various studies by our and other laboratories have shown that dysregulation of both β-catenin-dependent as -independent WNT signalling can contribute to the development and progression of chronic lung diseases, including idiopathic pulmonary fibrosis (IPF) (Chilosi et al., 2003; Konigshoff et al., 2008; Liu et al., 2009), asthma (Kwak et al., 2015), lung cancer (Akiri et al., 2009; Licchesi et al., 2008) and chronic obstructive pulmonary disease (COPD) (Jiang et al., 2016; Kneidinger et al., 2011; Uhl et al., 2015). In the next sections, we briefly highlight the current knowledge about the involvement of WNT signalling in general in lung development and disease.

Our knowledge about upstream events concerning (cell-specific) WNT-receptor (ligand-receptor) interactions (so-called proximal WNT signalling) recently began to broaden and in this review we assembled an overview on the involvement of diverse families of transmembrane receptors to WNT-mediated signal transduction in physiology and pathophysiology of the lung. Furthermore, we feature relevant proteins involved in endogenous regulation of WNT receptor signalling and we highlight the currently available biologicals and pharmacological tools that can modify proximal WNT signalling.

2. WNT signalling in lung development and pathology

2.1. Lung morphogenesis

The WNT signalling pathways are essential in lung organogenesis by tightly controlling cell proliferation, differentiation, polarity, and lineage specification. Several WNTs and receptors are expressed in the embryonic lung at different developmental stages (reviewed by De Langhe & Reynolds (2008) and Ota, Baarsma, Wagner, Hilgendorff, & Konigshoff (2016)). Endogenous WNT/β-catenin signal activity can be monitored in vivo in WNT-reporter mice carrying multimerized TCF binding sites, which drive the expression of either β-galactosidase (*LacZ*) or green fluorescent protein (GFP). By utilizing one of these reporter mice (i.e. TOPGAL mice), Okubo and colleagues showed dynamic changes in (β-catenin-dependent) WNT signalling during lung development at various embryonic stages (Okubo & Hogan, 2004). In addition, by using three distinct WNT reporter mice lines (TOP-GAL, BAT-GAL and $Axin2^{lacZ}$ mice) Al Alam and colleagues demonstrated that WNT/β-catenin signalling is in a spatiotemporal manner

active at various stages of lung development, particularly in the lung epithelium, mesenchyme and airway smooth muscle (Al Alam et al., 2011). Moreover, expression of each of the (β-catenin-dependent) WNT reporters was enhanced in adult mice during the repair phase after lung injury induced by naphthalene (Al Alam et al., 2011). Collectively, these studies demonstrate that WNT/β-catenin signalling is active during lung development and suggests that it contributes to tissue repair. Interestingly, both inhibition and excessive activation of WNT signalling pathways lead to abnormal development and/or maturation of the lung. For instance, cell-specific deletion of β-catenin in epithelial cells during embryogenesis disrupts lung morphogenesis (Mucenski et al., 2003), whereas ectopic expression of constitutively active β-catenin does not influence lung morphogenesis before birth, but leads to tumour development and airspace enlargement postnatally (Mucenski et al., 2005). The contribution of β-catenin-independent WNT signalling to lung development is less well understood, mainly due to the non-uniform and wide range of downstream effector proteins associated with β-catenin-independent WNT signalling. Nevertheless, in vivo studies in transgenic mice have demonstrated that alterations in WNT expression result in severe lung phenotypes. For instance, WNT-4$^{-/-}$ embryos develop hypoplastic lungs, display tracheal abnormalities and have reduced cell proliferation in the lung buds (Caprioli et al., 2015). On the other hand, the lungs of conventional WNT-5A$^{-/-}$ mice are characterized by accelerated cell proliferation leading to overexpansion of the distal airways and attenuated lung maturation and consequently these mice die shortly after birth (Li, Xiao, Hormi, Borok, & Minoo, 2002). Also (induced) overexpression of specific WNTs can influence lung development. For example, enhanced expression of WNT-5A influences both fibroblast growth factor 10 (FGF10) and Sonic hedgehog (SHH) signalling leading to disruption of epithelial-mesenchymal interaction and resulting in reduced epithelial branching and dilated distal airways (Li et al., 2005). The majority of the studies investigating WNT signalling in lung development have been performed in rodents, although recently a study demonstrated the spatiotemporal expression of WNT pathway components, including various receptors, signal transducers (e.g. Dishevelled proteins), transcription factors (i.e. TCF/LEF1) and effector molecules (i.e. β-catenin) in the developing human lung (Zhang, Shi, Huang, & Lai, 2012). This suggests that WNT signalling also plays an important physiological role in human lung development.

2.2. WNT signalling in lung pathology

In addition to lung development, WNT signalling, when aberrantly activated, also contributes to several lung pathologies, including asthma, IPF, COPD and lung cancer (Baarsma & Konigshoff, 2017). Over the last decade WNT signalling has gained extensive interest in the context of chronic lung diseases. Most of these studies thus far focused on the WNT/β-catenin signalling pathway (Kneidinger et al., 2011; Konigshoff et al., 2008; Selman, Pardo, & Kaminski, 2008; Uematsu et al., 2003; Uhl et al., 2015). However, recent reports suggest that WNT signalling independent of transcriptional co-activator β-catenin also significantly contributes to chronic lung pathologies (Baarsma et al., 2017; Boucherat et al., 2007; Durham et al., 2013; Heijink et al., 2013; Kumawat et al., 2013; Laumanns et al., 2009; Zhao et al., 2010).

2.2.1. Idiopathic pulmonary fibrosis (IPF)

Anomalies in the WNT signalling pathways and their potential as therapeutic targets in chronic lung diseases were initially identified in IPF (Chilosi et al., 2003; Konigshoff et al., 2008, 2009). IPF is a rare chronic lung disease where normal lung tissue is progressively replaced by scar tissue. On the cellular level, the disease is characterized by epithelial cell injury, epithelial cell reprogramming and (myo)fibroblast activation conjointly resulting in excessive extracellular matrix (ECM) deposition in the lung, which subsequently leads to distorted lung architecture and progressive loss of functional lung tissue (King, Pardo, & Selman, 2011). In both human and experimental IPF WNT signalling is aberrantly active as demonstrated by increased expression of various WNTs, enhanced nuclear β-catenin staining in various cell types, and augmented expression of WNT target genes (Chilosi et al., 2003; Konigshoff et al., 2008; Liu et al., 2009). Moreover, a variety of studies have demonstrated that pharmacological inhibition of the interaction between β-catenin and specific transcription factors reverses (Henderson et al., 2010) or attenuates (Kim et al., 2011) experimental pulmonary fibrosis in mice (Ulsamer et al., 2012).

2.2.2. Chronic obstructive pulmonary disease (COPD)

Contrarily to IPF pathogenesis, β-catenin-dependent WNT signalling is attenuated in emphysema, one of the pathological features of COPD (Jiang et al., 2016; Kneidinger et al., 2011; Uhl et al., 2015). COPD is characterized by airflow obstruction, chronic bronchitis, (small) airway remodelling and parenchymal tissue destruction called emphysema (Rabe et al., 2007). The relative contribution of each of these pathological features varies in individual patients who suffer from COPD. Similarly to IPF, limited pharmacological treatments halting disease progression are currently available. As indicated, WNT/β-catenin signalling is important in lung development and growth, and is therefore of tremendous interest for the initiation of tissue repair and regeneration. Indeed, several studies have demonstrated that reactivation or restoring of β-catenin signalling can attenuate emphysema development and progression in vivo (Baarsma et al., 2017; Jiang et al., 2016; Kneidinger et al., 2011). On the other hand, increased β-catenin-independent WNT signalling is observed in various cellular compartments of the lungs of COPD patients. For instance, enhanced WNT-4-mediated activation of p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun NH2-terminal kinase 1/2 is observed in bronchial epithelial cells of COPD patients and this contributes to increased (cigarette smoke-induced) inflammatory cytokine secretion by these cells (Durham et al., 2013; Heijink et al., 2013). Furthermore, cigarette smoke induces the expression of WNT-5B in the bronchial epithelium of COPD patients and this specific WNT protein enhances expression of remodelling-related genes in these cells (Heijink et al., 2016), whereas stimulation of lung fibroblasts with WNT-5B leads to an increased inflammatory response (van Dijk et al., 2016). Most recently, we have demonstrated that WNT-5A-mediated WNT signalling negatively regulates β-catenin signalling in alveolar epithelial type II (ATII) cells, thereby impairing endogenous lung repair processes and contributing to COPD pathogenesis (Baarsma et al., 2017). These studies collectively indicate that WNT signalling plays a pivotal role in both IPF and COPD pathogenesis.

2.2.3. Asthma

Asthma is a heterogeneous chronic inflammatory disorder of the airways, characterized by airway hyperresponsiveness and structural changes in the airways (airway remodelling). Aberrant expression and/or activation of WNT pathway components has been observed in asthmatic patients as well as in murine models of asthma, and both the β-catenin-dependent as well as -independent WNT signalling potentially contribute to asthma pathology (Baarsma & Konigshoff, 2017; Kumawat et al., 2013; Kumawat, Koopmans, & Gosens, 2014; Kwak et al., 2015). Pharmacological inhibition of β-catenin signalling dampens airway remodelling, including subepithelial fibrosis and airway smooth muscle thickening (Koopmans et al., 2016; Yao et al., 2017). Furthermore, ex vivo and in vitro evidence indicate that also β-catenin-independent WNT signalling, particularly mediated by WNT-5A, contributes to pathological features of asthma (Koopmans, Kumawat, Halayko, & Gosens, 2016; Kumawat et al., 2013). Thus, WNT signalling might be a potential therapeutic target for asthma treatment.

