The role of heat shock protein 90 in idiopathic pulmonary fibrosis: state of the art

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HSP 90 is a chaperonin involved in IPF pathogenesis, overexpressed in the lung tissue of IPF patients. It has been investigated as a potential therapeutic target and HSP 90 inhibitors have shown promising antifibrotic effects in preclinical studies. https://bit.ly/3VRk2Q2

Cite this article as: Monteleone G, Cameli P, Bonella F. The role of heat shock protein 90 in idiopathic pulmonary fibrosis: state of the art. *Eur Respir Rev* 2025; 34: 240147 [DOI: 10.1183/16000617.0147-2024].

Received: 25 June 2024
Accepted: 16 Dec 2024

Abstract

Heat shock protein 90 (HSP 90) and its isoforms are a group of homodimeric proteins that regulate several cellular processes, such as the elimination of misfolded proteins, cell development and post-translational modifications of kinase proteins and receptors. Due to its involvement in extracellular matrix (ECM) remodelling, myofibroblast differentiation and apoptosis, HSP 90 has been investigated as a key player in the pathogenesis of lung fibrosis. Idiopathic pulmonary fibrosis (IPF) is the most common and deadly interstitial lung disease, due to the progressive distortion of lung parenchyma related to the overproduction and deposition of altered ECM, driven by transforming growth factor-β (TGF-β) dependent and independent pathways. The inhibition or induction of HSP 90 is associated with a reduced or increased expression of TGF-β receptors, respectively, suggesting a role for HSP 90 as a biomarker and therapeutic target in IPF. Experimental drugs such as geldanamycin and its derivatives 17-AAG (17-*N*-allylamino-17-demethoxygeldanamicin) and 17-DMAG (17-dimethylaminoethylamino-17-demethoxigeldanamycin), along with AUY-922, 1G6-D7, AT-13387, TAS-116 and myricetin, have been found to reduce lung fibrosis in both *in vivo* and *in vitro* models, supporting the role of this emerging target. This review aims to illustrate the structure and biological function of HSP 90 in the context of IPF pathobiology, as well as perspective application of this molecule as a biomarker and therapeutic target for IPF.

Introduction

The chaperon system encompasses a variety of molecules which are responsible for maintaining cellular protein homeostasis and turnover [1]. It includes different types of proteins, such as chaperones and chaperones co-factors, including their receptors and ligands [2].

Heat shock proteins (HSPs) are a wide family of constitutive physiological chaperones, classified by their molecular weight and whose expression is upregulated under stress conditions, such as temperature variations, hypoxaemia and exposure to radiation or cytotoxic agents [3].

In the last two decades, chaperones have gained interest in oncology due to their potential role as carcinogenesis modulators [4]. An overexpression of HSPs has been correlated with cell growth, proliferation and apoptosis failure [5].

Among HSPs, the isoforms of heat shock protein 90 (HSP 90) are ubiquitous, highly preserved and regulate a broad range of cellular pathways including apoptosis, protein folding, proteostasis, cell development and differentiation [6–8]. They also appear to be involved in the direct and crossantigen presentation to $CD4^+$ and $CD8^+$ lymphocytes T-cells, the post-translational modifications of kinase proteins and their receptors, and in the degradation of proteasome-mediated peptides [9].

Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease characterised by fibrotic remodelling of the lung interstitium leading to a progressive and worsening clinical course until the exitus [10, 11].

A decade ago, nintedanib and pirfenidone were approved as antifibrotic treatments based on their efficacy in slowing down disease progression and prolonging survival [12]. Nevertheless, IPF remains fatal and deeply impacts the patient's quality of life.

Several studies from the last decade support a possible major role of HSP 90 as a potential pathogenetic driver and therapeutic target in IPF. HSP 90 is upregulated in type-II alveolar epithelial cells (AECIIs) and overexpressed within myofibroblasts and fibroblastic foci in IPF lung tissue [13–15]. Immunochemistry and western blot analyses demonstrated high expression of HSP 90α in vascular and bronchiolar smooth cells, while HSP 90β in hyperplastic AECIIs and interstitial lung fibroblasts in IPF lung [13, 14]. Both isoforms HSP 90α and HSP 90β are overexpressed in altered bronchiolar structures surrounding the fibroblastic foci and within the fibroblastic foci themselves [14]. In addition, higher levels of HSP 90 in serum and bronchoalveolar lavage fluid (BALF) of IPF patients compared to controls, as well as their correlation with lung function tests suggest an association with disease severity [16]. In this review, we aim to provide an overview of the structure of HSP 90 along with its role in IPF, including future perspectives in terms of potential therapeutic implications.

Methods

The literature search for this narrative review was conducted on the PubMed database using variants of several keywords, as follows: "heat shock protein", "HSP", "heat shock protein 90", "HSP 90", "idiopathic pulmonary fibrosis" and "IPF". G.M. and P.C. carried out an independent assessment of the literature search. G.M., P.C. and F.B. assessed the eligibility of the literature search and selected studies involving both animals and humans. Only full-text articles available in English were considered for data extraction and included in the reference list.

Physiologic role of HSP 90

Machinery and structure of HSP 90

The fundamental role of HSP 90 in maintaining the internal environment is demonstrated by its presence and high preservation in all living kingdoms, except for the Archea [17]. The mammals carry various isoforms of HSP 90, including HSP 90α, HSP 90β, HSP 90α2, glucose-regulated protein (GRP) 94 and tumour necrosis factor receptor-associated protein 1 (TRAP1). These isoforms recognise different cellular localisations. HSP 90α, HSP 90β and HSP 90α2 are localised in the cytoplasm, while GRP94 and TRAP1 are found in the endoplasmic reticulum (ER) and mitochondrial machinery, respectively [9, 18].

