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REVIEW

Cardiac Fibrosis in the Multi-Omics Era: Implications for Heart Failure

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ABSTRACT: Cardiac fibrosis, a hallmark of heart failure and various cardiomyopathies, represents a complex pathological process that has long challenged therapeutic intervention. High-throughput omics technologies have begun revolutionizing our understanding of the molecular mechanisms driving cardiac fibrosis and are providing unprecedented insights into its heterogeneity and progression. This review provides a comprehensive analysis of how techniques—encompassing genomics, epigenomics, transcriptomics, proteomics, and metabolomics—are providing insight into our understanding of cardiac fibrosis. Genomic studies have identified novel genetic variants and regulatory networks associated with fibrosis susceptibility and progression, and single-cell transcriptomics has unveiled distinct cardiac fibroblast subpopulations with unique molecular signatures. Epigenomic profiling has revealed dynamic chromatin modifications controlling fibroblast activation states, and proteomic analyses have identified novel biomarkers and potential therapeutic targets. Metabolomic studies have uncovered important alterations in cardiac energetics and substrate utilization during fibrotic remodeling. The integration of these multi-omic data sets has led to the identification of previously unrecognized pathogenic mechanisms and potential therapeutic targets, including cell-type-specific interventions and metabolic modulators. We discuss how these advances are driving the development of precision medicine approaches for cardiac fibrosis while highlighting current challenges and future directions in translating multi-omic insights into effective therapeutic strategies. This review provides a systems-level perspective on cardiac fibrosis that may inform the development of more effective, personalized therapeutic approaches for heart failure and related cardiovascular diseases.

Key Words: extracellular matrix ■ fibroblasts ■ fibrosis ■ heart failure ■ multiomics ■ precision medicine

Cardiac fibrosis is primarily mediated by activated cardiac fibroblasts (CFs), which drive the pathological deposition of excess extracellular matrix (ECM) proteins within the perivascular and interstitial cardiac spaces. CF activation represents the pivotal process in cardiac fibrosis and is initiated by a cascade of signals after injury.[1] This activation cascade involves multiple sequential steps: migration of fibroblasts to the site of injury, localized proliferation of these cells, and ultimately their transdifferentiation to myofibroblasts[2,3] (Figure 1). This maladaptive process leads to scarring, tissue stiffening, and progressive impairment of myocardial function, culminating in heart failure (HF). Fibrosis also commonly arises as a compensatory replacement in response to myocardial insult, such as ischemia or infarction, where it replaces cardiomyocytes in the poorly regenerative mammalian heart.[4] It may also develop in the absence of overt ischemic injury, driven reactively by aberrant signaling or mechanotransduction pathways in various cardiomyopathies.[5]

Interstitial fibrosis is characterized by diffuse collagen expansion throughout the myocardial interstitium, often driven by cardiomyopathic processes, systemic hypertension, metabolic dysfunction, or aging, among other causes, leading to ventricular stiffness and impaired diastolic function.[3] Perivascular fibrosis, seen, for example, in hypertensive heart disease, is characterized by collagen deposition in the coronary adventitia and can compromise coronary flow reserve and oxygen delivery, which exacerbates local ischemic or inflammatory stimuli,

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Nonstandard Abbreviations and Acronyms

α-SMA	α-smooth muscle actin
AUC	area under the curve
BCAA	branched-chain amino acid
BET	bromodomain and extra-terminal
CF	cardiac fibroblast
CFIRL	cardiac fibroblast-specific long noncoding RNA
DCM	dilated cardiomyopathy
DEP	differentially expressed protein
DNMT1	DNA methyltransferase 1
ECM	extracellular matrix
ECV	extracellular volume
GWAS	genome-wide association studies
HCF	human cardiac fibroblast
HCM	hypertrophic cardiomyopathy
HDAC	histone deacetylase
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
ICM	ischemic cardiomyopathy
IL	interleukin
LV	left ventricle
LVAD	left ventricular assist device
LVEF	left ventricular ejection fraction
MESA	Multi-Ethnic Study of Atherosclerosis
MI	myocardial infarction
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
NAD	nicotinamide adenine dinucleotide
NT-proBNP	N-terminal pro-B-type natriuretic peptide
RBP	RNA-binding protein
scRNA-seq	single-cell RNA sequencing
SGLT2	sodium-glucose cotransporter 2
snRNA-seq	single-nucleus RNA sequencing
TAC	transverse aortic constriction
TF	transcription factor
TGF-ß1	transforming growth factor-beta
TIMP	tissue inhibitors of metalloproteinase
TNF-α	tumor necrosis factor-α

perpetuating the cycle of fibrosis.[6–8] Both these forms of fibrosis frequently coexist. These fibrotic responses to mechanical and neurohormonal stress are initially adaptive but can become maladaptive when the stress persists and fibrosis progresses.

Cardiac fibrosis presents a clinical challenge as its presence is significantly associated with morbidity and mortality in HF.[9,10] Fibrosis has been implicated as an important etiological factor in heart failure with preserved ejection fraction (HFpEF), contributes to the progression of heart failure with reduced ejection fraction (HFrEF), and may predispose genetically susceptible cardiomyopathies to the development of cardiomyopathies.[11–13] Cardiac fibrosis is often a clinically insidious process, typically detected in the later stages of disease, and has not traditionally been a primary therapeutic target, as its role in supporting cardiac function has only recently been recognized. In the past 2 decades, the omics revolution has transformed the investigation of not only monogenic diseases but also complex, multifactorial diseases. High-throughput omics technology enables the analysis of multilayered biological data, which is especially useful in studying complex disease processes that are characterized by fibrosis, such as HF.[14] The spectrum of multi-omic approaches—encompassing genetic variants (genomics), dynamic gene expression profiles (transcriptomics), epigenetic modifications influencing transcription (epigenomics), protein interaction networks and posttranslational modifications (proteomics), and metabolic reprogramming (metabolomics)—has only recently been applied to unraveling the pathophysiology of cardiac fibrosis. Such multidimensional omics integration can provide a systems-level perspective on the molecular mechanisms driving cardiac fibrosis. This review aims to demonstrate the underlying drivers of cardiac fibrosis by using multi-omic approaches and data to inform its pathophysiology and highlight the potential identification and development of therapeutic targets that may mitigate or reverse fibrotic remodeling of the failing heart.