2.2.4. Lung cancer

Alterations and mutations in components of the WNT/β-catenin signalling pathway are common in human malignancies, in particular in cancer (Reya & Clevers, 2005). Strikingly, unlike in colon cancer, for

instance, β-catenin mutations are not frequently observed in lung cancers or in cells originating from lung cancers (Stewart, 2014). Moreover, the potential role of β-catenin in lung cancer (non-small cell lung cancer: NSCLC) is controversially discussed. β-Catenin is often highly expressed in NSCLCs and inhibition of the protein attenuates cell proliferation, suggesting that inhibition of β-catenin signalling may be beneficial in the treatment of lung cancer (Akiri et al., 2009). However, some clinical studies report that β-catenin expression is associated with improved prognosis for the patient, which may be due to the interaction of β-catenin with E-cadherin facilitating cell-cell contacts thereby limiting the possibility of metastasis (Jin et al., 2017). Moreover, a rising amount of evidence shows that also WNT signalling independently of β-catenin plays an important role in lung cancer, in part by regulating the WNT/β-catenin signalling activity (Li et al., 2015). WNT-5A suppresses proliferation and invasiveness of colon, thyroid and colorectal tumour cells (Dejmek, Dejmek, Safholm, Sjolander, & Andersson, 2005; Kremenevskaja et al., 2005). Conversely, the level of WNT-5A is increased in melanoma, breast and lung cancer (Iozzo, Eichstetter, & Danielson, 1995; Kikuchi & Yamamoto, 2008; Weeraratna et al., 2002). Moreover, WNT-5A expression was shown to correlate with the tumour aggressiveness in gastric cancer (Kurayoshi et al., 2006). Thus, it was proposed that alterations in WNT/β-catenin signalling might contribute to the tumour initiation, whereas changes in β-catenin-independent WNT signalling contribute to tumour progression (Kikuchi, Yamamoto, Sato, & Matsumoto, 2012). Although the impact of WNT signalling on lung cancer is currently not fully elucidated, the WNT pathways may serve as potential therapeutic targets and are therefore of great interest for further exploration.

WNT receptors represent potential therapeutic targets in the treatment of many lung diseases and therefore elucidating the alterations that potentially occur in chronic lung pathologies is of great interest. In the next sections, we will highlight the diverge expression of WNT receptors and co-receptors in the aforementioned chronic lung diseases. We start with the FZDs, which are WNT receptors involved in the both β-catenin-dependent and -independent WNT signalling. Subsequently, we highlight the LRP5/6 receptors, which are crucial in WNT/β-catenin signalling. Finally we describe the various other co-receptors (e.g. LGRs, ROR2, and VANGL2), which influence various WNT signalling cascades. In addition, we discuss the function and targetability of some of the endogenously expressed intracellular and extracellular WNT signalling modifying proteins (e.g. ZNFR3, RSPOs, DKKs, and sFRPs).

In addition to knowledge about receptor expression on WNT receiving cells it is of equal importance to know which cells in the lung are secreting the WNTs at (patho)physiological conditions. We have observed significant differences in WNT gene expression and secretion when comparing pulmonary epithelial cells to lung fibroblasts and airway smooth muscle cells (Baarsma et al., 2017; Baarsma, Meurs, Halayko, & Gosens, 2009). Moreover, alterations in WNT gene expression were observed when comparing pulmonary fibroblasts of individuals without and with COPD (Baarsma et al., 2011). These findings indicate cell-specific WNT gene expression profiles in the lung and shows that pathological conditions can change these expression profiles. If these alterations in WNT gene expression are a cause or consequence of the pathological condition needs to be elucidated. Nevertheless, all WNT processing and secretion by cells can be pharmacologically inhibited by small molecules called *Inhibitors of WNT Production* (IWP) (Chen et al., 2009). However, if inhibition of all WNT processing and secretion is beneficial or detrimental in the aforementioned chronic lung diseases is largely unknown. Taken together, which cells are the main source of WNTs in the lungs is to a great extent an unexplored field of research and is beyond the scope of this review.

3. Classical WNT receptors: Frizzled (FZD)

Frizzleds (FZDs), which were originally identified in *D. melanogaster* as WNT receptors, are seven-pass transmembrane receptors (FZD_1 through FZD_{10} and Smoothened: SMO) that belong to atypical G protein-coupled receptors (GPCRs) (Bhanot et al., 1996; Foord et al., 2005; Vinson, Conover, & Adler, 1989). These receptors share common characteristics and contain (a) the extracellular N-terminal signal sequence, followed by (b) a cysteine-rich domain (CRD: 10 cysteines) (c) the transmembrane and intracellular domains, giving rise to three extracellular loops and three intracellular loops and finally (d) the C-terminus domain (Schulte, 2010). The N-terminal signal sequence is responsible for proper insertion of the receptor in the membrane, whereas the CRD facilitates binding of WNT proteins (Xu & Nusse, 1998). The intracellular loops within each FZD contain several potential phosphorylation sites, which comprise an interaction surface for serine/threonine kinases, some tyrosine kinases and are essential for G protein coupling (Dijksterhuis, Petersen, & Schulte, 2014; Schulte, 2010). Moreover a number of specific amino acids within the intracellular loops of the receptors were shown to be required for the interaction of the receptor with DVL (Cong, Schweizer, & Varmus, 2004; Tauriello et al., 2012), including the highly conserved tyrosine at position 250 ($Y250^{2.39}$). The $Y250^{2.39}$ motif, within the first intracellular loop, specifies downstream signalling initiated by the receptor (Strakova et al., 2017). Lastly, the C-terminus (which is not well conserved between FZDs) contains a KTXXXW motif, which is essential for FZD signalling as it, next to the residues located in the intracellular loops, facilitates the interaction with specific domains within dishevelled (see Fig. 2) (Gao & Chen, 2010; Umbhauer et al., 2000; Vinson et al., 1989). The overall similarity between all members of the FZD family is approximately 11% (calculated by Uniprot); however, within specific clusters of FZDs the similarities vary between 50 and 75% (e.g. FZD_3/FZD_6: 49%, whereas FZD_2/FZD_7 share 77% similarity). The CRD domain is implicated as the WNT binding domain within the FZDs, although loss-of-function mutations within this domain do not completely abolish signal transduction by the receptors (Chen, Strapps, Tomlinson, & Struhl, 2004; Hsieh, Rattner, Smallwood, & Nathans, 1999). Moreover, distinct WNT proteins can bind with different affinities to individual FZDs, required for transducing both β-catenin-dependent and β-catenin-independent WNT signalling (Dijksterhuis et al., 2015; Grumolato et al., 2010). Accordingly, a biochemical study investigating binding affinities between WNT proteins and FZD-CRD domains (i.e. WNT binding domain) demonstrated that WNT-3A has intermediate to strong binding (<40 nM) to FZD_1, FZD_2, FZD_4 FZD_5, FZD_7 and FZD_8, whereas, for instance, WNT-4 hardly showed binding (>100 nM) to FZD_1 or FZD_2, but showed similar binding capacity as WNT-3A for FZD_5 (10–40 nM) and FZD_8 (<40 nM). Subsequent experiments showed that WNT-3A is able to induce LRP6 phosphorylation, hyperphosphorylation of DVL proteins and stabilization of β-catenin in cells with ectopic expression of FZD_2, FZD_4 or FZD_5. This indicates that these specific WNT-FZD pairs transduce their signal resulting in the activation of WNT/β-catenin signalling. However, in the same cells with ectopic expression of these specific FZDs, WNT-4 induces hyperphosphorylation of DVL proteins, but does not increase cellular expression of β-catenin (LRP6 phosphorylation in response to WNT-4 was not assessed). Thus, although WNT-4 binds with similar affinity as WNT-3A to these FZDs it does not result in the downstream activation of WNT/β-catenin signalling (Dijksterhuis et al., 2015). Collectively, these finding indicate that distinct WNTs can bind to the same subset of FZDs; however, also depending on the presence of different co-receptors (e.g. ROR2, RYK), this can result in differential activation of downstream signalling pathways, a phenomenon that can be of great importance for future development of drugs targeting the FZD family of receptors. Adding to the complexity of WNT-FZD interaction and activation of downstream signalling, WNT-5A is able to either activate or inhibit β-catenin signalling depending on the WNT receptors expressed on the cells (Mikels & Nusse, 2006). Consequently, a more comprehensive insight in receptor-ligand interactions in WNT signalling is required to augment our understanding of how WNT proteins exert their cellular function(s). Nevertheless, the interaction between a WNT and a FZD is the initial step of transducing