Physiologically, HSP 90 is a homodimer that dimerises *in vivo* [19]. The homodimer structure of HSP 90 consists of three components, namely a carboxy-terminal domain (CTD), an amino-terminal domain (NTD) and a middle domain (MD). These domains are highly conserved to ensure its constitutive and inducible activity [18, 20, 21].

The NTD contains an adenosine triphosphate (ATP) binding site that ensures the hydrolysis process where the ATP is converted into adenosine diphosphate (ADP) and inorganic phosphate (Pi). The MD consists of two alpha-helices with three irregular and six regular turns, respectively, and one three-layer alpha–beta–alpha sandwich arrangement. Additionally, the CTD interacts with other co-chaperones through tetratricopeptide repeat sites. Every constitutive component of the HSP 90 structure is part of a cycle that is strictly dependent on ATP hydrolysis [22, 23]. The NTD binds ATP molecules, the MD regulates ATP hydrolysis, while the CTD is necessary for the dimerisation process [19]. ATP hydrolysis is dependent on several effectors; in particular, the existing structural link between the MD and NTD leads to a stepwise switch of the HSP 90-NTD from an open to a closed conformation. The CTD and MD are also involved in the conformational shift [19, 24]. When the NTD binding site is inhibited or occupied, the CTD serves as an additional site for ATP hydrolysis [25] (figure 1).

Hence, the conformational change of both the NTD and CTD enhances the normally low binding affinity of HSP 90 for its targets and promotes the dimerisation process of NTD, inducing ATP hydrolysis. The domains return to open conformations after ATP hydrolysis from ATP to ADP+Pi had successfully ended [26]. The detailed knowledge of the HSP 90 structure and machinery has facilitated the development of selective chaperonin inhibitors, the majority of which target the ATP-binding domain of HSP 90. In contrast, the further HSP 90 inhibitors bind the CTD or MD of HSP 90 despite antifibrotic testing of those, which are believed to elicit a lesser heat shock response, remain poorly described [27, 28].

