Fibrotic disorders represent a major health problem in the United States, contributing to nearly 45% of deaths each year. Further, the incidence of fibrotic disease continues to rise. Despite numerous drug candidates that have advanced to clinical trials, there have been numerous clinical trial failures and treatment options for fibrotic disorders remain limited. It has become critical to re-evaluate the current paradigm by which treatment options have been developed. Although fibrotic disease predominately affects the elderly population, age-associated pathological mechanisms have not been targeted in the development of treatments for fibrotic disease. Nox4 is a well-validated target for fibrotic disease, and aberrant regulation of Nox4 in aging is associated with pathological fibrosis. Through transdisciplinary approaches, we have identified the first selective and effective small-molecule inhibitors targeting Nox4, which are currently in pre-clinical development as a novel therapeutic for age-associated pathological fibrosis.

Keywords: Fibrosis, drug discovery, Nox4.

1 Introduction

Fibrosis is the clinical term for scar tissue. Fibrosis or “scarring” of vital internal organs is an increasing cause of debilitation and death worldwide. Human fibrotic disorders affect many organ systems including the liver [1-3], skin [4], kidney [5, 6], heart [7, 8], and lung [9-11]. An estimated 45% of deaths in the U.S. are attributed to disorders that are characterized by varying degrees of fibrosis [12]. This alarming statistic is often underappreciated since the ‘cause of death’ is often end-stage organ failure; however, organ failure is often attributed to progressive fibrosis. Further, the incidence of fibrotic disease is increased with advancing age, accounting for a growing “epidemic” of fibrotic disorders in the aging U.S. population. However, there are no available therapies which can ‘reverse’ fibrosis. Despite efforts by numerous groups to develop treatments for fibrotic
disorders, progress has been aggravatingly slow. This paper will discuss some possible explanations for this apparent discrepancy and how transdisciplinary approaches to drug discovery and pre-clinical development may improve successful translation of more effective therapies.

2 Aging, Oxidative Stress, and Fibrosis

Fibrotic disorders represent a major health problem in the U.S. An estimated 45% of deaths in the U.S. are attributed to disorders that are characterized by varying degrees of fibrosis [12], with a rising incidence. This may be in part due to the growing elderly population; progressive fibrosis is a hallmark of aging in various organ systems, including the liver [13], kidney [14], pancreas [15] and lung [16].

The most severe fibrotic lung disease is idiopathic pulmonary fibrosis (IPF), a fatal and relentlessly progressive disorder. IPF affects approximately 200,000 people in the U.S. and five million worldwide. Although two drugs have recently gained FDA-approval for IPF, no drug treatment has been shown to definitively improve quality of life for IPF patients and they have only been shown to delay death by 6 months. The current drugs only moderately slow the progression of lung decline. There are no available therapies which can ‘reverse’ fibrosis. Further, these therapies (both orally administered) are associated with a number of significant and intolerable side-effects. Effective treatments for IPF and other fibrotic diseases are needed in order to improve the patient experience and outcomes.

Aging is a major risk factor for fibrotic disorders. This point is exemplified by IPF, which disproportionately affect the elderly population [17, 18]. IPF is now widely regarded as an age-related disease [19-21]. The incidence and prevalence of IPF increase with age; two-thirds of IPF patients are older than 60 years at the time of presentation with a mean age of 66 years at the time of diagnosis [17]. Further, the survival rate for IPF patients markedly decreases with age [19]. A better understanding of the contribution of aging to the cellular/molecular mechanism(s) involved in the pathogenesis of IPF is sorely needed.

Aging and fibrotic disease are both associated with cumulative oxidant burden, and lung tissue from IPF patients demonstrate “signatures” of chronic oxidative damage [22, 23]. It has been suggested that core pathways that mediate fibrosis in multiple organ systems may serve as better targets for anti-fibrotic drug development [24]; redox imbalance in the context of aging has been suggested to represent one of these core pathways [25]. Despite the well-recognized role of oxidative stress in aging and fibrosis [26], the ability to precisely target key mediators of this process have not been identified or developed. Anti-oxidant strategies have failed in clinical trials [27]. Strategies which directly target the source(s) of reactive oxygen species (ROS) generation are more likely to be more specific and effective in comparison to antioxidant interventions.

