

A Passion for Scleroderma Research

Galina S Bogatkevich*

Galina S Bogatkevich, Medical University of South Carolina, USA

My research experience in the field of scleroderma began in year 2000 when I was recruited as a postdoctoral fellow to the laboratory of Dr. Richard M. Silver. Since that time my research interests has focused on understanding the cellular and molecular mechanisms of pulmonary fibrosis in scleroderma patients. The ongoing research in the laboratory can be subdivided in three major projects: The first is thrombin signaling in scleroderma lung disease, the second is CTGF-interacting proteins in scleroderma-associated pulmonary fibrosis, and the third project concentrates on the role of genome in scleroderma.

Scleroderma (Systemic Sclerosis, SSc) is an autoimmune rheumatic disease, which affects about 300,000 Americans, primarily females who are 30 to 50 years old at onset. The leading cause of death in scleroderma patients is pulmonary dysfunction as a result of progressive interstitial lung fibrosis. The pathophysiology of pulmonary fibrosis is characterized by differentiation of fibroblasts into activated myofibroblasts, and enhanced synthesis and secretion of extracellular matrix (ECM) molecules by these cells. However, the mechanisms leading to the lung fibroblast activation and excessive deposition of collagen and other ECM proteins remain poorly understood.

In recent years, increasing evidence has accumulated to implicate involvement of the coagulation system in various fibrotic diseases, including SSc-ILD [1]. Activation of coagulation proteases, e.g. thrombin, is one of the earliest events following tissue injury. Thrombin modulates tissue repair responses by altering vascular permeability, stimulating fibroblast and neutrophil migration, and promoting adhesion and spreading of endothelial cells and fibroblasts [2]. It also activates various cell types and induces secretion of several pro-immune, profibrotic, and angiogenic factors [1-3]. Activation of these cells by thrombin is a likely mechanism for the development and progression of pulmonary fibrosis in general, and SSc-ILD in particular.

Our laboratory as well as others has demonstrated dramatically increased levels of thrombin in Broncho alveolar Lavage Fluid (BALF) from scleroderma patients with lung fibrosis and other fibrosing lung diseases [4,5]. It has been shown that BALF from normal subjects has a low level of thrombin activity, while BALF from SSc patients express up to 100-fold higher thrombin activity [4]. Elevated levels of thrombin activity have been also observed in bleomycin-induced pulmonary fibrosis in mice [6]. The majority of the cellular responses to thrombin are mediated via the G protein-coupled protease activated receptor-1 (PAR-1) [2,7]. In previous studies we demonstrated that PAR-1 expression is significantly increased in patients with SSc-ILD, notably in lung parenchyma associated with inflammatory and fibroproliferative foci [8]. PAR-1 is co-localized with myofibroblasts in SSc-ILD tissue and appears to decrease during later stages of pulmonary fibrosis when a decreased number of myofibroblasts is observed. Elevated expression of PAR-1 has been also shown in patients with IPF and in a murine model of bleomycin-induced lung fibrosis [1,6].

Previously, we demonstrated that thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype via the PAR-1 receptor and a protein kinase C-dependent pathway [9]. Thrombin is mitogenic for lung fibroblasts [4,8,9] and enhances the proliferative effect of fibrinogen on fibroblasts [10]. Thrombin is also a potent inducer of fibrogenic cytokines, such as transforming growth factor- β (TGF- β) [11] and connective tissue growth factor (CTGF) [12,13]. We recently showed that all this cellular effects of thrombin can be inhibited

by dabigatran *in vitro* and *in vivo* [14,15]. Dabigatran is a direct thrombin inhibitor that reversibly binds to the active site of thrombin preventing the conversion of fibrinogen to fibrin [16]. Recently, we have demonstrated that binding of dabigatran to thrombin prevents cleavage of the extracellular N-terminal domain of the PAR-1 receptor [14]. In the absence of dabigatran, thrombin binds to PAR-1, cleaves the peptide bond between residues Arg-41 and Ser-42, thereby unmasking a new amino terminus, SFLLRN, which then can bind to the second extracellular loop of PAR-1 and initiate receptor signaling [7,17]. Dabigatran-bound thrombin is unable to cleave and activate PAR-1 [14]. Further, we have shown that dabigatran inhibits thrombin-induced *in vitro* differentiation of normal lung fibroblasts to the myofibroblast phenotype and decreases CTGF, α -SMA, and collagen type I in scleroderma lung fibroblasts [14]. To test whether dabigatran attenuates lung injury in a murine model of SSc-ILD, we used dabigatran etexilate, the oral prodrug of dabigatran. The prodrug does not have antithrombin activity; however, after oral administration dabigatran etexilate is rapidly converted by ubiquitous esterases to the active moiety, dabigatran [16,18]. We observed that dabigatran etexilate significantly reduced thrombin activity and levels of TGF- β 1 in BALF, while simultaneously decreasing inflammatory cells and protein concentrations in BALF [15]. Histological lung inflammation and fibrosis were significantly decreased in dabigatran etexilate-treated mice. Additionally, treatment with dabigatran etexilate reduced collagen, CTGF, and α -SMA expression in mice with bleomycin-induced lung fibrosis, whereas it had no effect on basal levels of these proteins [15]. This data suggest that dabigatran etexilate may serve as a novel and attractive therapeutic agent for SSc-ILD especially for patients with over expression of thrombin.