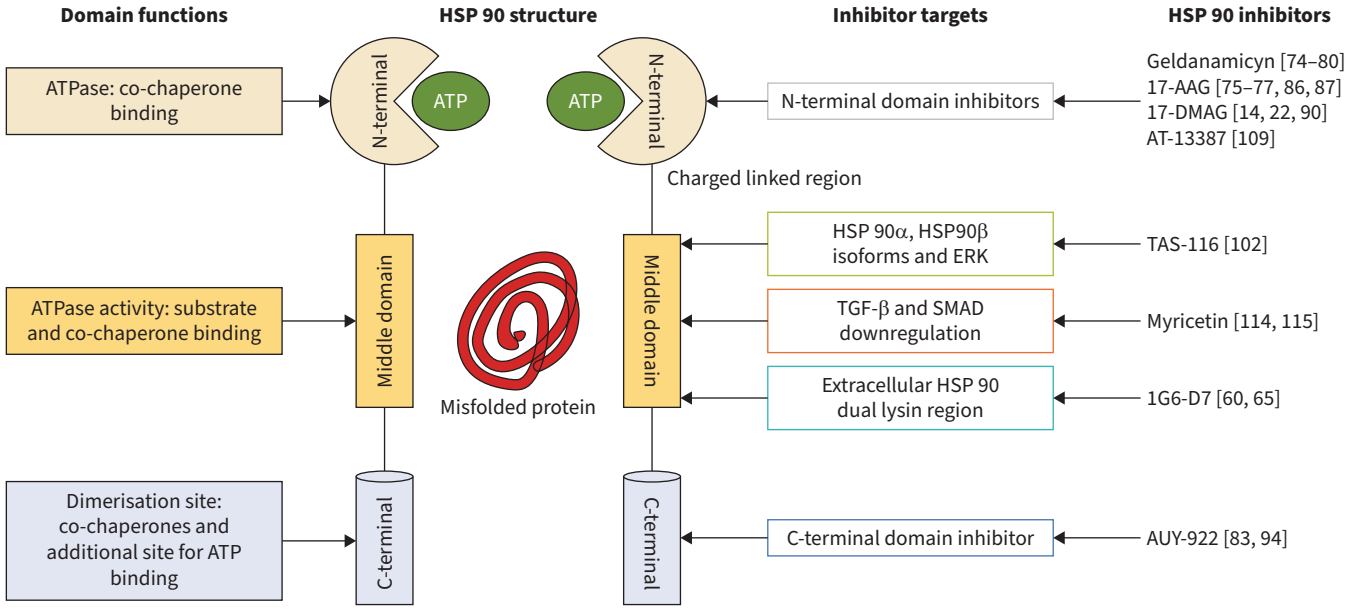

FIGURE 1 The structure, function and inhibitor targets of heat shock protein (HSP) 90. The chaperone consists of three domains: carboxy-terminal domain (CTD), amino-terminal domain (NTD) and a middle domain (MD). The CTD domain represents the site of dimerisation and interaction with co-chaperone sites. It also serves as additional pocket for the binding of adenosine triphosphate (ATP) and HSP 90 inhibitors (AUY-922). The NTD carries a binding site for both HSP 90 inhibitors including geldanamycin, 17-*N*-allylamino-17-demethoxygeldanamicin (17-AAG), 17-dimethylaminoethylamino-17-demethoxigeldanamycin (17-DMAG) and AT-13387 as well as ATP molecules, which are hydrolysed into adenosine diphosphate and inorganic phosphate. The MD embeds two-alpha helices with three irregular and six regular turns and exhibits a one-three-layer alpha–beta–alpha sandwich arrangement. As per the NTD, it is involved in the binding of co-chaperones and in ATPase activity. Furthermore, TAS-116, 1G6-D7 and myricetin are HSP 90 inhibitors that recognise different mechanisms of action. While TAS-116 exerts its inhibitory effect on HSP 90α and HSP 90β isoforms including extracellular signal-regulated kinase (ERK), 1G6-D7 and myricetin inhibits the chaperonin *via* extracellular HSP 90 dual lysin region binding and transforming growth factor-beta (TGF-β) and suppressor of mothers against decapentaplegic (SMAD) cytoplasmic proteins group downregulation.

In conclusion, the HSP 90 inhibition resulted in a compensatory effect with other HSPs, which is one of the essential mechanisms observed in HSP inhibition [29].

Function and regulation of HSP 90

The total amount of HSP 90 undergoes significant changes under stress conditions [3, 8, 30]. During the transition from physiological to stress conditions, the levels of HSPs may rise from 1–2% to 4–5% [31]. Under stress, the HSP intracellular concentration increases due to the release of a factor called heat shock factor-1 (HSF-1), which, in turn, is deactivated by a wide group of proteins, including HSP 90 isoforms. The presence of stressful factors, along with HSF-1 polymerisation, induces the transcription of nuclear genes encoding HSPs. This process is regulated by negative feedback *via* inhibition of HSF-1 polymerisation, induced by HSP 90 and other co-chaperones (*i.e.* P23 and FK506-binding protein 52) [19, 32]. The elevated levels of HSPs are functional in overseeing protein folding and eliminating misfolded proteins through the proteasome [33].

In terms of physiological functions, HSP 90α is involved in the regulation of retinal photoreceptor protection, spermatogenesis, DNA damage response and cardiomyocyte repolarisation. HSP 90β regulates embryonic development, the innate immune response, muscle cell differentiation and regeneration, hepatocyte formation, neuromuscular junction maintenance, and the control of glucose and cholesterol metabolisms [34]. Both HSP 90α and HSP 90β play key roles in cell differentiation and cellular interaction pathways, including the activity of cytoskeletal proteins such as actin and tubulin [35]. Moreover, extracellular HSP 90 is a secreted protein that can be released in response to internal or external stress factors. It may induce tissue repair or tumour formation, respectively, in physiological or pathological conditions. Preliminary findings from preclinical studies suggest that extracellular HSP 90 could be used to target wound healing, tumourigenesis, tumour cell migration and invasion, tissue fibrosis, wasting syndrome, and angiogenesis [36, 37].

Conversely, two other isoforms of HSP 90, namely GRP94 and TRAP1, are located in the ER and mitochondria, respectively. The GRP94 isoform plays a role in controlling intracellular calcium, monitoring protein folding and maintaining ER quality in both homeostasis and stress conditions [35]. The TRAP1 isoform serves as a defence mechanism against the oxidative stress induced by apoptosis in mitochondria. The inhibition of this pro-apoptotic protein causes the interruption of the transition of reactive oxygen species (ROS) through the mitochondrial pores. The TRAP1 isoform also regulates bioenergetic processes in mitochondria by hampering succinate dehydrogenase enzyme [35, 38, 39] (table 1).

Implication of HSP 90 in respiratory disorders

HSP 90 dysregulation is implicated in many respiratory disorders. First, an increased activity of HSP 90 has been demonstrated for many epithelial cancers, including nonsmall cell lung cancer (NSCLC) [4, 40]. An overexpression or uncontrolled activation of HSP-ATPase represents a typical feature of malignant cells, due to its anti-apoptotic and pro-angiogenic properties, expressed across multiple signalling pathways [41]. The highest levels of HSP 90 have been found in the lung tissue of patients with NSCLC by using protein pathway array analysis and a correlation with survival has been argued [42].

HSP 90 dysregulation is associated with other nonmalignant respiratory disorders, including asthma. Asthma is characterised by a chronic inflammation affecting all the structural cells of the airways, including epithelium, mucosal, submucosal and smooth muscle cells. In the majority of asthmatic subjects, the predominant inflammatory pattern is defined as type-2, due to the characteristic cytokine milieu and the cell profile of activation and secretion. Overstimulation of inducible nitric oxide (NO) synthase represents one of the key cellular pathways inducing and perpetuating inflammation in the airways. In asthmatic subjects, overexpressed HSP 90 binds heme-free soluble guanylate cyclase, making it unable to respond to NO exposure and, therefore, to induce bronchodilation [43]. Moreover, HSP 90 has been reported to be also involved in dysfunctional epithelial responses to allergen and/or irritating stimuli typical of asthmatic subjects, contributing to induce hyperplasia of goblet cells through the interleukin (IL)-4 and IL-13

TABLE 1 Different isoforms, localisations and functions of heat shock protein 90 (HSP 90)

