Egr-ly awaiting a “personalized medicine” approach to treat scleroderma

Andrew Leask

Received: 23 January 2012 / Accepted: 24 January 2012 / Published online: 15 February 2012
© The International CCN Society 2012

Abstract Scleroderma, an autoimmune disorder characterized by skin and organ fibrosis, has no treatment. Although over the past decade valuable insights into the molecular mechanisms underlying scleroderma have been generated, results in clinical trials have been disappointing. This issue is likely to arise due to the heterogeneity of scleroderma. Molecular insights into the heterogeneity of this disease have been provided by genome-wide expression profiling. In a recent paper, Bhattacharyya and colleagues (PLOS One 6:e23082, 2011b) to show that the overexpression of a range of “fibroproliferative” genes in diffuse cutaneous scleroderma patients are likely to be caused by the overexpression of transcription factor Early growth response (Egr)-1. Only a minority of Egr-1-regulated genes were also found to be regulated by TGF- β . Moreover, Greenblatt and colleagues (Am J Pathol., 2012) have shown that the overexpression of “inflammatory” genes overexpressed in “localized” scleroderma and a small subset of limited and diffuse scleroderma patients is likely to be due to the activity of interleukin-13 (IL-13). Intriguingly, at a gene expression level, murine sclerodermatous graft-versus-host disease (scGVHD) approximates this inflammatory subset of scleroderma. These data suggest that targeting Egr-1 expression/activity might be a novel therapeutic strategy to control fibrosis in a subset of diffuse scleroderma patients, and further emphasize that notion that elevated canonical TGF β signaling is insufficient to explain the fibrosis observed in scleroderma. Moreover, targeting IL-13 expression/activity might be a novel therapeutic strategy to target the inflammation leading to “localized” scleroderma.

Keywords Scleroderma · egr-1 · Graft versus host · IL-13 · Fibrosis

Scleroderma is a multisystem autoimmune disease that is characterized by the presence of auto-antibodies, vascular damage, and fibrosis (Abraham et al. 2009). A two-disease subset model of the systemic form of scleroderma (systemic sclerosis, SSc), consisting of limited and diffuse scleroderma (lSSc and dSSc, respectively) based on the extent of cutaneous involvement, is commonly used today (LeRoy and Medsger 2001). Localized scleroderma (LS) (also called morphea) affects mainly children and is restricted to a specific area. Unlike SSc, LS appears to have histopathologic similarities to chronic graft-vs-host disease (cGVHD) (Fleming et al. 2009; Zulian 2008).

Research conducted in the past decade has led to a much better appreciation of the pathogenic mechanisms underlying scleroderma. For example, both non-canonical transforming growth factor (TGF) β and endothelin-1 signaling is important for the activated fibrotic phenotype of the myofibroblasts found in the lesional areas of diffuse SSc patients (Leask 2008). However, translating this progress into positive results in clinical trials have proved to be difficult. The literature is filled with anecdotal observations that certain antifibrotic compounds are effective in isolated individuals; however, data often conflicts among different laboratories, and the results of randomly controlled clinical trials have been disappointing (Denton et al. 2007; Giordano et al. 2010; Seibold et al. 2010; Simms and Lafyatis 2010). A possibility for why large clinical trials have not yielded positive results may be due to the fact that SSc is a heterogeneous disease; clinical manifestations of SSc vary from patient to patient. This heterogeneity is likely to be masked by grouping these patients into the same clinical trial. That is, definitive conclusions regarding the

A. Leask (✉)
Departments of Dentistry and Physiology and Pharmacology,
Dental Sciences Building, University of Western Ontario,
London, ON, Canada N6A 5C1
e-mail: Andrew.leask@schulich.uwo.ca

efficacy of particular drugs cannot be made unless patient heterogeneity is considered.

Genome-wide expression profiling, which allows the examination of tens of thousands of genes at one time, is ideally suited for capturing the heterogeneity of scleroderma at a molecular level. This technique has been used by Whitfield and collaborators to show that the skin of SSc patients possess a gene expression profile that is reproducibly different than that of the skin of healthy individuals. Given that SSc is by definition systemic, it was not surprising that lesional and non-lesional skin possesses essentially identical gene expression profiles (Whitfield et al. 2003).

Whitfield and collaborators went on to use functional cluster analysis to further subcategorize the gene expression profile of SSc patients' skin. Healthy control skin (and some dSSc and lSSc patients) possessed a "normal" gene expression profile. LS and a small subset of lSSc and dSSc patients possessed an "inflammatory" signature. Finally, dSSc patients possessed a "diffuse-proliferation" (fibroproliferative) gene expression profile (Milano et al. 2008). As might be expected, given the notion that TGF β signaling and its downstream mediator endothelin-1 contribute to the fibrosis observed in dSSc (Leask 2008), a subset of genes in the "diffuse-proliferation" corresponded to a gene expression signature that was found in normal fibroblasts treated with TGF β ("TGF β -responsive" signature) (Sargent et al. 2010; Greenblatt et al. 2012).

In a recent study, the expression profile of dSSc patients was characterized further. The transcription factor early growth response (Egr)-1 appears to be important for fibrosis. Egr-1 is upregulated in fibrotic conditions including SSc and is required for bleomycin-induced fibrosis (Bhattacharyya et al. 2011a). When overexpressed in human dermal fibroblasts, Egr-1 elicited the overexpression of a set of genes that this 'Egr-responsive' signature corresponded with a group of genes also found in the "diffuse-proliferation" group of dSSc patients (Bhattacharyya et al. 2011b). Intriguingly, this profile was distinct from the 'TGF β -responsive' signature that was revealed by microarray analysis (Bhattacharyya et al. 2011b). These data provide yet another illustration that canonical TGF β signaling is insufficient to explain the SSc phenotype (Holmes et al. 2001; Ishida et al. 2006).

