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Hedgehog pathway and its inhibitors in chronic obstructive pulmonary disease (COPD)

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ABSTRACT

COPD affects millions of people and is now ranked as the third leading cause of death worldwide. This largely untreatable chronic airway disease results in irreversible destruction of lung architecture. The small lung hypothesis is now supported by epidemiological, physiological and clinical studies. Accordingly, the early and severe COPD phenotype carries the most dreadful prognosis and finds its roots during lung growth. Pathophysiological mechanisms remain poorly understood and implicate individual susceptibility (genetics), a large part of environmental factors (viral infections, tobacco consumption, air pollution) and the combined effects of those triggers on gene expression. Genetic susceptibility is most likely involved as the disease is severe and starts early in life. The latter observation led to the identification of Mendelian inheritance via disease-causing variants of *SERPINA1* – known as the basis for alpha-1 anti-trypsin deficiency, and *TERT*. In the last two decades multiple genome wide association studies (GWAS) identified many single nucleotide polymorphisms (SNPs) associated with COPD. High significance SNPs are located in 4q31 near *HHIP* which encodes an evolutionarily highly conserved physiological inhibitor of the Hedgehog signaling pathway (HH). HHIP is critical to several in utero developmental lung processes. It is also implicated in homeostasis, injury response, epithelial-mesenchymal transition and tumor resistance to apoptosis. A few studies have reported decreased *HHIP* RNA and protein levels in human adult COPD lungs. *HHIP*^{+/-} murine models led to emphysema. HH pathway inhibitors, such as vismodegib and sonidegib, are already validated in oncology, whereas other drugs have evidenced *in vitro* effects. Targeting the Hedgehog pathway could lead to a new therapeutic avenue in COPD. In this review, we focused on the early and severe COPD phenotype and the small lung hypothesis by exploring genetic susceptibility traits that are potentially treatable, thus summarizing promising therapeutics for the future.

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Abbreviations: AEC, airway epithelial cell; ASM, airway smooth muscle; AT2, alveolar type 2 cell; CCSP, club cell secretory protein; COPD, Chronic obstructive pulmonary disease; CS, cigarette-smoke; EMT, epithelial-mesenchymal transition; FEV1, forced expiratory volume in the first second; FGF, fibroblast growth factor; FVC, forced vital capacity; GLI, Glioma-associated oncogene family zinc finger; GWAS, Genome wide association studies; HH, Hedgehog; HHIP-AS1, HHIP antisense RNA 1; HHIP, Hedgehog interacting protein; IPF, idiopathic pulmonary fibrosis; LLN, lower limit of normal; MUC5AC, mucin 5AC; NSCLC, non-small cell lung cancer; PRS, polygenic risk scores; PTCH1, Patched 1; SCLC, small cell lung cancer; SCMF, secondary crest myofibroblast; SHH, Sonic hedgehog; SMO, Smoothened; SNP, single nucleotide polymorphism; SUFU, Suppressor of fused homolog.

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1. Introduction

In 2019, the World Health Organization ranked Chronic Obstructive Pulmonary Disease (COPD) as the third leading cause of global death, behind ischemic heart disease and stroke. Unlike the last two, COPD and its growing burden is poorly known to the general public. COPD is crudely pictured as a bronchial condition caused by over-consumption of tobacco in elderly people. Clinically, the disease presents as chronic dyspnea causing disability and acute respiratory life-threatening episodes triggered by infectious micro-organisms, even though some remain poorly explained (Barnes, 2000).

Lung function impairment characterized by post bronchodilatation airflow limitation is the common essence of the disease. At the functional level, spirometry measurements include forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC). COPD is defined by post-bronchodilator obstructive limitation (ratio FEV₁/FVC < Lower Limit of Normal, LLN) and progressive loss of lung function of growing severity assessed by FEV₁ (Vogelmeier et al., 2017). Until recently, airflow limitation in COPD was only seen as an accelerated decline in FEV₁ from a maximally attained peak at the beginning of adulthood. Peter Lange challenged this concept by showing variability of FEV₁ decline during life (Lange et al., 2015). Almost half of general population individuals with airflow obstruction at 60 years did not experience an accelerated decline. Indeed, they developed lung function impairment through a FEV₁ trajectory starting below average around age 30. Lung function in adulthood should be seen as the aggregate of processes occurring during gestation, birth, and early life, keeping in mind that lung growth continues even after birth until age 14–18 (Whitsett & Weaver, 2015). Any factor occurring during lung growth could lower the maximally attained lung function in adulthood (Allinson et al., 2017). Consequently, this could increase the risk of ending up with poor elder lung function, thus increasing the risk for COPD. This “small lung” theory supports the vision of COPD as an adult disease with pediatric roots (Martinez, 2016). Accordingly, new concepts have been raised: *early COPD* is now defined as a disease onset before age 50 in smokers with bronchial obstruction (FEV₁/FVC < LLN) (Rennard & Drummond, 2015). The prevalence of *early COPD* in The Copenhagen General Population Study reached 15% and was significantly associated with higher acute respiratory events and more premature deaths (Çolak, Afzal, Nordestgaard, Lange, & Vestbo, 2021). Recently, the Global initiative for chronic Obstructive Lung Disease (GOLD) introduced in their 2022 report new concepts (Venkatesan, 2022). They endorse *early COPD* as defined above, whereas *COPD in young people* is proposed for patients of age 20–50 who had never achieved normal peak lung function. However, such definitions remain challenging in clinical practice due to the scarcity of symptoms and limited health-care utilization in these populations.

COPD results from the combination of many interacting factors, of which cigarette-smoke (CS) exposition is the most prevailing in Western countries (Gershon, Warner, Cascagnette, Victor, & To, 2011). However, only 20% of active smokers develop the disease and an estimated 25–45% of COPD patients have never smoked (Salvi & Barnes, 2009). These findings suggest the involvement of other intrinsic and extrinsic factors. As for extrinsic factors, occupational exposures, such as in farmers or diggers, were the first to be identified. More recently, exposures to biomass fuel and outdoor air pollution have been strongly associated with an increased prevalence of COPD independently of smoking history (Postma, Bush, & van den Berge, 2015). Intrinsic factors such as genetic variations account for a substantial part of the occurrence of COPD. Epigenetics, i.e. post-translational modifications in histone proteins and DNA such as methylation and acetylation, as well as dysregulation of miRNAs expression, could also be strongly involved in COPD (Zhang et al., 2020), unraveling the link between genetic susceptibility and environmental factors (Probert, Miller, Kheirallah, & Hall, 2015).

Hence, COPD patients represent a heterogeneous group with a wide age range, an incompletely explained susceptibility and various clinical and pathophysiological characteristics. Structural and inflammatory

mechanisms resulting in the irreversible destruction of lung architecture remain poorly understood. At the cellular level, COPD features bronchial remodeling and thickening of the small airways with mucus luminal obstruction, peri bronchial fibrosis and immune cell recruitment (Hogg et al., 2004). Bronchial epithelium is characterized by multiple cells, of which the ciliated cells represent the most frequent type in healthy subjects (Verra et al., 1995). Two other major subtypes include goblet cells which produce mucus mainly in the proximal airways, and club cells which play an accessory progenitor role in small airways and secrete an anti-inflammatory protein named CCSP (club cell secretory protein) (Knabe, Fort, Chanez, & Bourdin, 2015). CCSP levels are decreased in COPD (Lange, Ahmed, Lahmar, Martinez, & Bourdin, 2021). The last subtype consists of basal cells, considered as the main progenitor that ontogenically differentiates sequentially into club, goblet or ciliated cells. CS exposure reduces the number of ciliated and club cells, while increasing the number of goblet cells (Harvey et al., 2007; Schamberger, Staab-Weijnitz, Mise-Racek, & Eickelberg, 2015). COPD lungs therefore have lower rates of both ciliated and club cells, whereas their goblet cell hyperplasia was associated with increased IL-13 induced mucus production (Kuperman et al., 2002). The alveolar epithelium is composed of two major cell subtypes: alveolar type 1 (AT1) and type 2 (AT2) cells. The latter are considered as stem cells responsible for repairing and maintaining lung tissue (Whitsett & Weaver, 2015). CS causes chronic inflammation, oxidative stress and apoptosis in the alveolar compartment (Tsuji, Aoshiba, & Nagai, 2006; Yuan et al., 2019). Emphysema is considered a hallmark of COPD and is characterized by alveolar rupture or destruction beyond the small airways. Emphysema has been linked to CS toxicity (Yuan et al., 2019), matrix remodeling with protease/antiprotease imbalance (Uemasu et al., 2020) or even directly due to chronic bronchiolar obstruction (Verra et al., 1995).

