Heat shock protein-90 toward theranostics: a breath of fresh air in idiopathic pulmonary fibrosis

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Heat shock proteins (HSPs), also known as stress proteins, constitute a complex network of proteins highly conversed across species that have been classified into different families according to their molecular weight: HSP110, HSP90, HSP70, HSP60, HSP40 and the small HSPs [1]. Although they are mainly known for their chaperone and cytoprotective properties, HSPs also participate in the regulation of many cellular signalling processes [2]. These proteins have been involved in various physiological as well as pathological conditions, including respiratory diseases such as asthma, chronic obstructive pulmonary disease, sarcoidosis or pulmonary hypertension pathogenesis. HSPs have been extensively studied in the cancer field thanks to their regulatory role for several proteins involved in carcinogenesis. Stress proteins are often overexpressed in cancer cells, promoting phenotypic hallmarks of cancer such as cellular reprogramming, sustaining proliferative signalling, supporting replicative immortality, evading growth suppressor or resistance to cell death. Therefore, several studies have focused on the potential use of HSP inhibitors concomitantly with chemotherapy in cancer patients [3, 4]. In particular, HSP90 has emerged as an important target in cancer and besides 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG), the first inhibitor targeting HSP90 tested in a clinical trial in 1999, several inhibitors targeting HSP90 have been developed and are now being tested in humans [5].

Idiopathic pulmonary fibrosis (IPF) and cancer share many features, such as cellular reprogramming and resistance to cell death [6, 7]. Several studies from our group and others described HSP overexpression in experimental and human pulmonary fibrosis [8–17]. A growing body of evidence suggests that HSPs might represent interesting therapeutic targets in lung fibrosis, mainly through transforming growth factor (TGF)-β signalling regulation [18]. In the context of pulmonary fibrosis, the potential of small HSP inhibition has been studied in different animal models of lung fibrosis. Mice deficient for the small HSP αB-crystallin are protected from experimental fibrosis induced by several agents [14, 15]. In the same

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manner, the local injection of OGX-427, an antisense oligonucleotide targeting HSP27 expression, interferes with TGF-β-induced subpleural fibrosis in rodent models [16]. Although several studies demonstrate a role for HSPs in fibrogenesis mechanisms *in vitro*, the effect of HSP inhibition in experimental lung fibrosis remains poorly investigated.

In this issue of the *European Respiratory Journal*, Bellaye *et al.* [19] nicely uncovered a sophisticated mechanism in which extracellular HSP90α regulates myofibroblast activation, highlighting a key role in lung fibrosis, notably in IPF. In addition, they demonstrated that inhibition of extracellular HSP90, which is increased in serum from patients with IPF compared with controls, hampers myofibroblast profibrotic activities and TGF-β1 induced lung fibrosis in rodent and thereby identified a potential new therapeutic target for IPF.

IPF is a progressive and lethal chronic lung disease from unknown origin [20]. Beside lung transplantation, two drugs, pirfenidone [21] and nintedanib [22], which slow down but are unable to hamper disease progression, have been approved for IPF management following decades of clinical trials [23]. Therapeutic option for patients with IPF is therefore limited and novel therapeutic approaches need to be developed. IPF patho-biology is complex and the most recent model proposes that repeated epithelial injuries result in stimulation of an abnormal epithelium that, following impaired cross-talk, promotes fibroblasts proliferation and activation leading to myofibroblast accumulation and subsequent collagen over-production [20]. TGF-β is a key cytokine in the fibrotic process and is involved in differentiation and activation of fibroblasts resulting in collagen accumulation and other extracellular matrix components production.

