

Commentary

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Towards a BETter understanding of cardiac fibrosis

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Abstract

Fibroblast activation is a hallmark feature of pathological remodeling of the heart and represents an attractive target for therapeutic intervention. Pharmacological inhibition of chromatin remodeling enzymes reduces cardiac fibrosis, but the underlying transcriptional regulatory mechanisms remain poorly understood. Using single-cell genomics to profile alterations in the transcriptional and chromatin landscape during stress-induced cardiac remodeling, Alexanian *et al.* discovered a critical role for Mesenchyme Homeobox 1 in the regulation of myofibroblast activation and cardiac fibrosis. We briefly review these important findings and comment on the significance of their work.

Keywords: Cardiac fibrosis, chromatin, fibroblast activation, heart failure

Despite advances in the clinical management of cardiovascular disease over the last 30 years, ischemic heart disease remains the leading cause of death globally with a rising trajectory^[1]. Cardiac fibrosis is a feature of numerous cardiac pathologies and contributes to ventricular dysfunction, which can lead to heart failure.



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Cardiac fibrosis is mediated by the activation and differentiation of resident cardiac fibroblasts into myofibroblasts in response to stress. The transcriptional mechanisms mediating myofibroblast activation are poorly understood. Single-cell genomic platforms have recently emerged as powerful technologies for mapping complex transcriptional networks with precise cellular resolution, thus providing unique opportunities to interrogate myofibroblast activation in the hope of identifying novel therapeutic targets for attenuating cardiac fibrosis.

In a pioneering study published in *Nature*, Alexanian *et al.*^[2] harnessed the power of a number of single-cell genomics technologies to identify DNA elements involved in coordinating fibrotic gene programs. The authors initially employed a murine model of heart failure induced by transverse aortic constriction (TAC) to study the anti-fibrotic effects of a small molecule bromodomain and extra terminal domain (BET) protein bromodomain inhibitor, JQ1. BET proteins are highly conserved acetyl-lysine reader proteins that function as transcriptional co-activators. Recent studies have shown that BET inhibition ameliorates heart failure in mouse models^[3,4]. BET inhibitors reversibly bind the bromodomains of the BET family, notably BRD2, BRD3, BRD4 and BRDT, to perturb their function. JQ1 has been shown to block both BD1 and BD2 domains of BET family proteins^[5]. Treatment of JQ1 attenuated fibroblast activation induced by TAC and subsequently reduced left ventricular fibrosis comparable to sham-operated mice [Figure 1]. Withdrawal of JQ1 treatment resulted in a regression in left ventricular systolic function, highlighting the reversibility of BET inhibition. Of the cell types identified by single-cell RNA sequencing (scRNA-seq) of this model, fibroblasts were found to be highly sensitive to JQ1 exposure. The effects of JQ1 on cardiomyocytes were modest [Figure 1].

In addition to scRNA-seq, Alexanian *et al.*^[2] also profiled the open chromatin landscape at single-cell resolution using the assay for accessible chromatin with sequencing (scATAC-seq). By integrating scRNA-seq and scATAC-seq data, dynamic changes in distal regulatory elements were assessed following TAC and JQ1 treatment to identify a catalog of sites exclusively regulated in fibroblasts that were responsive to injury and reversibly modulated by JQ1 exposure. Precision nuclear run-on sequencing (PRO-seq) was then used to pinpoint functionally relevant enhancers associated with fibroblast activation. These studies defined stress-responsive enhancers in cardiac fibroblasts and identified CCAAT enhancer-binding proteins (CEBP), Jun proto-oncogene (JUN) and Mesenchyme Homeobox 1 (MEOX1) as enriched transcription factor binding motifs in accessible chromatin regions following TAC that were also sensitive to JQ1 exposure. Of these elements, a large downstream enhancer of *Meox1* was shown to have the most significant effect. Further analysis showed that *Meox1* expression was induced by TGF β stimulation, suppressed by JQ1 and was most sensitive to BRD4 among BET proteins. Further, knock-down studies suggest MEOX1-dependent genes are involved in cell migration, motility, and proliferation, and included connective tissue growth factor (*Ctgf*) and periostin (*Postn*), which are highly associated with fibroblast activation. Importantly, MEOX1 was also found in activated fibroblasts in the adult human heart, which, together with previous reports^[6,7], identifies MEOX1 as a highly conserved master regulator of myofibroblast activation in the heart.

