



A healthy tension in translational research

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I would like to express my sincere appreciation to the American Society for Clinical Investigation and the Harrington Discovery Institute for the honor of being named the inaugural recipient of the Harrington Prize for Innovation in Medicine. I accept this distinction with a deep sense of gratitude and debt to my mentors, trainees, colleagues, collaborators, patients, and family. Moments of recognition such as this are rare, savored, and sustaining. They also invariably provoke personal reflection about what exactly is being recognized. If something innovative truly stands apart in my work, I have come to the conclusion that it relates less to the methods utilized to address questions and more to the questions I choose to ask, and when I ask them.

A somewhat simplistic but nevertheless informative categorization of science collapses the principles employed to two basic approaches: inductive and deductive reasoning. While both seek to derive truth, the former involves an iterative process of hypothesis and refinement based on experimental observation, while the latter applies rigorous logic to derive the inescapable consequences of fundamental principles that are deemed absolute. Given the daunting complexity of biological systems, it remains true that investigators in the biomedical sciences are largely restricted by necessity to the realm of inductive scientific reasoning. Absolute truths, while conceivable, remain elusive and inherently constrained by the nature of the system used to achieve them. Within this discipline, real and (sometimes) important distinctions are made between basic and translational biomedical scientists — those motivated strictly by the pursuit of knowledge versus those with a more practical (i.e., medical) goal in mind. In recent years, perhaps disproportionately fueled by a tightening funding environment and inadequately conceived or communicated expressions of priorities by funding agencies, this distinction seems to have become more discrete and more contentious than would reflect reality or a desire for progress should permit. Both basic and translational scientists are seeking a compass to steer them through the morass of biological complexity. Those in both camps achieve unan-

anticipated discovery, are neither inherently rigorous nor “fluffy,” seek validation with regard to both plausibility and relevance, embrace the concept of broad implications for their work, and maintain the potential to be informed by and to inform the other.

There are numerous examples of these principles in my own work. As a personal aside, in 1989 I made the deliberate decision to abandon a purely clinical training path in order to gain research experience and abilities that might be applied to improve the outcome of patients with Marfan syndrome (MFS), a condition that includes a strong predisposition for aortic aneurysm and dissection (tear) and death in early life. I was fortunate to receive a warm reception by mentors who were also invested in this goal, namely Clair Francomano, Reed Pyritz, Lynn Sakai, and Victor McKusick. By 1991, we had shown that mutations causing MFS occur in the gene *FBNI*, encoding the connective tissue protein fibrillin-1 (1). In collaboration with Francesco Ramirez, efforts in my own lab subsequently focused on the creation of mouse models to interrogate disease pathogenesis and explore treatment strategies.

Pursuing the causes of MFS

Prior to 2003, pathogenic models for MFS (and most other disorders caused by deficiency of a structural matrix protein) singularly invoked inherent weakness of the tissues as the driving force in disease progression. This suggested that children with these conditions are born with an obligate structural predisposition for their tissues to fail later in life, boding poorly for the development of productive postnatal treatments. Through clinical experience, we rec-

ognized that many of the manifestations of MFS — including bone overgrowth, low muscle mass and fat stores, and craniofacial dysmorphism — were difficult or impossible to reconcile with this “weak tissues” model. Through equal measure of inspiration and clinical desperation, we pursued this paradox in hope of finding a therapeutic angle. Ultimately, our data suggested that fibrillin-1 serves a second and important regulatory role by sequestering the large latent complex of the growth factor TGF- β and suppressing its activation and signaling (2). Importantly, this initiative was informed by and ultimately informed basic discoveries by the groups of Sakai, Ramirez, and Daniel Rifkin, among others, regarding the homology between fibrillin-1 and the latent TGF- β -binding proteins (LTBPs), their direct biochemical interaction, a genetic interaction between fibrillin-2 and bone morphogenetic proteins (BMPs; members of the TGF- β superfamily), and important principles regarding TGF- β bioavailability and activation (3–5). Using developmental emphysema as a readout, we showed that suppression of TGF- β activity using a neutralizing antibody could greatly attenuate or even prevent disease in a mouse model of MFS (2).

