Journal of
Clinical and Pharmaceutical Research
REVIEW ARTICLE OPEN ACCESS

MDM4: A Novel Molecular Target for Treating Idiopathic Pulmonary Fibrosis Associated with Aging

P. Krishna Mounica

Senior Associate, Chemical Safety and Regulatory Affairs, Freyr Global Regulatory Solutions and Services Company, Hyderabad, Telangana, India.

ARTICLE INFO

Article History:
Received: 09.03.2021
Revised: 18.04.2021
Accepted: 20.04.2021

Keywords :
Idiopathic Pulmonary Fibrosis
MDM4
Myofibroblast

Corresponding Author:
P. Krishna Mounica M.S (Pharm)
Senior Associate
Chemical Safety and Regulatory Affairs,
Freyr Global Regulatory Solutions and Services Company,
Hyderabad,
Telangana, India.
E mail id: mounica49padala@gmail.com

ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is the end stage of many diffuse parenchymal lung diseases typified by excessive matrix deposition leading to destruction of normal lung architecture and function of unknown etiology. Aging is considered as a strong risk factor and an independent prognostic factor for progression of IPF. However, the exact mechanisms that link the IPF with aging remained unknown, but a number of changes associated with aging revealed in IPF lungs. The p53 gene is a tumour suppressor gene that played a vital role in cancer and it is also known to have activity in fibrosis. MDM2 and MDM4 are the two major inhibitors of p53 .They only differ in their intrinsic E3-ligase activity and promote degradation of p53. MDM4 is a matrix stiffness -regulated negative regulator of p53 highly expressed in fibrotic lesions of IPF. Some of the invitro studies reported that MDM4-p53 pathway promoted lung fibrosis resolution in aged mice, this suggests that MDM4 can be better target against persistent lung fibrosis associated with aging. Also, better knowledge of the pathophysiological mechanisms linking aging to IPF may provide new therapeutic windows to treat this devastating disease.

Introduction

Pulmonary fibrosis is a group of chronic, irreversible and fatal interstitial lung disease associated with multitude of factors. Idiopathic fibrosis (IPF) is the most common type of pulmonary fibrosis that occurs by an unknown cause. It occurs in genetically susceptible individuals who are aging and those exposed to several environmental factors like cigarette smoking. Thus the disease is primarily seen in middle-aged and elderly people and is limited to the lungs [1, 2]. IPF is defined as aging-associated, progressive, irreversible lung diseases of unknown etiology.

It is characterised by persistence activation of myofibroblasts resulting in excessive deposition of extracellular matrix (ECM), lung remodelling, alveolar destruction and scarring of lungs leading to decline in lung function [3]. The average survival rate for IPF is only 2-4 years [4]. Thus, it is a alarming lung disease that affects human health. Though the incidence of pulmonary fibrosis is increasing in aging population, unfortunately the pathogenesis in pulmonary fibrosis is poorly understood and there are no effective therapeutic drugs for the rescue [5]. Pirferinidone and Nintedanib are the only two FDA approved drugs for the treatment of pulmonary fibrosis [6,7]. However, their mechanism of action is not well established but tend to have poor pharmacological effects with additional adverse effects showing no durable survival benefit [8,9].

Since, no pharmacological cure is available, lung transplantation is used to decrease mortality in patient with end stage disease. Hence, it is always important to understand the fundamental mechanisms underlying the disease pathogenesis and identify newer targets. Researchers had discovered newer target named mouse double minute 4 homolog (MDM4). It is a matrix stiffness regulated mechanosensitive inhibitor of p53. This MdM4-p53 dependent pathway shown to promote resolution of lung fibrosis associated with aging [10].

Epidemiology

The incidence of idiopathic pulmonary fibrosis across ten countries during the period of 1999 to 2012 was estimated by John P.et.al. The age standardised mortality ranged between 4 to 10 per 1,00,000 population. Lowest mortality rate was seen in Sweden (4.68 per 1,00,000), Spain (5.38 per 1,00,000) and New Zealand (5.55 per 1,00,000) whereas, the highest rate was observed in the United Kingdom (9.84 in England and wales, 10.71 in Scotland per 100,000 respectively) and Japan (10.26 per 1,00,000) [10]. Higher predominance of this disease is seen in men than in women with the ratio of 1.5 to 1.7:1 and the frequency is proportional to the age [2]. IPF occurs in middle-

aged and elderly adults >65 years. This proportion is estimated to double in age group of 65 and above, and over 2 billion individuals projected to surpass that mark by the year 2050 [11].