Fibroblasts isolated from SSc patients, also called activated fibroblasts or myofibroblasts, continue *in vitro* to generate the excess of ECM proteins and to produce various pro-inflammatory and profibrotic mediators [19]. One of the major cytokines constitutively elevated in scleroderma fibroblasts *in vivo* and *in vitro* is CTGF [13, 20,21]. Fonseca et al. [22] recently demonstrated a direct genetic association between CTGF and SSc, identifying a CTGF polymorphism within a novel SP3-dependent transcriptional repressor site that is significantly associated with the susceptibility to SSc [22]. Increased amounts of CTGF are found in scleroderma patients with more extensive skin involvement and severe pulmonary fibrosis [23]. In addition to increased tissue expression, CTGF levels are increased in the sera of patients with SSc compared with healthy controls and patients with other connective tissue diseases; also, patients with diffuse scleroderma exhibit higher levels of CTGF than those with the limited form of the disease [23]. Furthermore, broncho alveolar lavage fluid

*Corresponding author: Galina S Bogatkevich, Division of Rheumatology & Immunology, Medical University of South Carolina, USA, Tel: 843-792-7631; Fax: 843-792-7121; E-mail: bogatkev@musc.edu

Received May 25, 2012; Accepted May 28, 2012; Published May 30, 2012

Citation: Bogatkevich GS (2012) A Passion for Scleroderma Research. Rheumatol Curr Res 2:e109. doi:10.4172/2161-1149.1000e109

Copyright: © 2012 Bogatkevich GS. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

(BALF) from SSc patients with active lung fibrosis was found to contain much higher levels of CTGF as compared with patients without lung involvement [21].

Profibrotic properties of CTGF have been lately well established. It was shown that CTGF promotes deposition of several ECM proteins such as collagen, fibronectin, and tenascin C [20,24,25]. Using CTGF-specific small interfering RNA (siRNA), Xiao et al. [26] found that silencing of CTGF significantly decreases expression of collagen in SSc skin fibroblasts. In addition, CTGF was demonstrated to promote fibroblast proliferation, collagen gel contraction, cell adhesion and migration [13,20,25]. We recently identified a novel CTGF-induced protein, IQ motif containing GTPase activating protein-1 (IQGAP1), which is inherently elevated in lung fibroblasts isolated from SSc patients [27]. We demonstrated that expression of IQGAP1 in SSc lung fibroblasts correlates with the migration rate of cells in a wound healing assay and that IQGAP1 mediates cell migration induced by CTGF in normal lung fibroblasts [27]. The studies to understand the role of IQGAP1 in the pathogenesis of SSc-ILD are currently under progress in our laboratory.

The prognosis for pulmonary outcomes is much poorer and the mortality rate among African Americans with SSc is twice as high when compared with whites [28]. Such racial disparities in health may be explained to some extent by the effects of differences in health behaviors, socioeconomic status, and access to health care and biological factors. To understand the role of genome in scleroderma, one of our projects is focused specifically on hepatocyte growth factor (HGF) receptor, a genetic factor, which we have identified, that impacts upon the molecular mechanisms regulating the ability of HGF to control the expression of ECM proteins, such as collagen and CTGF, in the lung challenged by rheumatic disease.

We recently published the first study to demonstrate an association between deficiency of HGF and cellular mesenchymal-epithelial transition factor (c-MET) signaling and African American race. Our results demonstrated that HGF is reduced in BALF and plasma from African American SSc-ILD patients compared with white SSc-ILD patients. Moreover, in white SSc lung fibroblasts HGF down regulates extracellular matrix proteins, such as collagen and CTGF, whereas in African American SSc fibroblasts HGF is not functional due to a deficiency in phosphorylation of the HGF receptor [29].