Later work in my lab showed that the protection afforded by TGF- β antagonism extended to other phenotypes in MFS, including congenital mitral valve prolapse with myxomatous valve degeneration (6). The relevance of this mechanism to manifestations that more intuitively reflect failed tissue homeostasis (e.g., aneurysm) remained unknown. Using fibrillin-1-deficient mice, we demonstrated increased TGF- β signaling (nuclear accumulation of phosphorylated Smad2 and output of TGF- β -responsive genes) in the aortic wall (7). Systemic delivery of a TGF- β -neutralizing antibody attenuated aortic root enlargement and improved aortic wall architecture. In an attempt to become more clinically relevant, we turned our attention to losartan, an FDA-approved angiotensin II type 1 (AT1) receptor blocker that both lowers blood pressure and had been shown to inhibit TGF- β signaling in

Conflict of interest: Harry Dietz serves on the Scientific Advisory Board for GlaxoSmithKline.

Citation for this article: *J Clin Invest.* 2014; 124(4):1425–1429. doi:10.1172/JCI75840.



animal models of chronic renal disease. A long-term trial showed that losartan fully normalized TGF- β signaling, aortic root growth and size, and aortic wall architecture in a validated mouse model of MFS (7). This protection was independent of hemodynamic effects, as comparable rescue was not achieved in mice treated with β -adrenergic receptor blockers that showed an equivalent reduction in blood pressure. Furthermore, losartan rescued disease manifestations outside of the cardiovascular system in MFS model mice, including developmental emphysema (7) and skeletal muscle myopathy (8). This work revealed that the congenital myopathy seen in children with severe MFS reflects a TGF- β -induced failure of muscle regeneration and that the protection afforded by TGF- β antagonism extends to mouse models of other myopathic conditions, including Duchenne muscular dystrophy (8), a finding that has been substantiated by others (9–15). Other groups have subsequently shown the relevance of TGF- β and losartan to animal models of limb-girdle and congenital merosin-deficient muscular dystrophy and to muscle healing in general (16–19). As independent investigators, former members of my lab extended the relevance of losartan to mouse models of common human phenotypes, including smoking-induced emphysema (20) and disuse muscle atrophy (21).

Translating discoveries into the clinic

While the mouse data suggestive of therapeutic potential of losartan in MFS generated significant excitement, whether protection would extend to people remained unknown. Given that losartan is FDA approved, well tolerated, and widely used for hypertension in all age groups, we felt compelled to treat children with the most severe presentation of MFS on a compassionate basis, and to capture this experience in a scholarly manner. Historically, these individuals either die or require surgery in early childhood, despite optimized treatment with other antihypertensive agents. We started 18 children on losartan after long-term monitoring on conventional therapies. On average, there was an approximately 10-fold reduction in aortic root growth rate after starting losartan therapy (up to four years of follow-up), despite no change in blood pressure compared with prior treatment (22). Other aortic segments that are prone to dilatation in MFS showed reduced growth, while those that are spared

did not, which suggests that losartan does not induce generalized vessel hypoplasia, but rather restores the normal relationship between vessel growth and metabolic demands of the tissues for blood flow and nutrient delivery. Three additional clinical trials of losartan in children and adults with MFS have now reported similar protection (23–25). While the results of additional randomized trials that are underway will be required to fully elucidate therapeutic potential and refine therapeutic protocols (26–29), this experience heralds true progress in the decades-long effort to inform the treatment of MFS and related disorders through basic science inquiry.

The search for new clinical strategies continues

We have maintained an aggressive effort to further refine mechanistic understanding of MFS and related disorders for clinical gain. Angiotensin II signals through both AT1 and a type 2 receptor, AT2. AT1 signaling can accentuate TGF- β signaling through upregulation of TGF- β ligands, receptors, and activators, and AT2 can attenuate these AT1-mediated events, at least in some cell types. On this basis, we posited that selective AT1 blockers are attractive because they block the culprit pathway while preserving signaling through a potentially protective pathway (AT2). An alternative view held that AT2 signaling was detrimental because it can induce apoptosis, suggesting that use of angiotensin-converting enzyme inhibitors (ACEi) that effectively limit signaling through both receptor types would be more beneficial. This issue was addressed head-on by showing that (a) Marfan mice lacking AT2 showed accelerated aortic growth and died earlier from aortic dissection, (b) ACEi-treated mice did not show the robust protection seen with losartan, and (c) intact AT2 signaling was needed for the protective effect of losartan (30). In parallel work, we asked whether so-called canonical (Smad-dependent) TGF- β signaling was the primary culprit, or whether noncanonical cascades (e.g., ERK, JNK, or p38) contributed to disease. We found marked TGF- β -dependent activation of ERK signaling in the aortic wall and observed that selective ERK antagonists were as effective as losartan in suppressing aortic growth in MFS model mice (31). These studies converged when it was shown that while both losartan and ACEi suppress canonical TGF- β signaling in

MFS, losartan uniquely antagonizes ERK, and this effect is mediated through AT2. This work provided unequivocal evidence that AT2 signaling is protective in MFS and that AT1 blockers such as losartan offer the highest immediate therapeutic potential.