GENOMIC ARCHITECTURE OF CARDIAC FIBROSIS

Understanding the genetic basis of cardiac fibrosis has been a major challenge due to its complex pathogenesis. While some individuals develop severe fibrosis after cardiac injury, others show minimal scarring, suggesting genetic factors influence disease progression.

Monogenic Cardiomyopathy and Fibrosis

Cardiac fibrosis is a complex process influenced by interactions among multiple genetic variants, each exerting a modest effect on disease risk and progression. Unlike monogenic disorders, cardiac fibrosis arises from polygenic influences. Early genetic studies focused on Mendelian inheritance patterns to identify single-gene causes of familial cardiomyopathies.[15,16] The discovery of common and rare genetic variants associated with cardiomyopathies has enhanced our understanding of genetic

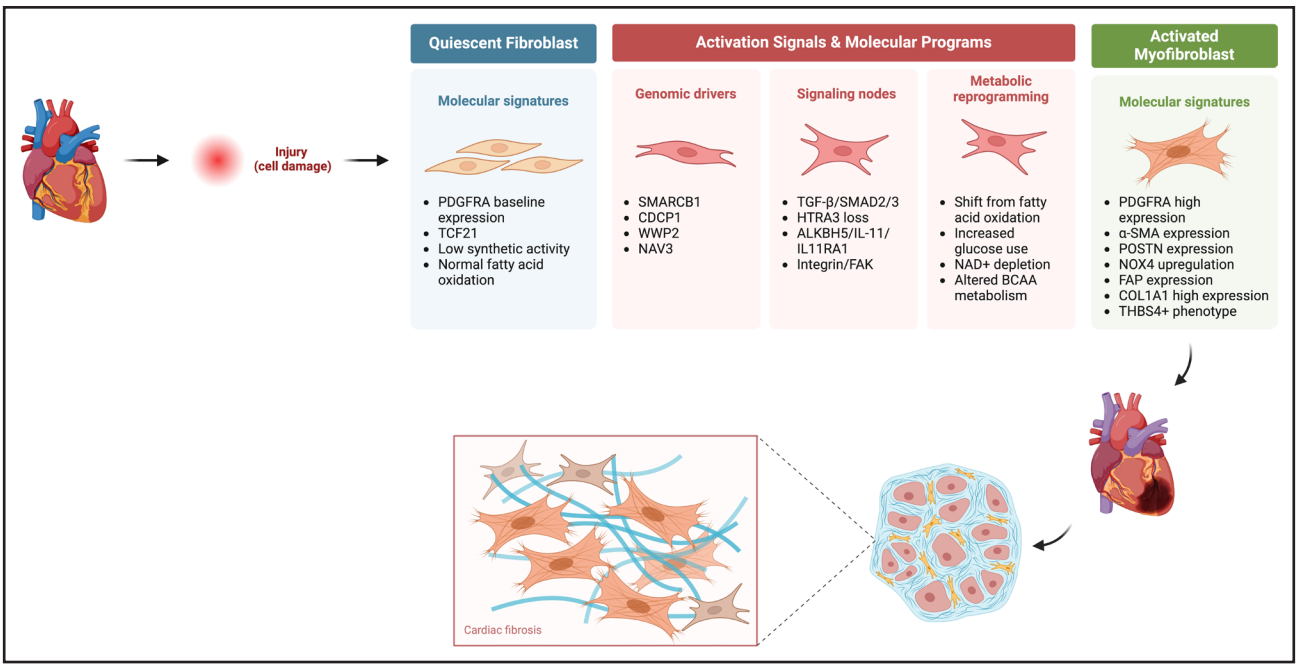

Figure 1. Omics of cardiac fibroblast activation and transdifferentiation.
Cardiac injury initiates stepwise molecular changes and converts quiescent fibroblasts to activated myofibroblasts. Quiescent fibroblasts show baseline expression of *PDGFRA*, *TCF21*, low synthetic activity, and normal fatty acid oxidation. Injury triggers genomic drivers including *SMARCB1*, *CDCP1*, *WWP2*, and *NAV3*, which regulate key signaling nodes such as TGF-β (transforming growth factor-beta)/SMAD2/3, HTRA3 (HtrA serine peptidase 3), and ALKBH5 (AlkB homologous 5, RNA demethylase)/IL-11 (interleukin-11)/IL11RA1 (interleukin 11 receptor subunit alpha 1) pathways alongside integrin/FAK (focal adhesion kinase) mechanotransduction. This activation involves significant metabolic reprogramming which is marked by decreased fatty acid oxidation, increased glucose utilization, nicotinamide adenine dinucleotide (NAD)$^+$ depletion, and altered branched-chain amino acid (BCAA) metabolism. The fully activated myofibroblast displays characteristic molecular signatures including α-SMA (α-smooth muscle actin) expression, POSTN (periostin) upregulation, NOX4 (NADPH oxidase 4) activation, FAP (fibroblast activation protein) expression, high COL1A1 (type I collagen) production, and acquisition of the *THBS4*$^+$ (thrombospondin-4) phenotype. This sequential activation culminates in pathological extracellular matrix (ECM) deposition and tissue-level fibrosis, illustrated by the characteristic cross-sectional cardiac tissue architectural disarray. Created in https://BioRender.com.