Risk factors of IPF

IPF results from a combination of genetic and nongenetic factors, where the nongenetic risk factor includes exogenous and endogenous elements that contribute to overall risk of the disease. Kaur et.al classified the genetic variants associated with predisposition to IPF into four caregories based on their role in pathogenesis of IPF. The first category of genes is those that effect alveolar stability, mostly genes enoding for surfactant proteins A and C like SFTPC, SFTPA1, SFTPA2. Second category of genes involve acceleration of cellular senescence through disrupted telomerase function like TERT, TERC, DKC1, PARN and RTEL1, while the other category include genes that effect host defense like MUC5B and TOLLIP [12]. However, results from genome-wide association studies (GWAS) found that MUC5B variant is major risk factor accounting for 30-35% of risk in IPF progression [13]. Nongenetic risk factors include life style habits like cigarette smoking that is associated with IPF. It mainly predisposes IPF by over expression of the genes linked to epithelial-mesenchymal transition upon exposure of cigarette smoke to alveolar epithelial cells. Nicotine which is the major component of the cigarette is a potent inducer of TGF-B that mediates fibrosis. It also produce other molecular effects like telomerase shortening, endoplasmic stress and oxidative stress by production of reactive oxygen species via mechanical stretch and impairs regeneration of lung tissue [14]. Certain occupational and environmental exposures are also associated to IPF which include organic dust from livestock, agriculture & farming, metal and mineral dust, wood dust, asbestos and ambient particulate matter [15]. There are drugs that cause pulmonary toxicity and linked to IPF namely antibiotics, anti-inflammatory drugs, antidepressents and cytotoxic drugs like Bleomycin and cyclophosphamide [16]. Among all the risk factors aging is considered as significant and independent risk factor of fibrotic disease [17]. Regardless of the strong association between aging and IPF, few investigations reported that it is due to redox imbalance and oxidative stress [18].

Aging an incentive for IPF

Aging is defined as the unavoidable time-dependent functional decline, distinguished by progressive loss of physiological integrity, reduced homeostatic control and increased susceptibility to environmental challenges and a growing risk of disease and death [19]. The process of aging is not restricted to single cell of the organism but effects different cell types to variable extent with varying impact to overall organism [20]. A series of landmark reports identified nine "hall marks" of aging namely genomic instability, telomere attrition, cellular senescence, stem cell exhaustion, epigenetic alterations, loss of proteases, deregulated and altered intercellular communication [21]. A new hall mark is also identified i.e. ECM dysregulation as shown in figure 1. There are various factors responsible for the dysregulation of ECM such as *de novo* synthesis and ECM deposition induced by profibrotic growth factors and proteolytic degradation of the MMPs and tissue inhibitors of metalloproteinases [2,22,23].

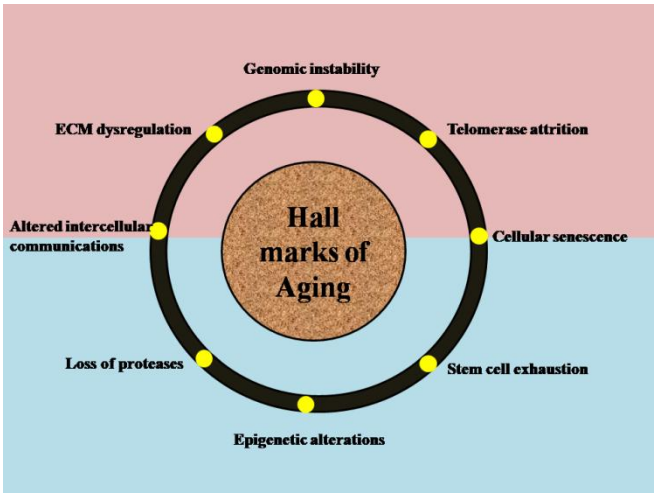

Figure 1: Hall marks of aging

MDM4 gene structure and function

In the year 1980, murine double minute 2 (MDM2) gene was identified as one of the three unknown genes (MDM1-3). Later, the oncogenic potential of MDM2 was demonstrated where it binds to inhibit p53, and the human gene homolog also known as MDM2 or HDM2 was found to amplify in human sarcomas. However, in the mid 90s, a new protein was identified sharing a structural homology with MDM2. It was first named as MDMX and later given the name MDM4. It is also known as MDM4, MDMX, HDM4 or HDMX [24]. It is over expressed in various tumours like lung, colon, stomach and breast cancers [25]. Human MDM2 and MDM4 contain 491 and 490 amino acids respectively with three domains: N-terminal domain is hydrophobic and binds to N-terminal part of p53, Zinc finger domain with an unknown function and a C-terminal RING domain. These two domains contain a central acidic region [26]. The C-terminal RING domains of MDM2 and MDM4 are essential for the formation of hetero and homo dimers [27]. The difference between MDM2 and MDM4 is that the RING domain of MDM2 is essential for its action as an E3-ubiquitin-ligase that targets p53 for ubiquitination and degradation whereas, MDM4 lacks intrinsic ubiquitin-ligase activity [28,29]. MDMX binds to N-terminal transcription activation domain of p53 and inhibits its function as transcription activator.