Understanding related clinical syndromes

Loeys-Dietz syndrome. The conclusion that dysregulated TGF- β signaling contributes to the multisystem manifestations of MFS and other important disease phenotypes was solidified upon description of mutations in *TGFBR1* or *TGFBR2* (which encode subunits of the TGF- β receptor) in patients that associate some features of MFS (arachnodactyly, scoliosis, chest wall deformity, dural ectasia, and aortic root aneurysm) with other unique characteristics (craniosynostosis, hypertelorism, cleft palate/bifid uvula, cervical spine malformation, club foot deformity, and congenital heart disease) (32–34). Most importantly, people with this condition (termed Loeys-Dietz syndrome [LDS]) show aneurysms throughout the arterial tree that tear at younger ages and smaller dimensions than aneurysms in those with other connective tissue disorders. Mutations that cause LDS substitute evolutionarily conserved residues in the kinase domains of the receptor subunits, and mutant receptors cannot support TGF- β signaling when expressed in cells naive for the corresponding TGF- β receptor subunit (34), implicating reduced TGF- β signaling as the relevant pathogenic mechanism, a model difficult to reconcile with increased TGF- β signaling in MFS. However, there was the clear signature of increased TGF- β signaling in aortic tissue from patients with LDS (32, 33). These data were the first to definitively implicate altered TGF- β signaling as the cause of many common human developmental phenotypes and highlighted the importance of consideration of chronic compensatory events to achieve comprehensive mechanistic insight. More rarely, LDS-like phenotypes can be caused by haploinsufficiency for SMAD3 or the TGF- β 2 ligand, both of which are positive effectors of TGF- β signaling (35–37). Notably, these heterozygous loss-of-function mutations in *TGFBR2* or *SMAD3* also associate with a strong signature for high TGF- β signaling in the aortic wall of patients and mouse models.

Other observations have contributed to ambiguity regarding mechanism. Some phenotypic features of LDS, such as cleft



palate, have historically been attributed to a low TGF- β state on the basis of cell type-specific targeting of genes encoding TGF- β effectors in mice (38, 39). Furthermore, organ culture experiments have implicated a specific deficiency of TGF- β 3 activity in the pathogenesis of premature skull fusion (40, 41). Conditional targeting experiments expected to attenuate or abrogate TGF- β signaling in mice have also shown altered vessel wall homeostasis, and pharmacologic antagonism of TGF- β signaling can exacerbate the inflammatory aneurysm phenotype observed after angiotensin II infusion in genetically predisposed mouse backgrounds (42–44). Together, these apparently contradictory data engendered considerable controversy in the field regarding the precise role of TGF- β in the pathogenesis of aortic aneurysm and other developmental phenotypes seen in connective tissue disorders.

We proposed mechanisms by which compensatory autocrine and/or paracrine events, initiated in response to a perceived deficiency of TGF- β signaling, might result in functional overshoot in a context-specific manner (45). In keeping with this hypothesis, both humans and mice with haploinsufficiency for *TGFB2* show upregulation of expression of TGF- β 1 in the aorta (36). Similarly, knockin mouse models of LDS show increased TGF- β ligand expression and a clear tissue signature for high TGF- β signaling in the aortic wall during postnatal aneurysm progression, and a remarkable clinical response to losartan correlates with normalization of both canonical and non-canonical TGF- β signaling (46).

We subsequently showed that Shprintzen-Goldberg syndrome (SGS), a systemic connective tissue disorder that includes virtually all the craniofacial, skeletal, skin, and cardiovascular manifestations of MFS and LDS with the additional finding of mental retardation, is caused by loss-of-function mutations in the Sloan Kettering Institute proto-oncogene *SKI*, a prototypical repressor of TGF- β activity (47). These data provide strong evidence that mutations predicted and observed to enhance TGF- β signaling are sufficient to cause human phenotypes that have variably been associated with low TGF- β signaling states, including craniosynostosis, altered palatogenesis, and aortic aneurysm, and support the conclusion that enhanced TGF- β activity contributes to the multisystem manifestations of multiple syndromic presentations of thoracic aortic aneurysm.