The role of HGF as a potent anti-fibrotic factor has been established in several animal models of fibrosis [30,31]. However, the therapeutic potential of HGF for the treatment of fibrosis in humans has yet to be explored. Several recent *in vitro* studies have characterized the anti-fibrotic effects of HGF on collagen, MMP-1, and CTGF in SSc skin fibroblasts [32,33], whereas our lab has demonstrated the mechanisms by which HGF down regulates collagen and CTGF in human lung fibroblasts [34]. The studies to validate the genetic alteration in the c-MET gene and to determine the correlations between HGF receptor loss or reduced function and the severity of SSc-ILD among African American and white scleroderma patients are under progress.

SSc-ILD is the most serious complication and a leading cause of mortality in scleroderma for which no proven therapy currently exists. Our laboratory, like hundreds other around the world, works hard toward the understanding of etiology and pathogenesis of this disease that will ultimately lead us to the development of novel, effective therapies. It is possible that one of the potential novel therapeutics for SSc-ILD will become dabigatran etexilate which has already demonstrated profound antifibrotic effects *in vitro* and *in vivo*.

References

- Ludwicka-Bradley A, Bogatkevich G, Silver RM (2004) Thrombin-mediated cellular events in pulmonary fibrosis associated with systemic sclerosis (scleroderma). *Clin Exp Rheumatol* 22: S38-S46.
- Howell DC, Laurent GJ, Chambers RC (2002) Role of thrombin and its major cellular receptor, protease-activated receptor-1, in pulmonary fibrosis. *Biochem Soc Trans* 30: 211-216.
- Chambers RC (2008) Procoagulant signalling mechanisms in lung inflammation and fibrosis: novel opportunities for pharmacological intervention? *Br J Pharmacol* 153: S367-S378.
- Ohba T, McDonald JK, Silver RM, Strange C, LeRoy EC, et al. (1994) Scleroderma bronchoalveolar lavage fluid contains thrombin, a mediator of human lung fibroblast proliferation via induction of platelet-derived growth factor alpha-receptor. *Am J Respir Cell Mol Biol* 10: 405-412.
- Hernández-Rodríguez NA, Cambrey AD, Harrison NK, Chambers RC, Gray AJ, et al. (1995) Role of thrombin in pulmonary fibrosis. *Lancet* 346: 1071-1073.
- Howell DC, Johns RH, Lasky JA, Shan B, Scotton CJ, et al. (2005) Absence of proteinase-activated receptor-1 signaling affords protection from bleomycin-induced lung inflammation and fibrosis. *Am J Pathol* 166: 1353-1365.
- Coughlin SR (1999) How the protease thrombin talks to cells. *Proc Natl Acad Sci U S A* 96: 11023-11027.
- Bogatkevich GS, Gustilo E, Oates JC, Feghali-Bostwick C, Harley RA, et al. (2005) Distinct PKC isoforms mediate cell survival and DNA synthesis in thrombin-induced myofibroblasts. *Am J Physiol Lung Cell Mol Physiol* 288: L190-L201.
- Bogatkevich GS, Tourkina E, Silver RM, Ludwicka-Bradley A (2001) Thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype via the proteolytically activated receptor-1 and a protein kinase C-dependent pathway. *J Biol Chem* 276: 45184-45192.
- Gray AJ, Bishop JE, Reeves JT, Laurent GJ (1993) A alpha and B beta chains of fibrinogen stimulate proliferation of human fibroblasts. *J Cell Sci* 104: 409-413.
- Bachhuber BG, Sarembock IJ, Gimble LW, Owens GK (1997) alpha-Thrombin induces transforming growth factor-beta1 mRNA and protein in cultured vascular smooth muscle cells via a proteolytically activated receptor. *J Vasc Res* 34: 41-48.
- Chambers RC, Leoni P, Blanc-Brude OP, Wembridge DE, Laurent GJ (2000) Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1. *J Biol Chem* 275: 35584-35591.
- Bogatkevich GS, Ludwicka-Bradley A, Nietert PJ, Silver RM (2006) Scleroderma Lung Fibroblasts: Contractility and Connective Tissue Growth Factor. In *Myofibroblasts* (Gabbiani G, Chaponnier C, Desmouliere A, eds), Landes Bioscience, Georgetown, TX 25-31.
- Bogatkevich GS, Ludwicka-Bradley A, Silver RM (2009) Dabigatran, a direct thrombin inhibitor, demonstrates antifibrotic effects on lung fibroblasts. *Arthritis Rheum* 60: 3455-3464.
- Bogatkevich GS, Ludwicka-Bradley A, Nietert PJ, Akter T, van Ryn J, et al. (2011) Anti-inflammatory and anti-fibrotic effects of the oral direct thrombin inhibitor dabigatran etexilate in a murine model of interstitial lung disease. *Arthritis Rheum* 63: 1416-1425.
- Sorbera LA, Bozzo J, Castaner J (2005) Dabigatran/ Dabigatran etexilate. *Drugs Fut* 30: 877.
- Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R (2001) Proteinase-activated receptors. *Pharmacol Rev* 53: 245-282.
- Wienen W, Stassen JM, Priepke H, Ries UJ, Huel N (2007) In-vitro profile and ex-vivo anticoagulant activity of the direct thrombin inhibitor dabigatran and its orally active prodrug, dabigatran etexilate. *Thromb Haemost* 98: 155-162.
- LeRoy EC (1974) Increased collagen synthesis by scleroderma skin fibroblasts in vitro: a possible defect in the regulation or activation of the scleroderma fibroblast. *J Clin Invest* 54: 880-889.
- Leask A, Holmes A, Abraham DJ (2002) Connective tissue growth factor: a new and important player in the pathogenesis of fibrosis. *Curr Rheumatol Rep* 4: 136-142.
- Shi-wen X, Pennington D, Holmes A, Leask A, Bradham D, et al. (2000)