Isoforms of HSP 90	Localisation	Regulated pathways	References
Intracellular HSP 90α	Cytoplasm	Retinal photoreceptor protection Spermatogenesis DNA damage response Cardiomyocyte repolarisation Cell differentiation Cellular interaction Activity of actin and tubulin in cytoskeleton	Maiti *et al.* [34] Basset *et al.* [35]
Intracellular HSP 90β	Cytoplasm	Embryonic development Innate immune response Muscle cell differentiation and regeneration Hepatocyte formation Neuromuscular junction maintenance Control of glucose and cholesterol metabolisms Cell differentiation Cellular interaction Activity of actin and tubulin in cytoskeleton	Maiti *et al.* [34] Basset *et al.* [35]
Extracellular HSP 90	Ubiquitous	Tumourigenesis Tumour cell migration and invasion Tissue repair Wound healing Tissue fibrosis Wasting syndrome Angiogenesis	Jay *et al.* [36] Sager *et al.* [37]
Glucose-regulated protein 94 (GRP94)	Endoplasmic reticulum	Control of intracellular calcium Monitoring protein folding Maintenance of endoplasmic reticulum integrity	Zininga *et al.* [9] Buchner *et al.* [18] Maiti *et al.* [34]
Tumour necrosis factor receptor-associated protein 1 (TRAP1)	Mitochondria	Defence against oxidative stress induced by apoptosis Control of bioenergetics processes in mitochondria	Zininga *et al.* [9] Buchner *et al.* [18] Basset *et al.* [35] Sager *et al.* [37] Berwin *et al.* [38]

pathway [43, 44]. Overall, these findings have paved the way to novel therapeutic targets in asthma and COPD, in which HSP 90 inhibitors appear to restore glucocorticoid sensitivity and reduce inhaled steroid resistance [45, 46].

HSP 90 and IPF: an intriguing connection

IPF pathobiology

The pathobiology of IPF encompasses multiple pathways and is not completely understood. The most accredited hypothesis is grounded upon the interaction between genetic predisposition (common variants and rare mutations) and environmental factors such as cigarette smoking, viral infections and inhaled noxae [47, 48]. There is growing evidence that cellular and immune senescence, telomere-related aging and epigenetic mechanisms are background mechanisms in IPF fibrogenesis [49].

Cellular senescence, one of the hallmarks of aging, is characterised by the aggregation of senescent cells, such as epithelial cells and fibroblasts, which are nonproliferative and resistant to apoptosis [48]. These cells carry an abnormal secretory pattern named the senescence associated secretory phenotype, secreting IL-1-β, which, in turn, leads to fibroblast–myofibroblast transition (FMT) and fibrosis progression [49, 50]. Further aging-related mechanisms are the accumulation of DNA damage, reduction of telomere length, the aberrant recapitulation of developmental pathways, alterations of DNA methylation and the up- and downregulation of noncoding microRNA (miRNA) [49–51]. Noncoding miRNA are short RNA sequences regulating transcriptional processes in both physiological and pathological conditions. In IPF, the increased expression of miR-154 and the reduced expression of miR-30a, miR-30d and miR-92a are likely to induce the Wnt/β-catenin pro-fibrotic pathway [49].

Both the innate and adaptive immune system act in the onset and perpetuation of IPF. Components of the innate immune system, including neutrophils, monocytes, macrophages, fibrocytes, type-II innate lymphoid cells (ILC2) and mast cells, are involved in both inflammation and fibrogenesis through different mechanisms [52, 53]. The recruitment of neutrophils from the bloodstream to the damaged lung tissue is followed by the secretion of a protease named neutrophil elastase, which initially triggers acute inflammation and then chronic fibrotic remodelling [52, 53]. Following lung injury, circulating monocytes infiltrate lung tissue where they differentiate into monocyte-derived alveolar macrophages and secrete inflammatory and pro-fibrotic cytokines [54]. When primed, alveolar macrophages and interstitial macrophages release IL-4, IL-13, transforming growth factor-β (TGF-β) and tumour necrosis factor alpha/beta [52–54]. Classically activated macrophages, compared to alternatively activated macrophages (M2), inhibit the fibrotic process by releasing metalloproteinases and C-X-C motif chemokine ligand-10. Conversely, M2, the most prevalent macrophage subtype in IPF, has the potential to accelerate the progression and exacerbation of IPF through the secretion of C–C motif chemokine ligand-18 and TGF-β [53, 54].

Mast cells are also present in lung tissue and BALF of patients with IPF. Their mediators, histamine and renin, promote proliferation, TGF-β secretion and collagen synthesis [55].

Furthermore, ILCs, in particular ILC2, exert profibrotic activity through the release of IL-4, IL-5, IL-9 and IL-13, as well as secreting TGF-β [52].

Within the immune adaptive system, lymphocytes T-cells can polarise towards the profibrotic T-helper 2 axis and induce an FMT by producing IL-4, IL-5, IL-9 and IL-13 [53].

Collagen accumulation in the ECM, driven by tyrosine kinases and pro-fibrotic cytokines, represents the end of the fibrotic cascade. TGF-β, produced by macrophages, alveolar epithelial cells (AECs), regulatory T-cells and platelets in response to injury, is considered as the pivotal pro-fibrotic cytokine by inducing an epithelial–mesenchymal transition (EMT), the differentiation of fibroblasts into myofibroblasts and ECM remodelling through collagen deposition [56, 57]. TGF-β receptors I and II are serine or threonine kinase receptors and mediate TGF-β intracellular signalling [57, 58]. While the former is inducible by TGF-β receptor II phosphorylation, the latter is constitutively activated. The TGF-β machinery induces the suppressor of mothers against decapentaplegic (SMAD) cytoplasmic proteins group whose translocation to the nucleus mediates the transcription of TGF-β-related genes [58]. Altogether, these pathways lead to an irreversible remodelling of the lung interstitium and ECM of the alveolus, *via* TGF-β pathways, causing a progressive distortion of pulmonary architecture and dynamics due to collagen deposition [10, 11].