More recently, studies in LDS have definitively implicated altered TGF- β signaling in the etiology of the full spectrum of pediatric allergic diseases, prominently including food allergy, asthma, eczema, allergic rhinitis, and eosinophilic gastrointestinal disease (48). Each of these phenotypes is dramatically increased in prevalence in LDS patients with primary mutations in *TGFB1/TGFB2* compared with the general population. LDS patients exhibited elevated IgE levels and eosinophil counts, as well as Foxp3⁺ Tregs that retained the ability to suppress effector T cell proliferation, but showed inappropriate expression of proinflammatory Th2 cytokines. Th2 cytokine-producing cells accumulated in cultures of naive CD4⁺ T cells from LDS subjects, but not controls, after stimulation with TGF- β , which suggests that LDS mutations support Th2 skewing in naive lymphocytes in a cell-autonomous manner. The monogenic nature of LDS demonstrates that altered TGF- β signaling can predispose to allergic phenotypes in humans and underscores a prominent role for TGF- β in directing immune responses to antigens present in the environment and foods. This paradigm also proved relevant to nonsyndromic presentations of allergic disease (48) and highlights the potential therapeutic benefit of strategies that inhibit TGF- β signaling.

Systemic sclerosis. In another line of investigation, we have taken an interest in scleroderma, defined as progressive hardening of the skin due to excessive collagen deposition. The most common and severe form, called systemic sclerosis (SSc), is observed in previously healthy young adults who show sudden onset of skin and visceral fibrosis in association with the production of autoantibodies. Obstacles to progress in SSc include a poorly defined genetic contribution, the lack of large families to support positional genetic approaches, and the absence of validated animal models. As an alternative, we initially focused on a rare but Mendelian presentation of scleroderma, called stiff skin syndrome (SSS), that shows congenital onset of skin fibrosis with secondary and severe joint contracture. Remarkably, all families with SSS harbor heterozygous missense mutations in *FBN1*, the MFS gene, despite the lack of phenotypic overlap between these conditions (49). Unlike in MFS, where mutations occur along the full length of the gene and protein, all mutations causing SSS occur in the sole domain of fibrillin-1

that contains an RGD sequence needed to mediate cell-matrix attachments through integrin binding.

We demonstrated that knockin mouse models of SSS harboring either a naturally occurring SSS mutation or an RGD-to-RGE substitution predicted to impose an obligate loss of integrin binding show complete replacement of dermal fat with collagen by 3 months of age (50). This associates with upregulation of α 5 β 1 and α v β 3 integrin expression in the dermis. Treatment of either model with β 1 integrin-activating antibody (in essence, “tricking” the cell into responding as if sensing its matrix ligand) normalized expression of β 3 integrin and prevented all clinical and histologic markers of skin fibrosis; the same rescue was seen upon genetic targeting of the β 3 integrin gene, and established skin fibrosis was reversed by systemic treatment with TGF- β -neutralizing antibody. SSc patient cells also showed increased integrin expression and collagen production that was normalized by treatment with antibodies that activate or block β 1 or β 3 integrins, respectively. Curiously, SSc fibroblasts show a fundamental difference in their acute-phase response to recombinant TGF- β , uniquely showing activation of ERK1/2 that was normalized upon treatment with β 1 integrin-activating or β 3 integrin-blocking antibody. ERK1/2 phosphorylation is seen within 5 minutes of TGF- β administration and is blocked upon treatment with SD208 (an inhibitor of TGF- β receptor type I kinase activity), suggesting a direct effect. An ERK1/2 inhibitor prevented skin fibrosis in SSS mouse models.

Remarkably, our SSS mouse models fully recapitulate all autoimmune and autoinflammatory events typical of SSc, including high titers of anti-nuclear and anti-topoisomerase antibodies (50). The cells expressing high levels of α 5 β 1 and α v β 3 integrins in the dermis are activated plasmacytoid DCs (pDCs) expressing type I IFN (predominantly IFN- α) and IL-6. This proinflammatory environment associates with enrichment for activated plasma cells and Th cell polarization in the skin, including IL-4- or IL-17-producing Th2 or Th17 cells, respectively. Virtually all of these findings are fully normalized upon treatment of SSS mice with β 1 integrin-activating antibody. Informatively, wild-type pDCs showed enhanced attachment and activation when plated on the matrix elaborated by SSS fibroblasts, when compared



with wild-type fibroblasts. These data suggest that the altered matrix environment in SSS (and perhaps SSc), devoid of any systemic influence, is sufficient to initiate proinflammatory and profibrotic events.

