Chaotic activation of developmental signalling pathways drives idiopathic pulmonary fibrosis

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ABSTRACT Idiopathic pulmonary fibrosis (IPF) is characterised by an important remodelling of lung parenchyma. Current evidence indicates that the disease is triggered by alveolar epithelium activation following chronic lung injury, resulting in alveolar epithelial type 2 cell hyperplasia and bronchiolisation of alveoli. Signals are then delivered to fibroblasts that undergo differentiation into myofibroblasts. These changes in lung architecture require the activation of developmental pathways that are important regulators of cell transformation, growth and migration. Among others, aberrant expression of profibrotic Wnt-β-catenin, transforming growth factor-β and Sonic hedgehog pathways in IPF fibroblasts has been assessed. In the present review, we will discuss the transcriptional integration of these different pathways during IPF as compared with lung early ontogeny. We will challenge the hypothesis that aberrant transcriptional integration of these pathways might be under the control of a chaotic dynamic, meaning that a small change in baseline conditions could be sufficient to trigger fibrosis rather than repair in a chronically injured lung. Finally, we will discuss some potential opportunities for treatment, either by suppressing deleterious mechanisms or by enhancing the expression of pathways involved in lung repair. Whether developmental mechanisms are involved in repair processes induced by stem cell therapy will also be discussed.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic respiratory disease characterised by a progressive destruction of lung parenchyma along with its replacement by a fibrotic scar tissue [1]. The disease usually affects elderly patients, and former or current smokers represent up to 85% of patients [2], making tobacco the main environmental risk factor associated with IPF. Genetic susceptibility is central to IPF pathophysiology, ranging from common polymorphisms to rare familial forms related to telomerase complex or surfactant protein gene mutations [3, 4].

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IPF histological hallmarks include alveolar remodelling with alveolar epithelial type 2 cell (AE2C) hyperplasia, bronchiolisation of the alveolar epithelium and formation of fibroblast foci (figure 1a). Although the precise cause of IPF is unknown, the disease is now considered to be epithelium-driven. In a predisposed aged lung, repeated injury (*i.e.* smoking, gastro-oesophageal reflux or chronic viral infection) leads to injury of type 2 pneumocytes with activation of endoplasmic reticulum stress and unfolded protein cellular response, and early apoptosis, followed by reactive AE2C hyperplasia and hypertrophy as a repair response [6]. Activated epithelial cells secrete cytokines and growth factors which promote the recruitment of inflammatory and immune cells, and the accumulation of fibroblasts with a profibrotic phenotype [7]. Accumulation of macrophages with a profibrotic phenotype contribute to the vicious circle, leading to progressive irreversible lung tissue destruction and subsequent impairment of lung function, and ultimately leading to death, with a median survival of 3–5 years [8]. Similarly to cancer, there is a body of evidence suggesting an aberrant re-activation of embryonic and developmental pathways in IPF that participate in remodelling and fibrosis [9], along with factors involved in epithelial-mesenchymal crosstalk [10].

Apparent disorder is also a characteristic of IPF, illustrated by the heterogeneity of lung parenchyma remodelling (figure 1a) and the unpredictable progression of the disease in patients (figure 1b). Those features could be just the consequence of stochastic events and disorganisation. However, several transcriptomic analyses showed that IPF could be classified and separated from other interstitial lung fibrotic diseases, suggesting that underlying patterns and laws do drive this disease [11]. In the present article, we will examine whether an aberrant transcriptional integration of those pathways may be involved