predispositions that contribute to variability in fibrosis severity among affected individuals. Whole-genome sequencing and whole-exome sequencing studies have identified rare pathogenic or likely pathogenic variants in cardiomyopathy-associated genes that predispose individuals to myocardial fibrosis, even in the absence of overt cardiac dysfunction. In 1135 asymptomatic participants from the MESA cohort (Multi-Ethnic Study of Atherosclerosis), 6 pathogenic or likely pathogenic variants in cardiomyopathy-associated genes such as *MYH7* (encoding the β-myosin heavy chain essential for sarcomere contraction),[17] *MYBPC3* (regulating contractile force through interaction with myosin and titin),[17] and *SCN5A* (encoding the cardiac sodium channel)[18] were enriched among participants with high myocardial fibrosis—as measured by cardiac magnetic resonance imaging (MRI)-derived extracellular volume (ECV) and native T1 mapping—compared with controls (1.1% versus 0.1%; P=0.03) even in the absence of overt cardiomyopathy.[19] Such variants therefore not only eventually disrupt cardiomyocyte contractile function but may also contribute to early fibrotic remodeling even before the onset of clinical symptoms. The molecular mechanisms linking these genetic variants to preclinical fibrosis and cardiac disease remain an important area for future investigation. The mechanistic diversity of the fibrotic drive is further demonstrated in monogenic cardiomyopathies, which can present with an inflammatory and fibrotic phenotype that mimics other cardiac conditions. In a study of 213 patients with presumed cardiac sarcoidosis, genetic testing revealed that 21% harbored pathogenic or likely pathogenic variants in cardiomyopathy-associated genes, suggesting that genetic cardiomyopathies can masquerade as inflammatory and fibrotic conditions.[20]

Common Genetic Variants, Risk of Cardiomyopathy, and Fibrosis

The search for genetic drivers of cardiac fibrosis extends beyond monogenic causes of HF. Whole-exome sequencing studies investigating sudden cardiac death in cases of cardiac fibrosis not attributable to secondary causes such as coronary artery disease have identified novel genetic variants in *CRTAC1*, *CAPN1*, *UNC45A*, and *UNC45B* that affect ECM integrity and sarcomere stability.[21] Disruptions in these genes may lead not only to pathological fibrosis but also, as a consequence, contribute to increased sudden cardiac death risk.

Large-scale genome-wide association studies (GWAS) have identified numerous genetic loci with significant associations to cardiomyopathy[22,23] and left ventricular (LV) volumes (end-diastolic and end-systolic) and ejection fraction (LVEF).[24–26] A multiancestry GWAS meta-analysis identified 47 risk loci associated with HF, including 34 novel loci, across diverse ancestries.[27] Polygenic risk scores have been derived from these variants and were associated with an increased risk of developing HF. Similarly, a cross-trait analysis of 50 common genetic variants previously associated with cardiomyopathy risk and LV traits in the MESA cohort identified variants in *SMARCB1*, which encodes a core subunit of the SWI (SWitch)/SNF (sucrose nonfermentable) chromatin-remodeling complex,[28] as significantly associated with increased cardiac fibrosis measured by cardiac MRI-derived ECV on T1-mapping—a noninvasive phenotypic marker of fibrosis.[29,30] These *SMARCB1* variants were found to be strong expression quantitative trait loci, with the risk alleles associated with decreased *SMARCB1* expression in human LV tissue. Functional validation through *SMARCB1* knockdown in human cardiac fibroblasts (HCFs) demonstrated enhanced TGF-β1 (transforming growth factor-β1)-mediated fibrosis, evidenced by increased expression of α-SMA (α-smooth muscle actin) and collagen.[29] In the fibroblast activation sequence, TGF-β is the central mediator, through the SMAD (mothers against decapentaplegic)-dependent pathway, that promotes the transition to a phenoconverted and activated myofibroblast expressing contractile filament proteins such as α-SMA.[31,32] Beyond canonical SMAD2/3 signaling, TGF-β activates parallel noncanonical pathways including MAP kinases (ERK [extracellular signal-regulated kinase], JNK [JUN N-terminal kinase], and p38), PI3K (phosphoinositide 3-kinase)/AKT (protein kinase B), and ρ-like GTPases that can either act independently or synergize with SMAD signaling.[33] Possessing both synthetic and contractile properties, the transdifferentiated myofibroblast secretes type I and III collagens, fibronectin, and other matricellular proteins in pathological excess.[34] The Wnt/β-catenin signaling pathway increases IL-11 (interleukin-11) production to sustain fibroblast activation and ECM production.[35] Under pressure overload conditions, mechanotransduction pathways, including integrin and focal adhesion kinase signaling, respond to increased mechanical stress.[36,37] The dysregulation of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs [tissue inhibitors of metalloproteinases]) leads to further progression of fibrosis.[38]

Pro-Fibrotic Gene Networks and Cardiac Fibrosis

Integration of genomic data has revealed regulatory mechanisms controlling pro-fibrotic gene expression. For example, the E3 ubiquitin ligase *WWP2* has been identified as a master regulator of a gene network associated with pathological fibrosis.[39] *WWP2* modulates SMAD2 signaling, a key pathway in TGF-β-mediated fibrotic responses.[40] In mouse models, deficiency of *WWP2* led to reduced myocardial fibrosis and improved cardiac function after injury, suggesting that targeting this ubiquitin ligase or its downstream signaling components could be a viable therapeutic strategy.

Network analysis through GWAS involving 686 patients with recent-onset dilated cardiomyopathy (DCM) on therapy identified protective loci near CDCP1 (a transmembrane glycoprotein regulating cell migration and anchorage independence) and NAV3 (shown to regulate myofibroblast transdifferentiation in kidney fibrosis).[41,42] Functional studies demonstrated that *CDCP1* knockdown in HCFs reduced *PDGF-BB*-induced proliferation and decreased levels of soluble *ST2*, a biomarker linked to cardiac fibrosis and HF prognosis.[43] Similarly, *NAV3* knockdown attenuated TGF-β1-mediated fibroblast-to-myofibroblast transdifferentiation, reducing the expression of α-SMA and collagen.[44,45]

The application of cardiac MRI in large cohorts has facilitated the identification of genetic variants associated with cardiac structure and function. In a study analyzing MRI data from over 36 000 individuals, 45 novel loci associated with LV traits were identified.[26] Recent advancements in noninvasive imaging and machine learning models have reinforced the notion of fibrosis as a systemic process influenced by shared genetic mechanisms. Quantification of T1 times—a surrogate for interstitial fibrosis[46,47]—in multiple organs revealed that fibrosis often cooccurs across the heart, liver, pancreas, and kidneys.[48,49] Genome-wide analyses in the studies identified shared fibrotic loci, including genes related to zinc transport (*SLC39A8*), iron metabolism (*HFE*), glucose metabolism (*PCK2*), and inflammatory signaling (*NFKB1*).