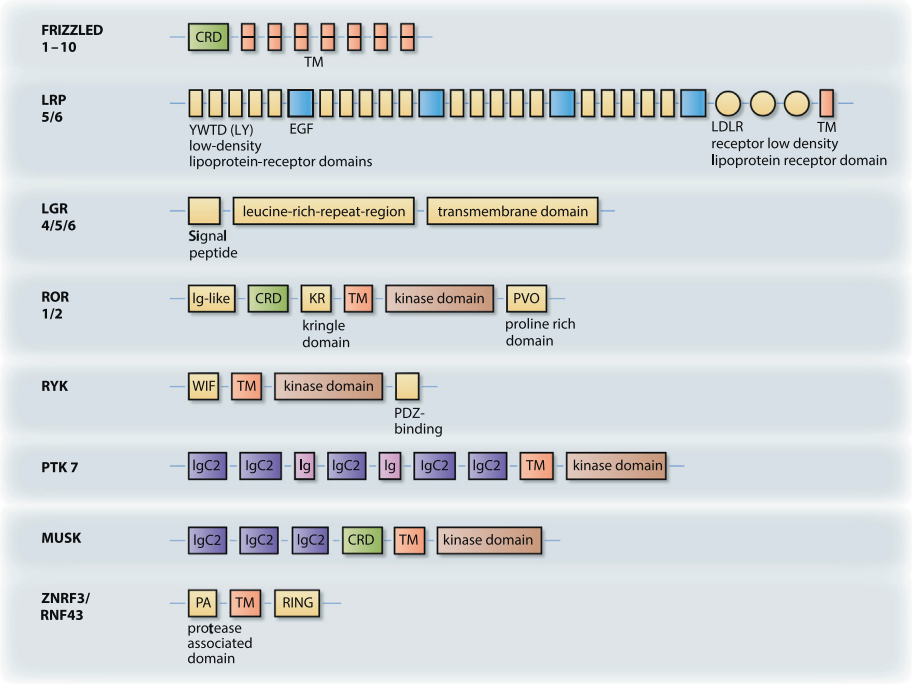

Fig. 2. Schematic representation of protein domains within WNT receptors and co-receptors. Abbreviations: Frizzled (FZD), low-density lipoprotein receptor related proteins (LRP) 5/6, leucine-rich repeat-containing G protein-coupled receptors (LGR) 4/5/6, Receptor tyrosine kinase-like orphan receptor (ROR), Related to receptor tyrosine kinase (RYK), Protein tyrosine kinase 7 (PTK7), MUSK, zinc and ring finger 3 (ZNRF3)/ring finger protein 43 (RNF43). Cysteine-rich domain (CRD), transmembrane domain (TM), epidermal growth factor-like domain (EGF), Immunoglobulin (Ig) WNT-inhibitory factor-1 domain (WIF).

an extracellular signal into an intracellular response. This makes FZDs attractive targets in many chronic (lung) diseases, where the WNT signalling pathways are aberrantly active. Knowledge about FZD alterations in specific diseases opens up new avenues to potential therapies. The current status of our knowledge regarding alterations in the expression of each of the FZDs in chronic lung diseases is summarized in the next sections (Fig. 3).

3.1. FZD_1

Cigarette smoke (CS), a well-known risk factor for various chronic lung diseases, to attenuates FZD_1 expression in bronchial epithelial cells (16HBE) (Guo et al., 2016; Heijink et al., 2013). Moreover, Kneidinger et al. reported that Fzd_1 was decreased in elastase- and CS-induced emphysema/COPD in mice (Kneidinger et al., 2011). In line with this, FZD_1 expression is decreased in small airway epithelium of smokers and COPD patients compared to non-smokers (Wang et al., 2011). Therefore, the downregulation of FZD_1 in human disease and in several animal models of COPD/emphysema potentially indicates that loss of the receptor in the pulmonary epithelium contributes to reduced expression of active β-catenin in the nucleus. Reduced expression of transcriptionally active β-catenin in the nucleus is a phenomenon observed in the lungs of individuals with COPD (Jiang et al., 2016; Kneidinger et al., 2011).

3.2. FZD_2

Cigarette smoke also reduces FZD_2 expression in bronchial epithelial cells (16HBE) (Guo et al., 2016; Heijink et al., 2013), however alveolar epithelial cells (A549) did not show an alteration in FZD_2 expression in response to this insult (Guo et al., 2016; Heijink et al., 2013). Nevertheless, pulmonary FZD_2 expression is decreased in the lungs of emphysematous mice, potentially suggesting that loss of FZD_2 contributes to COPD pathogenesis (Kneidinger et al., 2011). On the other hand, van Dijk and colleagues reported an increase in FZD_2 expression in lung tissue of COPD versus non-COPD patients and furthermore demonstrated that this FZD was involved in WNT-5B-induced inflammation in human lung fibroblasts (van Dijk et al., 2016). Although not significant, FZD_2 tended to be increased in primary human lung fibroblasts of individuals with moderate severe COPD (i.e. GOLD stage II), but not in pulmonary fibroblasts of individuals with very severe COPD (i.e. GOLD stage IV) (Baarsma et al., 2011). These latter studies suggest that in a subset of individuals with COPD, increased expression of FZD_2 in pulmonary fibroblasts may contribute to disease pathology perpetuating the inflammatory response. Also other mesenchymal cells, for example airway smooth muscle cells, abundantly express FZD_2 (Kumawat et al., 2013). Interestingly, FZD_2 has been linked to calcium mobilization in various biological systems (Vignola et al., 1997) and may therefore be of importance for smooth muscle contraction. Thus, although speculative, aberrant expression of FZD_2 may contribute to asthma pathogenesis. On the other hand, TGFβ, which is upregulated in asthma and contributes to airway remodelling (Redington et al., 1997; Vignola et al., 1997), negatively regulates FZD_1 and FZD_2 expression in human airway smooth muscle cells (Kumawat et al., 2013), suggesting divergent influence of different pathways on FZD expression. Regarding lung cancer, FZD_2 has been shown to be elevated in tumourous tissue and its expression correlated with the markers of epithelial-to-mesenchymal transition (EMT) (Gujral et al., 2014). Moreover, a FZD_2 neutralizing antibody (FZD_2 mAb) reduced cell migration and invasion of hepatocyte-derived carcinoma cells in vitro, and inhibited tumour growth in vivo (Gujral et al., 2014), which makes this receptor a promising target in further studies for cancer treatment. Another agent, UM206, has been reported to antagonize FZD_1 and FZD_2 in vitro and in vivo. UM206 is a synthetic peptide which design is based on the molecular structures of WNT-3A and WNT-5A. Although not yet investigated in the lung, UM206 antagonized both WNT-3A- and WNT-5A-induced migration of fibroblasts overexpressing FZD_1 and FZD_2 and it attenuated adverse tissue remodelling in vivo (Laeremans et al., 2011; Uitterdijk et al., 2016).

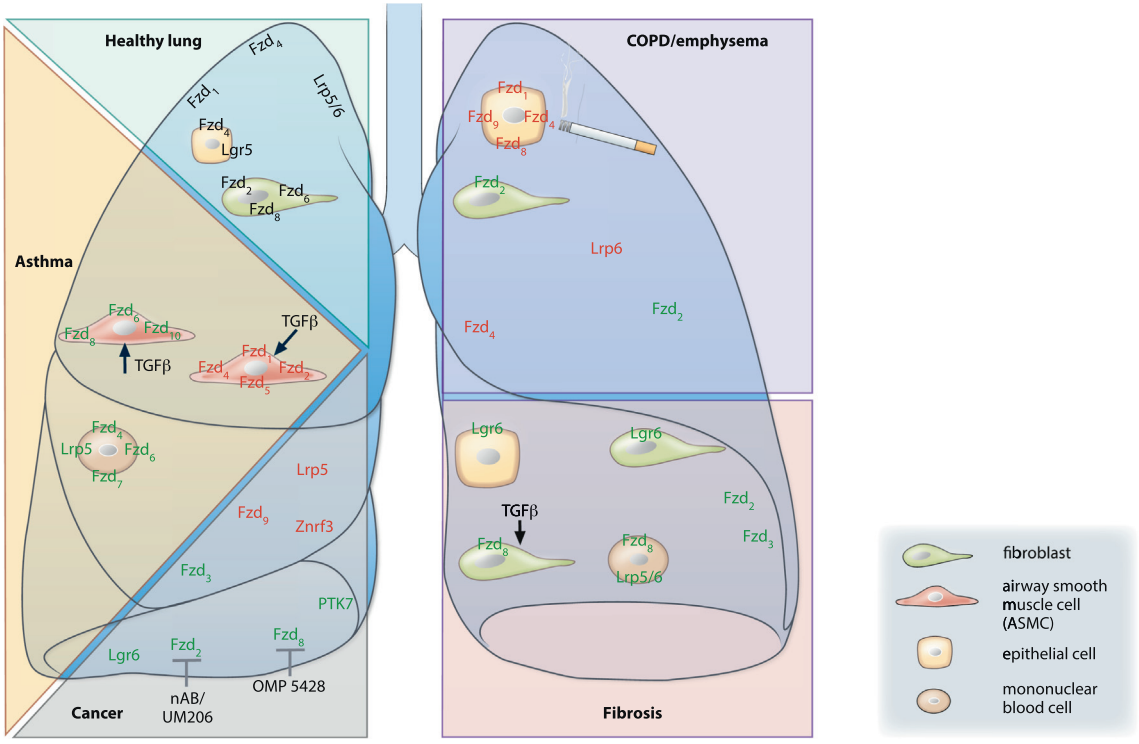

Fig. 3. Schematic representation of WNT receptor and co-receptor abundance in healthy and diseased lung. Abundance of WNT receptors and co-receptors in the healthy lung and in chronic lung pathologies, including asthma, COPD, IPF and lung cancer. Changes in the expression of these receptors by cigarette smoke exposure and/or TGF-β in specific cell types are indicated in red (downregulation) or green (upregulation). Biologicals targeting specific receptors are depicted: nAB is neutralizing antibody.