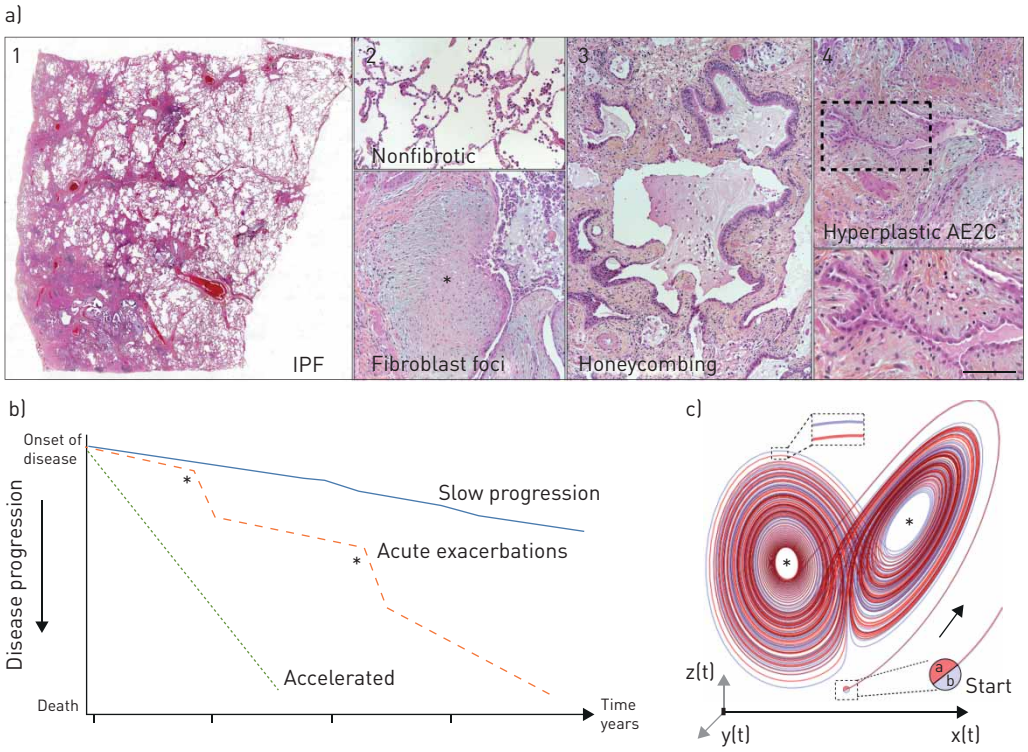

FIGURE 1 Chaos features in idiopathic pulmonary fibrosis (IPF). a) Haematoxylin eosin staining of lung paraffin sections showing: 1) the heterogeneity of parenchyma remodelling in IPF at low magnification; 2) sections from normal looking/nonfibrotic territories (upper image) and fibroblast foci (*, lower image); 3) honeycombing; and 4) hyperplastic alveolar epithelial type 2 cell (AE2C) (lower image is a magnification of the dashed box). Scale bar: 1) 10 µm; 2–4) 40 µm; lower image: 80 µm. Image in 1) courtesy of C. Danel (Bichat Hospital, APHP, Paris, France). b) Representation of the diverse clinical courses of IPF. Disease progression leading to patient's death may be rapid (green dashed line), slow (blue line) or mixed (orange dashed line) with acute exacerbations associated with acute decline (*). Adapted from [5]. c) Chaotic behaviour of the Lorenz model showing the trajectory of two orbits (red and blue lines) in phase space (axe x(t),y(t),z(t)) from two close initial positions (a and b) (see high magnification of starting points (lower dashed box)). This system is defined by three nonlinear, ordinary differential equations [25]. Note that the red and blue orbits are aperiodic (the trajectories never repeat) and get separated in the long term as shown in the upper dashed box (even though the two initial conditions were almost impossible to distinguish in the lower dashed box, showing sensitive dependency on initial conditions of the system) but they still evolve to the system attractors (*), meaning that these orbits are bounded. This schematic was generated with an R script.

in IPF through a chaotic pattern by comparison to normal early lung ontogeny. Finally, we will discuss whether some targets constitute potential opportunities for treatment, either by suppressing deleterious mechanisms or, alternatively, by enhancing the expression of pathways involved in lung repair; for instance, with the use of stem cell therapy.

Is IPF underlined by a chaotic re-activation of developmental pathways?

As reviewed recently [12, 13], the same developmental programmes are involved in both lung morphogenesis and fibrogenesis but lead to completely different outcomes. This different result (organogenesis *versus* fibrosis) might be underpinned by basal conditions.

The re-activation of developmental pathways in lung fibrosis was investigated using traditional but still powerful and highly relevant reductionist approaches by studying the small parts (*e.g.* fibroblasts, AE2C, *etc.*) to fully understand it as a whole (*e.g.* fibrotic lung). In such a reductionist approach, a biological system would also be considered as the sum of its different component properties and ruled by linear dynamics (meaning that a change in one of the baseline variables would trigger a proportionate response over time [14].

However, to better understand and reconcile this conundrum between the quite different effects of developmental pathways during development compared with IPF, a holistic approach should be also considered as previously advocated by others [15, 16]. In this case, the properties of such an integrative biological system would be more than the sum of its parts [14]. The rise of the system theory in biology was also intimately intricate with the emergence of nonlinear dynamics [17].

Indeed, biological systems are now theorised and described as opened and dissipative (meaning they perform exchanges with their environment) as well as driven by nonlinear dynamics (*i.e.* no proportional relationship between the input and the outcome) [18, 19]. Among those nonlinear dynamic systems, the behaviour of chaotic ones has been of interest in biology and biomedicine for several decades [18, 20–22] because of their high sensitivity to the initial conditions.

Even though the term "chaos" was first used in 1975 by Li and Yorke [23] to describe irregular behaviours in nonlinear mathematical systems, the idea of sensitivity to initial conditions in those dynamic systems was first unveiled by Poincaré at the beginning of 20th century: "a very small cause, which eludes us, determines a considerable effect that we cannot fail to see, and so we say that this effect is due to chance. If we knew exactly the laws of nature and the state of the universe at the initial moment, we could accurately predict the state of the same universe at a subsequent moment." [24].

In the 1960s and 1970s, the "butterfly effect" in meteorology, coined by Lorenz [25] widely popularised the notion of deterministic chaos with his famous seminar. Lorenz [25] observed in a numerical computer weather model that an infinitesimal change in the initial condition would lead to large differences at later state [24]. In the modern notion of chaos, the apparent erratic behaviour of a system is driven by underlying patterns and laws. As mentioned before, a slight change in the initial points will be amplified over time in chaotic systems and will produce a completely different outcome [19, 20], which may appear random at first glance. It is not possible to predict the long-term behaviour of a chaotic system, even though deterministic laws rule it (in which a given initial state will produce the same output). Interestingly, it has been shown that a chaotic behaviour can be easily achieved in "simple" systems [19, 20] as stated by May [26] in his seminal paper. For more about the history of the chaotic dynamic refer to [24].

A second key concept in chaotic systems is the existence of attractors as a consequence of its underlying deterministic nature. In other words, those different patterns generated by infinitesimal changes in the initial conditions will be preferentially attracted to specific regions called chaotic or strange attractors (figure 1c). These attractors will define the boundary of a given chaotic dynamic system. Thus, deterministic chaos is often quoted as a "form of order disguised as disorder" [18]. In addition, this schematic gives us the opportunity to summarise the four main characteristics of a system undergoing chaotic dynamic. As mentioned before, the evolution of such system is: 1) given by a deterministic function (three nonlinear, ordinary differential equations in this case); and 2) it has a "sensitive dependency on initial conditions" as illustrated by the divergent orbits of the two very close initial conditions shown in figure 1c, as the systems evolve. As shown in figure 1c, the orbit of a chaotic dynamic system also: 3) never repeats (aperiodic); and is 4) bounded, meaning its orbit evolves around attractor regions [27].