Genetic loci influencing diastolic heart function have also been identified, which is particularly relevant in HFpEF, a disease that is characterized by fibrosis. Genes near *NPPA*, *NPR1*, *NPR3*, and *PLN*—involved in natriuretic peptide signaling and calcium handling—show significant associations with diastolic dysfunction.[50–52] Sex-stratified GWAS and Mendelian randomization analyses identified sex-differential genetic variants and causal genes for HF. *NPR2* (encoding the natriuretic peptide receptor B) was found to increase LVEF and reduce HF risk in males, while *SORT1* (involved in protein trafficking and lipoprotein metabolism) was protective in females.[53] The natriuretic peptide system has been implicated in the pathophysiology of cardiac fibrosis.[54,55] Interestingly, the genetic architecture of HFpEF appears distinct from that of HFrEF. While multiple loci are associated with HFrEF, fewer genetic associations have been found in HFpEF. For example, only 1 significant locus near the *FTO* gene (involved in metabolic regulation) was associated with HFpEF, suggesting greater heterogeneity and the need for refined phenotyping.[56,57] Other genes,

such as *SPATS2L* (involved in cardiac remodeling) and *HSD17B12* (relating to myocardial energy homeostasis), have been implicated in HF and myocardial fibrosis.[58]

Analysis of the UK Biobank revealed that mosaic loss of Y chromosome in leukocytes was significantly associated with cardiovascular mortality, particularly HF, during an 11.5-year follow-up (hazard ratio, 1.76).[59] This observation led to mechanistic studies in mice where bone marrow reconstitution with Y chromosome-deficient cells resulted in accelerated cardiac fibrosis, dysfunction, and mortality. Detailed molecular characterization revealed that mosaic loss of Y chromosome promoted macrophage polarization toward a pro-fibrotic phenotype through enhanced TGF-β1 signaling. The causative role of this pathway was confirmed when TGF-β1 neutralizing antibody treatment reversed the cardiac phenotype in mosaic loss of Y chromosome mice.

EPIGENOMIC REGULATION OF CARDIAC FIBROSIS

Epigenomic regulation of the complex transcriptional networks driving myofibroblast activation and ECM deposition has shifted the paradigm in understanding the pathogenesis of cardiac fibrosis. High-throughput epigenomic profiling technologies, particularly chromatin immunoprecipitation sequencing and whole-genome bisulfite sequencing, have uncovered previously unappreciated layers of regulatory complexity in CF state transitions and revealed distinct epigenetic signatures. Epigenome-wide association studies have identified novel methylation quantitative trait loci that modulate cardiac fibrosis susceptibility, while technological advances in single-cell multi-omics enabled unprecedented resolution of cell-type-specific epigenetic landscapes during disease progression. The reversible nature of epigenetic modifications, such as posttranslational histone and covalent DNA modifications, has made them attractive therapeutic targets, and several epigenetic modulators have shown promise in preclinical models (Figure 2). Integration of epigenomic data sets with other omic modalities has begun to elucidate the intricate interplay between genetic variation, environmental factors, and epigenetic regulation in the fibrotic response to cardiac injury.

DNA Methylation Signatures in HF and Cardiac Fibrosis

DNA methylation, particularly within cytosine-phosphate-guanine-rich sites, regulates gene expression that maintains cardiac homeostasis.[60] Aberrant DNA methylation patterns have been linked to pathological cardiac remodeling and fibrosis progression. Large-scale methylation profiling has revealed distinct bulk-tissue methylation signatures across different HF subtypes. Targeted bisulfite sequencing identified 62 678 differentially methylated regions that distinguish between hypertrophic cardiomyopathy (HCM), DCM, and ischemic cardiomyopathy (ICM).[61–63] These methylation changes directly impact ECM remodeling genes critical for fibrosis. In patients with DCM, hypomethylation of the *MMP2* and *CTGF* (profibrotic growth factor) promoters increases their expression, and in ICM, hypomethylation of the *CTGF* promoter leads to a 3-fold increase in its expression.[61] Genome-wide methylation profiling has further revealed the breadth of methylation's influence on cardiac fibrosis. In infarcted heart tissue from a human epigenome-wide association studies in the Northern Swedish Population Health Study, distinct methylation signatures span 211 cytosine-phosphate-guanine sites across 196 genes, affecting key regulators of cardiac function and fibrotic remodeling such as *RYR2* (calcium handling), *NRG1* (cell survival), and *TGFB2* (fibrosis signaling).[64] Patients with ICM show widespread promoter hypermethylation, particularly affecting genes involved in oxidative metabolism. Furthermore, the interaction between the chromatin-modifying enzyme EZH2 and transcription factor (TF) FOXM1 provides another layer of control by regulating ECM-related genes through methylation-dependent mechanisms.[65]

The methylation machinery itself plays a critical regulatory role, particularly DNA methyltransferase 1 (DNMT1). In post-myocardial infarction (MI) cardiac tissue from a rat model, fibroblast-targeted DNMT1 upregulation leads to hypermethylation of the miR-133b promoter (Figure 2). This suppresses the antifibrotic microRNA (miRNA), resulting in increased expression of CTGF and enhanced fibrosis.[66] Pharmacological inhibition of *DNMT1* in mouse models improves cardiomyocyte metabolism and reduces ischemia-reperfusion injury markers. This cardioprotective effect can also be achieved through cardiomyocyte-specific adenosine kinase inhibition, which suppresses *DNMT1* under ischemia-reperfusion stress.[67] This methylation-fibrosis axis extends to inflammatory regulation, where *DNMT1*-mediated hypermethylation of the differentiation genes *NOTCH1*, *PU.1*, and *KLF4* in hematopoietic stem cells influences monocyte/macrophage lineage commitment and polarization and subsequent fibrotic responses in diabetes.[68]

Genetic background appears to influence methylation patterns in cardiac disease. Studies in different mouse strains revealed that susceptibility to cardiac disease correlates with specific methylation patterns. Following isoproterenol administration, susceptible strains showed widespread DNA demethylation in cardiac tissue, particularly in genomic regions controlling hypertrophic and fibrotic responses.[69] Moreover, epigenetic age acceleration, assessed through DNA methylation clocks in patients with end-stage kidney disease, showed significantly accelerated biological aging.[70] While no direct association with cardiovascular mortality was found, specific differentially methylated cytosine-phosphate-guanine