This landmark study by Alexanian *et al.*^[2] broadens our understanding of the transcriptional mechanisms controlling fibroblast activation and cardiac fibrosis. However, some outstanding questions remain. In particular, it should be noted that genetic loss-of-function studies were not performed in the current study, and it is unclear whether MEOX1 is required for fibroblast activation and cardiac fibrosis *in vivo*. The strong enrichment and induction of MEOX1 in cardiac fibroblasts following injury makes it a potentially attractive therapeutic target, but further studies are required to evaluate the therapeutic potential of targeting MEOX1 under pathophysiological conditions. Given the well-described challenges of drugging

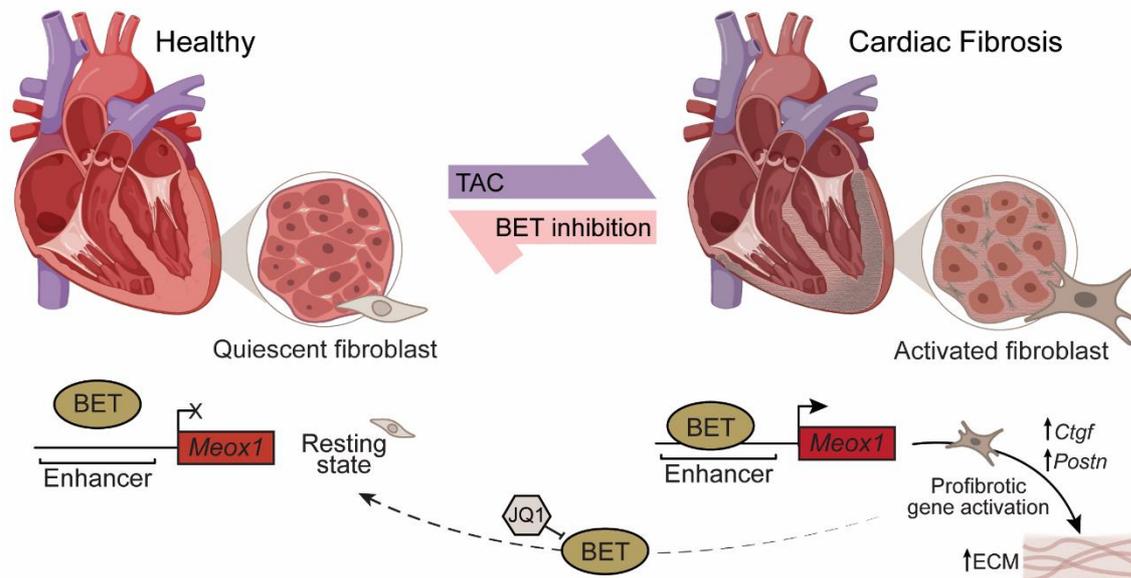


Figure 1. MEOX1 is a master regulator of fibroblast activation and cardiac fibrosis. Alexanian et al.^[2] identified MEOX1 as a key regulator of fibroblast activation in the heart. TAC induces cardiac remodeling and fibrosis, which results in chromatin remodeling and BET protein binding to MEOX1 enhancer elements and transcriptional activation of MEOX1. Treatment with the small molecule BET inhibitor, JQ1, disengages BET proteins from the MEOX1 enhancer and prevents downstream engagement of MEOX1 target genes implicated in fibroblast activation and ECM deposition including *Ctgf* and *Postn*. BET: Bromodomain and extra terminal domain protein. TAC: Transverse aortic constriction; *Meox1*: mesenchyme homeobox 1; *Postn*: periostin; *Ctgf*: connective tissue growth factor. Figures created with BioRender.com.

transcription factors, new technological developments may be required to pharmacologically target MEOX1 as an anti-fibrotic therapy. In this regard, advances in CRISPR/Cas9 technology^[8], may provide new opportunities for genome editing aimed at modulating the function of tissue-specific enhancers in cardiac fibrosis. Finally, although the current study provides new insights into the underlying anti-fibrotic mechanism of BET inhibitors, it is important to note that different BET inhibitors have varying efficacy, on-target selectivity, BD domain selectivity and safety profiles. It will be important to determine whether the MEOX1-dependent transcriptional mechanisms defined here for JQ1 are also conserved across other classes of BET inhibitors.

In summary, the current study by Alexanian and colleagues provides novel insight into the transcriptional landscape of cardiac fibrosis and heart failure. The authors have harnessed state-of-the-art single-cell genomic approaches to discover a master regulator of fibroblast activation, MEOX1, which appears to be critical for the anti-fibrotic effects of BET inhibitors. These findings open several future avenues of investigation into transcriptional mechanisms governing myofibroblast activation and provide a mechanistic basis for the development of much-needed anti-fibrotic therapies for heart failure.

DECLARATIONS

Authors' contributions

Conceived and wrote the paper: Stout ES, Elliott DA, Porrello ER

Availability of data and materials

Not applicable.

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Conflicts of interest

The authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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