Therefore, it could be appealing to revaluate the aberrant re-activation of developmental signalling pathways as well as IPF pathophysiology through the lens of deterministic chaos dynamics, already applied in translational research from enzymatic reactions [28] to cancer biology [20], as well as epidemiology [29]. In addition, oscillatory pathways displaying chaotic dynamics under certain circumstances have been already described in nuclear factor-κB signalling upon tumour necrosis factor-α stimulation [30]. We propose that small changes in initial conditions in which developmental pathways are reactivated in IPF would be

sufficient to trigger a long-term chaotic response. Ageing linked with senescence and epigenetic drifting could trigger such initial changes by influencing the dynamic of the signalling networks activated in cells exposed to chronic injuries and inflammation for years in IPF [13, 31]. Genetic mutations and polymorphisms associated with either epithelial senescence [5, 32] or stresses [33, 34] would achieve similar effects. Ageing was also recently associated with a heterogeneity in old fibroblasts to reprogramme induced pluripotent stem cells and to promote wound healing in mice compared with young fibroblasts, in an apparent stochastic way [35]. At the tissue scale, local mechanical constraints such as the recurrent traction peripheral lung injuries in IPF on a given set of progenitors could also influence those initial conditions (figure 2). A potential influence of the altered IPF lung microbiome [36] cannot be excluded as well.

Thus, small changes in the dynamic of developmental signalling networks in chronically injured lungs may have major impacts on the different lung cell populations leading to the generation of diverse and even atypical phenotypes. Indeed, single-cell transcriptomic methods applied to the fibrotic lung identified an alveolar epithelial cell population with mixed differentiation state [11, 37].

As stated at the beginning of this section, the activation of the same signalling pathways in lung development and fibrogenesis [12, 13] leads to quite different outcomes. The key difference between the physiologic state (*i.e.* ontogeny) and pathologic one (*i.e.* IPF) may lie in the transcriptional integration dynamic of those signalling pathways as we will discuss in the next sections.

Overview of reactivated embryonic pathways in IPF

Key embryonic signalling pathways and networks, namely Wingless Integration (Wnt), Sonic hedgehog (SHH), transforming growth factor (TGF)-β and fibroblast growth factor (FGF) are reactivated in IPF [12]. Those signalling networks allow cells to transduce and integrate internal and external clues to transcription factors, which then execute downstream target programmes by modulating gene expression. For example, the activation of transcriptional integrators such as β-Catenin, Gli, Smad2/3 and EtvTV transcription factors are respectively involved downstream of Wnt, SHH, TGF-β and FGF pathways during

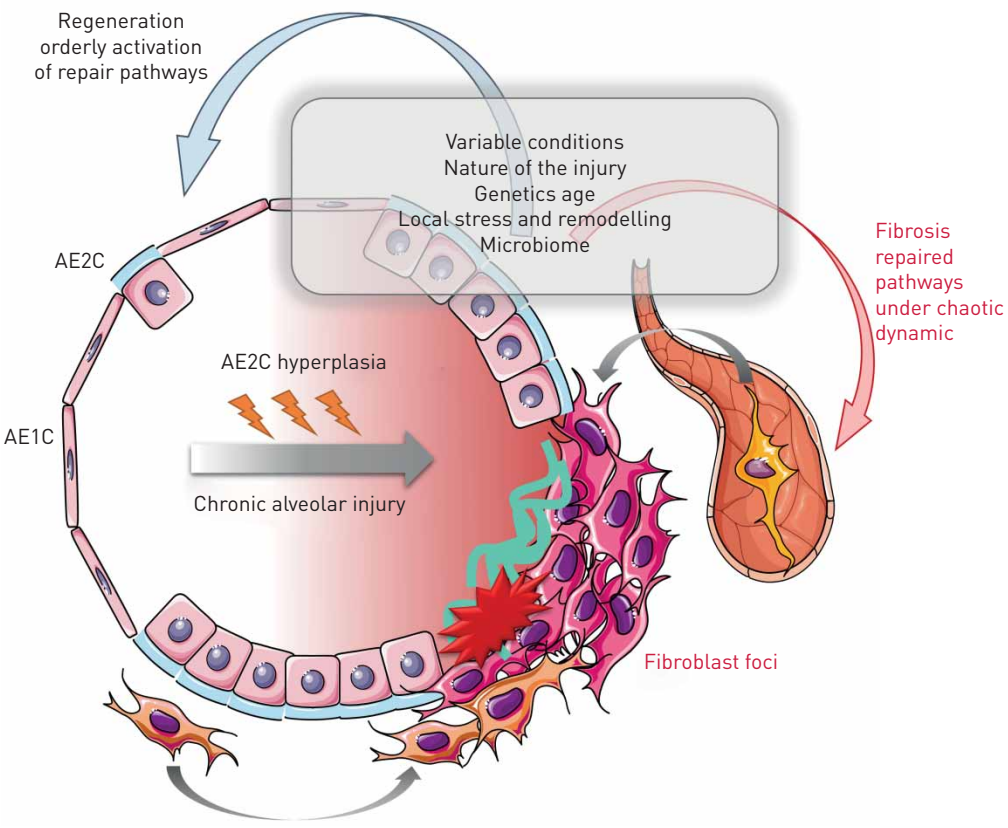

FIGURE 2 Synthetic view on the balance between normal alveolar repair/regeneration and deleterious overactivation of repair pathways, ultimately leading to fibrosis. Fate of chronically injured alveoli will depend on baseline conditions including nature of the injury, genetic background but also local conditions (microbiome, remodelling). AE1C: alveolar epithelial type 1 cell; AE2C: alveolar epithelial type 2 cell.