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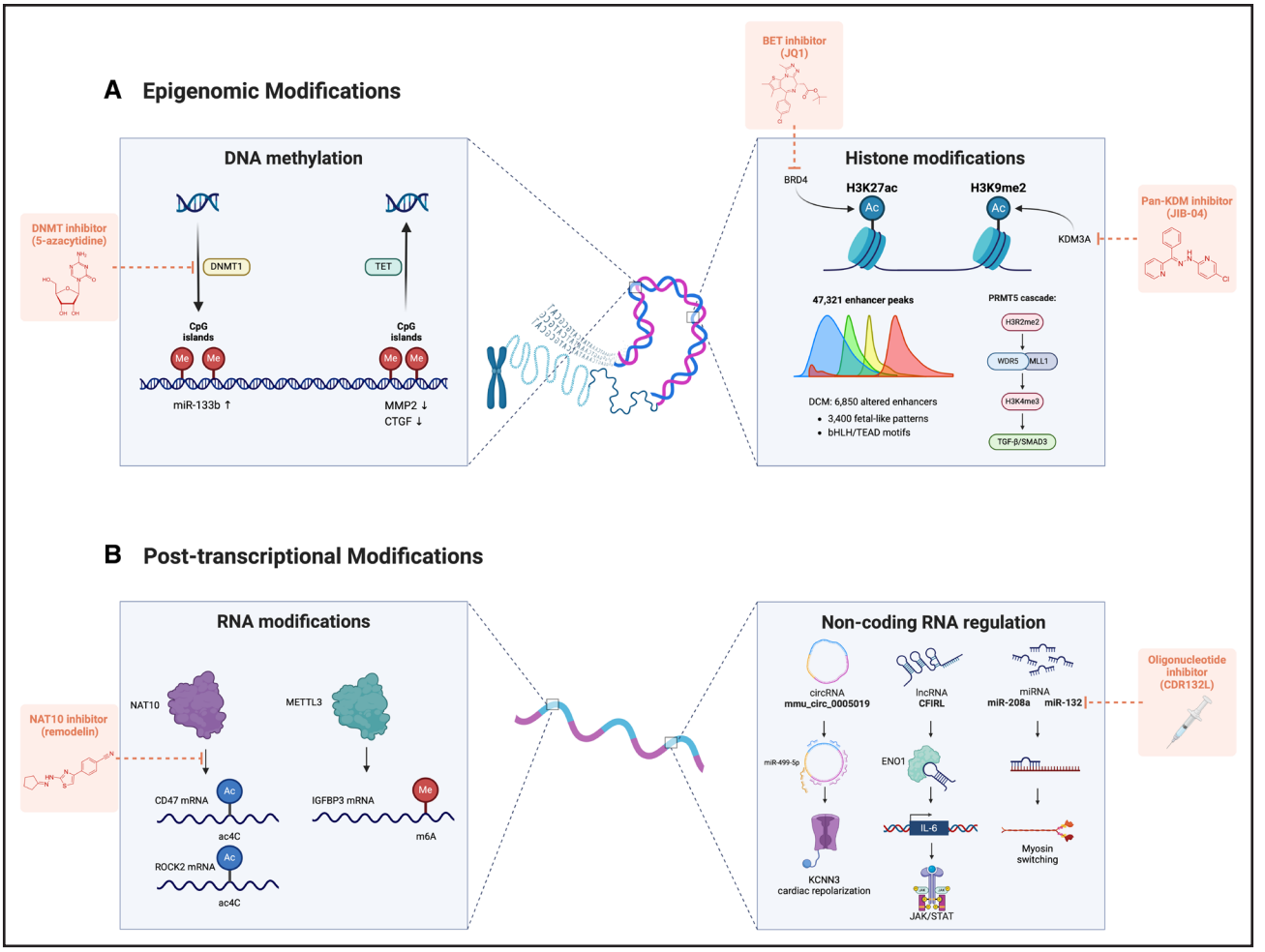

Figure 2. Epigenomic and transcriptional modifications as therapeutic targets in cardiac fibrosis.
A, Layers of epigenetic regulation present therapeutic opportunities in cardiac fibrosis. DNA methylation involving DNA methyltransferase 1 (DNMT1)-mediated regulation of miR-133b and CTGF (connective tissue growth factor) through cytosine-phosphate-guanine (CpG) methylation is targetable by DNMT inhibitors. TET (ten-eleven translocation)-mediated demethylation provides additional control of fibrotic genes. Histone modifications include BRD4 (bromodomain containing 4)-mediated reading of H3K27ac (histone 3 lysine 27 acetylation) marks at enhancers (inhibited by JQ1) and KDM3A (lysine demethylase 3A)-mediated H3K9me2 demethylation (blocked by JIB-04). In dilated cardiomyopathy (DCM), 6850 altered enhancers show fetal-like patterns with bHLH (basic helix-loop-helix)/TEAD (TEA domain transcription factor) motifs. The PRMT5 (protein arginine methyltransferase) cascade initiates H3R2 symmetrical dimethylation leading to WDR5/MLL1-mediated H3K4 trimethylation and eventual TGF-β/SMAD activation. **B**, Post-transcriptionally, NAT10 (N-acetyltransferase 10) mediates N4-acetylcytidine (ac4C) modification of CD47 (cluster of differentiation 47)/ROCK2 (a regulator of actin cytoskeleton) mRNAs (targeted by remodelin), and METTL3 (methyltransferase 3) regulates IGFBP3 (insulin-like growth factor binding protein-3) through m6A methylation. Non-coding RNA regulation includes circRNA mmu_circ_0005019 affecting cardiac repolarization, lncRNA CFIRL (cardiac fibroblast-specific long noncoding RNA) controlling IL-6 (interleukin-6) secretion through ENO1 (enolase 1), and therapeutic targeting of miRNAs (miR-208a and miR-132) through oligonucleotide inhibitors such as CDR132L. These mechanisms provide intervention points for antifibrotic therapy. Ac indicates acetyl group; BET, bromodomain and extra-terminal; JAK/STAT, Janus kinase/signal transducer and activator of transcription; KCNN3, potassium calcium-activated channel subfamily N member 3; Me, methyl group; and MMP2, matrix metalloproteinase. Created in https://BioRender.com.

sites linked to cardiovascular death were identified (eg, cg22305782 in *FBXL19*), suggesting epigenetic dysregulation in pro-fibrotic and proatherogenic pathways.