In the absence of disease modifying therapies, early severe COPD remains a largely untreatable condition. Currently marketed treatments mainly target the airway smooth muscle (ASM), such as long acting beta-2 agonists and long acting muscarinic antagonists (Vogelmeier et al., 2017). They mostly relieve symptoms in a palliative way. In severe end-stage COPD, lung transplantation represents the only curative strategy (Stavem, Bjørtuft, Borgan, Geiran, & Boe, 2006; Thabut et al., 2008). There is therefore a strong need to design approaches capable of restoring lung homeostasis and the mechanical disorders induced by COPD. Identifying the cellular pathways involved and understanding their diverse roles could reveal new targets and open new therapeutic avenues.

The Hedgehog pathway, and notably the main ligand Sonic Hedgehog (SHH), is a key developmental signaling pathway that targets the lungs in addition to central nervous system patterning or limb and digit development (Litingtung, Lei, Westphal, & Chiang, 1998). SHH is negatively regulated by the Hedgehog interacting protein HHIP. Genome Wide Association Studies (GWAS) identified polymorphisms near the *HHIP* gene associated with COPD conditions and respiratory features. In this review, we focused on the early and severe phenotype of COPD representative of genetic susceptibility through the Hedgehog cellular pathway. This pathway is likely involved in COPD through multiple mechanisms, and has promising potential as a target for pharmaceutical development.

2. Genetics in COPD

COPD aggregates in families and spirometric features are heritable (McCloskey et al., 2001; Polderman et al., 2015). Heritability can be defined as the relative contribution of a genotype to a phenotype. The heritability for COPD was measured at around 40% in the COPD Gene cohort (Zhou et al., 2013), while in a group of nearly 50,000 Danish and Swedish twin pairs, it reached over 60% (Ingebrigtsen et al., 2010). Phenotypes associated with COPD such as airway wall thickening on computed tomography (CT) or emphysema independently aggregate within families of individuals with COPD (Patel et al., 2008). Alpha-1 Antitrypsin (A1AT) deficiency remains the best described genetic association accounting

for 1% of COPD due to Mendelian inheritance of a single disease-causing variant in the *Serpin family A member 1* gene (*SERPINA1*) (Silverman & Sandhaus, 2009). This variant was associated with the onset of premature and severe emphysema. Other rare variants segregating in families were identified in 2014 near the *Telomerase reverse transcriptase* gene (*TERT*), which affects telomere length. The latter predisposes to COPD, pulmonary fibrosis and the combined emphysema-fibrosis phenotype (Nunes et al., 2014; Stanley et al., 2015). Beyond A1AT deficiency, and similar to other models of chronic heterogeneous complex diseases, COPD risk seems to be more the consequence of the combined contributions of multiple frequent variants with small effect sizes rather than rare disease-causing variants (Li, Cho, & Zhou, 2017).

The challenge of assessing disease risk motivated the creation of multiple cohorts in order to identify candidate-genes and create genetic risk scores. Investigators from the SPIROMICS, MESA Lung, ECLIPSE, UK Biobank or COPD Gene cohorts (among others) developed huge multicentric longitudinal studies to follow tens of thousands of active smokers (Cho, Hobbs, & Silverman, 2022). They were mainly non-Hispanic white Caucasians and African American individuals with a collection of plentiful clinical and biological characteristics including spirometry, CT imaging and biomarkers (Ragland et al., 2019). Genome wide association studies (GWAS) represent a strategy for detecting genetic variants involved in a trait as a result of genome-wide linkage and association methods. Their strength resides in high significance criteria, very large sample sizes and strong statistical power (Manolio, 2010). By screening the whole genome of two distinct groups based on a specific feature, GWASs were able to identify susceptibility variants and candidate genes without any *a priori* clinical evidence.

When assessing COPD risk in a population of smokers, confounding factors can exist. For example, polymorphisms in nicotinic acetylcholine receptor genes have been associated with smoking intensity (Mineur & Picciotto, 2008) and nicotinic dependence (Lassi et al., 2016) and more recently with COPD (Routhier et al., 2021). Assessing COPD risk in populations of smokers could remain in that case challenging.

Identifying susceptible genetic backgrounds can help understand COPD beyond exposition, underlining the importance of developing risk-measurement tools such as polygenic risk scores (PRS). These scores can evaluate the risk for developing the disease, identify subtypes of phenotype and measure prognosis in this complex heterogeneous condition. Recently, the data from the previously mentioned large cohorts allowed teams to construct PRSs able to identify a small subset of individuals at higher risk for COPD, emphysema and reduced lung growth (Moll et al., 2020). Complementation of PRS with clinical and blood-based data improved prediction of COPD and lung function decline (Moll et al., 2022). In this way, genetic studies have helped the scientific community understand new pathways that could be pharmacologically targetable.

2.1. Single nucleotide polymorphisms near *HHIP* in 4q31 and association with COPD

The common attitude in COPD consisted in genome-wide linkage to quantitative spirometric traits such as FEV1, FVC and FEV1/FVC ratios in large population samples. The first GWAS in COPD was published in 2009 and identified susceptibility to post-bronchodilator lung function impairment (FEV1/FVC < LLN) in loci near *HHIP* (*Hedgehog interacting protein*) in 4q31, but also in 15q25, a region of *CHRNA3/5* (*Cholinergic receptor nicotinic alpha 3/5 subunit*) and *IREB2* (*Iron-responsible element-binding protein 2*) (Pillai et al., 2009). The most recent GWAS in 2019 identified 82 loci significantly associated with either COPD or lung function impairment after comparing 35,735 cases to 222,076 controls (Sakornsakolpat et al., 2019).

Since 2009, multiple SNPs near *HHIP* in 4q31 were associated with high levels of significance with disease status, severity, emphysema, and lung function. Table 1 shows a selection of the most relevant SNPs associated with COPD or respiratory function in genome wide association studies (GWAS).

2.2. Functional validation of GWAS candidates

Very fashionable in the 2010's, GWAS have lost in popularity, primarily due to the poor ethnical-diversity in the investigations. GWAS conducted in westernized countries studied mainly Caucasians, whereas relevant polymorphisms are differentially represented in the Han Chinese for example (Xu et al., 2019). Most polymorphisms remain common as they can be found in nearly 30% of the population and would therefore have low individual effects. The major argument behind the trend to move beyond GWAS remains the scarcity of pathophysiological and/or clinical translation of the data. Many of the significant associations found do not have any perceivably meaningful link to clinical knowledge.

GWAS identified SNPs in the "gene desert", meaning regions devoid of protein coding genes. Interestingly, the majority of SNPs near *HHIP* are located in intergenic regions. These observations need to be analyzed while keeping in mind the three-dimensional structure of human chromatin, which allows contact between two distinct DNA regions. In 2012, X. Zhou proposed a functional validation with the demonstration of an interaction between a COPD-risk locus containing multiple SNPs in intergenic regions and the *HHIP* promoter (Zhou et al., 2012). The hypothesis suggested that a chromatin loop and functional variants within an enhancer could alter the binding of Sp3 transcription factor. Two SNPs within this enhancer region (rs6537296A and rs1542725C, confirmed as risk alleles by GWAS by Lutz et al., 2015) exhibited increased Sp3 binding, resulting in a repression of *HHIP* transcription. These *in vitro* experiments performed in heterologous cells should be considered with caution for *in vivo* extrapolation.

Beyond COPD risk, *HHIP* SNPs should be seen as an add-on to mechanistic studies as they highlight new actors that can complement the two major pathophysiological hypotheses: (i) the small lung theory with a defect in a crucial developmental process and (ii) the accelerated decline theory with a lack of a key inhibitor in a major signaling pathway imperative for lung homeostasis and mesenchymal-epithelial crosstalk in the mature adult lung. As for developmental process variants for lung function, height and COPD frequently overlap with regulatory regions of fetal and developmental genes (Fawcett et al., 2020; Sakornsakolpat et al., 2019). Furthermore, SNPs near *HHIP* were previously significantly associated with height (Weedon et al., 2008). Height is also an important predictor of FEV1 (Yang et al., 2015). Soler Artigas identified 180 loci associated with height, but only 3 were associated with lung function, with an increased effect in height resulting in a decrease in lung function (Soler Artigas et al., 2011). Moreover, they identified rs7689420, which is one of the strongest SNPs associated with height near *HHIP* (p-value = $6.2 \cdot 10^{-51}$), that lies within one of its introns but shows no association with lung function (FEV1 or FEV1/FVC ratio). However, these associations with lung function cannot be explained solely by incomplete adjustment for height; for example, rs1032296 was identified in that same study as significantly associated with lung function and height, with the same direction of effects.

To improve upon previous results, a new trend consists in combining them with transcriptomic and proteomic data (Liu et al., 2020) (see Table 2). However, up to now, previous studies do not reveal expression quantitative trait loci (eQTL) associated with *HHIP* SNPs. This may be because most RNAseq experiments are performed on whole tissues (i.e. bulk RNAseq) without individualization of *HHIP*-productive cells. Single-cell RNAseq (scRNAseq) may solve this problem; however, this powerful but very expensive technique is still lacking the depth needed to quantify small changes associated with low effect SNPs in large cohorts.