lung development and fibrogenesis [38]. Additional developmental transcription factors have been also implicated in IPF pathogenesis over the past decades, such as members of the Forkhead family (FOXF1 [39], FOXO3 [40]) and the T-box (TBX) transcription factor (TBX4) [41, 42]. Epithelial to mesenchyme transition (EMT) plays a crucial role in embryogenesis during gastrulation and neural crest cell migration [43]. EMT is regulated by a set of transcription factors that are also reactivated in IPF (TWIST-1, SNAI1 and FOXM1) [44, 45]. Lung parenchyma remodelling and stiffening is a salient feature of IPF. Such microenvironmental changes trigger mechanotransduction signals that are integrated through key transcriptional co-activator such as Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding protein (TAZ). Similarly to lung ontogeny, both mechanotransduction and hippo pathway activation may also account to YAP/TAZ transcriptional upregulation in IPF [46]. Thus, a plethora of secreted factors, aberrantly triggered in IPF, and next integrated at the cellular levels by a multitude of transcription factors could give rise to new and potentially deleterious downstream regulatory networks as compared with lung development. This will raise questions regarding the factors driving the transcriptional integration of these pathways in IPF. When applying chaos dynamic principles to IPF, we could hypothesise that a minimal change in the activation of repair and developmental pathway would trigger a fibrotic cascade, ultimately leading to lung fibrosis.

Activation of developmental signalling pathways and transcription factors in IPF: comparison with embryonic life

There is a striking histological resemblance of some remodelled lung parenchyma territories in IPF with embryonic lung during the pseudoglandular stage (figure 3). IPF may be the consequence of a chronic recapitulation of developmental programme, with a deregulated re-activation of developmental pathways. As opposed to fetal lung development, where these developmental pathways are spatially and temporally integrated, IPF lung is characterised by a disorderly sequence of pathways activation. We will discuss and compare the interplay between the FGF and SHH pathways; two key developmental pathways/modules during lung branching morphogenesis and during IPF. In this model, we will also introduce a third player, the TGF-β pathway, which is a well-characterised and important pathway known to be involved in fibrogenesis. For the sake of the demonstration and of the discussion, this simplistic model will exclude other critical developmental pathways during pseudoglandular lung development such as the Wnt, Notch and YAP pathways, which have been involved in IPF pathophysiology. The interplay of the later signalling pathways during either lung development or fibrogenesis have been already reviewed [47–49].

Overview of bud outgrowth during the pseudoglandular stage

Lung bud growth is tightly controlled, in part by the interplay between the FGF and SHH pathways during the pseudoglandular stage (figure 3a). During mouse lung development, FGF10 secreted by the distal mesenchyme will stimulate epithelial budding and SHH epithelial expression through the action of the ETV4/5 transcription factors [50]. In a feedback loop, SHH will then inhibit mesenchymal *Fgf10* expression. Meanwhile, the activation of the hedgehog/GLI canonical pathway is also required for the proliferation and survival of the embryonic lung mesenchyme [51, 52]. Another FGF, FGF9, is also required for proper early lung development [53]. *Fgf9*, expressed by both the epithelium and mesothelium, is thought to stimulate mesenchyme proliferation through a feedback loop involving the Wnt/β-catenin pathway. Both sources of *Fgf9* also promote epithelial budding. Epithelial FGF9 directly stimulates branching morphogenesis in an autocrine way, while mesothelial FGF9 would enhance the process by increasing mesenchymal *Fgf10* expression levels [54, 55]. Positive transcriptional regulators such as *Tbx4* and *Foxf1* are also involved in maintaining *Fgf10* mesenchymal expression during early fetal lung development [56–58]. In this dynamic system, mesenchyme-specific deletion of TGF-β receptor II (TβRII) showed that the activation of TGF-β receptors dampens both hedgehog/GLI and *Fgf10* expression in mesenchymal cells [59]. Whether the canonical SMAD pathway downstream of TβRII mediates those phenomena is not known. Thus, the TGF-β pathway would rather negatively regulate mesenchymal proliferation and survival as well as epithelial branching during pseudoglandular lung development [59].

Aberrant activation of developmental networks in IPF?

In IPF, the re-activation of the FGFs, SHH and, of course, TGF-β pathways has been reported and demonstrated by several groups [60–63]. Some regulatory networks seem to be conserved between the embryonic stage and IPF (figure 3b). For instance, FGF9 from both mesothelium (reactivated in IPF pleura) [64, 65], and epithelium would rather promote the proliferation/survival of mesenchymal cells in IPF [64] with detrimental long-term effects since those cells are the main fibrosis effectors. Since SHH inhibits *Fgf10* in the distal lung mesenchyme during pseudoglandular development [66], the hedgehog pathway upregulation in IPF could also explain, in part, the downregulation of FGF10 expression in IPF [62]. Thus SHH/FGF10 balance is impaired in IPF; whether a dysregulation of ETV transcription factors in IPF may be involved in this imbalance is not known. In addition, the increase in epithelial SHH