3.3. *FZD*$_3$

Expression of *FZD*$_3$ has been demonstrated to positively associate with β-catenin-driven target gene expression (e.g. *Cyclin D1* and *c-Myc*), which might indicate that FZD$_3$ is positively regulating WNT/β-catenin signalling (Ueno, Hirata, Hinoda, & Dahiya, 2013). Interestingly, Lee and colleagues demonstrated in a subset of patients with lung tumours that *FZD*$_3$ is overexpressed in cancerous tissue compared to matched control tissue, with 8 out of 20 patients having a ≥2-fold increase in transcript levels of this particular receptor (Lee et al., 2008). Additionally, also in IPF, a chronic lung disease characterized by increased nuclear β-catenin signalling in various cells types (e.g. myofibroblasts and hyperplastic alveolar epithelial type II cells), the expression of both *FZD*$_2$ and *FZD*$_3$ is increased, providing further indirect evidence that these receptors might be positive regulators of β-catenin signalling (Konigshoff et al., 2008).

3.4. *FZD*$_4$

A recently performed comprehensive analysis of FZD expression in lung tissue identified FZD$_4$, a highly expressed FZD in human lung during development (Zhang et al., 2012), as a potentially interesting candidate for therapeutic intervention in the context of COPD (Skronska-Wasek et al., 2017). We and others have shown that *FZD*$_4$ is directly downregulated by CS exposure in alveolar (Skronska-Wasek et al., 2017) and bronchial (Guo et al., 2016) epithelial cells in vitro as well as in elastase- and CS-induced emphysema in vivo (Skronska-Wasek et al., 2017). In agreement, *FZD*$_4$ expression is decreased in the whole lung homogenate (Guo et al., 2016; Skronska-Wasek et al., 2017) and in small airway epithelium (Wang et al., 2011) from smokers and COPD patients compared to healthy donors. Interestingly, we have shown that overexpression of FZD$_4$ positively regulates WNT/β-catenin signal activity in the pulmonary epithelium, as shown by increased phosphorylation of LRP6 and enhanced β-catenin-mediated gene transcription. Furthermore, overexpression of FZD$_4$ increased pulmonary/alveolar epithelial cell proliferation and repair, whereas blocking of the receptor with FzM1 (Generoso et al., 2015) had opposite effects (Skronska-Wasek et al., 2017). FzM1 is an allosteric ligand of FZD$_4$, which hampers the FZD$_4$-DVL complex formation required for β-catenin activation and nuclear translocation (Generoso et al., 2015). As such, FzM1 could be useful in the treatment of β-catenin-driven pathological processes, such as cell proliferation in cancer. All the more, as FZD$_4$ expression was linked to aberrant WNT/β-catenin activation in acute myelogenous leukaemia (Tickenbrock et al., 2008) and prostate cancer pathogenesis involving EMT (Acevedo et al., 2007; Gupta et al., 2010). Moreover, FZD$_4$ predisposes tumour cells towards EMT thereby facilitating tumour progression. A study investigating early-stage NSCLC revealed single nucleotide polymorphisms (SNPs) in *FZD*$_4$ and patients carrying the variant allele of SNP rs10898563 showed a 2- to 3-fold increase in recurrence of cancer and death risk (Coscio et al., 2014); however, this association requires further in depth investigation. To date not much is known about FZD$_4$ regulation and function in asthma pathogenesis. Nevertheless, increased *FZD*$_4$ expression has been reported in nuclear cells from peripheral blood of asthma patients as assessed by microarray analysis and reduced *FZD*$_4$ expression has been shown in airway smooth muscle cells upon TGF-β treatment (Kumawat et al., 2013; Lee, Bae, Choi, & Yoon, 2012). Furthermore, *FZD*$_4$ has extensively been investigated in processes involved in angiogenesis in a variety of organs (Z. Wang, Shu, Lu, & Morrisey, 2005; Wright, Aikawa, Szeto, & Papkoff, 1999). The receptor is expressed in mesenchymal cells, vascular precursor cells and endothelial cells (Favre et al., 2003; Robitaille et al., 2002; Wang et al., 2005; Wright et al., 1999; Xu et al., 2004). Aberrant angiogenesis and vascular remodelling can occur in chronic lung diseases such as asthma and COPD and are important contributors to disease pathogenesis (Harkness, Kanabar, Sharma, Westergren-Thorsson, & Larsson-Callerfelt, 2014; Matarese & Santulli, 2012). Further in-depth studies are required to investigate if targeting of FZD$_4$ has a therapeutic

potential for the treatment of pulmonary vascular changes in these lung diseases. Taken together, FZD_4 appears to be positive regulator of WNT/β-catenin signalling in, for instance, the pulmonary epithelium, although the involvement of FZD_4 in alternative WNT signalling cascades in the lung has not yet been addressed. Most recently, Riccio and colleagues designed and synthesized an allosteric agonist of FZD_4. The small molecule called FzM1.8, which is derived from FzM1, interacted with FZD_4 and activated WNT signalling by promoting TCF/LEF dependent gene transcription (Riccio et al., 2018). Both FzM1 and FzM1.8 could be useful pharmacological tools to target and study FZD_4 in lung physiology and pathology.

3.5. FZD_5

To date, limited knowledge exists about the function of the FZD_5 in the lung; however, FZD_5 has been implicated to contribute to inflammatory responses. For example, FZD_5 protein expression has been detected in granulomatous lesions in the lungs of patients with Mycobacterium tuberculosis infection. Moreover, FZD_5 activated by WNT-5A regulated the microbial-induced interleukin-12 (IL-12) response of antigen-presenting cells and interferon-γ production by T cells (Blumenthal et al., 2006). Interestingly, an antibody against FZD_5 reduced IL-6 and IL-15 expression in rheumatoid arthritis (Sen, Chamorro, Reifert, Corr, & Carson, 2001). Additionally, Kumawat et al. have shown that the expression of *FZD5* is negatively regulated by TGF-β treatment in airway smooth muscle cells (Kumawat et al., 2013) suggesting potential role of this receptor in asthma pathogenesis. FZD_5 has been reported to be up-regulated in cancer, for instance in renal carcinoma (Janssens, Andries, Janicot, Perera, & Bakker, 2004), and to be highly expressed in K-562 cells derived from chronic myelogenous leukaemia (Saitoh, Hirai, & Katoh, 2001). Moreover, blocking of FZD_5 by specific antibodies inhibited proliferation of pancreatic ductal adenocarcinoma cells (Steinhart et al., 2017), whereas blocking of the receptor with Box5 (a peptide derived from WNT-5A) antagonized metastasis in melanoma (Jenei et al., 2009). Interestingly, activation of FZD_5 by Foxy5 (a hexapeptide that mimics WNT-5A) decreased breast cancer cell migration (Prasad, Sodergren, & Andersson, 2017), which indicates that also activation of the receptor might be beneficial in specific types of cancer. Nevertheless, expression of FZD_5 in lung cancer has not yet been investigated. Together these studies indicate a potential role of FZD_5 as a therapeutic target in the treatment of cancer as well as inflammation driven diseases.

3.6. FZD_6

FZD6 can transduce both β-catenin-dependent (Frojmark et al., 2011) and -independent WNT signalling, however most reports indicate a role of this receptor in the β-catenin-independent signalling pathways (Golan, Yaniv, Bafico, Liu, & Gazit, 2004; Heinonen, Vanegas, Lew, Krosl, & Perreault, 2011). Recently, a GWAS study analysis was performed to classify genes that are associated with asthma susceptibility and this study identified a locus close to *FZD6* as a novel asthma susceptibility locus. Three SNPs in close proximity to the *FZD6* locus were discovered. The minor allele of one of these SNPs (i.e. G allele of rs3133805) was directly associated with asthma susceptibility (Barreto-Luis et al., 2017). However, from this study it could not be concluded if the SNPs had a functional role and if they affected *FZD6*, as also two other genes (i.e. *CTHRC1* and *SLC25A32*) are in vicinity to these SNPs and may contribute to asthma susceptibility (Barreto-Luis et al., 2017). Nevertheless, *FZD6* transcript level was increased in nuclear cells from peripheral blood of asthmatic patients and the expression of the receptor was positively regulated by profibrotic cytokine TGF-β in airway smooth muscle cells (Kumawat et al., 2013; Lee et al., 2012). Remodelling processes in airway smooth muscle activated by TGF-β are dependent on the activation of β-catenin, but also on WNT signalling independent of β-catenin (Baarsma et al., 2011; Kumawat et al., 2013). Thus, it is possible that enhanced *FZD6* expression contributes to airway remodelling processes mediated by airway smooth muscle in reaction to this cytokine. Furthermore, Piga and colleagues aimed to elucidate the function of FZD_6 in bronchial epithelial cells (BEAS-2B) and could demonstrate that 199 genes were differentially expressed (149 upregulated and 50 downregulated) in cells lacking *FZD6* (FZD_6^- BEAS-2B) compared to their wild type counterparts (FZD_6^+ BEAS-2B). Subsequent ontology enrichment analysis revealed that the differentially expressed genes were clustered to mitochondria, cell cycle and cell death, nucleoside metabolic processes and, to a lesser extent, to proteolysis and nucleotide binding (Piga, van Dartel, Bunschoten, van der Stelt, & Keijer, 2014). Aberrant activation or impairment of these clusters has detrimental consequences and may contribute to several chronic pathologies in the lung; however, more work is needed to answer if FZD_6 might be involved in the pathogenesis of chronic lung disease, such as asthma, and if targeting of the receptor could serve as a therapeutic option.