3 Development of Age-relevant Animal Models for Pre-clinical Testing

One major limitation in the pulmonary fibrosis scientific field is the lack of animal models that reliably predict therapeutic efficacy of agents in clinical trials [28, 29]. The most widely used murine model utilizes bleomycin to induce lung fibrosis. However, despite promising pre-clinical efficacy of numerous therapeutic agents using this animal model (>240 experimental drugs evaluated), clinical translation has been poor [28]. Thus, the use of this model for pre-clinical evaluation of drug candidates has been questioned. One potential reason for the lack of clinical translation is not the model per se, but the failure to account for the widely acknowledged concept that IPF is an age-related disease [19-21]. Pre-clinical animal models of lung fibrosis are largely employed in young rodents (8-12 weeks); in this model, injury-induced fibrosis is self-limited, with resolution of injury [30]. Thus, pre-clinical treatment interventions employed are largely preventative (dosing before or at the time of injury), rather than curative [28]. Although this model is a tremendously useful tool for identifying therapeutic targets involved in fibrotic responses to lung injury, its utility as a pre-clinical efficacy model has proven to be substandard.

Previous studies in our lab evaluated reparative responses to lung injury in young (2m) and aged (18m) mice. Mice were administered intra-tracheal bleomycin, and sacrificed at 0d (no injury), 3w, 2m, and 4m post-injury. Our studies revealed that severity of fibrosis at 3w post-injury (the peak fibrotic
phase) was similar in both young and aged mice. However, young mice demonstrate significant resolution of fibrotic injury (~60% resolution at 4m post-injury), whereas aged mice exhibited an impaired capacity to resolve fibrosis (with little to no resolution of fibrosis at 4m post-injury) [10]. The resolving nature of bleomycin-induced lung injury in young mice supports reversibility of fibrosis – a property that is lost with aging.

An age-relevant model offers the following advantages over the current prevailing pre-clinical model:

1. Fibrosis that better mimics the persistent/progressive nature of fibrosis seen in IPF patients.
2. A more representative animal model for a disease that predominantly affects the elderly population.
3. The ability to implement more clinically relevant testing protocols (i.e. determine the effect of a drug candidate on reversibility of established/persistent fibrosis).
4. It permits long-term examination of physiological parameters (i.e. survival, body weight, lung compliance) which are more appropriate indicators of the potential for therapeutic success.

Overall, the use of this model in pre-clinical efficacy approaches is more likely to result in improved accuracy of predicting therapeutic potential in clinical trials, and may prevent time-consuming, costly, and ultimately unsuccessful clinical trials.

4 Novel Pro-fibrotic Mechanisms in Age-associated Pathological Fibrosis

The pathogenesis of IPF remains poorly understood. Fibro-proliferation has been implicated as a key mechanism for the persistence (vs. initiation) of fibrosis; however this is inconsistent with the clinical observation of an increased risk of fibrotic disease with advancing age. Recent studies from our group (using the aged mouse model described above) offer new insight into how aging leads to a predisposition to fibrosis.

Our group was the first to identify a novel role for the oxidant generating enzyme, NADPH oxidase-4 (Nox4), in mediating lung fibrosis (2009); since then, Nox4 has also been implicated in a variety of fibrotic diseases, including the liver, skin, kidney, and heart. In resolving fibrosis in young mice, lung myofibroblasts (the key ‘scar tissue generating’ cells) eventually undergo apoptosis (programed cell death) to promote healing. In contrast, in aged mice with non-resolving fibrosis, myofibroblasts acquire a senescent and apoptosis-resistant phenotype, which contributes to myofibroblast accumulation and ultimately persistent fibrosis. Specifically, age-dependent alterations in Nox4 results in a sustained redox imbalance, which promotes senescence and apoptosis-resistance of myofibroblasts [10]. Thus, the ultimate fate of these normally reparative cells is altered in the context of aging, where they acquire an apoptosis-resistant phenotype that contributes to the persistence of fibrosis (whereas apoptosis of these cells is a hallmark of fibrosis resolution). In support of this concept, we have also demonstrated that human IPF lung myofibroblasts are predominantly non-proliferative and demonstrate features of senescence and apoptosis-resistance [10]. Importantly, we demonstrated that Nox4 mediates senescence and apoptosis in vitro, and that therapeutic targeting of Nox4 in an aging model of persistent fibrosis resulted in decreased senescence and susceptibility to apoptosis in vivo [10]. This previously unknown pro-fibrotic mechanism may help to explain why IPF develops more frequently in older individuals. However, therapeutic targeting of age-associated pathologic mechanisms in the development of IPF treatments remains unexploited.