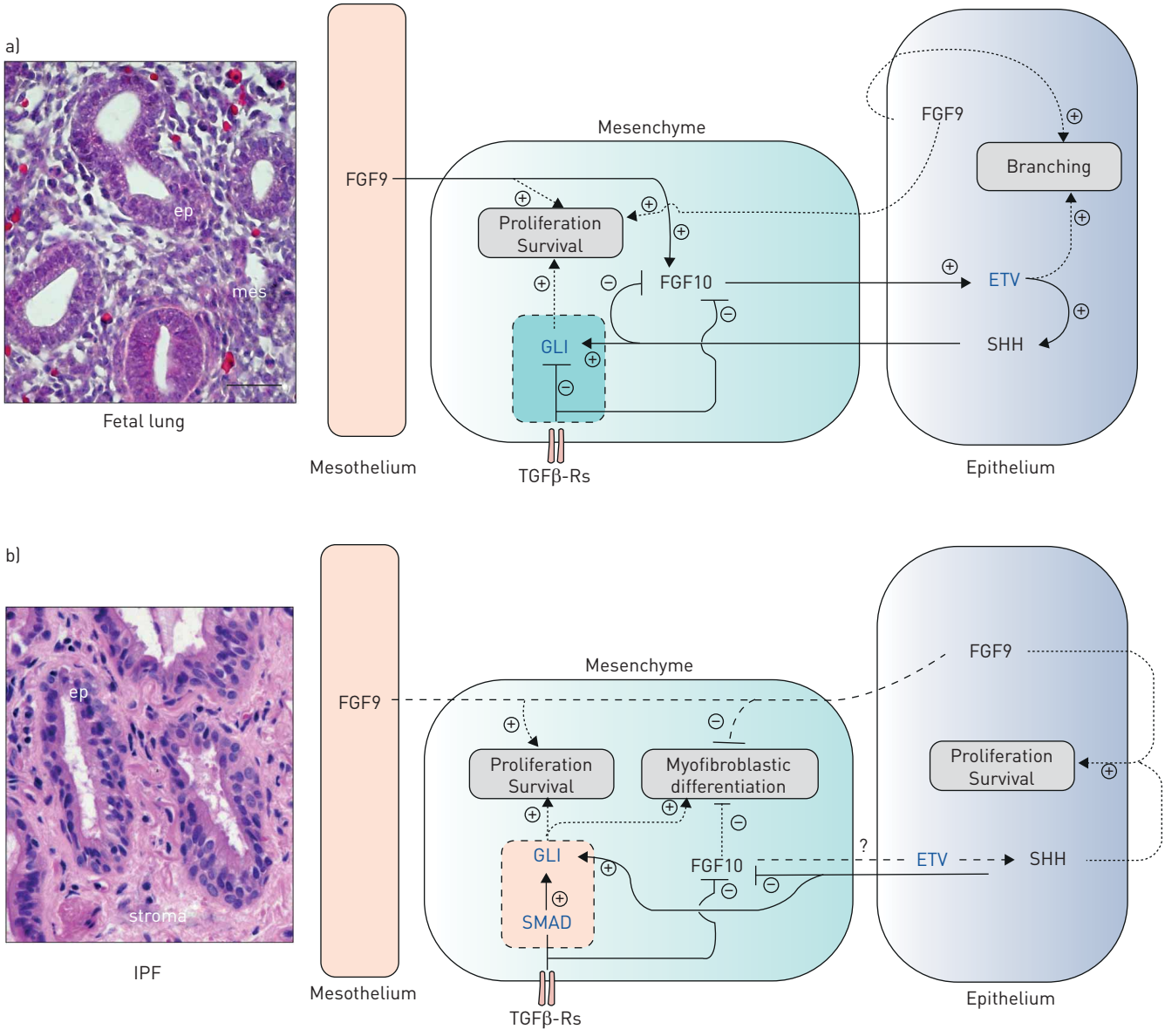

FIGURE 3 Model of the interplay between the fibroblast growth factor (FGF), SHH and transforming growth factor (TGF)-β pathway during embryonic lung branching morphogenesis and fibrogenesis in patients with idiopathic pulmonary fibrosis (IPF). a) Model of the interactions between the mesothelial, mesenchymal and epithelial cells during lung pseudoglandular stage. The arrow shows the interactions between the different genes of interest. Transcription factors are in blue. The dashed arrows depict the biological effects of these genes of interest. Note the negative regulation of GLI by TGF-β receptors in the light green box. See the main text for further details. Haematoxylin eosin staining of a mouse embryonic mouse lung at E14.5 during the pseudoglandular stage is shown. Scale bar=80 µm. b) Model of the interactions between the mesothelial, mesenchymal and epithelial cells during lung fibrogenesis in adult. Note the positive regulation of GLI by TGF-β receptors in the light orange box. See main text for further details. Haematoxylin eosin staining of an epithelial metaplasia region in a human lung of a patient with IPF is shown. ep: epithelium; mes: mesenchyme.

secretion concomitantly with FGF9 may also enhance epithelial proliferation in an autocrine/paracrine fashion, which may account for the epithelial hyperplasia observed in IPF [67].

Furthermore, fibrosis is characterised by a huge activation of the TGF-β pathway [63]. Similarly to fetal lung, TGF-β1 still downregulates FGF10 expression in adult lung mesenchymal cells [38]. In contrast to fetal lung, the canonical TGF-β/SMAD pathway strongly upregulates the hedgehog/GLI pathway in mesenchymal cells promoting their survival and myofibroblastic differentiation as well [61, 68]. This synergic profibrotic effect of the SHH and TGF-β pathways is probably inefficiently counteracted by FGF9 secreted by both mesothelium and remodelled epithelia in IPF. Indeed, FGF9 partially reverted the effects of TGF-β in both control and IPF primary lung fibroblasts [64].