3.7. FZD_7

In mice it was demonstrated that maternal smoking negatively affects the mRNA expression of the *Fzd7* and *Ctnnb1* (gene symbol of β-catenin) in lung tissue of the offspring (113). Although not investigated in humans, impairment of these WNT pathway components may have implications not only for lung development, but also later in life, as these WNT components regulate neoangiogenesis and lung branching morphogenesis (Blacquiere et al., 2010). Limited to no data is available regarding the expression and function of FZD_7 in lung physiology and pathology in adults. Interestingly, *FZD7* is one of the few WNT pathway components that was investigated at early stages of human lung morphogenesis. In situ hybridization was performed to visualize the expression of *FZD7* in the developing human lung, which confirmed the transcript data obtained by PCR, and located the expression of the receptor predominantly to alveolar and bronchial epithelial cells. The expression pattern of *FZD7* in the human lung is distinct from the expression of the receptor in the developing murine lung, where it is restricted to the mesenchyme (Winn et al., 2005). Moreover, similarly to *FZD6* also the expression of *FZD7* is increased in nuclear cells from peripheral blood of patients suffering from asthma (Lee et al., 2012).

3.8. FZD_8

Recently increasing knowledge has been gained regarding the expression and function of the FZD_8 receptor in lung (patho)physiology. *FZD8* is decreased in small airway epithelium of smokers and COPD patients compared to epithelium of non-smokers (Wang et al., 2011), but elevated in peripheral blood mononuclear cells of IPF patients (Lam et al., 2014). Furthermore, expression of the receptor is increased by TGF-β in primary lung fibroblasts (Baarsma et al., 2011) and airway smooth muscle cells (Kumawat et al., 2013). Interestingly, mice lacking *Fzd8* ($FZD_8^{-/-}$) were partially protected against bleomycin-induced lung fibrosis and had improved lung architecture in comparison to respective control mice (Spanjer et al., 2016). Furthermore, the absence of FZD_8 (partially) prevented CS-induced airway inflammation in vivo (Spanjer et al., 2016). Moreover, a significant association was discovered between SNP rs663700 in the *FZD8* region and chronic mucus hypersecretion, a major pathological feature of chronic bronchitis in COPD (Spanjer, Menzen, et al., 2016). Collectively, these studies indicate that FZD_8 contributes to pathological features of IPF and COPD.

Enhanced expression of FZD_8 is observed in human lung cancer tissue and cell lines derived from lung cancers (Bravo et al., 2013; Wang, Xu, Ma, Zhang, & Xie, 2012). The receptor was proposed to be a putative therapeutic target for human lung cancer, as siRNA-mediated silencing of FZD_8 sensitized cancer cells to chemotherapy (Wang et al., 2012). Preclinical studies with blocking FZD_8 are ongoing. OMP-54F28 (Ipafricept, OncoMed), which is a fusion protein consisting of the

cysteine-rich domain of FZD_8 and the immunoglobulin Fc domain. OMP-54F28 acts as a decoy receptor, which competes with native FZD_8 for its ligands and antagonizes WNT signalling, and reduces tumour growth and decrease cancer stem cell (CSC) frequency as a single agent and in combination with other chemotherapeutics (Le, McDermott, & Jimeno, 2015). Interestingly, OMP-54F28 also attenuated metastasis formation of pancreatic cancer to the lung (Hoey, 2013).

3.9. *FZD*$_9$

Cigarette smoke downregulates the expression of *FZD*$_9$ in bronchial epithelial cells (16HBE) in vitro (Winn et al., 2005) and in mouse lung tissue in vivo (Tennis et al., 2016). Moreover, *FZD*$_9$ is decreased in NSCLC (Tennis et al., 2016; Winn et al., 2006). The decrease of *FZD*$_9$ by cigarette smoke as well as reduced expression of the receptor in human lung tumour and dysplastic tissue, suggest that loss of the receptor may contribute to the development of premalignant lesions and tumour establishment (Tennis et al., 2016). Therefore, it is proposed that FZD_9 plays an important role in maintaining normal lung epithelium and in preventing tumour development (Tennis et al., 2016).

3.10. *FZD*$_{10}$

To date, no study investigating the role of FZD_{10} in the lung diseases exists. However, *FZD*$_{10}$ is increased by TGF-β treatment in airway smooth muscle cells, although baseline expression of the receptor in these cells is relatively low (Kumawat et al., 2013). Moreover, FZD_{10} is linked to cancer pathogenesis as its expression was increased in synovial sarcoma (Nagayama et al., 2005). An antibody against FZD_{10} (Mab 92-13) coupled to yttrium-90 has been shown to reduce tumour growth in synovial sarcoma in a xenograft mouse model (Fukukawa et al., 2008).

3.11. Therapeutics targeting FZDs

Distinct FZDs are altered in different diseases and they represent attractive therapeutic targets for some of these diseases. Several drugs targeting FZDs (Table 1) are or have been tested in clinical trials. In this regard, the monoclonal antibody OMP-18R5 (Vantictumab, OncoMed), which was initially identified to bind and block FZD_7 but is now recognized to block FZD_1, FZD_2, FZD_5 and FZD_8, attenuates β-catenin-independent WNT signalling activated by multiple WNT family members (Gurney et al., 2012; Smith et al., 2013). Treatment of individuals with OMP-18R5 led to inhibition of growth in several types of human tumours in several organs, including breast, pancreatic, colon and lung. Additionally, synergy was observed when OMP-18R5 was combined with several standard-of-care chemotherapeutic agents, including Taxol in NSCLC. OMP-18R5 exhibited its inhibitory effect in 7 of 8 NSCLC tested (Gurney et al., 2012).

Table 1
Small molecules and biologicals targeting WNT receptors.

Compound	Target receptor
FZD_2 neutralizing AB	FZD_2
UM206	FZD_1, FZD_2
FzM1 (allosteric ligand)	FZD_4
FzM1.8 (activator)	FZD_4
FZD_5 neutralizing AB	FZD_5
Foxy-5 (WNT-5A mimic)	FZD_5
Box-5 (WNT-5A/FZD_5 inhibitor)	FZD_5
OMP-54F28 (Ipafricept)	FZD_8
OMP-18R5 (Vantictumab)	FZD_1, FZD_2, FZD_5, FZD_7, FZD_8
Mab 92–13 (coupled to yttrium-90)	FZD_{10}
DKK-1 (recombinant protein)	LRP5/6

4. Low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6)

LRP5 and 6 belong to the superfamily of at least ten LRPs (Schulte, 2010) that are involved in a wide variety of biological processes, including endocytosis, cellular communication, lipid homeostasis and embryonic development (Li, Cam, & Bu, 2001; May, Woldt, Matz, & Boucher, 2007). LRP5/6 are crucial for WNT/β-catenin signalling (Cheon, Nadesan, Poon, & Alman, 2004; Goel et al., 2012). The extracellular domain (ECD) of LRP5/6 mediates the interaction with FZD and a WNT, which leads to the formation of a ternary complex (WNT/FZD/LRP5/6). LRP5/6 have multiple (independent) WNT binding sites, which enables binding of multiple different ligands simultaneously (Bourhis et al., 2010). Binding of specific WNTs leads to the phosphorylation of specific serine/threonine (Ser/Thr) residues in the PPP(S/T)P motif of intracellular domain (ICD) of LRP5/6, an essential step in β-catenin-dependent WNT signal transduction (Tamai et al., 2004; Zeng et al., 2005). Activated LRP5/6 forms signalosomes (Bilic et al., 2007), which leads to the recruitment of AXIN to the membrane, and in turn inhibits the β-catenin destruction complex (Fig. 1) (Piao et al., 2008). Interestingly, WNT-5A can physically interact with LRP6. This does not result in activation of β-catenin signalling, but leads to the decreased activation of the WNT-5A downstream target RAC1 (a small GTPase). Thus, LRP6 can also act as a negative regulator of β-catenin-independent WNT signalling activated by WNT-5A, by acting as an extracellular scavenger. Moreover, due to the physical interaction of WNT-5A with LRP6, other WNT (i.e. WNT-3A) are less capable of inducing phosphorylation (i.e. activation) of the receptor. This is one of the mechanisms by which WNT-5A can act as negative regulator of β-catenin signalling. This mechanism is also operational in chronic lung diseases, as we have recently demonstrated in emphysema/COPD that WNT-5A impairs β-catenin driven endogenous lung repair, in part, by decreasing WNT-3A-induced LRP6 activation in alveolar epithelial cells (Baarsma et al., 2017).

In a mouse model of elastase-induced emphysema, LRP6 expression was downregulated, however this was not recapitulated in individuals with COPD (Kneidinger et al., 2011). This might be due to the nature of the human disease in which both loss of tissue (emphysema) and gain of tissue (small airway disease) can occur. Expression of both *LRP5* and *6* is not altered in lung tissue of IPF patients compared to healthy donors (Konigshoff et al., 2008), but is elevated in peripheral blood mononuclear cells of IPF patients. Moreover, *LRP5* was associated with disease progression (diffusion capacity of the lung for carbon monoxide (DL_{CO}) % and Composite Physiologic Index (CPI)) (Lam et al., 2014). In agreement, mice lacking LRP5 were protected against development of bleomycin-induced fibrosis, an effect which was mimicked by blockade of WNT/β-catenin signalling (Lam et al., 2014). Regarding lung cancer; *LRP5* has been shown to be decreased in squamous cell lung cancer (Lee et al., 2008). SNPs in *LRP5* have been reported to be significantly associated with an increased risk of developing NSCLC (SNP rs3736228) and squamous cell carcinoma (SCC) (SNP rs64843) (Wang, Zhang, Fang, Bao, & Deng, 2016). A SNP in *LRP6* (i.e. rs10845498) has been shown to be associated with a reduced risk of lung SCC, whereas *LRP6* rs6488507 polymorphism increased the risk of NSCLC in tobacco smokers (Deng, Zhang, Bao, & Kong, 2014). Regarding asthma, *LRP5* is upregulated in nuclear cells from peripheral blood of patients as assessed with microarray (Lee et al., 2012), but more studies are required to determine potential beneficial or detrimental role of this receptor in disease.