Binding of MDMX to this domain prevent interaction of p300 resulting in reduced acetylation of p53 and activation of p53. Interestingly, p300 acetylates various lysine residues at the C-terminal region of p53 that are also targeted by Mdm2-mediated ubiquitination which infere that MDMX indirectly stimulates the MDM2-mediated ubiquitination by reducing acetylation of residues [26]. This states that MDM2 mainly regulates p53 stability and that MDM4 has role in regulating p53 activity.

Role of p53 in fibrosis

In the year 1979, p53 (tumour suppressor gene) was first identified with its ability to co-precipitate with the large T Antigen of simian virus 40 (SV40) [30]. The human p53 is localised on chromosome 17 (17p13), composed of 11 exons and 10 introns. It consists of 393 amino acids with four major functional domains namely the N-terminus domain with 1-42 amino acids contains transactivation domain (TAD) and within the central part of p53 is the sequence-specific DNA binding domain containing 102-292 amino acids. The C-terminus portion contains an oligomerisation domain with 323-356 amino acids and a regulatory domain with 360-393 amino acids [31]. The p53 is considered as a "cellular gatekeeper" or "the guardian of the genome" that plays a vital role in normal cell growth, inhibition of malignant tumour growth and regulation of cell cycle [32]. Upon extensive research p53 was found to exhibits its role not only in cancer but also in the regulation of pulmonary fibrosis. Researchers when collected lung tissues of 10 patients with pulmonary fibrosis concluded that nine mutations were found in ten tissue samples and most of these mutations occurred in the central area of the p53 gene [33]. In another study, researchers found that wild type p53 gene is highly expressed in the patients with IPF [34]. Studies have also demonstrated that p53 expression was elevated in lung tissues of mice after the Bleomycin induced pulmonary fibrosis model is established [35]. However, when this Bleomycin is injected intratracheally to WT and p53 deficient mice it was found that lung tissue injury and collagen deposition in p53-deficient mice is significantly reduced compared to that of wild type mice. This suggests that p53 expression inhibition can slow down the progression of pulmonary fibrosis [36].

MDM4-p53 pathway in resolution of pulmonary fibrosis

The lung is an organ with the capacity of resolving fibrotic repair. This process of resolution involves degradation of excessive ECM, removal of myofibroblast and regeneration of normal lung tissue by stem cells.

The p53 expression is initially suppressed and reoccurs in the healing phase, and reaches peak level at the completion of reepithelialisation in wound healing process [37]. However, myofibroblasts that are effectors of tissue fibrosis produce in response to tissue injury and undergo apoptosis at the wound closure [38]. These observations conclude that p53 expression and myofibroblast are inversely correlated during tissue repair after injury. Jing Qu et al. demonstrated that MDM4 is highly expressed in the fibrotic lesions of both human IPF and Bleomycin-induced experimental lung fibrosis in aged mice. This MDM4 acts as a matrix stiffness-regulated negative regulator of p53.

Gain of p53 function activates the gene program that sensitizes lung myofibroblast to apoptosis and promotes efferocytosis of myofibroblasts through recruitment of macrophages through the release of paracrine signal. Destiffening of the fibrotic ECM by targeting non enzymatic glycation cross-linking or genetic ablation of Mdm4 in collagen I-producing myofibroblasts reverses persistent lung fibrosis in aged mice. These observations suggest that mechanosensitive MDM4 is a molecular target having potential against persistent lung fibrosis associated with aging [39].

Conclusion

IPF is a devastating lung disease whose incidence and prevalence increases with aging. Though the course is heterogeneous the median survival rate is only about 3 years after the diagnosis. Currently only two drugs were approved by FDA however they didn't effectively increase the life span of the patients. Hence, it is always essential to identify a newer target for IPF. Researchers have identified a novel molecular target namely MDM4 having therapeutic potential for targeting non-enzymatic AGE-cross linking to resolve persistent lung fibrosis associated with aging. Hence, developing chemical entities targeting MDM4 gene can be a future therapeutic intervention in treating age related IPF.