Bellaye *et al.* [19] present a novel mechanism impacting myofibroblast differentiation and suggest that HSP90 might be an interesting target in pulmonary fibrosis. HSP90 is, with HSP70, the main chaperone system for protein folding. Two isoforms of HSP90 have been described: HSP90α and HSP90β, and within the cell, HSP90 chaperone acts as a dimer. Although the role of each isoform remains poorly understood at the moment, two recent studies have highlighted the beneficial role of HSP90 global inhibition using geldanamycin-derived compounds in pulmonary fibrosis. Previously this year in the *European Respiratory Journal*, the therapeutic potential of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, a 17-AAG analogue) was tested in a murine model of pulmonary fibrosis [13]. This approach interfered with bleomycin-induced lung fibrosis. Mechanistically, HSP90β stabilised TGF-β receptor II, thus contributing to enhanced TGF-β profibrotic activities. These findings were later confirmed using 17-AAG in TGF-α overexpressing transgenic mouse model [17]. In that study, authors showed using siRNA approaches that HSP90β (but not HSP90α) was required to drive migratory properties and proliferation of fibroblasts. Bellaye *et al.* [19] have confirmed the importance of HSP90β in myofibroblast activation through stabilisation of the LRP1 surface receptor. Further, this study nicely demonstrated a key role for the other HSP90 isoform, HSP90α, in myofibroblast biology and provides evidence that HSP90α (but not HSP90β) is secreted by myofibroblasts upon mechanical stress *ex vivo*. The authors observed that extracellular HSP90α activates myofibroblast differentiation in a LRP1-dependent manner.

Importantly, the work from Bellaye *et al.* [19] also shows that circulating levels of HSP90α increased in patients with IPF compared with controls. Moreover, patients with severe IPF have a significantly higher level of circulating HSP90α compared to those with moderate IPF with a negative correlation between serum level of HSP90α and lung function in these patients. Altogether, the demonstration that HSP90α levels increase in blood and bronchoalveolar lavage fluid of patients with IPF and that this extracellular HSP90 has an important role in IPF development, opens the possibility of its use both as a biomarker for diagnostic purposes and as a therapeutic target. Nevertheless, further studies are needed before suggesting the potential use of extracellular HSP90α as biomarker for IPF progression. Theranostic medicine has already been proposed for other HSPs in different pathological contexts. In this way, circulating HSP70 plasma level is a marker of metastasis and inhibition of this extracellular HSP, by blocking its function activating myeloid-derived suppressive cells, induces the development of an efficient anticancer immune response [24, 25]. Similarly, circulating HSP27 serum levels increase during myelofibrosis development and its inhibition blocks medullary fibrosis [26].

Many different inhibitors of HSP90 are available and some are in advanced clinical trials in cancer [5]. Most of these molecules are geldanamycin derivatives (such as 17-AAG or 17-DMAG) that block both isoforms of HSP90. Given the fact that HSP90 has a broad spectrum of activity impacting several signalling and physiological processes, the caveat will be that HSP90 inhibition will most likely come along unwanted side-effect. Furthermore, histological staining clearly shows that HSP90 expression is not only localised in fibroblasts but also in hyperplastic epithelium in IPF lung. One answer to these concerns might come from the study from Bellaye *et al.* [19]. Contrary to other studies demonstrating the potential effect of HSP90 inhibition using geldanamycin derivatives, Bellaye *et al.* [19] used the non-permeable