Perhaps most promising for clinical applications is the discovery of cross-tissue conservation in methylation signatures, with 3798 dysmethylated regions conserved between heart and blood.[62] This conservation has promising biomarker potential, as 3 specific methylation markers (cg24884140, cg12115081, and cg25943276) demonstrated noninferiority to NT-proBNP (N-terminal pro-B-type natriuretic peptide) in distinguishing DCM from controls. Similar approaches could conceivably extend to attempting to detect cardiac fibrosis.

Histone Modifications and Chromatin Remodeling in Fibrosis

Histone modifications alter chromatin structure, which influences gene accessibility and transcriptional activity. Dysregulation of histone-modifying enzymes and

chromatin remodelers can also drive pathological cardiac remodeling and fibrotic progression. Recent high-throughput chromatin immunoprecipitation sequencing profiling of 70 human hearts mapped 47 321 H3K27ac peaks as putative heart enhancers, with 3897 differential acetylation peaks enriched in HF-associated pathways.[71] In DCM, genome-wide chromatin immunoprecipitation sequencing analysis identified ≈6850 enhancers showing altered activity.[72] Nearly half of these enhancers (3400) demonstrate fetal-like activation patterns (fetalization), particularly affecting the genes involved in ECM remodeling and TGF-β signaling. This enhancer reprogramming is characterized by the enrichment of *bHLH* and *TEAD* TF binding motifs, demonstrating that fetal-like gene expression programs are reestablished in cardiac fibrosis.

Chromatin immunoprecipitation sequencing analysis of the lysine demethylase KDM5A in angiotensin II-treated CFs from patients with DCM identified 13 152 binding peaks at promoter and intergenic regions of fibrosis-related genes.[73] KDM5A, activated through PI3K/AKT signaling, directly regulates the ECM-related genes *IGF1* (a pro-fibrotic growth factor), *MYH11* (smooth muscle myosin), and *TGFB3* (a TGF-β family member), controlling ECM organization through chromatin modification. Another key player, the histone lysine demethylase KDM3A, promotes hypertrophy by demethylating H3K9me2 at the *TIMP1* promoter, as demonstrated in a transverse aortic constriction (TAC) mouse model.[74] Pharmacological inhibition of *KDM3A* with JIB-04 restores H3K9me2 levels and attenuates fibrosis. The Y chromosome-encoded histone demethylase UTY influences cardiac fibrosis through modulation of macrophage phenotypes. Loss of UTY in hematopoietic cells promotes a switch from inflammatory to fibrogenic transcriptional programs, accelerating cardiac dysfunction through increased TGF-β signaling.[75] This sex-specific transcriptional regulation may partly explain the increased HF susceptibility observed in men with age-related Y chromosome loss.

Similarly, the regulation of autophagy gene promoters involves *MYST1*-dependent H4K16 acetylation, which becomes suppressed during TGF-β-induced fibroblast activation.[76] HDACs (histone deacetylases) remove acetyl groups from histones. This leads to chromatin condensation and gene repression. SIRT3, a class III HDAC, inhibits fibrosis through histone H3K27 deacetylation at the FOS (fos proto-oncogene, AP-1 transcription factor subunit) promoter, reducing the pro-fibrotic and proinflammatory FOS/AP-1 signaling.[77] In hypertensive and aging rodent models, treatment with the HDAC inhibitor ITF2357 improved cardiac relaxation, and thus diastolic function, through increased acetylation, independent of changes in myosin isoforms or calcium sensitivity.[78]

Histone acetylation is mediated by histone acetyltransferases, which generally promote gene transcription by relaxing chromatin. BRD4 is a chromatin reader protein and member of the BET (bromodomain and extra-terminal) domain family that is recruited dynamically to cardiac enhancers in both immune and fibroblast cells. In macrophages, BRD4 enhances inflammatory signaling by binding the IL1b enhancer to increase IL-1β secretion, whereas in fibroblasts, it drives pro-fibrotic gene expression through TGF-β-responsive enhancer activation.[79,80] This pathological gene expression is fine-tuned by miRNA-9, which negatively regulates BRD4's recruitment to stress-responsive super-enhancers.[81] Inhibition of BRD4 with JQ1 reduces fibrosis and improves cardiac function in vivo.[80] Moreover, the significance of enhancer regulation is further highlighted by the pro-fibrotic NLRP3 inflammasome, which possesses super-enhancer regions with high H3K27ac and H3K4me1 levels.[82]

In CFs, protein methylation adds another dimension through PRMT5 (protein arginine methyltransferase), which initiates a methylation cascade beginning with H3R2 symmetrical demethylation, as shown in HCFs. This is followed by WDR5 (WD repeat domain 5)/MLL1 (lysine methyltransferase 2A)-mediated H3K4 trimethylation, ultimately activating pro-fibrotic TGF-β/SMAD3 signaling.[83] Accordingly, PRMT5 deficiency attenuates cardiac fibrosis and improves LVEF.

TRANSCRIPTOMIC LANDSCAPES IN CARDIAC FIBROSIS

By examining RNA transcripts in fibrotic cardiac tissue, transcriptomic technologies, particularly single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq), have highlighted critical gene expression changes and provided a unique transcriptomic fingerprint related to ECM production, fibroblast activation, and inflammatory responses—all central to the fibrotic process. It is important to note that while some recent studies use single-cell or spatial omics to identify markers at the cell population level, many of the studies cited in this review report bulk-tissue or plasma-based analysis. As a result, proposed fibrosis markers often represent integrated signals from distinct cell types reflecting the myocardial microenvironment as a whole.

