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The potential of targeting epigenetic regulators for the treatment of fibrotic cardiac diseases

“...we strongly believe that clinical testing of histone deacetylase and BET inhibitors in patients with cardiac fibrosis is justified.”

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Cardiac fibrosis: the clinical problem

Fibrosis of the heart can have devastating consequences, and scientists and clinicians from diverse disciplines are finally beginning to fully appreciate the pathophysiological significance of this scarring process. At the beginning of the 21st century, a PubMed search for ‘cardiac fibrosis’ yielded 394 results. This number increased to 737 in 2005, 1254 in 2010 and in 2015 alone, there were 2022 cardiac fibrosis-related papers published. The reasons for this surge in interest are fourfold: (i) increased ability to non-invasively detect cardiac fibrosis through advanced imaging tools; (ii) establishment of clear correlations between cardiac fibrosis and clinical outcomes; (iii) essentially a complete lack of effective anti-fibrotic treatment options, creating a heightened awareness of this massive unmet medical need; and (iv) increasing recognition that the heart is a complex mixture of heterogeneous, interacting cell types, rather than simply a pump consisting only of myocytes [1]. It is our view that epigenetic regulators, which govern gene expression via modifications of nucleosomal DNA and protein, are among the most auspicious [2] and underappreciated [3] therapeutic targets for the treatment of pathological cardiac fibrosis.

Cardiac fibrosis is defined as the existence of excess collagen-rich fibrotic tissue in the myocardium, which leads to adverse outcomes such as fatal arrhythmias, and heart failure via abnormal muscle relaxation and contraction. Until recently, attempts to correlate fibrosis with cardiac disease were limited

to histological detection of fibrotic lesions in explanted or cadaveric hearts. However, major advances in imaging now enable quantification of cardiac fibrosis in patients, with cardiac magnetic resonance imaging (CMR) as the current gold-standard modality for non-invasive evaluation of cardiac fibrosis [4].

The ability to detect cardiac fibrosis before a patient has overt clinical manifestations, such as a life-threatening arrhythmia or severe heart failure, will undoubtedly facilitate the development of anti-fibrotic therapies for the heart. However, reliance on imaging alone for diagnosis (or assessment of therapeutic efficacy) is inadequate due to cost, availability and risks. CMR is currently only available at large, typically regional, medical centers for use in a select population of patients. Patients with kidney disease, metal implants, obesity, claustrophobia or advanced heart disease, who may not be able to safely lie flat for a prolonged period or be administered heart rate lowering drugs, are not candidates for CMR. In addition, cost is a significant limitation, with a single CMR study typically ranging from US\$1500 to US\$3500 [5]. Advances in echocardiography, such as speckle tracking and strain imaging, will address some of these issues, but access and cost will remain significant barriers. To circumvent these limitations, surrogate biomarkers of cardiac fibrosis have been proposed, such as Galectin-3; however, thus far none have been found that reliably correlate with the presence of cardiac fibrosis across disease states [6,7].

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Clinical trials that have been performed with numerous therapies aimed at reversing cardiac fibrosis in a variety of disease states have largely been unsuccessful. Aldosterone blockade with spironolactone in the TOPCAT trial is perhaps the most widely publicized example. Although small clinical trials with spironolactone were initially promising, results of the large multicenter, randomized controlled trial in patients with heart failure with preserved ejection fraction, a disease tightly linked to cardiac fibrosis, showed no effect on the primary endpoints of death from cardiovascular causes, aborted cardiac arrest or hospitalization for heart failure [8]. Thus, cardiac fibrosis remains a major unmet medical need, and the elucidation of novel mechanisms involved in fibrogenesis in the heart is required for development of new therapies for this prevalent and deadly process.

“Obtaining the necessary heart tissue for pharmacodynamics studies, while not jeopardizing the health of study patients, may not be an insurmountable task.”

Targeting epigenetics to treat cardiac fibrosis: promising preclinical data

Acetylation of nucleosomal histone tails plays a crucial role in epigenetic control, and experiments with small molecules that target histone deacetylases (HDACs) and acetyl-histone ‘readers’ have shown promise in pre-clinical models of cardiac fibrosis. Pan-, hydroxamic acid-based HDAC inhibitors have long been known to suppress cardiac fibrosis [9] and, more recently, isoform-selective HDAC inhibitors were employed to demonstrate a role for class I HDACs (HDAC1, -2 and -3) in the control of cardiac fibrosis [2,10–11]. In one example, the benzamide class I HDAC inhibitor mocetinostat blunted progression of pre-existing cardiac fibrosis in a rat myocardial infarction model [10]. Importantly, mocetinostat did not alter the integrity of the structural scar that formed post-myocardial infarction; this is an important consideration when developing anti-fibrotic therapies, since agents that weaken the structural scar could cause ventricular rupture and sudden death.