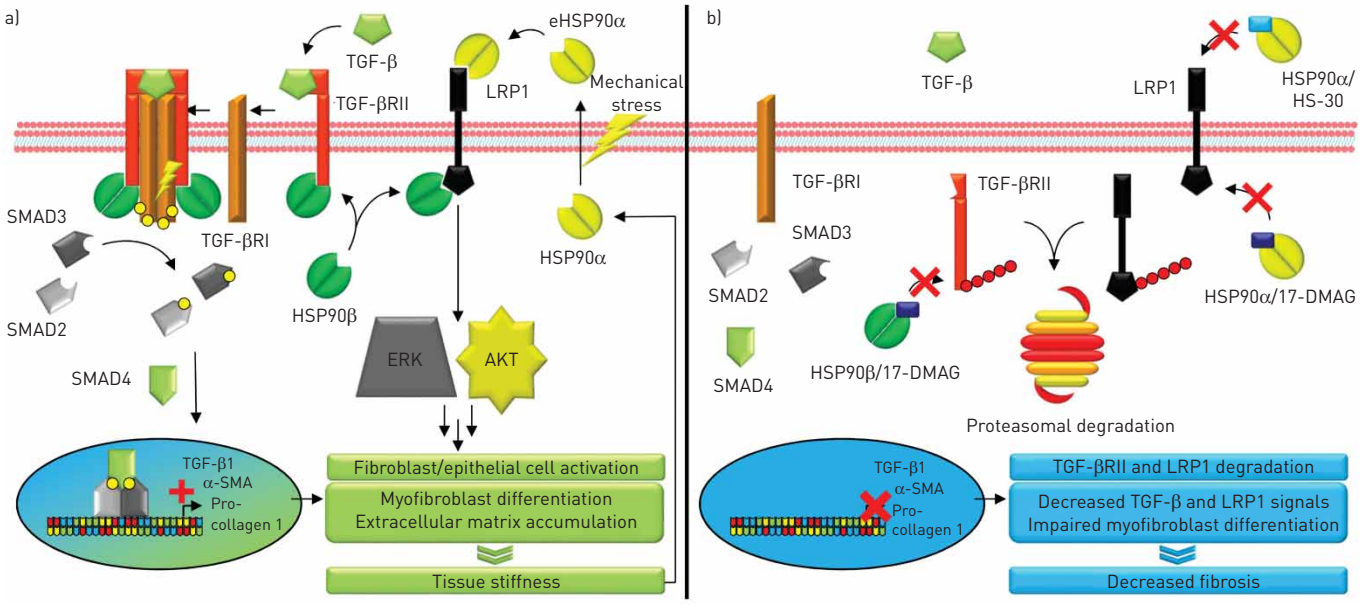

FIGURE 1 Heat shock protein (HSP)90 inhibition hampers profibrotic signalling at different levels. a) HSP90β stabilises type II TGF-β receptor (TGF-βRII) promoting transforming growth factor (TGF)-β profibrotic signalling. HSP90 also interferes with LRP1 proteasomal degradation. Tissue stiffness induces secretion of HSP90α in the extracellular milieu. Extracellular HSP90α further signals through LRP1 to enhance myofibroblast differentiation and profibrotic properties. b) HSP90 inhibition by geldanamycin derivatives (such as 17-DMAG) interferes with TGF-βRII and LRP1 stabilisation by intracellular HSP90, triggering their proteasomal degradation and decreasing both TGF-β and LRP1 fibrotic signals. In addition, the specific inhibition of extracellular HSP90α using the non-permeable HSP90 inhibitor HS-30 blocks HSP90α interaction with LRP1.

inhibitor of HSP90 HS30 to target the extracellular form of HSP90α. They showed that specific inhibition of extracellular HSP90α has an antifibrotic effect in an *ex vivo* precision-cut lung slice model of TGF-β1-induced lung fibrosis. Although HSP90 extracellular activities are not fully understood at the moment, the approach taken by Bellaye *et al.* [19] might have several advantages in case of long-term treatments with HSP90 inhibitors, which will be most likely the case in chronic conditions such as IPF. Moreover, these findings have recently been confirmed in mice with 1G6-D7, a cell impermeable antibody raised against HSP90α, which hampered bleomycin-induced pulmonary fibrosis [27]. However, the authors injected 1G6-D7 starting the following day post-bleomycin thus before lung fibrosis has occurred. Therefore, the potential therapeutic role of extracellular HSP90α inhibition remains to be fully demonstrated *in vivo*.

Despite the crucial need for more complete studies in animal models to confirm the recent findings, we witness the emergence of a growing body of evidence suggesting HSPs, and notably HSP90, as interesting targets for potential future therapies in pulmonary fibrosis (figure 1). As shown by Bellaye *et al.* [19], HSP90α can be measurable both in the blood and bronchoalveolar lavage fluid of the patients and correlates with clinical parameters. This pioneering study enlarges the landscape of HSPs in pulmonary fibrosis and opens the way to their use as biomarkers, which will be useful to select the population of patients which might benefit the most from this targeted therapy in the modern era of personalised medicine.

References

1 Kampinga HH, Hageman J, Vos MJ, *et al.* Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* 2009; 14: 105–111.
2 Kim YE, Hipp MS, Bracher A, *et al.* Molecular chaperone functions in protein folding and proteostasis. *Annu Rev Biochem* 2013; 82: 323–355.
3 Jego G, Hazoume A, Seigneuric R, *et al.* Targeting heat shock proteins in cancer. *Cancer Lett* 2013; 332: 275–285.
4 Mbofung RM, McKenzie JA, Malu S, *et al.* HSP90 inhibition enhances cancer immunotherapy by upregulating interferon response genes. *Nat Commun* 2017; 8: 451.
5 Sidera K, Patsavoudi E. HSP90 inhibitors: current development and potential in cancer therapy. *Recent Pat Anticancer Drug Discov* 2014; 9: 1–20.
6 Horowitz JC, Osterholzer JJ, Marazioti A, *et al.* "Scar-cinoma": viewing the fibrotic lung mesenchymal cell in the context of cancer biology. *Eur Respir J* 2016; 47: 1842–1854.
7 Vancheri C. Common pathways in idiopathic pulmonary fibrosis and cancer. *Eur Respir Rev* 2013; 22: 265–272.
8 Burgy O, Bellaye PS, Causse S, *et al.* Pleural inhibition of the caspase-1/IL-1beta pathway diminishes profibrotic lung toxicity of bleomycin. *Respir Res* 2016; 17: 162.