Fibroblast Subpopulation Heterogeneity and Activation

Advanced transcriptomic analyses have uncovered significant heterogeneity among CFs and identified distinct subpopulations that contribute differently to fibrotic remodeling (Figure 3). In HF-induced TAC mouse models, scRNA-seq and fate tracing of the vascular and perivascular niche identified a fibroblast subpopulation expressing THBS4 (thrombospondin-4, a matricellular protein that mediates cell-matrix interactions and promotes fibrosis)[84] that expanded significantly during late-stage fibrosis (28 days post-TAC).[85,86] These *THBS4*$^{+}$ fibroblasts express high levels of ECM genes, including

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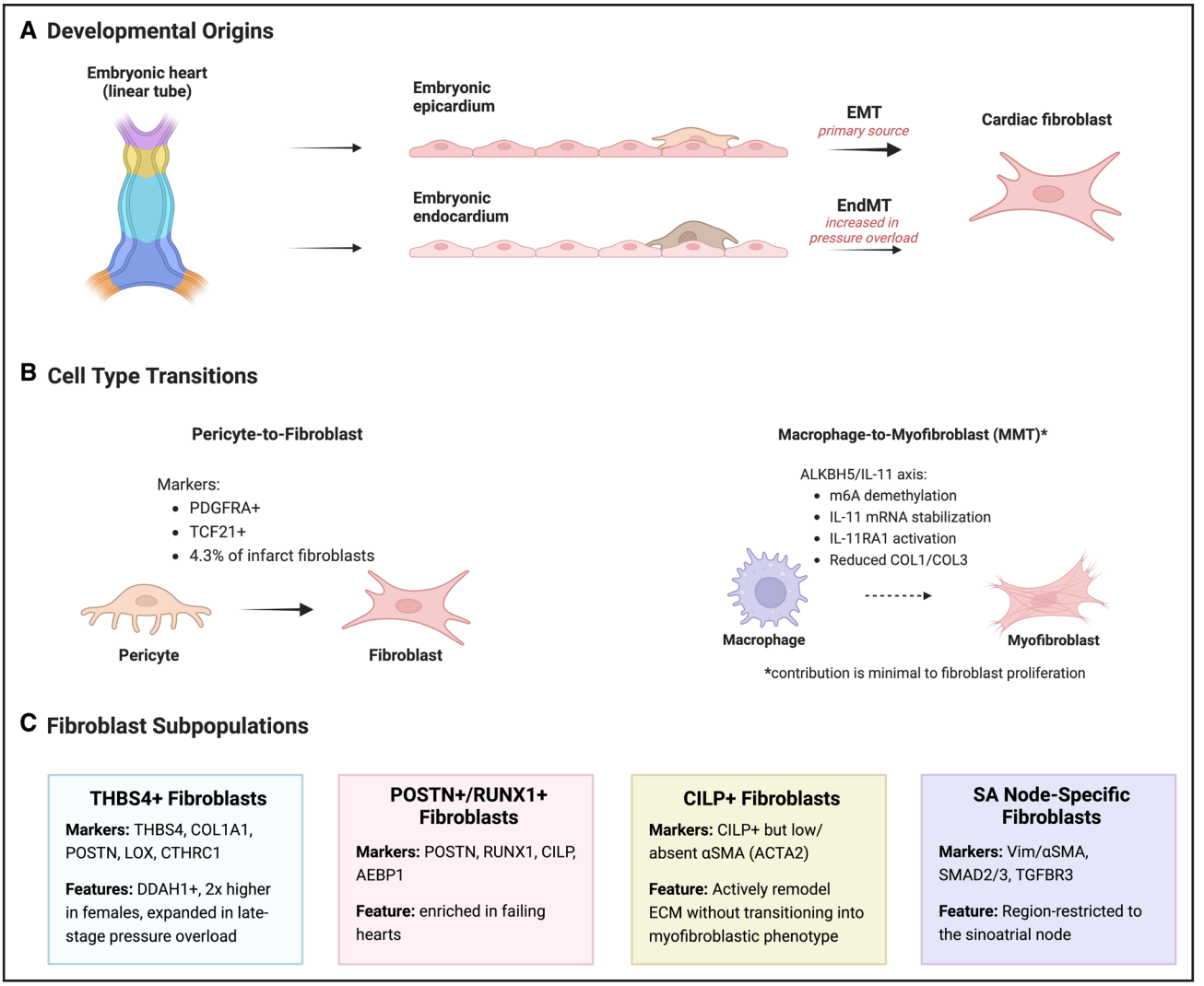

Figure 3. Origins and transcriptional heterogeneity of cardiac fibroblasts.
A, Adult CFs show diverse developmental origins. During development, epicardial-to-mesenchymal transition (EMT) serves as the primary source of CFs, while endocardial–to-mesenchymal transition (EndMT) represents a likely limited source of fibroblasts in adult cardiac injury, characterized more by transient endothelial plasticity than complete transition. Particularly important during pressure overload conditions. **B**, Recent studies uncovered novel cellular plasticity pericyte-to-fibroblast transition contributes about 4.3% of infarct fibroblasts, marked by *PDGFRA*$^+$ and *TCF21*$^+$ expression. MMT is regulated by *ALKBH5*-mediated m6A demethylation of IL-11 mRNA, leading to its stabilization and enhanced IL-11/IL11RA1 signaling, though its contribution to overall fibroblast numbers appears minimal. **C**, Single-cell transcriptomics revealed 4 distinct fibroblast subpopulations: THBS4$^+$ (thrombospondin-4) fibroblasts expressing THBS4, COL1A1 (type I collagen), POSTN (periostin), LOX (lysyl oxidase), and CTHRC1 (a pro-fibrotic gene), characterized by DDAH1 (dimethylarginine dimethylaminohydrolase 1) positivity and 2-fold higher abundance in females during late-stage pressure overload; *POSTN*$^+$*/RUNX1*$^+$ fibroblasts expressing POSTN, RUNX1, CILP (cartilage intermediate layer protein), and AEBP1 (adipocyte enhancer-binding protein 1), which are enriched in failing hearts; *CILP*$^+$ fibroblasts that maintain CILP expression but low/absent αSMA (α-smooth muscle actin; ACTA2), actively remodeling extracellular matrix (ECM) without myofibroblastic transition; and SA node-specific fibroblasts expressing vimentin (Vim)/αSMA and regional SMAD2/3 and TGFBR3 (TGF-β receptor III) activation. Created in https://BioRender.com.