Concluding remarks

While my early adoption of “What if?” translational types of experiments has provided some enlightenment and optimism, there is no denying that mechanistic holes remain to be filled. Is enhanced TGF-β signaling in MFS and related disorders primary and pathogenic, secondary and partially compensatory, or both? What is the exact link between AT1 signaling and disease progression? What dictates the balance between canonical and noncanonical TGF-β signaling cascades? What is the basis for the low signaling/high signaling paradox in LDS? Are both relevant to disease at different stages of pathogenesis? Will the answers to such questions improve treatments? Undoubtedly so. I also note eroding enthusiasm for the use of mice to model human disease in development of therapeutics, fueled in part by lack of uniform predictive value for conditions such as cancer and the inflammatory response to acute insults. Is it surprising that divergent species with distinct physiologies and survival needs have different adaptations to common forms of chronic evolutionary pressure? I think not. Will the same be (as) true for evolutionarily irrelevant rare disorders – especially when the same genetic insult results in full phenotypic concordance between species? I bet not.

I would be the first in line to acknowledge the reliance of my work on purely knowledge-driven discoveries and the most adamant proponent that such work is essential for progress in more goal-oriented endeavors. Indeed, some paths to medical application do not wander far from such fundamental observations. For example, the realization of imatinib as a potent treatment option for chronic myelogenous leukemia (CML) built deliberately upon the demonstrated transforming potential and tyrosine kinase activity of the product of the viral oncogene *v-abl* (reviewed in ref. 51). Recognition of particular relevance of this finding to CML, however, derived from pioneering translational work demonstrating that CML is associated with a translocation between chromosomes 22 and 9 (the Philadelphia chromosome), resulting in a fusion transcript derived from the *BCR* and *ABL* genes that generates a pro-

tein with elevated and constitutive kinase activity. Basic advances in medicinal chemistry and small-molecule screening and translational studies in animal models and patients culminated in an effective therapy. Examples of such glaring successes are rare and notably enriched for phenotypes that logically and demonstrably manifest cell-autonomous events that are robustly modeled in cell culture systems such as cancer. We are faring less well with slowly evolving phenotypes in complex tissues that integrate dynamic interplay among underlying predisposition, time, different cell types, genetic and biochemical individuality, and chronic productive and nonproductive compensatory events. Here, reliance solely on experimental systems that have been defined (and hence simplified) in a sufficient manner to achieve clarity might lead to either false-positive or false-negative conclusions – in essence, *reductio ad absurdum* – in a full physiologic and pathologic context. While advances in human genetics promise to inform the issue of predisposition, and parallel advances in the basic sciences should and will contribute to initial pathogenic hypotheses, early and frequent translational provocations in experimental systems that best approximate the physiologic complexity of humans seem critical to the mix. Net assessment of clinical consequence (it got better or it got worse), considered in conjunction with attendant changes in tissue and cellular phenotype, will provoke and inform basic inquiry for reconciliation and ultimate modification or refinement of translational hypotheses.

The stated goal of the Harrington Discovery Institute is to enable physician-scientists to make breakthroughs that will change the standard of care for patients. My advice to young people entering the field is to (a) take full advantage of their clinical observations, insights, and intuition in crafting therapeutic hypotheses; (b) firmly ground such hypotheses with fundamental basic science principles; (c) frequently and boldly test these hypotheses in relevant preclinical systems, using phenotypic readouts as a prominent – if not exclusive – guide; and (d) establish a network of the best and brightest colleagues, collaborators, and trainees with diverse questions, expertise, and immediate scientific priorities.

Acknowledgments

The author acknowledges funding from the Howard Hughes Medical Institute,

the NIH (grant nos. R01-AR41135 and P01-AR49698), the Bloomberg Fund of the Marfan Foundation, the Scleroderma Research Foundation, the Leducq Foundation, and the William S. Smilow Foundation for Marfan Syndrome Research.

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