5. Leucine-rich repeat-containing G protein-coupled receptors (LGR)

The LGR receptor family is a unique class of GPCRs characterized by a large extracellular domain (ectodomain) harbouring leucine-rich repeats (LRR) recognizing ligands and modulating downstream intracellular signalling (Luo & Hsueh, 2006).The LGR family consists

of 9 members and, according to the sequence similarities, is divided into 3 subgroups; A, B and C (Barker, Tan, & Clevers, 2013; Luo & Hsueh, 2006). LGR4, LGR5 and LGR6 (all members of subgroup B) have recently been identified as R-spondin receptors crucial for WNT/β-catenin signalling activation (Barker et al., 2013; Carmon, Gong, Lin, Thomas, & Liu, 2011; de Lau et al., 2011; Glinka et al., 2011; Ruffner et al., 2012).

R-spondins (RSPOs) are secreted proteins that can enhance WNT/β-catenin signalling (Jin & Yoon, 2012). They consist of (a) a N-terminal putative signal sequence for secretion, (b) two cysteine-rich furin-like domains, (c) a single thrombospondin type 1 repeat (TSR1) and (d) a C-terminus (Hankenson, Sweetwyne, Shitaye, & Posey, 2010; Yoon & Lee, 2012). The exact mechanism of action was not known for a long time, but recently RSPOs were shown to exhibit their potential to induce WNT signalling by binding to LGRs and the E3 ligases zinc and ring finger 3 (ZNRF3) and ring finger protein 43 (RNF43). ZNRF3 and RNF43 are discussed in more detail in the next chapter. To exert their biological function, RSPOs need to interact via the furin-like domains with both LGR4/5 (engagement receptor) and ZNRF3/RNF43 (effector receptor) (Xie et al., 2013). In addition, RSPOs are postulated to induce WNT/PCP signalling via the TSR1 domain, which requires interactions with syndecans as coreceptors (Ohkawara, Glinka, & Niehrs, 2011). Limited information is available regarding the regulation of RSPO in the lung. There are indications that they might play a role in lung fibrosis, as *RSPO2* was shown to be upregulated in fibroblasts and epithelial cells of IPF patients (Munguia et al., 2016). *RSPO2* and *RSPO3* are increased in lung tumour cells and treatment with antibody against RSPO3 decreased tumour growth in vivo in mice (Chartier et al., 2016). Interestingly, RSPO2 is crucial for lung development, as knockout mice display lung hypoplasia and branching defects (Bell et al., 2008; Jin, Turcotte, Crocker, Han, & Yoon, 2011; Yamada et al., 2009).

LGRs, aside of being newly discovered receptors for RSPOs, are known as stem cell markers. More specifically, LGR4/5 are markers for stem cells in the intestine and hair follicles (Barker et al., 2010; Jaks et al., 2008), whereas LGR6 was reported as a marker of progenitor cells localized in the lung (Oeztuerk-Winder, Guinot, Ochalek, & Ventura, 2012; Ruiz, Oeztuerk-Winder, & Ventura, 2014). A current hypothesis is that malignant transformations originate from adult stem cells. In this context, LGR6 is enriched in NSCLC during malignant progression and $LGR6^{+}$ cells displayed both self-renewal and high tumourigenic potential (Guinot, Oeztuerk-Winder, & Ventura, 2016). LGR5 (protein) has been localized in the alveolar region and basal layer in bronchi of normal mouse lung (Zhang et al., 2016). Importantly, lung morphogenesis is highly regulated by LGR5 as shown in $LGR5^{+/-}$ mice, which displayed structural irregularities and abnormalities in lung architecture (X. Zhang et al., 2016). In addition, LGR5 has been linked to tumour size and state of the disease in lung adenocarcinoma (Zhang et al., 2016). Regarding (adeno)squamous cell carcinoma or large cell carcinoma divergent reports exist, which either show no differences (Ryuge et al., 2013) or increased abundance (169) of LGR5 in these types of cancer. Interestingly, LGR5 has been proposed as a candidate marker for NSCLC cells with stem cell-like properties, as LGR5 mRNA and protein expression was shown to be frequently increased in these patients (Gao et al., 2015). Contrarily, Gautam et al. showed that LGR5 is present in both non-cancerous human tissue and in lung cancer, which makes it unspecific as a cancer marker, and therefore LGR5 should rather be considered as a signature protein of regenerating tissue (Gautam et al., 2015).

The contribution of LGR signalling to other lung pathologies is largely unknown. There are indications for a role of LGRs in lung fibrosis development, as LGR6 along with RSPO2 are upregulated in fibroblasts and epithelial cells from these patients (Munguia et al., 2016). Given the role of LGRs in RPSO-mediated potentiation of WNT/β-catenin signalling, one could imagine their therapeutic potential in lung diseases with aberrant WNT/β-catenin signalling.

6. Zinc and ring finger 3 (ZNRF3)/ring finger protein 43 (RNF43)

ZNRF3 and RNF43 belong to Goliath family of transmembrane E3 ligases and represent a recently discovered group of negative regulators of WNT signalling (Hao et al., 2012; Koo et al., 2012). They consist of (a) an extracellular domain (signal peptide), (b) a transmembrane domain and (c) an intracellular RING domain. ZNRF3 and RNF43 bind and promote ubiquitination and subsequent degradation of FZDs and LRP5/6 and are therefore negative regulators of WNT signalling (Hao et al., 2012; Koo et al., 2012). Binding of RSPO to LGRs promotes auto-ubiquitination of these E3 ligases and in turn leads to restoration of FZDs and LRPs on the membrane (Hao et al., 2012; Koo et al., 2012).

Moreover, ZNRF3 contributes to WNT/PCP signalling, as embryos of ZNRF3 knockout mice exhibit neural tube closure defects, which is dependent on PCP activity (Hao et al., 2012). Several other studies proposed alternative mechanisms of action. One study showed that RNF43 physically interacts with TCF4 in the nucleus. This interaction silences the transcriptional activity of TCF4 resulting in inhibition of WNT signalling (Loregger et al., 2015), whereas other studies suggested that DVL associates with ZNRF3/RNF43 and serves as adaptor protein targeting these ligases to FZD to promote FZD ubiquitination and degradation (Jiang, Charlat, Zamponi, Yang, & Cong, 2015). Very recently, it was shown that expression of *ZNRF3* is reduced in lung carcinoma and positively correlates with survival of the patients (Shi et al., 2016). One might hypothesize that restoration of ZNRF3 expression could be beneficial in the treatment of the cancers which depend on WNT signal activity. All the more, since it was shown that ZNRF3 protein abundance is reduced in gastric adenocarcinoma and that overexpression of ZNRF3 induces apoptosis and suppresses proliferation via negatively affecting WNT signalling (Zhou et al., 2013). In addition, RNF43 inhibits gastric cancer cell proliferation (Niu et al., 2015). To date, no information is available regarding the regulation of ZNRF3 and RNF43 in asthma, IPF or COPD. However, given that WNT signalling activity is altered in these diseases and the potential of ZNRF3 and RNF43 ligases to modulate WNT signalling, it would be of a great interest to investigate the role of these ligases in the context of these diseases.

7. Non-Frizzled WNT receptors involved in β-catenin-independent signalling

7.1. Receptor tyrosine kinase-like orphan receptors (ROR)

RORs represent a family of 2 receptor tyrosine kinases, termed ROR1 and ROR2, of which only ROR2 has demonstrable intrinsic tyrosine kinase activity (Green, Nusse, & van Amerongen, 2014). RORs are characterized by an extracellular cysteine rich domain able to bind to WNTs, although other ligands including Resistin (for ROR1) have also been described (Sanchez-Solana, Laborda, & Baladron, 2012). The role of ROR1 in WNT signalling is very poorly characterized in contrast to that of ROR2, particularly as it relates to lung biology.

ROR2 (receptor tyrosine kinase-like orphan receptor 2) is among the best described non-FZD WNT receptors and can function as a receptor homodimer or as co-receptor with FZD. Both WNT-3A and WNT-5A form complexes with ROR2, but only WNT-5A leads to receptor homodimerization and activation of receptor-intrinsic tyrosine kinase activity (Liu, Rubin, Bodine, & Billiard, 2008). Nonetheless, ROR2 binding by WNT-3A does functionally interact with FZD_2 activation leading to positive cooperativity in WNT/β-catenin signalling. In the human lung carcinoma cell line H441, overexpression of ROR2 and FZD2 had cooperative effects on TCF reporter activation (Li et al., 2008). In contrast, WNT-5A activation of ROR2 homodimerization drives the serial activation of TGF-β-activated kinase-1 (TAK1) and nemo-like kinase (NLK), which functions to antagonize WNT/β-catenin signalling by phosphorylating TCF/LEF (Kumawat & Gosens, 2016) (Fig. 1). ROR2 is expressed in abundance by epithelial cells in the lung, but is virtually absent from mesenchymal cells such as lung

fibroblasts and airway smooth muscle cells (Baarsma et al., 2011; Kumawat et al., 2013). Presumably, this accounts for why WNT-5A functions to antagonize WNT/β-catenin signalling in the alveolar epithelium (Baarsma et al., 2017), yet co-exists and even cooperates with active β-catenin signalling in airway smooth muscle and lung fibroblasts to downstream functional effects on matrix protein expression (Kumawat et al., 2013; Kumawat, Menzen, et al., 2014).