3. Hedgehog pathway and implications for COPD

3.1. Human Hedgehog pathway

In 1980, Pr Wieschaus and Pr Nüsslein-Volhard discovered *Hedgehog* gene orthologs during genetic research on fruit fly development. *SHH*-

Table 1The most relevant polymorphisms in 4q31 near *HHIP* identified in multiple GWAS.

SNP	Location (hg38)	Risk allele	RAF	P-value	Reported trait	Beta coefficient	Odds Ratio [CI 95%]	Reference
rs13109426	4:144409476	A	0.59	1×10^{-11}	FEV1	0.0161 unit decrease		(Shrine et al., 2019)
rs12510916	4:144451096	C	0.69	1×10^{-7}	COPD		1.27 [1.16–1.39]	(Lee et al., 2014a)
rs13116999	4:144521212	A	0.54	1×10^{-66}	FEV1	0.0404 unit increase		(Shrine et al., 2019)
				7×10^{-111}	FEV1/FVC ratio	0.0535 unit increase		
rs1512282	4:144510345	G	0.41	3×10^{-8}	FEV1/FVC ratio		0.58 [0.36–0.8] % increase	(van der Plaats et al., 2016)
rs1032295	4:144513432	T	0.32	3×10^{-20}	FEV1/FVC ratio	0.052 unit increase		(Hancock et al., 2010)
rs1032296	4:144513536	T	0.43	7×10^{-7}	FEV1		1.14 [1.08–1.20]	(Wain et al., 2015)
rs1032297	4:144513592	A	0.64	4×10^{-9}	FEV1/FVC ratio	0.01537 unit increase		(Lutz et al., 2015)
rs12504628	4:144515172	T	0.37	1×10^{-9}	FEV1	0.044 unit decrease		(Soler Artigas et al., 2011)
				6×10^{-19}	FEV1/FVC ratio	0.063 unit decrease		
				3×10^{-7}	FEV1	0.027 unit decrease		
rs138641402	4:144524627	T	0.38	4×10^{-11}	FEV1	0.046 unit increase		(Repapi et al., 2010)
				5×10^{-33}	FEV1/FVC ratio	0.082 unit increase		
		A	0.62	4×10^{-9}	COPD		1.3 [1.19–1.42]	(Lee et al., 2014a)
				1×10^{-8}	Emphysema pattern measurement	0.03 unit decrease		(Castaldi et al., 2014)
rs13147758	4:144539078	G	0.34	8×10^{-11}	FEV1/CVF ratio	0.1 unit increase		(Wilk et al., 2009)
rs6828982	4:144549452	C	0.73	2×10^{-13}	FEV1	0.142 unit decrease		(Wain et al., 2015)
rs13107665	4:144551492	A	0.46	8×10^{-10}	FEV1		1.16 [1.10–1.22]	(Wain et al., 2015)
rs13140176	4:144567946	A	0.61	3×10^{-59}	COPD		1.18 [1.16–1.20]	(Sakornsakolpat et al., 2019)
rs1489759	4:144553321	A	0.59	3×10^{-8}	FEV1/CVF ratio	0.01439 unit increase		(Lutz et al., 2015)
rs11100860	4:144557987	G	0.43	4×10^{-9}	FEV1	0.041 unit increase		(Soler Artigas et al., 2011)
				7×10^{-20}	FEV1/FVC ratio	0.064 unit increase		
rs1828591	4:144559628	G	0.41	1×10^{-7}	COPD		1.38 [1.17–1.63]	(Pillai et al., 2009)
rs1980057	4:144564586	T	0.31	3×10^{-15}	FEV1/FVC ratio	0.047 unit decrease		(Hancock et al., 2010)
				4×10^{-9}	FEV1	0.042 unit increase		(Soler Artigas et al., 2011)
				1×10^{-19}	FEV1/FVC ratio	0.063 unit increase		
				2×10^{-8}	FEV1/FVC ratio	0.0146 unit increase		(Lutz et al., 2015)
rs7655625	4:144564763	C	0.36	2×10^{-8}	FEV1/FVC ratio	0.01471 unit increase		(Lutz et al., 2015)
rs13118928	4:144565237	A	0.32	9×10^{-7}	COPD		0.80 [0.61–0.86]	(Pillai et al., 2009)
				9×10^{-8}	COPD		0.77 [0.70–0.85]	(Cho et al., 2010)
				6×10^{-7}	COPD		0.76 [0.65–0.89]	(Cho et al., 2012)
rs10029430	4:144577260	A	0.68	3×10^{-7}	FEV1/FVC ratio	0.01 unit increase		(Lutz et al., 2015)
rs6537297	4:144580877	T	0.67	8×10^{-7}	FEV1	0.045 unit increase		(Lutz et al., 2015)
				6×10^{-8}	FEV1/FVC ratio	0.01 unit increase		
rs13141641	4:144585304	T	0.32	9×10^{-41}	COPD		1.22 [1.19–1.26]	(Hobbs et al., 2017)
				8×10^{-10}	Emphysema imaging phenotype (Perc15)	2.2 unit decrease		(Cho et al., 2015)
				6×10^{-18}	Emphysema distribution (ratio950)	0.12 unit increase		(Bouez et al., 2017)
				4×10^{-48}	FEV1	0.0348 unit decrease		(Shrine et al., 2019)
				4×10^{-184}	FEV1/FVC ratio	0.0704 unit decrease		
				4×10^{-15}	Severe COPD		1.39 [1.28–1.51]	(Cho et al., 2014)
				4×10^{-14}	FEV1	0.072 unit decrease		(Lutz et al., 2015)
				3×10^{-7}	FEV1/FVC ratio	0.012 unit decrease		
				3×10^{-7}	COPD		1.32 [1.14–1.54]	(Cho et al., 2012)
				3×10^{-6}	Chronic bronchitis in COPD		1.27 [1.15–1.41]	(Lee et al., 2014b)
				2×10^{-12}	Emphysema imaging phenotype (%LAA–950)	0.12 unit increase		(Cho et al., 2015)
				2×10^{-12}	Moderate to severe COPD		1.27 [1.19–1.36]	(Cho et al., 2014)
				2×10^{-11}	Emphysema distribution (diff950)	0.09 unit increase		(Bouez et al., 2017)
				1×10^{-19}	FEV1/FVC ratio	0.018 unit decrease		(Lutz et al., 2015)
rs6537298	4:144585719	T	0.57	3×10^{-9}	FEV1	0.051 unit increase		(Lutz et al., 2015)
				2×10^{-10}	FEV1/FVC ratio	0.011 unit increase		
rs7670758	4:144590723	A	0.39	6×10^{-9}	FEV1/FVC ratio	0.011 unit decrease		(Lutz et al., 2015)
rs1542726	4:144594617	A	0.38	7×10^{-7}	FEV1	0.047 unit decrease		(Lutz et al., 2015)
				3×10^{-9}	FEV1/FVC ratio	0.012 unit decrease		
rs1489765	4:144595226	T	0.61	4×10^{-7}	FEV1	0.047 unit increase		(Lutz et al., 2015)
				2×10^{-8}	FEV1/FVC ratio	0.011 unit increase		
rs2353940	4:144819746	T	0.75	5×10^{-19}	FEV1/FVC ratio	0.0246 unit increase		(Shrine et al., 2019)
				5×10^{-10}	FVC	0.0169 unit increase		
				2×10^{-24}	FEV1	0.0275 unit increase		

COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; RAF: risk allele frequency; SNP: single nucleotide polymorphism. We provide a curated version of an extraction from the GWAS catalog with the following parameters: MAPPED_GENE *HHIP* and/or *KRT18P51*. Curation includes implementation of non-listed SNPs found as relevant in the literature, and the allele risk if available, correction of p-values, odds ratios and confidence intervals using original papers and supplemental data. Full data is available in the online GWAS catalog (<https://www.ebi.ac.uk/gwas/>). Beta coefficients represent the per unit increase or decrease in the reported trait.

Table 2
Core Hedgehog pathway components in bronchial epithelium.