The most well-characterized proteins that ‘read’ acetyl-lysine marks on histone tails are the bromodomain and extraterminal (BET) family of proteins, which includes BRD4. BRD4 activates RNA Polymerase II (Pol II) by recruiting CDK9 to gene promoters, resulting in Pol II phosphorylation and transcription elongation [12]. JQ1, a small molecule that disrupts binding of BET bromodomains to acetyl-histones, was shown to block interstitial cardiac fibrosis in mouse models of heart failure [13,14]. This was accompanied by decreased Pol II activity at pro-fibrotic genes (e.g., α -SMA, PAI-1), establishing a role for BET proteins in cardiac fibrosis [13].

BET inhibitor-mediated blockade of cardiac fibrosis may involve disruption of BRD4-enriched, cell state-specific enhancers, referred to as superenhancers (SEs). SEs are thought to signal to gene promoters via long noncoding RNAs known as enhancer RNAs, thereby facilitating Pol II phosphorylation. We recently defined the first genome-wide map of BRD4-enriched SEs in cardiac muscle cells (cardiomyocytes) and, strikingly, ~30% of these SEs were associated with pro-fibrotic genes, including genes encoding secreted factors such as transforming growth factor β -2 [15]. These findings suggest the possibility that BRD4-enriched SEs in cardiomyocytes regulate expression of paracrine factors that crosstalk with fibroblasts (the major extracellular matrix-producing cell type in the heart) to elicit cardiac fibrosis.

Epigenetic therapies for cardiac fibrosis: translational considerations

It is fortunate that most epigenetic therapies are being clinically evaluated with an initial therapeutic focus on various cancers (ClinicalTrials.gov). Consequently, there will be considerable human pharmacokinetic (PK) data available for the design of Phase I trials focused on secondary fibrotic indications like heart failure (i.e., cardiac fibrosis). As a complement to PK data it will be important to obtain evidence for dose-dependent target engagement in the heart (also known as pharmacodynamics or PD) in order to establish the PK/PD relationship for a given epigenetic therapy. This is easily accomplished in preclinical animal studies, but poses a challenge for Phase I human clinical trials.

Obtaining the necessary heart tissue for PD studies, while not jeopardizing the health of study patients, may not be an insurmountable task. One option that has been used previously is to obtain endomyocardial biopsies as part of the trial design (e.g., BORG/NCT01798992) [16]. However, endomyocardial biopsies would not be justified in healthy subjects due to unwarranted risk. We recently proposed a potentially attractive solution that would pose less risk to patients [2]. Patients scheduled to undergo surgery to implant a left ventricular assist device (LVAD) as a ‘bridge-to-transplant’ measure would be attractive candidates for Phase I clinical trials of epigenetic therapies. LVAD surgery involves the removal of a small amount of tissue from the heart that could be used for subsequent PD studies. This allows for minimal risk to the patient since they typically undergo heart transplants within several months of the LVAD surgery. At last, it may be possible to collaborate with the study sponsor for epigen-

etic therapy cancer trials to obtain heart tissue from patients who were treated but succumb to their illness during the trial. One caveat to this approach is that, depending on the clinical phase of development, the study may be blinded and the samples would not be able to be analyzed until after the trial was complete. Nonetheless, this approach could provide a valuable head start for subsequent clinical trials with epigenetic therapies for nononcology indications, such as heart failure.

Another translational challenge that may be more pronounced with epigenetic therapies is that caution must be observed in the search for highly coveted diagnostic or prognostic serum biomarkers for fibrosis. By their very nature, epigenetic therapies will alter the expression of many genes, which may include a proposed biomarker. It will be very important to demonstrate that the changes in biomarker that are observed are therapeutically meaningful and not ancillary to the disease state.

Conclusion

Many investigators are concerned about targeting global epigenetic regulators, such as HDACs and BET proteins, for the treatment of cardiac fibrosis. Undeniably, the therapeutic benefit of any agent must be carefully weighed against the potential risk of toxicity. In cancer patients, who typically receive maximum tolerated doses of HDAC and BET inhibi-

tors, gastrointestinal disturbance, fatigue, hematologic toxicity and electrical QT interval prolongation have been reported [17,18]. However, based on our unpublished preclinical studies of cardiac fibrosis, anti-fibrotic activity of HDAC and BET inhibitors is observed at doses far lower than the maximum tolerated doses. Furthermore, we have found that episodic dosing of these compounds is sufficient to elicit beneficial effects in the heart, presumably due to transient ‘tweaking’ of epigenetic events resulting in sustained inhibition on profibrotic gene expression. Given these findings, we strongly believe that clinical testing of HDAC and BET inhibitors in patients with cardiac fibrosis is justified.

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