With regards to those three signalling pathways, IPF would be characterised by an upregulation of profibrotic and survival factors such as SHH and TGF-β1 concomitantly with an inefficient secretion or effect of epithelial protective and antifibrotic factors such as FGF10 or FGF9 [38, 63, 64, 69].

Signal integration through a chaotic dynamic in IPF?

The fetal and fibrotic lungs are dynamic systems undergoing morphogenetic or pathological remodelling, respectively. The stereotypical pattern and periodicity clock of branching morphogenesis strongly suggest that oscillating networks (oscillators) involving those developmental pathways as well as their downstream transcriptional integrations drive this process. One could assume that these oscillators and routines are probably also reactivated in IPF but in an aberrant way. The dynamic of a transcriptional response to a given stimulus can be influenced by several factors such as concentration, nuclear translocation of the transcription factor itself, as well as specific co-factors. Thus, two main and prevalent transcriptional dynamics have been described: sustained and pulsatile signals that elicit differential cellular responses by a given transcription factors in a context or cell-dependent manner [70, 71]. For example, a sustained extracellular signal-regulated kinase (ERK) signal dynamic in response to epidermal growth factor will promote cell proliferation, while a pulsatile ERK signal upon nerve growth factor stimulation will elicit cell differentiation in the same cell type [72, 73]. Transcriptional network regulation and integration are probably different in IPF compared with fetal lung. For instance, the switch from a negative to positive effects of TGF-β1/ALK5 upon SHH/GLI pathway in adult mesenchymal cells compared with fetal mesenchymal cells illustrates such transcriptional rewiring in IPF. Furthermore, it has been shown that developmental pathway such as Wnt/β-CAT, SHH/GLI or FGFs are usually reactivated broadly in all the IPF lung regions including territories of normal aspects [64, 68, 74]. This may suggest that local perturbations could influence the transcriptional dynamic of those pathways and the outcome in term of cellular phenotype in IPF.

The concept of a chaotic dynamic in IPF could also be supported by the recent discovery of an abnormal transcriptional feedback loop that occur within fibrotic lung. Yeo *et al.* [75] recently demonstrated that TWIST1 directly increased paired-related homeobox (PRRX)-1 transcription factor which subsequently induced Tenascin-C that itself stimulated TWIST1 activity, constituting a positive feedback loop that continuously activates fibroblasts.

Altogether, we propose that the heterogeneity of IPF cellular ecosystem could be the consequence of a chaotic transcriptional integration of those developmental pathways. Those different cell phenotypes/responses could represent the different strange attractors generated by the chaotic signalling and downstream transcriptional dynamics in IPF.

Key genetic variations (*i.e.* the activating variant of the MUC5B promoter gene) favour cell differentiation towards fibrosis and thus enhance the effect of those "attractors". This is illustrated by the recent discovery of Gli^{+} perivascular progenitors that resemble mesenchymal stem cells. Their homeostatic function is elusive, but in disease, these cells contribute to early fibrosis following organ injury. Conversely, their depletion prevented *in vivo* fibrotic remodelling in kidney, heart and lungs [76]. One could thus speculate that in predisposed individuals, early activation of these cells would underlie lung fibrosis. Interestingly, the heterogeneity of old fibroblasts to reprogramme induced pluripotent stem cells and to support wound healing was linked to the expression level of the transcription factor EBF2 [35]. The potential involvement of the EBF2 transcription factor in the abnormal transcriptional network in IPF remains to be determined.

Developmental pathways and transcription factors in IPF therapy

In the following section, we will review recent advances in the field of emerging IPF therapy. By modulating key developmental pathways, future treatments might reverse their apparent chaotic activation.

Targeting deleterious developmental pathways

To date, attempts to treat lung fibrosis through the Wnt-β-catenin pathway have been limited to *in vitro* and *in vivo* studies (table 1). Konigshoff *et al.* [77] demonstrated that preventive administration of a monoclonal antibody directed against WISP1 could hamper fibrosis development in bleomycin mice. Using similar experimental settings, Kim *et al.* [78] administered siRNA directed against β-catenin. This resulted, as expected, in a lower expression of β-catenin in the lungs but also in lower collagen deposition, type 2 matrix-metalloprotease (MMP2) and TGF-β1. Henderson *et al.* [79] demonstrated that the small molecule ICG-001 was able to prevent and partially reverse bleomycin-induced fibrosis. The drug targets cyclic AMP response-element binding protein (CBP)-dependent transcription. Wnt inhibition by the pharmacological agent XAV939, administered intraperitoneally, results in dampened lung fibrosis in bleomycin mice through TGF-β1 and FGF-2 inhibition [84].

TABLE 1 Targeting developmental pathways in idiopathic pulmonary fibrosis

Pathway	Drug/molecule	Target	Effect	[Reference]
Wnt-β-catenin	Anti-WISP1 antibody	WISP1	Decreases fibrosis in bleomycin-induced fibrosis in mice	[76]
	siRNA against β-catenin	β-catenin		[77]
	ICG-001	CBP		[78]
Sonic Hedgehog	GDC-0449 (Vismodegib)	Smoothened		[79]
	Cyclopamine	Smoothened		[80]
	GANT-61	GLI		[79]
	Pirfenidone	GLI-2	Slow lung function decline, improves survival	[6, 81]
FOXO3	UCN-01	Protein kinase C	Improves lung function, survival and decreases fibrosis in bleomycin-induced fibrosis in mice	[39]
YAP/TAZ	Verteporfin	TEAD/YAP interaction	Decreases fibrosis in mouse model of kidney fibrosis	[45, 82]
	Fasudil/KD205	ROCK	Decreases fibrosis in bleomycin-induced fibrosis in mice	[83]

siRNA: small interfering RNA; CBP: cyclic AMP response-element binding protein; YAP: Yes-associated protein.