	Healthy airways		Bronchial diseases	
	Murine	Human	Murine	Human
HHIP	<i>Hhip</i> ^{-/-} mice develop severe lung hypoplasia (Chuang, 2003). Single cell RNAseq analyses identify <i>Hhip</i> co-expressed with myofibroblast elastin confirmed in FISH (Travaglini et al., 2019).	Single cell RNAseq analyses identify <i>HHIP</i> in the alveolar compartment expressed in AT2 cells (Travaglini et al., 2019), and in the mesenchymal compartment in ASMC (Danopoulos et al., 2020).	<i>Hhip</i> ^{+/-} mice develop spontaneous age-related emphysema, higher CS-induced emphysema than controls and CS-induced ASMC hyperplasia (Lao et al., 2015, 2016; Li et al., 2021).	HHIP protein and mRNA decreased in COPD whole lung samples (Zhou et al., 2012). HHIP represses cell proliferation in COPD-derived ASMCs (Li et al., 2021).
SHH	<i>Shh</i> is expressed in embryonic alveolar and bronchial epithelium (Bellusci et al., 1997) in almost all subgroups of epithelial cells. Coimmunostaining with <i>Ccsp</i> at E18 in bronchiolar airways and in club cells in adult proximal airways (Peng et al., 2015) and distal alveolar epithelium (Wang et al., 2018).	SHH is produced by AEC during fetal development (Belgacemi et al., 2022) and during differentiation in an ALI culture (Belgacemi et al., 2020)	<i>Shh</i> floxing in murine airway epithelium increases proliferation in the surrounding mesenchyme. (Peng et al., 2015).	SHH and CCSP co-immunostaining in BALFs from children with asthma (Xu et al., 2018). <i>SHH</i> transcripts are reduced in COPD whole lungs (Ancel et al., 2020) (Zhou, Qiu, et al., 2013). SHH is overexpressed in lung fibroblasts from COPD smokers compared to non-smokers (Figeac et al., 2015).
PTCH1	A <i>Ptch1</i> ^{-/-} mice model is lethal before lung development (Bellusci et al., 1997).	<i>PTCH1</i> is identified in myofibroblast clusters through single cell RNAseq analyses (Travaglini et al., 2019).	<i>Ptch1</i> ^{+/-} mice were protected from HDM-induced mucous expression (Xu et al., 2018).	PTCH1 protein and mRNA increased in COPD lung tissue and bronchial brushings (Tam et al., 2019). PTCH1-siRNA treated human bronchial cells (1HAE0 and NCI-H292) experienced delayed wound repair and attenuated EGF induced MUC5AC upregulation (Tam et al., 2019).
GLI	<i>Gli2</i> ^{-/-} mice develop hypoplastic lungs lethal at birth (Chuang, 2003) (Motoyama et al., 1998). Fibroblasts are Gli-1 positive cells in alveolar and peribronchiolar perivascular zones in developing mouse tissue (Liu et al., 2013), increased with recombinant <i>Shh</i> and decreased with AB5E1.	Single cell RNAseq analyses identifies <i>GLI 1, 2, 3</i> in the alveolar compartment (Travaglini et al., 2019).	<i>Muc5ac</i> gene serves as a direct target of Gli proteins in asthma models of ovalbumine sensitized mice (Xu et al., 2018).	<i>GLI 1</i> or <i>GLI2</i> shRNA lentivirus knockdown attenuated HH induced MUC5AC promoter activity in a bronchial cell model (16HBE) (Xu et al., 2018). GLI1, 2 and 3 are decreased in lung tissue samples from COPD subjects compared to samples from control smokers (Zhou, Qiu, et al., 2013). GLI proteins are overexpressed in lung fibroblasts derived from COPD smokers compared to COPD non-smokers (Figeac et al., 2015).

Summary of the roles and mechanisms of Hedgehog pathway components studied in murine and human models over the last decades. Protein labels are in lower cases for murine and upper cases for human. Gene labels in italics respect the same rule. AEC: airway epithelial cells; ALI: air liquid interface; ASMC: Airway Smooth Muscle Cells; BALFs: broncho-alveolar liquid fluids; CCSP: club cell secretory protein; EGF: epithelial growth factor; HDM: house dust mite; MUC5AC: mucin 5AC

mutated fruit fly larva displayed a spiky exoskeleton, hence the “hedgehog” name. Three differentially expressed human HH ligands exist: SHH, IHH for Indian and DHH for Desert (Beachy, Hymowitz, Lazarus, Leahy, & Siebold, 2010). In the lungs, SHH is the most expressed.

Hedgehog (HH) remains unusual among cellular signaling cascades due to its series of inhibitory interactions (Fig. 1). The way signals are transmitted between the main actors remains unclear and is not entirely evolutionarily conserved between invertebrates and vertebrates (Beachy et al., 2010).

Glioma-associated oncogene homolog kruppel family members (GLI) are zinc finger proteins first identified as glioma oncogenes in the late 1980's (Kinzler et al., 1987). They act as bipotential and switchable transcriptional regulators inducing activation or repression of HH target genes.

The HH ligand's main receptor is a 12-pass transmembrane protein entitled Patched (PTCH1). PTCH1 inhibits specifically and not through direct contact the activity of a G-protein coupled receptor known as smoothened (SMO) (Briscoe & Théron, 2013; Calderon, 2000; Varjosalo & Taipale, 2008). In the inactive state of SMO, two other components, the protein kinase A (PKA) and the suppressor of fused (SUFU), both restrain GLI protein transcriptional activity by phosphorylation and direct association, respectively. Then GLI proteins undergo partial proteolysis into a C-terminally truncated repressor (GLI-R) translocating into the nucleus to repress downstream gene transcription.

SHH is an activator of HH signaling that can be inhibited by interaction with HHIP. HHIP was identified in a mouse cDNA expression library after screening for proteins able to interact with SHH (Chuang & McMahon, 1999). HHIP binds to SHH through multimodal mechanisms with either direct interaction on two specific sites or by a cluster of glycosaminoglycans (GAG) at the proteoglycan layer which in turn can inhibit SHH lipoprotein multimers (Griffiths et al., 2021). Indeed, heparan sulfate proteoglycans have been implicated in modulating HH activity in both vertebrates and invertebrates (Beachy et al., 2010). HHIP interacts with all three HH ligands (Bishop et al., 2009). In mice, the ectopic expression of *Shh* leads to ectopic *Hhip* expression, indicating that it is both induced by and serves as a negative regulator of HH signaling (Chuang & McMahon, 1999).

SHH undergoes multiple processing steps before its secretion from the producing cell via protein dispatched homolog (DISP1), exovesicles or multimeric protein complex. Its binding and inhibition of PTCH1 receptor releases PTCH1-mediated inhibition of SMO. Activated SMO transduces HH signal through a not entirely clear mechanism that involves kinesin family member 3A (KIF3A) and beta-arrestin, leading to primary cilium localization. The ciliary complex of SMO, SUFU, kinesin family member 7 (KIF7) and GLI proteins results in full length GLI processing in its active forms: GLI1, GLI2, or GLI3. Activated GLI can translocate to the nucleus to mediate HH signaling outputs. GLI1 acts exclusively as a transcription activator while GLI2 and 3 can act either as repressor or activator. HH signaling activation modulates multiple

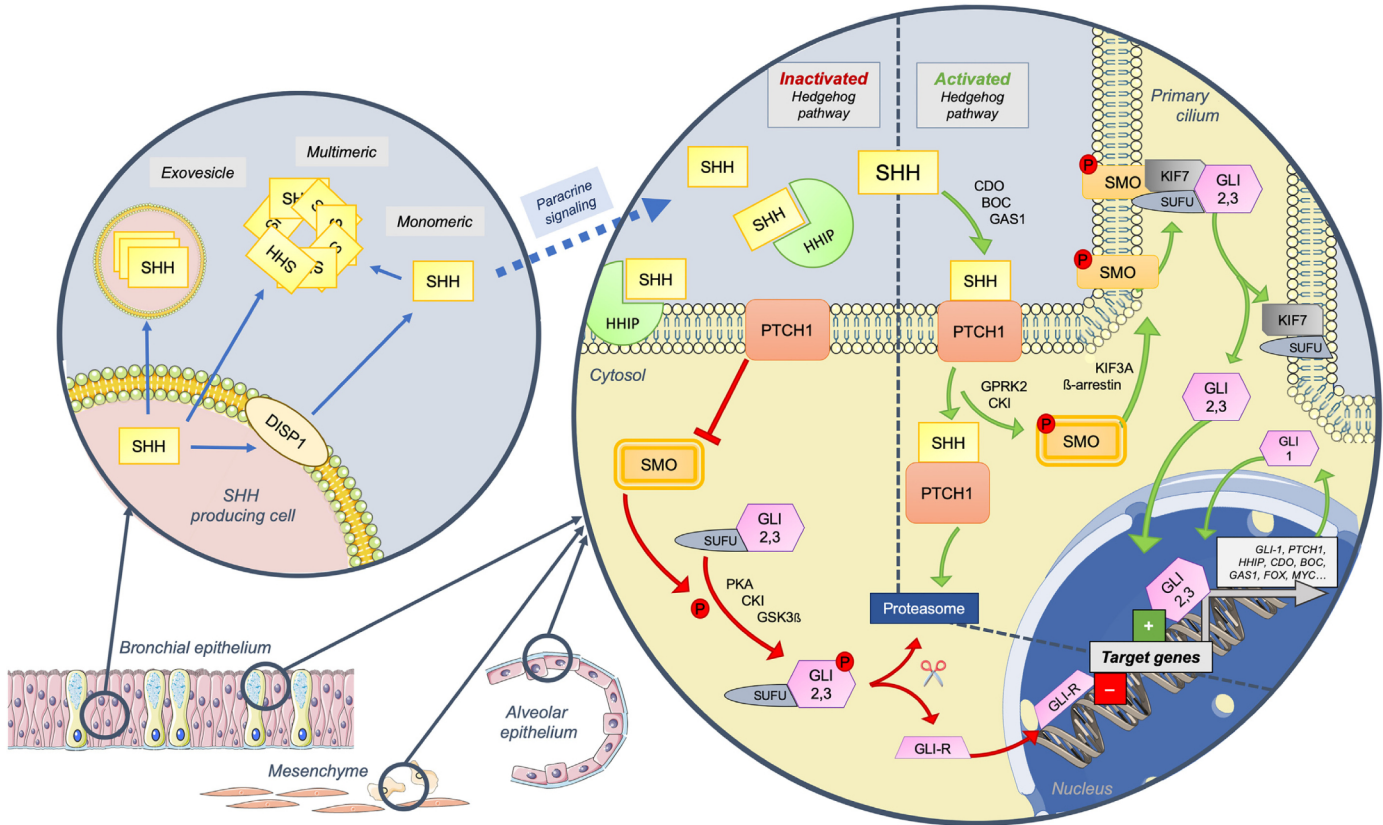


Fig. 1. Simplified depiction of Hedgehog canonical pathway in human airways.