5 Selective Nox4 inhibitors have not been previously identified

Although Nox4 is considered to be among the most promising targets for fibrotic disease, no selective Nox4 inhibitors are clinically available. Nox4 drug development has proved challenging for several reasons. The crystal structure of Nox4 is not known, which precludes traditional rational drug design approaches. Further, screening methods for Nox inhibitors typically utilize ROS detection-based screening assays that have limited specificity. Thus, it may be difficult to discern whether a putative inhibitor is acting directly on Nox versus inhibition of a signaling pathway(s). One study reported that of >350 ‘Nox inhibitors’ described, a majority of
these did not directly block Nox enzymatic activity, but rather they showed interference with upstream signaling pathways or demonstrated ROS scavenger activity [31]. Genkyotex (Geneva, Switzerland) is developing a candidate Nox1/4 inhibitor (GKT137831) for diabetic nephropathy. However, selectivity of GKT137831 for Nox4 is low relative to other Nox isoforms; it has been reported to inhibit Nox4 (82%), Nox1 (86%), and Nox2 (60%) [32]. There are industry concerns regarding the specificity of this compound for drug development aimed at fibrotic disorders; particularly since Nox2 plays well-described roles in inflammation, and anti-inflammatory strategies have been shown to lead to worse outcomes for IPF patients [33]. Identification of small-molecule inhibitors that selectively target Nox4 has been a major challenge.

6 Transdisciplinary Approaches to Drug Discovery and Pre-clinical Development

Despite efforts by numerous groups to develop IPF treatments, progress has been aggravatingly slow. We offer two possible explanations for this discrepancy:

1. Although IPF is widely regarded as an age-related disease, drug treatments have not targeted age-associated pathologic mechanisms of IPF, and 
2. current pre-clinical animal models fail to reliably predict the success of drug candidates in human clinical trials. We believe that transdisciplinary approaches to drug discovery and pre-clinical development are critical to the development of effective therapies for IPF (i.e. age-relevant animal models and therapeutic targeting of age-associated pathological mechanisms).

Given the accumulating data on Nox4 as a “core pathway” in diverse fibrotic disorders, the search for safe, specific and effective Nox4 inhibitors continues. An overall goal of our laboratory is to identify a lead drug candidate that is highly selective and effective in inhibiting Nox4, with favorable pharmacokinetic/pharmacodynamic properties for subsequent clinical development as a therapy for IPF. We have screened over 30,000 compounds and through our medicinal chemistry and hit-expansion efforts, we have identified 2 novel classes of small-molecule inhibitors that are highly effective and selective in inhibiting Nox4. Our biophysical characterization studies demonstrate favorable qualities of our leads for subsequent clinical development. Two U.S. patent applications have been filed. Pre-clinical development of these novel inhibitors remains ongoing in our laboratory. Current and future success of our mission is highly dependent on the transdisciplinary nature of our team, including researchers, physicians, collaborators, consultants, and an advisory board with specialized expertise in key areas (intellectual property, commercialization, successful clinical translation, and patient advocacy).

7 Conclusion

It has been suggested that core pathways that mediate fibrosis in multiple organ systems may serve as better targets for anti-fibrotic drug development [24]; Redox imbalance in the context of aging has recently been highlighted as one of these core pathways [25]. However, despite the well-recognized role of oxidative stress in fibrosis and aging, the ability to precisely target key mediators of this process has proved difficult. Nox4 represents a well-validated therapeutic target for fibrotic disease. Further, recent studies support an age-associated defect in Nox4 regulation, thus targeting of Nox4 represents a plausible strategy for age-associated pathological fibrosis. However, despite the identification of this well-validated target, the successful clinical translation of treatments for fibrotic disease will require transdisciplinary approaches to drug discovery and pre-clinical development.

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References


About the Author

Dr. Hecker’s broad research background and training has been rooted in regenerative biology, with experience in development, tissue engineering, and mechanisms of injury-repair. Her research team previously identified a novel role for NADPH oxidase-4 (Nox4), an oxidant-generating enzyme, in mediating myofibroblast functions and scar tissue formation (fibrosis), published in Nature Medicine. Since this discovery, her research interests have expanded to include understanding the role of aging/senescence in lung injury-repair responses. Dr. Hecker’s current research interests also encompass translational aspects, including drug discovery for Nox4 and the development of preclinical animal models of acute lung injury and fibrosis.