The role of HSP 90 in fibrogenesis

HSP 90 appears to be directly involved in many of the key molecular pathways associated with IPF onset and progression. HSP 90 stabilises the expression of TGF-β receptor I and TGF-β receptor II and preserves

their integrity by direct binding [59–61]. On the other hand, HSP 90 inhibition is associated with reduced TGF-β receptor expression through Smurf2 E3 ligase dependent ubiquitination or SMAD inhibition [60, 61]. HSP 90 inhibitors have been shown to reduce collagen deposition in animal models of pulmonary, renal, hepatic and myocardial fibrosis [62–64].

HSP 90 has a regulatory function in non-SMAD mediated proteins such as AKT, P38 and extracellular signal-regulated kinase (ERK), which are required for TGF-β signalling responses [65]. AKT, also known as protein kinase B, is a key molecular target of HSP 90. AKT binds the phophatidylinositol-3-kinase protein (PI3K) to form the PI3K–AKT axis, which, in turn, enhances AEC apoptosis, EMT, fibroblast survival and proliferation, and increases the expression of α-smooth muscle actin [66, 67]. HSP 90 can also bind a co-chaperone called cell division cycle 37 (CDC37), forming the complex HSP 90–CDC37, which, in turn, inhibits ERK degradation and induces the intranuclear movement of the SMAD 4 phosphorylated form [22]. Overall, HSP 90 acts as an AKT stabiliser, blocking apoptosis and prolonging cell survival [68, 69]. AKT–HSP 90 binding requires testis-specific serine-threonine kinase 4 (TSSK4) to ensure a complete phosphorylation process [69] (figure 2). TSSK4 is a cell apoptosis inducer in the spermatogenesis process by exerting kinase activity [70]. In a bleomycin mouse model of pulmonary fibrosis, Chen et *al.* [71] observed that inflammation can enhance TSSK4 expression on the surface of AECIIs. TSSK4, in turns, blocks the activity of HSP 90–ATPase by phosphorylating HSP 90β, leading to the suppression of AKT and the subsequent apoptosis of AECIIs.

In a mouse model of fibrosis, obtained by intratracheal instillation of hydrochloric acid (HCl), Marinova *et al.* [72] found an increased expression of phosphorylated HSP 90 and ERK 44/42 in lung tissue at the 10th