BOC: Brother of CDO; CDO: Cell Adhesion Molecule Down-regulated by Oncogenes; CKI: Choline Kinase Alpha; DISP1: Dispatched RND Transporter Family Member 1; FOX: Forkhead box; GAS1: Growth Arrest-Specific Protein 1; GLI-R: repressor form of GLI; GLI: Glioma-Associated Oncogene Family Zinc Finger; GPRK2: G Protein-Coupled Receptor Kinase 2; GSK3 β : Glycogen Synthase Kinase 3 Beta; HH: Hedgehog; HHIP: Hedgehog Interacting Protein; KIF3A: Kinesin family member 3A; KIF7: Kinesin family member 7; MYC: MYC proto-oncogene; PKA: Protein Kinase A; PTCH1: Patched 1; SHH: Sonic Hedgehog; SMO: Smoothened; SUFU: Suppressor of Fused Homolog.

The SHH producing cell in the bronchial epithelium secretes HH ligand by different methods including a monomeric form requiring DISP1, a multimeric form and exovesicles. HH receiving cells in bronchial epithelium, alveolar epithelium and their surrounding mesenchyme are stimulated by SHH through paracrine signaling. In the inactivated HH state, SHH does not bind its membranous receptor PTCH1. Membranous or extracellular HHIP can link SHH to prevent its action on PTCH1. Inactivated PTCH1 sequesters SMO in intracellular vesicles. Sequestered SMO facilitates SUFU-mediated phosphorylation of GLI proteins (GLI2,3) which requires kinases (PKA, CKI and GSK3 β). The complex formed by SUFU and phosphorylated GLI proteins undergoes proteolytic cleavage into the repressor form of GLI (GLI-R), which dissociates and translocates into the nucleus to repress target gene expression. In the activated HH state, the binding of SHH to PTCH1 is facilitated by the cell-surface co-receptors CDO, BOC and GAS1. The cellular internalization of the SHH-PTCH1 complex releases SMO from its sequestration, which undergoes GPRK2 and CKI mediated phosphorylation. Phosphorylated SMO moves to the primary cilium membrane under the influence of KIF3A and β -arrestin. The accumulation of phosphorylated SMO in the primary cilium membrane allows KIF7 to recruit KIF7-SUFU complexes. GLI proteins experience conversion to activated forms that dissociate and translocate to the nucleus to activate target gene transcription (including CDO, BOC, and GAS1) and key proteins of the cellular cycle (such as FOX and MYC). They also provide feedback loops: positive with *GLI1* and negative with *PTCH1* and *HHIP*.

gene transcription including *FOX*, *MYC*, *CYCLIN E* and *CYCLIN D*, involved in cell proliferation and differentiation, survival, migration, epithelial-mesenchymal transition (EMT), and stem cell maintenance (Robbins, Fei, & Riobo, 2012). *HHIP* and *PTCH1* promoters are direct targets of GLI1 activation identified in genome-wide ChIP studies, suggesting that they play a role in upregulating repressive pathway members leading to a negative feedback loop that prevents HH activation (Lee et al., 2010; Vokes et al., 2007; Vokes, Ji, Wong, & McMahon, 2008). *CDO* (Cell Adhesion Molecule Down-regulated by Oncogenes), *BOC* (Brother of *CDO*), and *GAS1* (Growth Arrest Specific 1), represent three downstream GLI target genes encoding for their eponymous proteins, which may play a positive role in HH signaling (the first two through synergistic binding of SHH and the latter by interacting with HHIP (Akhshi, Shannon, & Trimble, 2022)).

Nevertheless, some studies suggest non-canonical pathway regulation as SHH-independent variations in HH transcriptional targets, such as *PTCH1* and *GLI1*, have been identified (Regan et al., 2017) (Fig. 2). Moreover, alternate HH signaling may exist since not every cell has primary cilia such as CD4 T cells for example (de la Roche et al., 2013). Recent studies reinforced the role of the primary cilia as the key element in the crossroad between canonical and non-canonical HH pathways. However, the emergence of examples of their crosstalk could change the paradigm of their division (Akhshi et al., 2022) and reveal more relevant targets for therapies, limiting side effects (see section 4. Targeting the HH pathway).

3.2. Roles in airway diseases

SHH signaling is expressed in the lung from embryo to adulthood and its role from airway development to COPD has been reviewed by Kugler (Kugler, Joyner, Loomis, & Munger, 2015). Here, we highlight how SHH signaling in COPD can reconcile the apparent dichotomy currently opposing the “small lung” theory with the “accelerated decline” theory. The main findings regarding HH component involvement in various COPD models are summarized in Table 2.

HHIP expression at the protein and mRNA levels is decreased in COPD compared with control lung tissues (Zhou et al., 2012). Interpretation of these findings is not obvious as it may emphasize a decrease in HH pathway activation since HHIP is a direct transcriptional target, but may also indicate an activation runaway due to its inhibiting role.

Nonetheless, SHH should not be seen as the only player in airway diseases either during lung growth or during adulthood relative lung quiescence. SHH interacts and cross-talks with many other imperative but not entirely understood pathways, such as Notch and Wnt (Zepp & Morrissey, 2019).

3.2.1. Developmental processes

In mice, Hhip-regulated dynamic interactions between Shh and fibroblast growth factor signaling controls early lung branching (Chuang, 2003). During normal airway development, neuroendocrine precursors respond to an adjacent epithelial Shh signaling (Watkins et al., 2003). Shh expressed in the distal airway epithelium interacts in a paracrine manner with Ptch1 in the juxtaposed mesenchyme. Complete knock-out of the HH components such as *Shh*, *Hhip*, *Ptch1*, *Gli2*, *Gli3* are not viable or lethal at birth (Kugler et al., 2015). The *Gli1*^{-/-} mice are viable unless one copy of *Gli2* is missing, supporting an overlapping function of both proteins in mice with partial compensation in lung development (Bai, Auerbach, Lee, Stephen, & Joyner, 2002; Park et al., 2000).

Homozygous invalidation of *Shh*^{-/-} murine lungs led to a primitive respiratory organ with few, large, poorly vascularized airways as a consequence of a significant defect in branching (Pepicelli, Lewis, & McMahon, 1998). *Shh*^{+/-} mice are viable with no reported abnormalities (Kugler et al., 2015). In contrast, *Shh*-overexpression induces mesenchymal cell proliferation resulting in hypoplastic lungs characterized by an overly abundant mesenchyme without any functional alveoli

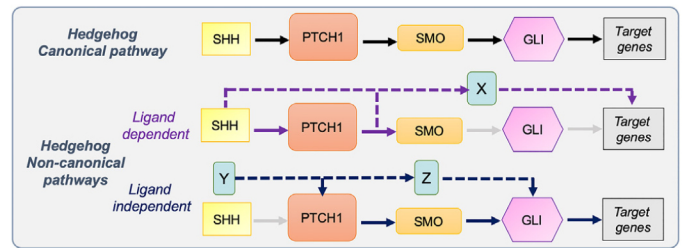


Fig. 2. Canonical and non-canonical Hedgehog pathways.

GLI: Glioma-Associated Oncogene Family Zinc Finger; HH: Hedgehog; PTCH1: Patched 1; SHH: Sonic Hedgehog; SMO: Smoothened.

Canonical HH pathway signaling involves SHH, which binds the cell membrane receptor PTCH1 to release SMO and activate GLI proteins acting as transcriptional factors. Non-canonical HH pathways have been identified and not fully explained. HH ligand dependent signaling could involve an alternate pathway from SHH (X) with or without PTCH1-SMO involvement to directly induce target gene transcription. A HH ligand independent pathway is an alternate ligand-mediated signaling (Y) which can involve either PTCH1-SMO or alternate mediators (Z) to activate GLI proteins.

