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

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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 categories based on their role in pathogenesis of IPF. The first category of genes is those that affect alveolar stability, mostly genes encoding 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 affect 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 lifestyle 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, antiprotein 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](image)

### 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 homodimers [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 infers 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 mechanosensititive 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.

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