The functional role of ROR2 in the lung has not been studied in great detail thus far. Chick pulmonary WNT-5A expression appears to mediate, at least in part via ROR2 activation, airway branching and alveolar development in avian embryos (Loscertales, Mikels, Hu, Donahoe, & Roberts, 2008). In mice, ROR2 knock-out leads to major defects in lung development (Oishi et al., 2003). At E18.5, embryos of ROR2 knockout-mice exhibited severe abnormalities in the lungs with shortened trachea along the proximal-distal axis and a reduced number of cartilage rings, which is similar to what is observed in the lungs of WNT-5A deficient mice (Oishi et al., 2003). This indicates that WNT-5A/ROR2 signalling plays crucial roles in regulating epithelial cell proliferation and plasticity in the developing lung, possibly by negatively modulating WNT/β-catenin signalling. Later in life, this regulatory function remains active and may contribute to normal lung repair, while when dysregulated, to lung cancer development. Thus, ROR2 supports lung repair induced by mesenchymal stem cells in a mouse model of LPS-induced lung injury, with ROR2 overexpressing cells being more effective in supporting alveolar type II cell differentiation and restoration of alveolar barrier function (Cai et al., 2016). In lung cancer, however, ROR2 functions as a tumour suppressor that is found frequently methylated in carcinoma, leading to disinhibition of β-catenin and AKT signalling (Li et al., 2014). Tumours that develop in spite of ROR2 expression on the other hand, may use it in concerted action with WNT-5A, possibly to support metastasis in non-small cell lung cancer patients with unfavourable outcome (Kikuchi et al., 2012; Lu et al., 2015).

7.2. Related to receptor tyrosine kinase (RYK)

RYK (related to receptor tyrosine kinase) represents another non-FZD WNT co-receptor that mediates β-catenin-independent signalling. Unlike ROR2, RYK does not have functional intrinsic tyrosine kinase activity in its C-terminal domain, although it shares homology with receptor tyrosine kinases and is therefore classified as such (Green et al., 2014; Inoue et al., 2003; Yoshikawa, Bonkowsky, Kokel, Shyn, & Thomas, 2001). It remains incompletely understood how RYK functions as a WNT receptor, and like ROR2, both direct receptor signalling and signalling induced by heterodimerization with the FZDs has been observed. Since the intracellular domain of RYK (LIN-18 in *C. elegans*) is not required for signalling (Inoue et al., 2003), and since RYK has been shown to functionally interact with FZD signalling in regulating neurite outgrowth in Drosophila (Lu, Yamamoto, Ortega, & Baltimore, 2004), it is assumed that RYK functions primarily as a co-receptor with FZD and not as a catalytically active receptor tyrosine kinase homodimer (Green et al., 2014). Both WNT/β-catenin-dependent and -independent functions have been attributed to RYK, and unfortunately its downstream signalling intermediates are not well established (Cheyette, 2004). The expression pattern of RYK is quite different from that of ROR2 with substantial expression in mesenchymal cells, transcript levels being among the highest of all WNT receptors (Kumawat et al., 2013). RYK knockdown in smooth muscle produces similar effects to WNT-5A or FZD_8 knockdown, reducing effects on extracellular matrix protein production by TGF-β (Kumawat et al., 2013; Spanjer, Baarsma, et al., 2016). Similar functions of RYK have been reported in the mammary gland, in which WNT-5A represses tumourigenesis by facilitating RYK and TGF-β pathway activation (Borcherding et al., 2015). Whether this is also the case for lung cancer is unknown; RYK is abundantly expressed in small cell lung cancer, but its functional role is not well understood (Hamilton, Rath, Klameth, & Hochmair, 2015).

7.3. Protein tyrosine kinase 7 (PTK7)

Protein tyrosine kinase 7 (PTK7) is a highly conserved but, similar to RYK, catalytically inactive receptor tyrosine kinase. Like RYK, PTK7 has been ascribed both positive and negative modulating roles in WNT/β-catenin signalling and likely supports WNT/PCP signalling during development (Bin-Nun et al., 2014; Hayes, Naito, Daulat, Angers, & Ciruna, 2013). The functional role of PTK7 in the lung has only been minimally investigated. It has been described as a tumour suppressor in lung squamous cell carcinoma inhibiting ERK and AKT activation (Kim et al., 2014). In contrast, elevated expression of PTK7 has been reported in non-small cell lung cancer and inhibition of PTK7 by a PTK7-targeted antibody-drug conjugate was recently shown to repress tumour growth (Damelin et al., 2017).

8. WNT planar cell polarity receptor signalling in lung diseases

Planar cell polarity signalling is an essential component of epithelial cell structure and function (Cui et al., 2013; Damelin et al., 2017). It regulates cell polarity and thus allows for proper directional position and cellular transport. Induction of PCP signalling can be mediated by WNT binding to receptors, as part of the β-catenin-independent pathways. In the lung, PCP signalling is particularly important for ciliogenesis, cilia orientation and beating, as well as for directed cell migration and possibly convergent extension (Vladar, Lee, Stearns, & Axelrod, 2015; Vladar, Nayak, Milla, & Axelrod, 2016; Yates, Papakrivopoulou, et al., 2010). The majority of core PCP components were identified first in Drosophila, which until today is also the most used organism to study PCP function. Nevertheless and not surprisingly given its biological role, more recently PCP has been studied in the context of homeostasis and disease in the mammalian system (Yates & Dean, 2011). PCP core components in the mammalian system include WNTs, transmembrane proteins, such as Frizzled receptor, Van Gogh-like protein (VANGL) 1 and 2 as well as Cadherin EGF LAG Seven-Pass G-Type Receptor (CELSR) 1 and 2, and the intracellular signalling intermediates DVL and Dishevelled-associated activator of morphogenesis (Daam). Here, we will discuss the potential role of the PCP receptors in lung disease. Both *Vangl* and *Celsr* have been investigated in genetic (knockout) models, are perinatal lethal, and do exhibited a developmental lung phenotype (Yates, Schnatwinkel, et al., 2010). *Vangl* and *Celsr* showed a distinct spatial expression in the lung epithelium with discontinuous distribution of CELSR1 around the basal side of the airway. Loss-of function of CELSR1 and/or VANGL2 led to smaller lungs, hypoplasia, and reduced epithelial cell branching. Interestingly, cellular proliferation and apoptosis was normal in mutant lungs compared to wild-type. Instead, both knockout mice displayed differences particularly in airway cell morphology with deranged cell positioning as well as cytoskeletal structures. This is likely mediated by disturbed Rho kinase (ROCK) signalling, as the phenotype of the *Celsr* knockout lungs was partially rescued by a Rho activator, while Rho kinase inhibition led to a very similar phenotype in ex vivo lung cultures (Yates, Schnatwinkel, et al., 2010). Altogether, the study provided evidence that CELSR1 and VANGL2 regulate lung branching morphogenesis and as such, might further be involved in the development of adult lung disease. In line with this, a more recent study investigated the phenotype of adult heterozygous *VANGL2* mice (Poobalasingam et al., 2017). Single copy mutations in *Vangl2* showed similar but milder changes compared to the homozygous knockout. The heterozygous *Vangl2* mice exhibited a significant reduction of airways in the lung, with many of them showing a narrow or closed lumen. Postnatally, at 10 weeks of age, the lungs developed emphysema like structures with enlarged airspaces, increased linear intercept and reduced lung function (as measured by elastance). The authors did not observe any differences in cellular proliferation or apoptosis in these mice, but found disturbed actin cytoskeletal organization and less membranous expression of β-catenin in vivo, suggesting defects in epithelial cell positioning. In

vitro experiments demonstrated that VANGL2 knockdown led to impaired wound repair and cell migratory potential along with a reduced number of polarized alveolar epithelial cells (Poobalasingam et al., 2017). Similarly, a recent study further investigated another transmembrane component of the PCP pathway, the atypical cadherin Dachsous 1, in the mammalian system and found that this protein is exclusively expressed in the cilial base and aberrantly expressed on transcript level in lung cancer (Dau et al., 2016). Collectively, this strongly suggests that disturbed PCP signalling contributes to lung injury and impairs repair processes. This is furthermore supported by the notion that misaligned PCP components, including the VANGL receptor, have been found in injured airway epithelial cells from cystic fibrosis or chronic rhinosinusitis patients. These cells display misaligned cilia and exhibit defective differentiation along with disrupted PCP (Vladar et al., 2016). Another study suggests that WNT/β-catenin signalling might be involved in blocking proper PCP signalling, which will need further corroboration (Boscke et al., 2017; Costa & Konigshoff, 2017). In summary, the PCP pathway represents a growing area of investigation, in particular with the recognition of the potential impairment of cilia function (Tilley, Walters, Shaykhiev, & Crystal, 2015; Vladar et al., 2016; Yang et al., 2013) and WNT signalling (Baarsma & Konigshoff, 2017; Konigshoff et al., 2008) in a number of chronic lung disease. Future studies, further dissecting the different components of PCP signalling, including other receptors such as FAT (Katoh, 2012), will be essential to advance our knowledge and potentially identify novel therapies targeting this pathway.