With respect to the hedgehog/GLI pathway, it has been shown that GLI inhibition is more potent than Smoothened (SMO) inhibition to decrease myofibroblastic differentiation of IPF primary lung fibroblast *in vitro* and lung fibrosis *in vivo* in the bleomycin mouse model [68, 80]. Similarly, a SHH blocking antibody did not protect mice from lung fibrosis in this model [60]. Thus, GLI transcription factors seem to be better potential therapeutic targets in IPF than SMO. Unfortunately, GLI specific inhibitors have been in the pre-clinical stage of development for the past decade. Pirfenidone, a pyridine molecule, was approved in 2012 for the treatment of IPF [85]. Although the molecular target of pirfenidone is unknown, several mechanisms of action have been unveiled. *In vitro*, the drug interferes with fibroblast development, differentiation and survival [86]. *In vivo*, it dampens experimental fibrosis in rodents [87]. A transcriptomic approach demonstrated that the drug exerted its effect through interaction with several pathways and cell types [88]. Didiasova *et al.* [82] recently demonstrated that pirfenidone, although at a high concentration, interacts with the activator of hedgehog-driven gene transcription GLI2. The authors showed an overall decrease of hedgehog target genes including GLI1, hedgehog receptor Patched-1, a-smooth muscle actin and fibronectin. Those features were linked to reduced cell migration and proliferation. It would be important to confirm these results at a dose range closer to the one delivered to patients with IPF [82]. However, the alkaline cyclopamine (derived from the plant *Veratrum californicum*) has anti-proliferative and anti-tumoural effects that are driven by its ability to directly bind Smoothened [81]. A cyclopamine derivative, vismodegib, has been recently tested in a Phase 1b clinical study in combination with pirfenidone (clinicaltrials.gov identifier: NCT02648048) [89]. In future studies, CXCL14, a molecule induced by SHH stimulation, could be also a biomarker reflecting the activity of SHH inhibitors [90].

TYAP/TAZ pathway, a mechanosensitive pathway reactivated in response to matrix stiffening in IPF, could be inhibited using verteporfin, a small molecule that negatively influences YAP transcription [46]. Verteporfin efficiency has been already proven in experimental models of kidney fibrosis [83]. Verteporfin could also be tested in clinical trials in IPF since it is currently used for the treatment of macular degeneration in combination with photodynamic therapy [91, 92]. Inhibiting ROCK, which regulates actin filament assembly, could also indirectly target YAP activation by the molecular mechanotransduction pathway. ROCK inhibition by fasudil has been shown to impact lung fibrogenesis in experimental models [93]. Interestingly, a phase 2 clinical trial using a ROCK inhibitor (KD205) is ongoing in patients with IPF (clinicaltrials.gov identifier: NCT02688647).

Of the four mammalian FOXO proteins [94], which are all phosphorylated by PI3K/AKT leading to the exclusion of the FOXO's transcription factor from the nucleus and their inactivation [95], FOXO3 has been recently identified as a new player in IPF. Interestingly, the phosphorylated FOXO3 form was increased in the lungs of people with IPF. Unlike the other transcription factors discussed above, FOXO3 transcription factor bears antifibrotic properties *in vitro* and *in vivo* [96]. Thus, FOXO3 re-activation in IPF would rather dampen fibrogenesis and could be an interesting therapeutic avenue. In fact, the reversion of FOXO3 inhibition in IPF could be achieved in patients with IPF using UCN-01, a staurosporine analogue. UCN-01 inhibits FOXO3 phosphorylation and cytoplasmic translocation preventing its inactivation. UCN-01 efficiency to prevent fibrosis development in experimental model of lung fibrosis has been already positively evaluated [40]. UCN-01 is under evaluation in cancer (clinicaltrials.gov identifier: NCT00082017).

As illustrated by several *in vivo* studies, targeting developmental pathways and their downstream transcriptional integrators might be a promising approach in fibrosis. However, a major hurdle will be that treatments directed against these pathways will probably interfere with normal wound healing or regeneration, exposing patients to potentially serious adverse events. This is illustrated by the worsening of lung fibrosis observed after epithelial-specific removal of β-catenin in murine fibrosis. A broad canonical Wnt inhibition approach should be taken even more cautiously as a recent study showed that lung alveolar epithelial type 2 stem cells display active Wnt signalling [97]. Similarly, inhibition of SHH with cyclopamine impacted skin wound healing [98]. Finally, even though *Smad3*$^{-/-}$ mice are protected against TGF-β mediated pulmonary fibrosis, ageing *Smad3* null mice display an emphysema-like phenotype, indicative that SMAD3 transcriptional activator downstream of TGF-β1 signalling is also required to maintain alveolar integrity [99].

The ideal situation would be to identify developmental pathways involved in lung fibrosis but not in wound healing, meaning that they are exclusively deleterious. In this regard, the recent identification of the embryonic TBX4 transcription factor as a key driver of myofibroblast differentiation is promising. TBX4 expression is strongly associated with the lung mesenchymal cell lineages as compared with other organs. As mentioned before, the authors demonstrated that depletion of TBX4-expressing cells or inhibition of TBX4-related signalling leads to attenuated fibrosis in bleomycin-induced fibrotic mice [42]. This study also suggests that targeting transcription factors associated with mesenchymal cells in IPF could be an interesting therapeutic avenue as these cells are thought to be one of the major effectors during fibrosis. Furthermore, a single-cell transcriptomic study in the bleomycin experimental model of lung fibrosis [100] recently classified the heterogeneous lung fibroblast populations. This kind of unbiased approach will help to better identify transcription factors associated with the different fibroblast populations in normal and fibrotic lungs.