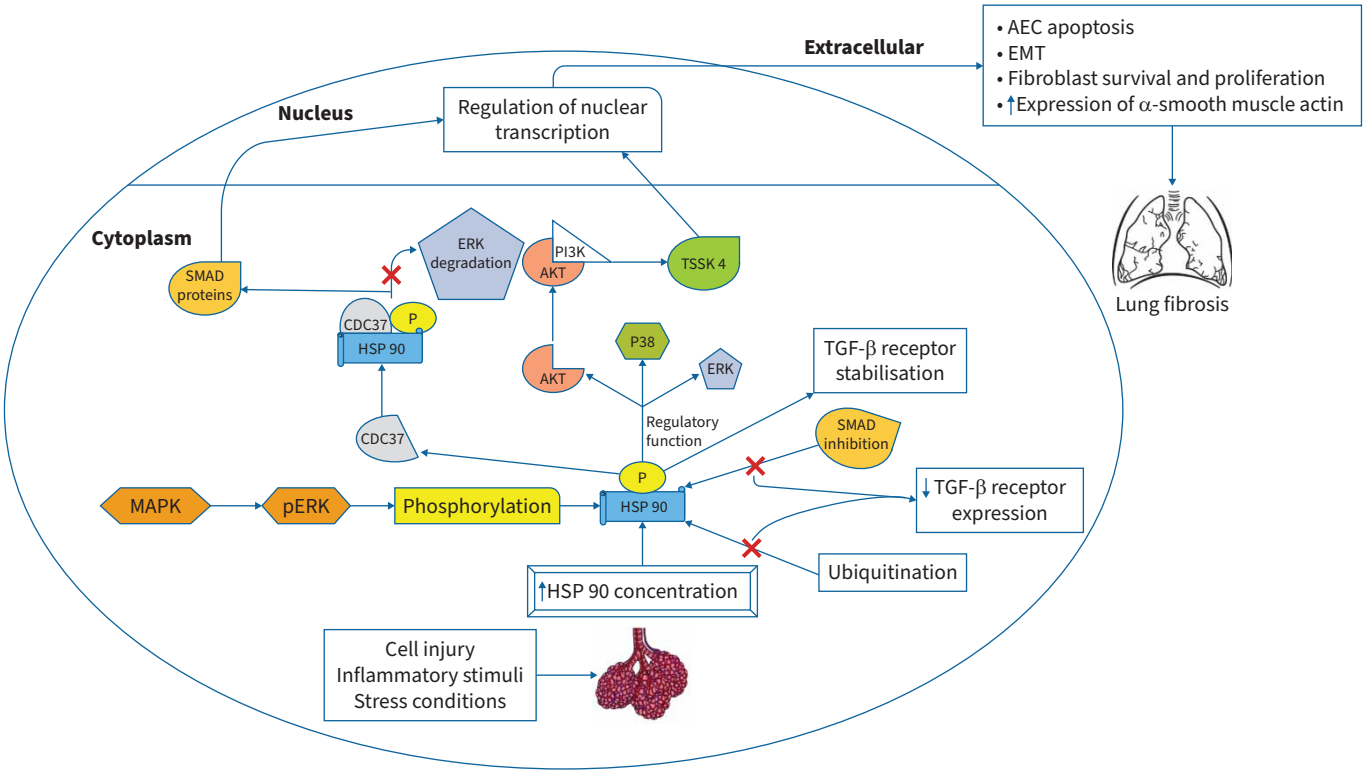

FIGURE 2 The role of heat shock protein (HSP) 90 in fibrosis molecular cascade. Throughout inflammation, stress conditions or cell injury, HSP 90 concentration increases. This chaperonin is activated by a phosphorylation process mediated by mitogen-activated protein kinase (MAPK). HSP 90 activated form binds cell division cycle 37 (CDC37), enhances extracellular signal-regulated kinase (ERK), protein kinase B (AKT) and P38 and stabilises TGF-β receptors. The complexes HSP90–CDC37 and AKT–phophatidylinositol-3-kinase protein (PI3K) activate suppressor of mothers against decapentaplegic (SMAD) proteins and testis-specific serine-threonine kinase 4 (TSSK4) which, in turn, regulate gene expression after translocation into the nucleus. All in all, the regulation of nuclear transcription results in alveolar epithelial cell (AEC) apoptosis, epithelial–mesenchymal transition (EMT), fibroblast survival and proliferation, and increased expression of α-smooth muscle actin leading to lung fibrosis. Conversely, both ubiquitination and SMAD inhibition suppress HSP 90, by reducing transforming growth factor-β (TGF-β) receptor expression potentially acting as an antifibrotic.

and 30th days after HCl instillation, but not earlier. These findings suggest that HSP 90 overexpression occurs at a later stage of fibrosis development, but further confirmation is needed.

In 2021, Solopov *et al.* [73] investigated lung fibrosis development in mice following the administration of intratracheal HCl or nitrogen mustard (NM) for up to 30 days, in comparison to mice receiving saline solution. Unexpectedly, collagen deposition and the development of fibrotic areas were more pronounced in male rather than female mice. In BALF from male mice treated with either HCl or NM, an increased expression of phosphorylated HSP 90 and ERK was also observed.

Taken together, all the preclinical data support the role of this chaperonin in the development and perpetuation of lung fibrosis.

HSP 90 as a biomarker of fibrosis

HSP 90 and its isoforms have been shown to be elevated in blood and lung tissue samples of IPF patients [13, 15, 16]. Korfei *et al.* [13] performed proteomic analysis *via* gel electrophoresis and matrix-assisted laser desorption time-of-flight mass spectrometry to examine the differential expression of proteins in lung tissue samples from 14 patients with IPF and 10 controls. A total of 203 protein spots were overexpressed in both IPF and controls, but significantly higher (two folds or more) in IPF patients. After statistical correction, 89 proteins remained differentially expressed and western blot analysis confirmed the upregulation of HSP 90α in IPF lungs but not in controls. HSP 90β was overexpressed in AECIIs, particularly around the areas of fibrosis in IPF patients [13]. Whether the distinct distribution of HSP 90 isoforms within lung tissue has functional implications requires further investigation.

Tian *et al.* [74] conducted a proteomic study on tissue samples obtained from 20 explanted IPF lungs and from patients undergoing lung cancer surgery (controls). They were able to identify 662 differentially/differently expressed proteins between IPF and controls by using isobaric tags for relative and absolute quantitation, as well as liquid chromatography tandem mass spectrometry. The isoform HSP 90 AA1, a protein contributing to ECM remodelling, was found to be upregulated in lung tissue samples from transplanted IPF patients. However, its precise role in IPF development remains unclear.

Bellaye *et al.* [16] measured the circulating levels of HSP 90α and HSP 90β isoforms in the sera of 31 IPF patients and nine controls by using ELISA. IPF patients had higher serum HSP 90α levels compared to controls, whereas no differences were seen for HSP 90β. In addition, HSP 90α serum levels inversely correlated with total lung capacity, forced vital capacity and forced expiratory volume in 1 s, suggesting a potential role as a severity marker. At present, there are no published studies on the prognostic value of serum HSP 90 in IPF.

The increased levels of HSP 90 in serum, BALF and, in parallel, its augmented expression in bronchial walls, myofibroblasts, fibroblastic foci and AECIIs from IPF lung tissue samples, suggest a spill-over of HSP 90 from the interstitial/alveolar space to the bloodstream through a concentration gradient. To date, no data are available in the literature to corroborate this hypothesis. Further studies are warranted to confirm the role of chaperonin as blood and tissue biomarker of IPF.

Targeting HSP 90 for IPF treatment

HSP 90 is still under investigation as a potential therapeutic target in IPF in preclinical studies (table 2). A compound named geldanamycin, along with its derivatives 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-dimethylaminoethylamino-17-demethoxigeldanamycin (17-DMAG), acts as competitor of the ATP binding site of HSP 90. Geldanamycin has an antifibrotic effect by reducing HSP 90 kinase activity, which stabilises folded proteins, but can also enhance the expression of specific stress-related proteins (*i.e.*, GRPs and HSP 90) through the induction of the heat shock genes involved in pro-fibrotic pathways [75–79]. Remarkable hepatotoxicity, the induction of oxidative stress and ROS as well as its poor bioavailability and solubility represent significant limitations to its application in clinical trials [79, 80].