9. Modulators of WNT signalling

9.1. Soluble Frizzled-related proteins (sFRP)

The Soluble Frizzled-related protein (sFRP) family consists of 5 members (sFRP1–5). Similarly, to the FZDs the sFRPs contain a CRD domain at the N-terminus (Forrester, Dell, Perens, & Garriga, 1999). sFRPs can bind to WNTs as well as to FZDs, which can paradoxically results in the potentiation (Xiao et al., 2015) or antagonism of WNT signalling (Kawano & Kypta, 2003) (206). Xavier and colleagues showed that this dual role of sFRPs depends on the concentration of specific sFRPs, the cellular context and, most likely, the expression pattern of FZDs at the membrane of cells (Xavier et al., 2014). The sFRPs are implicated in controlling WNT signal activity in various tissues and are frequently downregulated in carcinomas and upregulated in degenerative diseases (Marsit et al., 2005; Suzuki et al., 2004). sFRP1, expressed during lung development, is elevated in the lungs of emphysema/COPD patients as well as in experimental models of emphysema (Foronjy et al., 2010). Furthermore, sFPR1 can induce apoptosis of pulmonary epithelial cells and endothelial cells in vitro (Imai & D'Armiento, 2002). Moreover, mice lacking sFRP1 exhibit dysregulated WNT signalling with rapid repair responses leading to aberrant mesenchymal proliferation and impaired alveoli formation (Foronjy et al., 2010). Collectively, these findings suggest a divergent and crucial role of the protein during development, but upregulation of the protein in the adult lung may have detrimental effects (Foronjy et al., 2010; Shiomi, Sklepkiewicz, Bodine, & D'Armiento, 2014). Moreover, sFRP1 might play a role in maintenance of adult murine bronchial alveolar progenitor cells in their undifferentiated state (Shiomi et al., 2014), once more showing the potential role of sFPR1 to modulate the WNT pathway and to affect repair processes in the lung. Furthermore, enhanced sFRP2 expression can promote cancer development in the lung and could serve as a promising anticancer target (Xiao et al., 2015). On the other hand, *sFRP3* is decreased in lung adenocarcinoma and has been proposed as a prognostic marker and a putative tumour suppressor (Schlensog et al., 2016). Taken together, aberrant expression of sFRPs is observed in emphysema/COPD and lung cancer and they are participatory factors in these diseases.

9.2. Dickkopf (DKK)

The DKK protein family, consisting of 4 members (DKK-1 through DKK-4), represents another group of extracellular WNT signalling modifiers. Structurally, they contain two CRD domains (CRD1 and CRD2), where CRD2 is highly conserved among all members (Glinka et al., 1998). The mechanism of WNT inhibition differs though from the one exhibited by sFRPs. DKKs bind to LRP5/6 receptors, rather than to WNT proteins, thereby preventing formation of ternary WNT-FZD-LRP5/6 complexes (Semenov et al., 2001). Also an alternative mechanism of action has been described in which DKKs act via binding with another receptor, called Kremen (KRM), leading to the internalization of LRP from the surface membrane, resulting in attenuation of WNT signalling (Mao et al., 2002). DKK-1, DKK-2 and DKK-3 are expressed distally in the epithelium of the developing lung (De Langhe et al., 2005).The role of DKK-1 seems to be cell- and/or organ-dependent. DKK-1 expression is decreased in melanoma, colon, and gastric cancer (Gonzalez-Sancho et al., 2005; Kuphal et al., 2006; Mikata et al., 2006), but overexpressed in cancers in various other organs, including the lung (Dong, Qu, Chu, Zhang, & Liu, 2014). Interestingly, cigarette smoke decreases the expression of DKK-1 in lung cancer cells in vitro, which is accompanied by (enhanced) activation of WNT signalling (Hussain et al., 2009). Moreover, DKK-1 and DKK-4 expression is enhanced in the epithelium of IPF patients and, in the case of DDK-1, was shown to regulate epithelial cell proliferation (Pfaff, Becker, Gunther, & Konigshoff, 2011). Additionally, knock down of DKK-3 in NSCLC cells leads to apoptosis, which could indicate that DKK-3 is an anti-apoptotic agent (Jung, Kang, Kim, & Kim, 2010). However, at the same time DKK-3 has been shown to be silenced by promoter hypermethylation, frequently inactivated in lung cancers, and to have a role in suppressing lung cancer cell growth via inhibition of WNT/β-catenin signalling (Yue et al., 2008). Interestingly, in several clinical trials DKKs are being used as target (i.e. inhibition via neutralizing antibodies) or as therapeutic to activate or inhibit WNT signalling, respectively (B. Lu, Green, Farr, Lopes, & Van Raay, 2016).

10. Concluding remarks

The comprehensive family of WNT proteins can activate transcriptional or non-transcriptional responses within cells by activating β-catenin-dependent or -independent signalling pathways. The signal initiated by an extracellular WNT is transduced by binding of the ligand to a wide variety of receptors and co-receptors present at the cell membrane. We gained more insight in the different receptors involved in WNT signalling over the last decades; however our knowledge about the biological function of each individual receptor, in particular in lung physiology and pathology, is limited and remains largely to be elucidated. Chronic lung diseases represent a major social economical health burden and, as current treatment options are not sufficient, alternative treatment options are required. Several preclinical studies have addressed the therapeutic potential of targeting WNT signalling in chronic lung diseases, mainly focusing the role of β-catenin, and the majority of these studies have reported beneficial results (Henderson et al., 2010; Kneidinger et al., 2011; Koopmans, Crutzen, et al., 2016). The underlying cause of aberrant activation of WNT in these pathologies remains unknown, but can have different origins including changes in WNT expression and secretion or diverged expression of WNT receptors and co-receptors (Baarsma et al., 2017; Skronska-Wasek et al., 2017). In this review we have highlighted that in various chronic lung diseases (IPF, COPD, asthma and lung cancer) aberrant expression of WNT receptors is commonly observed. Therefore, with the notion that also downstream WNT signalling events are abnormally active in these diseases, deviations in WNT receptors seem to be the key factor in the development and progression of pathologies in the lung and, thus, of interest for pharmacological intervention and future research. The number of small molecules and biologicals targeting specific ligand-receptor

interactions involved in WNT signalling is growing, which may gain momentum with the recent elucidation of the crystal structure of some FZD domains (DeBruine et al., 2017; Nile, Mukund, Stanger, Wang, & Hannoush, 2017). Targeting WNT signalling could potentially result in unwanted side-effects due to the importance of these signalling cascades in various cells, most importantly stem cells, with the risk of developing pathologies such as cancer. Prudence is advised with targeting WNT signalling pharmacologically. Nevertheless, in animal models of COPD, asthma and IPF it was shown that modulation of WNT signalling by small molecules and biologicals (e.g. antibodies) is safe and has beneficial effects in the lung (Baarsma et al., 2017; Henderson et al., 2010; Kneidinger et al., 2011; Koopmans, Crutzen, et al., 2016). Hence, this may also be of interest for future treatment of patients suffering from these diseases. Inhalation offers a non-invasive route for drug delivery and is suitable for delivery of small molecules, peptides and proteins with good bioavailability (Labiris & Dolovich, 2003a, 2003b). Recent advances in device technology have led to the development of very efficient delivery systems that are capable of delivering large doses of active compounds to specific regions of the lung. Hence, the lung is a very attractive organ for drug delivery, especially for treatment of local diseases (Labiris & Dolovich, 2003a, 2003b). A challenge remains how to target specific branches of WNT signalling by using pharmacologically active compounds directed against WNT receptors. The difficulty with targeting of the WNT-FZD interaction is that this may not result in discrimination between β-catenin-dependent versus –independent WNT signalling, as these receptors are required for WNT signalling in general. Nevertheless, recent insights in signalling events mediated by distinct WNTs and FZD-CRD domains (i.e. WNT binding domain) revealed that functional ligand- and receptor-selective signalling occurs (Dijksterhuis et al., 2015). Videlicet, distinct WNTs (e.g. WNT-3A and WNT-4) can bind with similar affinity to a subset of FZDs, however; this can result in differential downstream signalling events and consequently result in distinctive biological responses. A challenge for the future will be to develop small molecules or biologicals that modulate the interaction of distinct FZDs with a subset of selected WNTs. This is an exciting opportunity for drug discovery, and future pharmacological intervention targeting FZD receptors or any other WNT receptor for that matter can only be successful when we understand the molecular code and mechanism(s) of signal specification. Another approach would be to target WNT receptors and/or co-receptors that are (primarily) associated with only one branch of WNT signalling. For instance, LRP5 and LRP6 appear to be exclusively involved in β-catenin-mediated WNT signalling, whereas ROR2, RYK and PTK7 predominantly activate β-catenin-independent signalling pathways. The DKK family of secreted proteins target LRP5/6 to attenuate WNT/β-catenin signalling, demonstrating the possibility to influence specific WNT signalling branches by targeting specific receptors. This approach has been applied in vitro and ex vivo to study the role of WNT/β-catenin signalling in the lung (De Langhe et al., 2005; Flozak et al., 2010). However, pharmacological targeting of the latter group of receptors that are involved in β-catenin-independent signalling is currently not yet possible. Collectively, the WNT receptors and co-receptors are of great interest in chronic lung diseases characterized by aberrant WNT signalling. Although limited in number, the currently available biologicals and pharmacological tools to modify proximal WNT signalling hold great promise for treatment of chronic lung diseases in the future.

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Conflict of interest statement

All the authors declare that there are no conflicts of interest.

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