(Bellusci et al., 1997). Alveologenesis is driven by mesenchymal remodeling processes (among multiple other mechanisms) compelled by secondary crest myofibroblasts (SCMF) with a Shh-mediated contraction that helps in shaping the alveolus (Zepp & Morrissey, 2019). Shh expression is high in the embryonic respiratory epithelium with a majority of epithelial cells expressing Shh after birth, whereas it is more heterogeneous in the alveolar compartment (Kugler et al., 2015). In humans, the expression of HH pathway components was recently documented as spanning between the mid-pseudoglandular to mid-canalicular stages of development (i.e. between 11.5 to 18.5 weeks of gestation): *SHH* is expressed in epithelial cells, whereas *PTCH1* and *HHIP* are widely expressed in mesenchymal cells, such as ASM cells (Belgacemi et al., 2022).

Mesenchyme regulation and its role in the development of COPD has been recently reviewed by Nasri et al. (Nasri et al., 2021). Fibroblast Growth Factor (FGF) proteins are implicated in a bidirectional epithelial-mesenchymal crosstalk during lung development (Shannon & Hyatt, 2004). SHH expressed in human distal airway epithelial cells acts as a dual player in its mesenchymal influence. SHH can either downregulate FGF10 via PTCH1 through HH canonical activation, or activate FGF10 through the Wnt pathway (Nasri et al., 2021). Supporting the “small lung” theory in COPD, several studies identified associations between disease susceptibility, *FGF10*, and developmental abnormalities. *FGF10* polymorphisms were significantly linked to various bronchial anatomic variations, narrower airway lumens and greater central airway bifurcation density assessed by CT in COPD lungs versus healthy controls (Smith et al., 2018).

3.2.2. Epithelial homeostasis and epithelial-mesenchymal crosstalk

Airway epithelium turnover is slow at steady-state with a constant SHH-mediated repression of peribronchiolar mesenchymal proliferation (Zepp & Morrissey, 2019). There is a continuous epithelial self-renewal of the basal cell population with a preferred differentiation towards the secretory phenotype over the Notch-signaling regulated ciliary phenotype (Zepp & Morrissey, 2019). ASM cells and SCMF represent both HH and WNT responsive cells as they are GLI-1 and Axin2 positive (axin like protein 2 positive, involved in WNT signaling) (Danopoulos, Bhattacharya, Mariani, & Al Alam, 2020). They restrict airway tube diameter, regulate extracellular matrix, and support homeostasis turnover. Additionally, they could modulate epithelial-mesenchymal transition (EMT) in smokers and COPD patients (Eapen et al., 2021).

The alveolar compartment contains AT2 cells which are considered as the stem cells responsible for repairing and maintaining alveolar tissue (Zepp & Morrissey, 2019). A subtype of alveolar cells, Axin2^{+/+}/FGF10^{+/-} identified in mice, seems to control the proliferation and differentiation of the alveolar niche (Taghizadeh et al., 2021). Recent

work showed that HH-responsive PDGFR α (+) cells (also referred to as SCMF) serve as a reservoir of differentiating AT1 and AT2 cells (Gao et al., 2022). By analysis of scRNAseq profiles of parenchymal tissue from explanted lungs, HHIP is mainly expressed in AT2 cells (Travaglini et al., 2019). Eighty percent of AT2 from advanced COPD-lung cells express HHIP (Sauler et al., 2022). Sauler identified a subpopulation of HHIP-expressing AT2 cells expressing abnormal cellular stress response genes as a reduction of the stress regulator *NUPR1* (nuclear protein 1).

In 2019, Wang reviewed the role of HH in adult lung maintenance and proposed a model of asymmetric HH airway activation (Wang, Cassandras, & Peng, 2019). A gradient of HH activation should progressively decrease from the distal airway to the alveoli. In the distal airways, SHH interacts with the proximal mesenchyme compartment through FGF10 and Wnt pathways, while HHIP coordinates the alveolar compartment through ASM and myofibroblast interactions. Thus, variation in HH pathway signaling could disrupt this physiological asymmetry and disrupt the compartmental identity and explain some of the structural alterations seen in homeostasis and injury response characteristic of airway diseases.

3.2.3. Injury response

Airway injury leads to recruitment of inflammatory cells, stimulates multiple cell types to regenerate and releases the mesenchymal compartment from its quiescence (Zepp & Morrissey, 2019). In turn, the activated mesenchyme increases epithelial cell proliferation. Among others, FGF10 derived from ASM and myofibroblasts stimulate secretory cell proliferation and differentiation.

Whether HH pathway set up during embryonic lung development is re-activated in response to injury is still debated. In mice, Watkins demonstrated its extensive activation within the airway epithelium during repair of acute airway injury in pulmonary neuroendocrine precursor cells (Watkins et al., 2003), whereas Peng showed that Hh signaling is rather downregulated and removed cell quiescence (Peng et al., 2015). In humans, C. Xu and colleagues hypothesized that an auto- or paracrine activation of SHH secreted by club cells was able to induce mucin 5AC (MUC5AC) as a marker of goblet cell metaplasia and mucous hypersecretion (Xu et al., 2018). The assumption was made after showing higher ELISA-measured SHH levels and a co-immunolocalization with CCSP in bronchoalveolar lavage fluids of children with asthma compared to controls. This was then confirmed in an ovalbumine-sensitized mice model of asthma. Nevertheless, it is rather unclear at this stage if mechanisms in asthma can be transposed to COPD, and murine small airway bronchial epithelium do not contain basal nor goblet cells (Travaglini et al., 2019).

CS exposition and COPD are characterized by basal (Crystal, 2014) and secretory cell hyperplasia (Innes et al., 2006). This is related to squamous epithelial metaplasia that contributes to airway wall thickening. It has been evoked that metaplasia could be attributed to epithelial-mesenchymal dysregulation after a stimulation by injury (Araya et al., 2007). Squamous metaplasia may also induce peribronchiolar fibrosis through increased IL-1 β secretion and TGF- β activation, which correlated with the severity of COPD.

To support the idea that a genetic polymorphism-related uncontrolled HH activation after an injury could contribute to COPD pathogenesis, teams developed murine models of *Hhip* haploinsufficiency. *Hhip*^{+/-} mice exposed to CS develop emphysema faster than those exposed to room air (Lao et al., 2015). In other models, *Hhip*^{+/-} mice develop spontaneous age-related emphysema (Lao et al., 2016) and form obvious lymphoid CD8 aggregates with age, even in the absence of CS exposure (Yun et al., 2017), suggesting a constitutional susceptibility toward exaggerated inflammation through upregulation of IL-18. Another COPD model of *Hhip*^{+/-} mice suggested an enhanced susceptibility to CS-induced chronic oxidative damage due to an alteration in macronutrient metabolism and a reduced antioxidant capacity (Wan et al., 2017). More recently, *Hhip*^{+/-} mice exposed to CS demonstrated an

increased α -SMA (smooth muscle alpha-actin) staining around their airways and extracellular matrix remodeling (Li et al., 2021), confirming similar observations achieved in COPD-derived ASM cell cultures. Accordingly, it can be concluded that HHIP seems to protect from airway remodeling by repressing ASM hyperplasia.

HHIP silencing by shRNA in human bronchial epithelium Beas-2B cells, led to the differential expression of nearly 296 genes involved in the extracellular matrix (Zhou et al., 2013). Seven of those genes had significant differential expression between COPD and control lung tissues. HHIP may therefore alter extracellular matrix and cell growth pathways contributing to the imbalance of proteinases and proteinase inhibitors as well as abnormal tissue repair, all these mechanisms being likely involved in the pathogenesis of COPD (Gill & Parks, 2008).

Considering its role in mesenchymal homeostasis, the HH pathway, and its key inhibitor HHIP, may therefore be involved in peribronchiolar fibrosis.

3.2.4. Tumorigenesis

In small cell lung cancer (SCLC), the malignant in vitro and in vivo phenotype is maintained by SHH-dependent HH activation (Watkins et al., 2003). Similarly, in non-small cell lung cancer (NSCLC), an inhibition of HH signaling via lentiviral-shRNA knockdown of GLI-2 suppresses tumor growth (Huang, Walter, Hayes, & Onaitis, 2014). Moreover, HH seems to reduce sensitivity to treatments in NSCLC through EMT induction. In a mesenchymal phenotypic cancer cell model, siRNA-mediated SHH blockade led to sensitization to erlotinib and cisplatin (Ahmad et al., 2013). Other HH partners such as CDO should be required in HH-mediated tumor survival since its inhibition by siRNA reduced proliferation and tumorigenicity in human NSCLC cells (Leem, Ha, Bae, Baek, & Kang, 2014). Another example is the overexpression of DISP1 in NSCLC, which is associated with poorer clinical outcomes (Rodriguez-Blanco et al., 2013).