The compound 17-AAG, also known as tanespimycin, is a modified water-insoluble derivative of geldanamycin that targets the NTD-ATP binding domain of HSP 90. 17-AAG inactivates the HSP 90 multi-chaperone complex, which includes CDC37, P23, HSP 40, HSP 70, HSP 70-HSP 90 organising protein and immunophilins, *via* a concentration gradient [81, 82]. This is associated with a decrease of ECM deposition and fibrosis development [82, 83]. Concentration-dependent HSP 90 inhibition is an alternative mechanism to the classical degradation of Erb-B2 receptor tyrosine kinase 2 and AKT, which leads a complete loss of AKT signalling [83]. This compound has undergone evaluations in phase I/II trials and found to exhibit antineoplastic properties in breast cancer, multiple myeloma and melanoma [81, 84, 85]. Although 17-AAG

TABLE 2 Mechanisms of action and biological effects of heat shock protein 90 (HSP 90) inhibitors

Drug	Mechanism of action	Biological effects	References
17-AAG	Competitor of HSP 90 NTD–ATP binding site Inhibition of HSP multi-chaperone complex *via* concentration gradient	Inhibition of fibroblastic activity, migration and invasion Reduction of collagen I post-translational secretion process and ECM gene expression Attenuation of EMT	Roque [75] Bonniaud *et al.* [76] Bonniaud *et al.* [77] Wong *et al.* [86] Sontake *et al.* [87]
17-DMAG	Competitor of HSP 90 NTD–ATP binding site	Reduction of fibroblastic activation and TGF-β-driven myofibroblast transformation	Sibinska *et al.* [14] Colunga Biancatelli *et al.* [22] Mohammed *et al.* [90]
1G6-D7	Inhibition of HSP 90α dual lysin region	Diminished fibroblastic activity Attenuation of lung fibrosis Improvement of lung function	Zhong *et al.* [60] Dong *et al.* [65]
AUY-922	Inhibition of HSP 90 CTD	Decreased ECM deposition Reduction of fibrosis development	Solopov *et al.* [94] Jensen *et al.* [83]
TAS-116	Inhibition of HSP 90α, HSP 90β and ERK	Diminished collagen type I deposition, ECM and fibronectin Increased lung resistance and elastance	Solopov *et al.* [102]
AT-13387	Inhibition of HSP 90 NTD–ATP binding site	Reduction of elastin and collagen miRNA Diminished TGF-β levels Decreased phosphorylated ERK1/2 quantity Increased lung resistance and elastance	Colunga Biancatelli *et al.* [109]
Myricetin	Downregulation of TGF-β and SMAD signalling pathways	Inhibition of TGF-β, fibroblast activation and EMT	Zhang *et al.* [114] Li *et al.* [115]

17-AAG: 17-*N*-allylamino-17-demethoxygeldanamicin; 17-DMAG: 17-dimethylaminoethylamino-17-demethoxigeldanamycin; CTD: carboxy-terminal domain; ECM: extracellular matrix; EMT: epithelial–mesenchymal transition; ERK: extracellular signal-regulated kinase; miRNA: microRNA; NTD: amino-terminal-domain; SMAD: suppressor of mothers against decapentaplegic; TGF-β: transforming growth factor-beta.

demonstrated a relatively weak binding affinity for HSP 90, it highlighted lower toxicity and controllable side-effects such as diarrhoea, dizziness, fatigue and headache compared to geldanamycin [79].

In terms of antifibrotic effects, the administration of 17-AAG to fibroblasts derived from mice and human IPF lungs suppressed fibroblast migration and invasion and reduced collagen I post-translational secretion [76, 86, 87]. Similarly, the application of a knockdown short interfering RNA selective for HSP 90 AA or AB to cultured fibroblasts led to a reduction in migration. Although HSP 90 AA and HSP 90 AB isoforms are involved in fibroblast migration, only the loss of HSP 90 AB reduced ECM gene expression [87, 88].

The 17-DMAG, also known as alvespimycin, is a second-generation water-soluble geldanamycin derivative that demonstrates superior bioavailability and efficacy compared to 17-AAG, as well as a more precise affinity for HSP 90 in cancer cells [79, 89]. This compound, especially in combination with oleuropein, a proteasome inductor derived from *Olea europea*, is effective in reducing fibroblastic activation and TGF-β-driven myofibroblast transformation in both *in vivo* and *in vitro* models [14, 22, 90]. 1G6-D7 is a monoclonal antibody that targets the dual lysine region of the secreted HSP 90α [60, 65, 91]. It has been found to inhibit tumour formation and growth in mice, enhance tumour cell death in human breast cancer cells, and ameliorate epithelial airway barrier dysfunction in induced asthmatic animal models [92, 93]. In bleomycin-mice models of lung fibrosis, 1G6-D7 reduced fibroblastic activity attenuating lung fibrosis [60, 65].

AUY-922 is a third-generation small molecule HSP 90 inhibitor that binds to the CTD of HSP90 [83, 94]. The compound attenuated lung tissue fibrosis induced by NM in mice and alleviated endothelial dysfunction caused by spike protein subunit 1 of severe acute respiratory syndrome coronavirus 2 in cell cultures of human lung microvascular endothelial cells [95–97]. In a phase I study, AUY-922 administration was associated with ocular toxicity, with visual disturbances being the primary adverse effect [95]. Promising data have emerged from the application of AUY-922 in pancreatic, breast and lung cancer cell cultures; this compound seems to enhance the effect of anti-programmed death-ligand 1 and anti-cytotoxic T-lymphocyte antigen-4 drugs [98–101].