Favouring repair pathways: transcriptional modifications induced by stem cell therapy

Mesenchymal stem cells (MSCs) are multipotent cells, defined by their ability to differentiate, their capacity to self-renew and their clonality [101]. Current evidence on IPF development suggest that the disease is characterised by impaired wound healing, alveolar injuries, failure of re-epithelialisation [102] and hyperplasia of aberrantly reprogrammed alveolar epithelial cells [103]. Therefore, the idea emerged that treating patients with MSCs could restore a normal healing process and prevent fibrosis extension through inhibition of Wnt-30, TGF-β1 and FGF [104]. In human disease, the first trial evaluating the safety and tolerability of MSC treatment in IPF was recently published. Glassberg *et al.* [105] conducted a phase I study using allogeneic human MSCs in patients with IPF (AETHER trial). Several studies suggest that stem cells exert their effect by regulating the transcription of profibrotic pathways: induced pluripotent stem cells inhibit fibroblast to myofibroblast differentiation and TGF-β1 dependent proliferation through a downregulation of SMAD2 and SMAD3 [106]. Similarly, human bone marrow-derived stem cells have the ability to sensitise TGF-β1 downstream signals that regulate interleukin (IL)-6/STAT3 activation, ultimately promoting regulatory T-cell expansion and production of the antifibrotic molecule IP-10 [107]. Moreover, Dinh *et al.* [108] have recently developed a method allowing isolation of lung spheroid cells from transbronchial biopsies. The authors were able to demonstrate angiogenic potential and lung distribution following cell transfer to athymic mice. This new method paves the way for future application in humans; for example, by interrupting aberrant reprogramming of alveolar epithelial type 2 cells. Finally, Guo *et al.* [109] have recently demonstrated that a careful reprogramming of AE2C generated induced progenitor-like cells that dampen bleomycin-induced fibrosis when transferred to mice. Of course, whether those promising pre-clinical results will apply to clinical world is elusive, as architectural changes that occurred in IPF lung might themselves preclude stem cell efficacy; also it is likely that stem cells display transient systemic antifibrotic effects through secretion of prostaglandin E2 and IL-10 [110] rather than true effective re-epithelialisation. In line with this concept, in systemic sclerosis, allogeneic stem cell therapy resulted in improved lung function, extend of high-resolution computed tomography lesions, decreased anti-SCL-70 antibody concentration and a diminution in skin sclerosis; the latter demonstrating the effect of the therapy on disease progression [111].

Conclusion and perspectives

In this review, we described developmental pathways and their downstream transcriptional integrators aberrantly recapitulated in IPF. Aberrant activation of developmental pathways is a common feature of cancer [112], and other lung diseases [113, 114] such as pulmonary hypertension and COPD. It will be interesting to determine in these diseases, and for a given cell type, whether the transcriptional integration of those signalling pathways may undergo similar or different maladaptive rewiring. For instance in IPF, from the *primum movens* of the disease, namely AE2C injury, to end-stage fibrosis, several major developmental pathways are reactivated. A key point is the timing of resurgence and the influence of

baseline conditions, which might result in a chaotic re-activation. In normal development, developmental pathways are activated following a precise time and space configuration, allowing proper organ development [115]. Similarly, normal wound healing requires tightly regulated expression of developmental pathway compounds [116]. Conversely, in disease, the re-activation of development pathway compounds seems to occur at first glance in an anarchic way. We propose that a chaotic dynamic may in fact govern this process in IPF, which explains at least in part the propagation of the disease. To determine whether the re-activation of developmental pathways is chaotic during lung fibrosis will require further unveiling of the rewired transcriptional dynamic of the networks driving the aberrant phenotype of mesenchymal and epithelial cells in IPF.

So far, a possible involvement of chaos in pulmonary diseases has not been reported to our knowledge. However, chaos signatures have been previously spotted in other biomedical fields. For example, cardiac arrhythmia and its control have been linked to chaotic dynamics [117]. Study of cancers as chaotic systems have been also proposed over the years as their unpredictability pose a threat to personalised cures [20]. Regarding chaos in IPF and since we will be dealing with dynamic systems, it will be crucial to acquire accurate time series and a lot of data when interrogating clinical material to determine whether the evolution of a given parameter might be driven by such a dynamic. A similar approach could be also undertaken in cellular/*in vitro* systems [19, 30] which are more fitted to perform high-throughput acquisition of data than *in vivo* pre-clinical models of lung fibrosis. Indeed, the current development of high-throughput imaging and transcriptomic approaches at the single-cell scale will greatly help to investigate such hypotheses. A future challenge for researchers and clinicians will be to find more complex models of fibrosis than the classical bleomycin model, as it fails to recapitulate salient IPF features such as AE2C hyperplasia and bronchiolisation [118]. If the aberrant integration of development pathways in IPF is chaotic, it could be potentially controlled as a consequence of its deterministic underlying nature. From a therapeutic perspective, chaos control in biomedicine is an interesting and still potentially promising avenue [117, 119].

In the meantime, targeting transcription factors activated in mesenchymal cells during lung fibrosis could represent another interesting therapeutic avenue in IPF. Such an approach would minimise the potential caveat associated with the targeting of broad/general developmental transcription factors also expressed in the lung epithelium as well as alveolar stem cell niche. In addition, stem cell therapy is a huge step forward in the field, offering the perspective of regenerative treatment in IPF. Phase I studies have led to encouraging results, but whether those treatment will benefit to patients with advanced IPF is so far elusive.

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