The association between COPD and lung cancer has been debated for decades. With an incidence in COPD patients of 16,7/1000 lung cancer per year, several cohort studies have identified strong significant associations between these two entities (de Torres et al., 2011). Incidence increases with age, smoking (more than 30 pack-years), mild airflow obstruction (GOLD Stage 1 and 2) and lower body mass index. Nevertheless, COPD should be seen as an independent risk factor for lung cancer (Takiguchi, Sekine, Iwasawa, Kurimoto, & Tatsumi, 2014; Young et al., 2009). Emphysema was also reported as an independent risk factor irrespective of the presence of airflow obstruction (de Torres et al., 2011; Sanchez-Salcedo et al., 2015). Indeed, COPD and lung cancer share other mechanisms beyond tobacco exposure. Genetic susceptibility, epigenetic modifications in DNA methylation, local pulmonary inflammation and impaired lung repair represent common contributors to both diseases. As presented earlier, the HH pathway plays a role in all the latter mechanisms in the adult lung (Lemjabbar-Alaoui et al., 2006). The hypothesis of its common overactivation in both COPD and lung cancer is another argument supporting the use of same pharmacological modulators.

4. Targeting the Hedgehog pathway

4.1. Pharmacological inhibitors of the Hedgehog pathway

Most HH modulators were developed for treating advanced or metastatic cancers and are currently used in combination with other active molecules. Until now, three HH modulators are approved by both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Table 3). Multiple HH negative modulator mechanisms can be classified as: (i) extracellular blockade of the SHH ligand; (ii) inhibition of SMO at the cellular membrane, (iii) alteration in the intranuclear GLI action, and (iv) other undetermined or non-canonical mechanisms (Table 3).

Table 3
Modulators of the sonic hedgehog pathway.

Name	Properties	Target Conditions or Diseases	References and/or trials
(i) extracellular blockade of SHH ligands			
AB5E1	Binds SHH to prevent SHH-PTCH1 interaction	Human bronchial epithelial cell model	(Belgacemi et al., 2020)
Robotnikinin	Binds SHH-N terminal to prevent SHH-PTCH1 interaction	Human skin model* Human cell lines*	(Stanton et al., 2009; Stanton & Peng, 2010)
(ii) SMO inhibitors (SMOi)			
Cyclopamine	Natural steroidal alkaloid, direct inhibitor of SMO Many adverse effects Teratogenic Stem-like cancer cell depletion and tumor engraftment blockade	Melanoma* Pancreatic cancer* Prostatic cancer* Ovarian cancer* Glioblastoma* Murine lung cancer model (CyclinE transgenic mouse)*	(Feldmann et al., 2007; Kandala & Srivastava, 2012; Keeler, 1975; Shaw & Bushman, 2007; Stecca et al., 2007) (Galimberti et al., 2012)
GDC-0449	Direct inhibitor of SMO	Advanced or metastatic BCC\$ (phase 2, vismodegib 150mg)	NCT00833417 (Prasse et al., 2019)
Vismodegib	Acquired resistance through adaptive SMO mutations	IPF (phase 1 in combination with anti-fibrotic pirfenidone) Recurrent glioblastoma (phase 2)	NCT02648048 NCT00980343 (Yauch et al., 2009)
LDE225	Direct inhibitor of SMO	Locally advanced BCC\$ (phase 2, sonidegib 200mg)	(Kieran et al., 2017)
Sonidegib	Acquired resistance through adaptive SMO mutations	Refractory paediatric MB (phase 1/2, oral sonidegib 233-800 mg/m ²) SCLC (phase 1 in combination with cisplatin and etoposide)	NCT01125800 NCT01579929 (Danial, Sarin, Oro, & Chang, 2016)
BMS-833923	Direct inhibitor of SMO	SCLC (phase 1 in combination with carboplatin and etoposide)	NCT00927875 NCT00670189
XL-139	Direct inhibitor of SMO	BCC (phase 1) CML (phase 2 in combination with dasatinib)	NCT01218477
PF-5274857	Direct inhibitor of SMO	Human bronchial epithelial cell model (Beas-2B)*	(Zhou et al., 2016)
Env-101	Direct inhibitor of SMO	IPF (phase 2)	NCT04968574
Taladegib	Direct inhibitor of SMO	SCLC (phase 1b)	NCT01722292
LY2940680	Direct inhibitor of SMO	Advanced or metastatic BCC (Phase 1b)	(Bendell et al., 2018)
(iii) GLI's intranuclear function alteration			
GANT 61	Inhibitor of GLI1- and GLI2-mediated transcription by preventing their binding to DNA	Prostatic cancer cells* Breast cancer cells* T-cell lymphoma* Paediatric malignancies* IPF	(Arnhold, Boos, & Lanvers-Kaminsky, 2016; Benvenuto et al., 2016; Geng et al., 2017; Lauth, Bergström, Shimokawa, & Toftgård, 2007)
Glabrescione B (GlaB)	A naturally found isoflavone that binds to GLI1 in its zinc finger domain, to reduce DNA interaction	Cancer stem cells*	(Ingallina et al., 2017)
Arsenic trioxide (ATO)	Inhibition of GLI's transcriptional activity	Acute promyelocytic leukemia\$ (phase 3, in combination with ATRA)	(Owonikoko et al., 2016; Platzbecker et al., 2017), NCT01470248
AS203	Inhibition of GLI's transcriptional activity	SCLC (phase 2)	
ATRA	Attenuates Hh pathway components	Murine lung fibrosis model (bleomycin)* Murine emphysema model (elastase)*	(Eleraky, Helal, Elshafie, & Ismail, 2021; Uniyal, Tyagi, & Muyal, 2020)
ABBV-075	BRD4 inhibitor	Advanced solid tumors (breast, SCLC, NSCLC, prostate) with AML, NHL and MM (phase 1)	NCT02391480
Mivebresib	Interaction with GLI	Metastatic colorectal cancer (phase 1/2, combined to oxaliplatin-5-FU with or without bevacizumab)	(Piha-Paul et al., 2019) NCT01985763 NCT02567799
Genistein	Natural isoflavone	NSCLC (phase 1/2, combined to concurrent chemoradiotherapy)	NCT01036321
Prunetol	GLI inhibitor (unknown mechanism)	Prostate cancer (phase 2)	(Citrin et al., 2017; Pintova et al., 2019)
BIO 300	GLI inhibitor (unknown mechanism)	Cancer stem cells*	
(iv) other, undetermined or non-canonical mechanisms			
Itraconazole	Downregulator of AKT with HH pathway involved in itraconazole-mediated autophagy Strong inhibitor of CYP3A4	Esophageal cancer (phase 1) NSCLC (phase 1 neoadjuvant, completed)	NCT02749513 NCT02357836 (Deng et al., 2020; Zhang et al., 2021)
CX 4945	CK2 Inhibitor	Severe covid-19 (phase 2, oral silmitacertib 1g twice a day)	NCT04668209
Silmitacertib	CK2 Inhibitor	MB (phase 1/2, oral silmitacertib 200mg)	NCT03904862
Turmeric curcuma	Natural compound that inhibits the Shh-Gli1 signaling pathway. Triggers apoptosis	Metastatic colorectal cancer (phase 1, combined with irinotecan) NSCLC (phase 1, combined with gefitinib or erlotinib)	NCT01859858 (Doello et al., 2018) NCT02321293
HL2-m5	Macrocyclic peptide inhibitor of the Shh/Ptch1 interaction	Mouse fibroblast embryo cell line*	(Owens et al., 2017)
Vorinostat	HDAC inhibitor	NSCLC (phase 1, in combination with radiotherapy)	NCT00821951, (Chun et al., 2009)
Mocetinostat	HDAC inhibitor	NSCLC (phase 1, combined with pembrolizumab-guadecitabine)	NCT03220477
MGCD0103	Inhibition of GLI1-GLI2 deacetylation	Leiomyosarcoma (phase 2, combined with gemcitabine)	NCT02303262 (Coni et al., 2017)
MG-0103 (selective HDAC1/HDAC2 inhibitor)	Inhibition of GLI1-GLI2 deacetylation		

(continued on next page)

Table 3 (continued)

Name	Properties	Target Conditions or Diseases	References and/or trials
HHIP-AS1 (GeneSymbol: NR_037595)	Long non-coding RNA that has been shown as positively associated with HHIP mRNA stability. Increasing HHIP protein expression	Human bronchial cancer cell model (A549)* Human hepatocellular carcinoma cells*	(Bo et al., 2019; Zhou, Lai, Cao, Zhuo, & Tang, 2021b)

Therapeutic inactivation of HH signaling offers potential treatments for cancer, with various actions on different levels.

*: preclinical studies; \$: FDA- and EMA-approved drug; 5-FU: 5-fluorouracil; AML: acute myeloid leukemia; ATRA: all-trans retinoic acid; BCC: basal cell carcinoma; BRD4: bromodomain-containing protein 4; CML: chronic myeloid leukemia; HH: hedgehog; IPF: idiopathic pulmonary fibrosis; MB: medulloblastoma; MM: multiple myeloma; NHL: non-hodgkin lymphoma; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; SMO: Smoothened.

Genetic SHH pathway alterations in cancer consist mainly in loss-of-function of suppressors (PTCH1, SUFU) or gain-of-function in HH promoters (SMO, GLI). The first attempts to therapeutically target HH focused on SHH itself, followed by the SMO and GLI proteins (Carpenter & Ray, 2019).