- Autocrine overexpression of CTGF maintains fibrosis: RDA analysis of fibrosis genes in systemic sclerosis. *Exp Cell Res* 259: 213-224.
22. Fonseca C, Lindahl GE, Ponticos M, Sestini P, Renzoni EA, et al. (2007) A polymorphism in the CTGF promoter region associated with systemic sclerosis. *N Engl J Med* 357: 1210-1220.
 23. Sato S, Nagaoka T, Hasegawa M, Tamatani T, Nakanishi T, et al. (2000) Serum levels of connective tissue growth factor are elevated in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. *J Rheumatol* 27: 149-154.
 24. Gore-Hyer E, Shegogue D, Markiewicz M, Lo S, Hazen-Martin D, et al. (2002) TGF-beta and CTGF have overlapping and distinct fibrogenic effects on human renal cells. *Am J Physiol Renal Physiol* 283: F707-F716.
 25. Grotendorst GR, Rahmanie H, Duncan MR (2004) Combinatorial signaling pathways determine fibroblast proliferation and myofibroblast differentiation. *FASEB J* 18: 469-479.
 26. Xiao R, Liu FY, Luo JY, Yang XJ, Wen HQ, et al. (2006) Effect of small interfering RNA on the expression of connective tissue growth factor and type I and III collagen in skin fibroblasts of patients with systemic sclerosis. *Br J Dermatol* 155: 1145-1153.
 27. Bogatkevich GS, Ludwicka-Bradley A, Singleton CB, Bethard JR, Silver RM (2008) Proteomic analysis of CTGF-activated lung fibroblasts: identification of IQGAP1 as a key player in lung fibroblast migration. *Am J Physiol Lung Cell Mol Physiol* 295: L603-L611.
 28. Nietert PJ, Mitchell HC, Bolster MB, Shaftman SR, Tilley BC, et al. (2006) Racial variation in clinical and immunological manifestations of systemic sclerosis. *J Rheumatol* 33: 263-268.
 29. Bogatkevich GS, Ludwicka-Bradley A, Highland KB, Hant F, Nietert PJ, et al. (2007) Impairment of the antifibrotic effect of hepatocyte growth factor in lung fibroblasts from African Americans: possible role in systemic sclerosis. *Arthritis Rheum* 56: 2432-2442.
 30. Dai C, Liu Y (2004) Hepatocyte growth factor antagonizes the profibrotic action of TGF-beta1 in mesangial cells by stabilizing Smad transcriptional corepressor TGIF. *J Am Soc Nephrol* 15: 1402-1412.
 31. Mizuno S, Matsumoto K, Li MY, Nakamura T (2005) HGF reduces advancing lung fibrosis in mice: a potential role for MMP-dependent myofibroblast apoptosis. *FASEB J* 19: 580-582.
 32. Jinnin M, Ihn H, Mimura Y, Asano Y, Yamane K, et al. (2005) Effects of hepatocyte growth factor on the expression of type I collagen and matrix metalloproteinase-1 in normal and scleroderma dermal fibroblasts. *J Invest Dermatol* 124: 324-330.
 33. Sherriff-Tadano R, Ohta A, Morito F, Mitamura M, Haruta Y, et al. (2006) Antifibrotic effects of hepatocyte growth factor on scleroderma fibroblasts and analysis of its mechanism. *Mod Rheumatol* 16: 364-371.
 34. Bogatkevich GS, Ludwicka-Bradley A, Highland KB, Hant F, Nietert PJ, et al. (2007) Down-regulation of collagen and connective tissue growth factor expression with hepatocyte growth factor in lung fibroblasts from white scleroderma patients via two signaling pathways. *Arthritis Rheum* 56: 3468-3477.