References

1. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. American journal of respiratory and critical care medicine. 2011 Mar 15; 183(6):788-824.

2. King Jr TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. The Lancet. 2011 Dec 3; 378(9807):1949-61.

3. Habiel DM, Hogaboam C. Heterogeneity in fibroblast proliferation and survival in idiopathic pulmonary fibrosis. Frontiers in pharmacology. 2014 Jan 23; 5:2.

4. Lederer DJ, Martinez FJ. Idiopathic pulmonary fibrosis. New England Journal of Medicine. 2018 May 10; 378(19):1811-23.

5. Oldham JM, Ma SF, Martinez FJ, Anstrom KJ, Raghu G, Schwartz DA, Valenzi E, Witt L, Lee C, Vij R, Huang Y. TOLLIP, MUC5B, and the response to N-acetylcysteine among individuals with idiopathic pulmonary fibrosis. American journal of respiratory and critical care medicine. 2015 Dec 15; 192(12):1475-82.

6. Margaritopoulos GA, Vasarmidi E, Antoniou KM. Pirfenidone in the treatment of idiopathic pulmonary fibrosis: an evidence-based review of its place in therapy. Core Evidence. 2016; 11:11.

7. Raghu G, Selman M. Nintedanib and pirfenidone. New antifibrotic treatments indicated for idiopathic pulmonary fibrosis offer hopes and raises questions. American Journal of Respiratory and Critical Care Medicine, 2015; 191(3): 252-4.

8. Hughes G, Toellner H, Morris H, Leonard C, Chaudhuri N. Real world experiences: pirfenidone and nintedanib are effective and well tolerated treatments for idiopathic pulmonary fibrosis. Journal of clinical medicine. 2016 Sep;5(9):78.

9. Sakai N, Tager AM. Fibrosis of two: Epithelial cell-fibroblast interactions in pulmonary fibrosis. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2013 Jul 1; 1832(7):911-21.

10. Hutchinson JP, McKeever TM, Fogarty AW, Navaratnam V, Hubbard RB. Increasing global mortality from idiopathic pulmonary fibrosis in the twenty-first century. Annals of the American Thoracic Society. 2014 Oct; 11(8):1176-85.

11. Gulati S, Thannickal VJ. The aging lung and idiopathic pulmonary fibrosis. The American journal of the medical sciences. 2019 May 1; 357(5):384-9.

12. Kaur A, Mathai SK, Schwartz DA. Genetics in idiopathic pulmonary fibrosis pathogenesis, prognosis, and treatment. Frontiers in medicine. 2017 Sep 25; 4:154.

13. Evans CM, Fingerlin TE, Schwarz MI, Lynch D, Kurche J, Warg L, Yang IV, Schwartz DA. Idiopathic pulmonary fibrosis: a genetic disease that involves mucociliary dysfunction of the peripheral airways. Physiological reviews. 2016 Oct; 96(4):1567-91.

14. Zaman T, Lee JS. Risk factors for the development of idiopathic pulmonary fibrosis: a review. Current pulmonology reports. 2018 Dec; 7(4):118-25.

15. Trethewey SP, Walters GI. The role of occupational and environmental exposures in the pathogenesis of idiopathic pulmonary fibrosis: A narrative literature review. Medicina. 2018 Dec; 54(6):108.

16. Rosenow III EC, Myers JL, Swensen SJ, Pisani RJ. Drug-induced pulmonary disease: an update. Chest. 1992 Jul 1; 102(1):239-50.

17. Duffield JS, Lupher M, Thannickal VJ, Wynn TA. Host responses in tissue repair and fibrosis. Annual Review of Pathology: Mechanisms of Disease. 2013 Jan 24; 8:241-76.

18. Kurundkar A, Thannickal VJ. Redox mechanisms in age-related lung fibrosis. Redox biology. 2016 Oct 1; 9:67-76.

19. Budinger GS, Kohanski RA, Gan W, Kobor MS, Amaral LA, Armanios M, Kelsey KT, Pardo A, Tuder R, Macian F, Chandel N. The intersection of aging biology and the pathobiology of lung diseases: A joint NHLBI/NIA workshop. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2017 Oct 12; 72(11):1492-500.

20. Jones OR, Scheuerlein A, Salguero-Gomez R, Camarda CG, Schaible R, Casper BB, Dahlgren JP, Ehrlen J, Garcia MB, Menges ES, Quintana-Ascencio PF. Diversity of ageing across the tree of life. Nature. 2014 Jan; 505(7482):169-73.