Greenblatt and colleagues (2012) also used genome-wide expression profiling to show that murine sclerodermatous graft-versus-host disease (scIGVHD) approximates the "inflammatory" expression signature that is especially important in LS. These two subsets showed IL-13 cytokine pathway activation. Of the genes coordinately up-regulated in scIGVHD, the human inflammatory subset, and IL-13-treated fibroblasts, the chemokine CCL2 emerged. Finally, anti-CCL2 antibodies prevented scIGVHD. Collectively these data suggest that an inflammatory subset of scleroderma (most notably LS patients) is driven by IL-13.

These observations illustrate the heterogeneity of SSc at a molecular level and suggest that targeting Egr-1 expression or activity might be a novel therapeutic strategy to control fibrosis in specific SSc subsets. Blockade of IL-13/CCL2 may be important especially in LS. These results point to the potential usefulness of a 'personalized medicine' approach to treat SSc.

References

- Abraham DJ, Krieg T, Distler J, Distler O (2009) Overview of pathogenesis of systemic sclerosis. *Rheumatology (Oxford)* 48(Suppl 3):iii3–iii7
- Bhattacharyya S, Wu M, Fang F, Tourtellotte W, Feghali-Bostwick C, Varga J (2011a) Early growth response transcription factors: key mediators of fibrosis and novel targets for anti-fibrotic therapy. *Matrix Biol* 30:235–242
- Bhattacharyya S, Sargent JL, Du P, Lin S, Tourtellotte WG, Takehara K, Whitfield ML, Varga J (2011b) Egr-1 induces a profibrotic injury/repair gene program associated with systemic sclerosis. *PLoS One* 6:e23082
- Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, Silliman N, Streisand J, Powell J, Akesson A, Coppock J, Hoogen F, Herrick A, Mayes MD, Veale D, Haas J, Ledbetter S, Korn JH, Black CM, Seibold JR, Cat-192 Study Group, Scleroderma Clinical Trials Consortium (2007) Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis Rheum* 56:323–333
- Fleming JN, Shulman HM, Nash RA, Johnson PY, Wight TN, Gown A, Schwartz SM (2009) Cutaneous chronic graft-versus-host disease does not have the abnormal endothelial phenotype or vascular rarefaction characteristic of systemic sclerosis. *PLoS One* 4:e6203
- Giordano N, Puccetti L, Papakostas P, Di Pietra N, Bruni F, Pasqui AL, Acampa M, Bocchi V, Donati V, Voglino M, Fioravanti A, Montella A, Auteri A, Nuti R, Pastorelli M (2010) Bosentan treatment for Raynauds phenomenon and skin fibrosis in patients with Systemic Sclerosis and pulmonary arterial hypertension: an open-label, observational, retrospective study. *Int J Immunopathol Pharmacol* 23:1185–1194
- Greenblatt MB, Sargent JL, Farina G, Tsang K, Lafyatis R, Glimcher LH, Whitfield ML, Aliprantis AO (2012) Interspecies Comparison of Human and Murine Scleroderma Reveals IL-13 and CCL2 as Disease Subset-Specific Targets. *Am J Pathol*. doi:10.1016/j.ajpath.2011.11.024
- Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A (2001) CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. *J Biol Chem* 276:10594–10601
- Ishida W, Mori Y, Lakos G, Sun L, Shan F, Bowes S, Josiah S, Lee WC, Singh J, Ling LE, Varga J (2006) Intracellular TGF-beta receptor blockade abrogates Smad-dependent fibroblast activation in vitro and in vivo. *J Invest Dermatol* 126:1733–1744
- Leask A (2008) Targeting the TGFbeta, endothelin-1 and CCN2 axis to combat fibrosis in scleroderma. *Cell Signal* 20:1409–1414
- LeRoy EC, Medsger TA Jr (2001) Criteria for the classification of early systemic sclerosis. *J Rheumatol* 28:1573–1576
- Milano A, Pendergrass SA, Sargent JL, George LK, McCalmont TH, Connolly MK, Whitfield ML (2008) Molecular subsets in the gene expression signatures of scleroderma skin. *PLoS One* 3:e2696

- Sargent JL, Milano A, Bhattacharyya S, Varga J, Connolly MK, Chang HY, Whitfield ML (2010) A TGFbeta-responsive gene signature is associated with a subset of diffuse scleroderma with increased disease severity. *J Invest Dermatol* 130:694–705
- Seibold JR, Denton CP, Furst DE, Guillemin L, Rubin LJ, Wells A, Matucci Cerinic M, Riemekasten G, Emery P, Chadha-Boreham H, Charef P, Roux S, Black CM (2010) Randomized, prospective, placebo-controlled trial of bosentan in interstitial lung disease secondary to systemic sclerosis. *Arthritis Rheum* 62:2101–2108
- Simms RW, Lafyatis R (2010) Rituximab: a potential therapeutic advance in scleroderma: what is the evidence? *Rheumatology (Oxford)* 49(2):201–202
- Whitfield ML, Finlay DR, Murray JI, Troyanskaya OG, Chi JT, Pergamenschikov A, McCalmont TH, Brown PO, Botstein D, Connolly MK (2003) Systemic and cell type-specific gene expression patterns in scleroderma skin. *Proc Natl Acad Sci U S A* 100:12319–12324
- Zulian F (2008) New developments in localized scleroderma. *Curr Opin Rheumatol* 20:601–607