9 Decologne N, Kolb M, Margetts PJ, *et al.* TGF-beta1 induces progressive pleural scarring and subpleural fibrosis. *J Immunol* 2007; 179: 6043–6051.
10 Decologne N, Wettstein G, Kolb M, *et al.* Bleomycin induces pleural and subpleural fibrosis in the presence of carbon particles. *Eur Respir J* 2010; 35: 176–185.
11 Kakugawa T, Yokota S, Ishimatsu Y, *et al.* Serum heat shock protein 47 levels are elevated in acute interstitial pneumonia. *BMC Pulm Med* 2014; 14: 48.
12 Korfei M, Schmitt S, Ruppert C, *et al.* Comparative proteomic analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF) and lung transplant donor lungs. *J Proteome Res* 2011; 10: 2185–2205.
13 Sibinska Z, Tian X, Korfei M, *et al.* Amplified canonical transforming growth factor-beta signalling via heat shock protein 90 in pulmonary fibrosis. *Eur Respir J* 2017; 49: 1501941
14 Bellaye PS, Burgy O, Colas J, *et al.* Antifibrotic role of alphaB-crystallin inhibition in pleural and subpleural fibrosis. *Am J Respir Cell Mol Biol* 2015; 52: 244–252.
15 Bellaye PS, Wettstein G, Burgy O, *et al.* The small heat-shock protein alphaB-crystallin is essential for the nuclear localization of Smad4: impact on pulmonary fibrosis. *J Pathol* 2014; 232: 458–472.
16 Wettstein G, Bellaye PS, Kolb M, *et al.* Inhibition of HSP27 blocks fibrosis development and EMT features by promoting Snail degradation. *FASEB J* 2013; 27: 1549–1560.
17 Sontake V, Wang Y, Kasam RK, *et al.* Hsp90 regulation of fibroblast activation in pulmonary fibrosis. *JCI Insight* 2017; 2: e91454.
18 Bellaye PS, Burgy O, Causse S, *et al.* Heat shock proteins in fibrosis and wound healing: good or evil? *Pharmacol Ther* 2014; 143: 119–132.
19 Bellaye PS, Shimbori C, Yanagihara T, *et al.* Synergistic role of HSP90α and HSP90β to promote myofibroblast persistence in lung fibrosis. *Eur Respir J* 2018; 51: 1700386.
20 Martinez FJ, Collard HR, Pardo A, *et al.* Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers* 2017; 3: 17074.
21 Noble PW, Albera C, Bradford WZ, *et al.* Pirfenidone for idiopathic pulmonary fibrosis: analysis of pooled data from three multinational phase 3 trials. *Eur Respir J* 2016; 47: 243–253.
22 Collard HR, Richeldi L, Kim DS, *et al.* Acute exacerbations in the INPULSIS trials of nintedanib in idiopathic pulmonary fibrosis. *Eur Respir J* 2017; 49: 1601339.
23 Raghu G. Idiopathic pulmonary fibrosis: lessons from clinical trials over the past 25 years. *Eur Respir J* 2017; 50: 1701209.
24 Gobbo J, Marcion G, Cordonnier M, *et al.* Restoring anticancer immune response by targeting tumor-derived exosomes with a HSP70 peptide aptamer. *J Natl Cancer Inst* 2016; 108: djv330.
25 Chalmin F, Ladoire S, Mignot G, *et al.* Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J Clin Invest* 2010; 120: 457–471.
26 Sevin M, Kubovcakova L, Pernet N, *et al.* HSP27 is a new partner of JAK2/STAT5 and plays a role in myelofibrosis. *Nat Commun* 2017; in press.
27 Dong H, Luo L, Zou M, *et al.* Blockade of extracellular heat shock protein 90alpha by 1G6-D7 attenuates pulmonary fibrosis through inhibiting ERK signaling. *Am J Physiol Lung Cell Mol Physiol* 2017; 313: L1006–L1015.