Two HH activators exist as *in vitro* modulators but are not yet supported by clinical trials. They could potentially bring promising data on reparation after an acute injury. Purmorphamine acts *in vitro* as a neuroprotectant in acute experimental ischemic stroke, as demonstrated in various pathological states of brain and spinal cord wound (Liu et al., 2020). SAG (smoothened agonist) is also able to help regenerate tissues after injury (Dunaeva & Waltenberger, 2017).

In light of the SHH dysregulation demonstrated in COPD, targeting the HH pathway appears as an attractive strategy aiming at correcting the above mentioned aberrant cellular courses. HH modulators may affect peribronchiolar fibrosis (Zepp & Morrissey, 2019) and goblet cell hyperplasia (Xu et al., 2018). A recent work presented a systemic Smo inhibition that reduces T cell pulmonary infiltration and ameliorates Th2 inflammation in a murine allergic model (Yáñez et al., 2021). More than 130 clinical trials are currently referenced (active or completed) with HH inhibitors, mainly as adjuvant therapies in cancer, but also in Idiopathic Pulmonary Fibrosis (IPF). IPF is a progressive type of interstitial pneumonia with an unknown cause that is characterized by deposition of extracellular matrix and fibrotic tissues in the lungs, leading to destruction of the alveolar structures (Raghu et al., 2011). Epithelium damage causes the abnormal communication of the epithelial-mesenchymal cell, which inhibits epithelial healing, enhances mesenchymal cell proliferation and activation, and ultimately stimulates angiogenesis (Fernandez & Eickelberg, 2012). In IPF in human lungs, the increased expression of many developmental pathway genes is detected in microarray data, including PTCH1 (reviewed in (Froidure et al., 2020)). Two SMO inhibitors (SMOi), taladegib and vismodegib have been evaluated in IPF. A phase 1b open-label trial study evaluated vismodegib in combination with Pirfenidone and did not show any significant clinical benefit at 6 months on lung function parameters, with side effects (Prasse et al., 2019). In a mouse model, Vismodegib did not perform well in the bleomycin model of lung fibrosis (Moshai et al., 2014). Despite evidence of HH pathway dysregulation in COPD, and also in asthma (Zeng et al., 2022), there is no current clinical trial with HH inhibitors in these two airway disorders.

Evidence of the adverse effects of HH inhibitors reported with those currently available on the market in the treatment of cancer join those currently observed with other antitumoral therapies including QT elongation, tachycardia (serious and life-threatening adverse effect, only for ATO) and various effects in circulatory musculoskeletal or gastrointestinal systems. All drugs targeting the HH pathway are teratogen. Dyspnea, cough and sore throat have also been associated with ATO exposure (Carpenter & Ray, 2019). Further studies are needed to determine whether current cancer-fitted doses can be extrapolated to COPD.

4.2. HHIP: a potential therapeutic target in COPD?

The promotion of HHIP expression in COPD airways could restore a physiological and more subtle negative regulation of HH pathways.

Though various approaches can be postulated, no past or ongoing clinical trials exist in this domain.

4.2.1. Changes in epigenetic patterns of HHIP promoters

Drugs targeting epigenetic modifications seem promising for efficiently promoting HHIP expression in HH responsive cells. The demethylation of the *HHIP* promoter reduces proliferation and migration of tumoral cells (Zuo et al., 2018). 5-aza-2'-deoxycytidine is not specific to a single promoter, but a beneficial demethylation effect was observed in facilitating *HHIP* overexpression in a human gastric cell proliferation model, even if the precise mechanism remains to be understood. Histone Deacetylase inhibitors (HDACi) are able to suppress cell growth and are already used in cancer therapies. The combination of vorinostat and the SMOi SANT-1 may induce cooperative suppression of HH pathway activity, with an upregulation of *HHIP* expression attributed to vorinostat (Chun, Zhou, & Yee, 2009). HDACi can also downregulate the HH pathway through inhibition of GLI1 and GLI2 deacetylation. GLI acetylation is catalyzed by the histone acetyl-transferase p300 and reduced by HDAC1 and HDAC2. By preventing recruitment to target promoters, GLI acetylation inhibits its transcriptional activity (Coni et al., 2017).

4.2.2. Activation of HHIP co-regulators

Restoring positive *HHIP* regulators could also be considered. The lncRNA antisense *HHIP-AS1* seems to be an interesting candidate since it is transcribed in the opposite way but shares a common promoter with *HHIP*. In a hepatocarcinoma cell culture model, *HHIP-AS1* transcript stabilized *HHIP* mRNA by direct interaction, in order to suppress cell proliferation, migration and invasion, while promoting apoptosis (Bo et al., 2019). *HHIP-AS1* was also shown to inhibit proliferation, invasion and clonal formation in lung cancer cell cultures by regulating the microRNA (miR)-153-3p/PCDHGA9 axis (Zhou, Lai, Cao, Zhuo, & Tang, 2021a). Further studies are needed to understand the genomic and post-transcriptional positive regulation loop linking *HHIP* and *HHIP-AS1*, and figure out lncRNA stabilization and delivery, before being considered as a therapeutic tool.

Dozens of RNA-based therapeutics were FDA- or EMA-approved in 2021, focusing mainly on short oligonucleotides. Currently, no lncRNA-based approaches are clinically investigated in COPD or other diseases, but they are gaining more and more interest (Winkle, El-Daly, Fabbri, & Calin, 2021). The main challenging limitations consist in stabilization of the lncRNA and HH-responsive cell targeting. Chemical modifications are usually required to stabilize short oligonucleotides (leading to peptide nucleic acids, phosphorodiamidate morpholino oligomers, and locked nucleic acids). But in turn, they may increase cell-toxicity if transposed to lncRNAs.

4.2.3. Direct delivery of HHIP recombinant protein

An ongoing study provides promising preliminary results concerning HHIP's ability to improve the repair of a damaged respiratory model and in restoring lung function (poster by Hanna et al., posted in the 2020 American Thoracic Society Convention (Hanna et al., 2020) and personal discussion). *HHIP* overexpression induced by adenoviral transfection or exogenous supplementation of a recombinant HHIP

protein treatment in 3-dimensional human COPD stem cell derived air liquid interface (ALI) cultures improved mucociliary clearance by reducing mucin production. Moreover, intra-tracheal HHIP exposition in mice repaired CS-induced damage and prevented inflammatory immune recruitment. To be druggable, the HHIP recombinant protein formulation should be optimized to be highly airway specific and delivered to HH responsive cells. Exogenous intra-tracheal delivery would avoid enzymatic cleavages. Furthermore, cell surface GAG-mediated clustering represents an opportunity to facilitate robust delivery. HHIP presents an N-terminal GAG-binding site facilitating oligomerization to form clusters on the proteoglycan layer (Griffiths et al., 2021). This physiological process increases its avidity to inhibit SHH and might be used as a tool to reinforce its effect on HH responsive cells.

4.2.4. Challenges for drug targeting and delivery

Systemic delivery often induces off-target effects. Thus, nebulization (using inhaled dry powders for example) could be considered as a credible alternative to systemic treatments as they are already widely used for treating airway diseases (Vogelmeier et al., 2017). A submucosal diffusion of inhaled drugs would be an essential feature in COPD given the compartmental airway distribution of the HH pathway: the target to reach would be either the HH-responsive mesenchymal cells or the SHH-productive epithelial cells.

Huge efforts have already been made to optimize lung epithelial cell targeting with the development of exogenous vectors (liposomes, viruses, cell penetrating peptides), endogenous vectors (exosomes), and other combined nanoparticles. Some of these systems can be upgraded with surface modifications like aptamer to increase target-specificity (Xiao et al., 2022). Anti-EGFR aptamer can specifically target EGFR-expressing lung cancer cells (Li, Nguyen, Byrom, & Ellington, 2011). Nevertheless, as all cells harboring a primary cilium may act as HH-responsive cells, the targeting and delivery to lung tissues (from the airways to reach basal cells or stroma; from the blood to reach the stroma or even the airway epithelial cells) remain highly challenging in airway diseases such as COPD.

5. Conclusion

Canonical HH pathways involved in lung development and repair are deregulated in COPD, leading to aberrant airway regeneration. Genetic analyses support strong knowledge of HH pathway implication in COPD. HHIP is a key regulator during airway development, whose altered expression in human COPD lungs is based on limited data. The mapping of HH component expressions in epithelial and mesenchymal compartments is emerging, building up a very attractive potential for pharmacological modulation. Animal models provide evidence that the HHIP/SHH axis is a reachable target and may represent a unique opportunity to enhance airway repair in COPD. Consistent with the small lung theory in which the HH pathway seems deeply implicated, the growing awareness for early COPD could place emphasis on new therapeutic modalities and maybe even prevent this disease before its clinical manifestation.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

Z. Lahmar, E. Ahmed, A. Fort, I. Vachier, A. Bourdin, A. Bergougoux declare that there are no conflicts of interest.

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