TAS-116, an oral direct inhibitor of HSP 90α, HSP 90β and ERK, differs from geldanamycin, 17-AAG and 17-DMAG in terms of its molecular structure, displaying antineoplastic activity [89, 102]. This compound has shown to be effective in preclinical studies on T-cell lymphoma/leukaemia and clinical

trials in patients with metastatic colorectal cancer, gastrointestinal stromal tumour (GIST), gastric tumour, sarcoma, melanoma and NSCLC [103–107]. The administration of TAS-116 in mice with lung fibrosis, provoked by HCl instillation, was associated with a reduction of collagen type I, ECM and fibronectin deposition, with a potential antifibrotic effect [102]. Although the drug displays a satisfactory safety profile, with reduced ocular toxicity in comparison to AUY-922, diarrhoea, fatigue, weight loss, as well as renal and liver dysfunction were observed following administration in both preclinical studies and humans [104, 107].

AT-13387 is a nongeldanamycin-derived inhibitor of HSP 90 directed towards the NTD-ATP binding site of HSP 90 and is administered subcutaneously [108]. It has proven to be effective in reducing elastin and collagen microRNA, TGF-β, and phosphorylated ERK1/2 levels in cell cultures and BALF from HCl-instilled mice, leading to improvements in lung resistance and elastance [109]. Due to its antineoplastic activity, this compound has been associated with the standard of care in phase I/II clinical trials in patients with advanced and metastatic solid tumours, including resistant stromal tumours such as GIST [110–112]. AT-13387 seems to have more controllable gastrointestinal side-effects such as diarrhoea, nausea and dry mouth, as well as ocular disturbances and transient AST and ALT increases [108].

Myricetin, a natural flavonoid derived from the tree known as *Myrica rubra*, exerts antineoplastic, antioxidant, anti-inflammatory, analgesic and immunomodulating effects [113]. In cultures of human cutaneous fibroblasts and in lung fibrosis bleomycin mice models, myricetin reduced collagen deposition *via* downregulation of the TGF-β and SMAD signal pathways [114, 115]. A dose-dependent inhibition of fibroblast activation and EMT has been observed [115]. Its unfavourable pharmacokinetic properties and poor bioavailability are significant challenges in performing translational research in humans and clinical trials [113, 116].

Overall, the administration of HSP 90 inhibitors induces relevant side-effects, probably due to the interference with the physiological functions of HSP 90 and a lack of specificity for HSP 90 isoforms. Further studies are warranted to develop more selective and better-tolerated HSP inhibitors.

Future perspectives

Although HSP 90 has emerged as a promising biomarker and molecular treatment target in IPF, there are several concerns. This chaperonin contributes to a wide array of physiological processes. Inhibiting HSP 90 can trigger the formation of a multi-chaperone complex leading to significant off-target and adverse effects. Further data from *in vivo* and *in vitro* studies in IPF are needed.

Regarding its potential role as a biomarker, the evidence supporting a correlation of HSP 90 serum and BALF levels with lung function, disease progression and outcome in IPF, and other interstitial lung diseases (ILDs), is still scarce and needs validation in larger cohorts. Whether patients with higher levels of HSP 90 represent a sub-phenotype which could benefit from treatment remains to be elucidated.

Given the recent advances in IPF drug development, the pleiotropic role of HSP 90 as a modulator of inflammatory, fibrotic and neoplastic processes could be advantageous in combined treatment strategies for patients with IPF, lung cancer or autoimmune/inflammatory ILD.

Overall, a better knowledge of the specific role of HSP 90 in the pathogenesis of IPF and fibrotic ILDs is mandatory to guide the development of more targeted drugs.

Conclusions

Chaperonins, in particular HSP 90 and its isoforms, seem to play a central role in the development and perpetuation of pulmonary fibrosis. In experimental models, compounds that inhibit its overexpression have shown to reduce pulmonary fibrosis *via* the TGF-β pathway, providing new insights into IPF pathogenesis. Chaperonin modulators can potentially become a new class of antifibrotic drugs if the promising results from preclinical studies are confirmed in clinical trials.

Points for clinical practice

- HSP 90 and its isoforms are involved in IPF pathogenesis and progression.
- HSP 90 could become a serum and tissue biomarker for IPF detection and severity assessment.
- A wide array of HSP 90 inhibitors are under investigation. They have provided promising results in preclinical studies.

Questions for future research

- HSP 90 expression in the tissues of non-IPF fibrotic ILDs.
- Combined intra- and extracellular inhibition of HSP 90 to slow down/stop IPF progression.
- HSP 90 expression and IPF outcome, in particular survival and acute exacerbation.

Provenance: Submitted article, peer reviewed.

Author contributions: G. Monteleone and P. Cameli conducted the literature review and drafted the manuscript. F. Bonella was critically involved in revision and edited the manuscript. All authors assessed, revised and approved the final version of manuscript.

Conflict of interest: G. Monteleone has nothing to disclose. P. Cameli reports payment for lectures, presentations, manuscript writing, or educational events from Boehringer Ingelheim, Sanofi, GlaxoSmithKline and AstraZeneca, and travel support from GlaxoSmithKline. F. Bonella reports consulting fees from Boehringer Ingelheim, Sanofi, BMS, and Savara Pharma; payment or honoraria for lectures, presentations, and manuscript writing from Boehringer Ingelheim and Sanofi; support for attending meetings from Boehringer Ingelheim, AstraZeneca, Atyr and Savara Pharma; and participation on a Data Safety Monitoring Board or Advisory Board with Boehringer Ingelheim, Sanofi and BMS.

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