21. Meiners S, Eickelberg O, Konigshoff M. Hallmarks of the ageing lung. European Respiratory Journal. 2015; 45(3):807-27.

22. Chilosi M, Doglioni C, Murer B, Poletti V. Epithelial stem cell exhaustion in the pathogenesis of idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2010 Jul 1; 27(1):7-18.

23. Laurent GJ. Dynamic state of collagen: pathways of collagen degradation in vivo and their possible role in regulation of collagen mass. American Journal of Physiology-Cell Physiology. 1987 Jan 1; 252(1):C1-9.

24. Toledo F, Wahl GM. MDM2 and MDM4: p53 regulators as targets in anticancer therapy. The international journal of biochemistry & cell biology. 2007 Jul 1; 39(7-8):1476-82.

25. Toledo F, Krummel KA, Lee CJ, Liu CW, Rodewald LW, Tang M, Wahl GM. A mouse p53 mutant lacking the proline-rich domain rescues Mdm4 deficiency and provides insight into the Mdm2-Mdm4-p53 regulatory network. Cancer cell. 2006 Apr 1; 9(4):273-85.

26. Lenos K, Jochemsen AG. Functions of MDMX in the modulation of the p53-response. J Biomed Biotechnol. 2011; 2011: 876173.

27. Sharp DA, Kratowicz SA, Sank MJ, George DL. Stabilization of the MDM2 oncoprotein by interaction with the structurally related MDMX protein. Journal of Biological Chemistry. 1999 Dec 31; 274(53):38189-96.

28. Kawai H, Wiederschain D, Yuan ZM. Critical contribution of the MDM2 acidic domain to p53 ubiquitination. Molecular and cellular biology. 2003 Jul 15; 23(14):4939-47.

29. Meulmeester E, Frenk R, Stad R, De Graaf P, Marine JC, Vousden KH, Jochemsen AG. Critical role for a central part of Mdm2 in the ubiquitylation of p53. Molecular and cellular biology. 2003 Jul 15; 23(14):4929-38.

30. Choisy-Rossi C, Reisdorf P, Yonish-Rouach E. The p53 tumor suppressor gene: structure, function and mechanism of action. Apoptosis: Biology and Mechanisms. 1999; 23:145-72.

31. May P, May E. Twenty years of p53 research: structural and functional aspects of the p53 protein. Oncogene. 1999 Dec; 18(53):7621-36.

32. Kaur RP, Vasudeva K, Kumar R, Munshi A. Role of p53 gene in breast cancer: focus on mutation spectrum and therapeutic strategies. Current pharmaceutical design. 2018 Aug 1; 24(30):3566-75.

33. Hojo S, Fujita J, Yamadori I, Kamei T, Yoshinouchi T, Ohtsuki Y, Okada H, Bandoh S, Yamaji Y, Takahara J, Fukui T. Heterogeneous point mutations of the p53 gene in pulmonary fibrosis. European Respiratory Journal. 1998 Dec 1; 12(6):1404-8.

34. Lok SS, Stewart JP, Kelly BG, Hasleton PS, Egan JJ. Epstein–Barr virus and wild p53 in idiopathic pulmonary fibrosis. Respiratory medicine. 2001 Oct 1; 95(10):787-91.

35. Zaafan MA, Haridy AR, Abdelhamid AM. Amitriptyline attenuates bleomycin-induced pulmonary fibrosis: modulation of the expression of NF-κβ, iNOS, and Nrf2. Naunyn-Schmiedeberg's archives of pharmacology. 2019 Mar; 392(3):279-86.

36. Nagaraja MR, Tiwari N, Shetty SK, Marudamuthu AS, Fan L, Ostrom RS, Fu J, Gopu V, Radhakrishnan V, Idell S, Shetty S. p53 expression in lung fibroblasts is linked to mitigation of fibrotic lung remodeling. The American journal of pathology. 2018 Oct 1; 188(10):2207-22.

37. Antoniades HN, Galanopoulos T, Neville-Golden J, Kiritsy CP, Lynch SE. p53 expression during normal tissue regeneration in response to acute cutaneous injury in swine. The Journal of clinical investigation. 1994 May 1; 93(5):2206-14.

38. Desmouliere A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. The American journal of pathology. 1995 Jan; 146(1):56.

39. Qu J, Yang SZ, Zhu Y, Guo T, Thannickal VJ, Zhou Y. Targeting mechanosensitive MDM4 promotes lung fibrosis resolution in aged mice. Journal of Experimental Medicine. 2021; 218(5): e20202033.