COL1A1 (type I collagen), *POSTN* (periostin), and *LOX* (lysyl oxidase), facilitating ECM cross-linking and tissue stiffening. These fibroblasts are regulated by the *TEAD1* TF, which correlates with increased fibrosis-related gene expression, such as *CTHRC1* (a pro-fibrotic gene) and *DDAH1* (involved in nitric oxide signaling). Similarly, high-resolution transcriptomic profiling in angiotensin II-treated mouse hearts identified fibroblast subpopulations such as fibroblast-*CILP* and fibroblast-*THBS4* as major contributors to ECM remodeling during chronic stress.[86] These fibroblasts promote fibrosis without transitioning into traditional *ACTA2*$^+$ (α-SMA-expressing) myofibroblasts. This fibroblast heterogeneity extends to human HF. Persistent fibroblast activation in hypertrophic and failing human hearts was characterized using snRNA-seq of LV myocardium samples from patients with aortic stenosis and HFrEF, which identified a distinct fibroblast subcluster (FB1) enriched in diseased hearts. This subcluster expresses POSTN, RUNX1, CILP, and AEBP1 (adipocyte enhancer-binding protein

1),[87] reflecting its activated state. This enrichment of FB1 fibroblasts, along with a decrease in FB4 fibroblasts (a more quiescent population), appears to be a hallmark of failing hearts.[88] The pathogenic role of this shift is supported by the finding that elevated circulating levels of AEBP1 correlate strongly with cardiac fibrosis severity, as confirmed by T1 mapping on cardiac MRI, and its silencing in HCFs reduces proliferation, migration, contraction, and α-SMA expression, linked to decreased TGF-β pathway activity.[89] In both DCM and HCM, single-nucleus profiling has identified activated fibroblast populations expressing an array of fibrotic markers, including *POSTN*, *NOX4* (NADPH oxidase 4, which generates pro-fibrotic reactive oxygen species),[90] *FAP* (fibroblast activation protein), and *COL1A1*, while simultaneously downregulating *PDGFRA*.[91] This activated population is largely absent in nonfailing LV tissue, and pseudotemporal trajectory analysis suggests a progressive transition from quiescent to activated states, marked by increasing expression of SLC44A5 (a solute carrier involved in choline transport), POSTN, and AEBP1 along the activation pathway. Functional validation using clustered regularly interspaced short palindromic repeats-knockout screens identified PRELP (a leucine-rich repeat protein that regulates collagen fibrillogenesis), JAZF1 (a transcriptional repressor), and COL22A1 as critical regulators of this activation process.[91] This regulatory complexity extends to disease-specific responses, where scRNA-seq of ischemic mouse hearts revealed that fibroblast subpopulations highly express markers of activation such as POSTN, TNC (tenascin C, an ECM glycoprotein induced during injury), WISP1 (WNT1-inducible signaling pathway protein 1, a matricellular protein), and CKAP4 (cytoskeleton-associated protein 4).[92] Intriguingly, CKAP4 appears to act as a molecular brake on fibroblast activation—while upregulated in diseased hearts, its inhibition in vitro actually enhanced fibroblast activation in response to TGF-β, indicating a previously unrecognized negative feedback mechanism. These data collectively demonstrate that the activation of fibroblasts is marked by core pro-fibrotic gene signatures (eg, *THBS4*, *POSTN*, and *ECM* gene expression) but also demonstrate that context-specific variation exists. In the pressure overload (TAC or angiotensin II) and chronic human HF settings, THBS4$^+$ or CILP$^+$ fibroblast clusters are observed that remain relatively ACTA2$^-$ yet produce abundant ECM. By contrast, in acute postinfarction models, α-SMA (ACTA2) is significantly upregulated in activated fibroblasts, resulting in the classic myofibroblast phenotype. And thus, although the core pro-fibrotic signature is broadly shared, the relative prominence of ACTA2$^+$ myofibroblasts versus ACTA2$^-$ ECM-secreting fibroblasts varies with the pathophysiologic context and timeline of injury.

CF heterogeneity also extends beyond disease-specific patterns to include both sex-specific and regional variations. Sexual dimorphism in cardiac remodeling is evident in the transcriptomic landscape, observed in angiotensin II-treated mouse hearts, where female hearts show a 2-fold higher abundance of fibroblast-*THBS4* compared with males and higher expression of ECM remodeling genes including *FMOD* (fibromodulin, which regulates collagen fibrillogenesis) and *CILP2* (cartilage intermediate layer protein 2).[86] ScRNA-seq analyses of cellular diversification in human HF identified multiple fibroblast subpopulations, including disease-associated fibroblasts significantly enriched in DCM samples. These subpopulations express key fibrotic markers *POSTN*, *TNC*, and *SERPINE1*.[93] Within the heart itself, fibroblast activation shows regional specificity, contributing to localized patterns of fibrosis under pathological conditions. This is particularly evident in the sinoatrial node, where human samples of failing hearts demonstrate a unique population of Vimentin$^+$/α-SMA$^+$ myofibroblasts.[94] These specialized myofibroblasts, found exclusively in failing sinoatrial nodes, drive pathological fibrosis through a coordinated program of pro-fibrotic gene expression, including upregulation of *SMAD2/3* (key TGF-β signaling mediators), *TGFBR3* (TGF-β receptor III), *COL1A2*, and *POSTN*. This regional specificity of fibroblast activation may help explain the often-heterogeneous patterns of cardiac fibrosis observed in HF.

Lineage Transitions and Nonfibroblast Contributors to Fibrosis

Genetic lineage tracing and advanced in vivo studies have demonstrated that adult CFs predominantly originate from mesodermal progenitors in the embryonic epicardium, particularly through epithelial-to-mesenchymal transition of epicardial-derived cells, which migrate into the myocardium and differentiate into fibroblasts.[95] Recent work further highlights that fibroblasts originating from the endocardium may preferentially expand via endothelial-to-mesenchymal transition under pressure overload conditions[96,97] (Figure 3). The role of endothelial-to-mesenchymal transition in adult cardiac fibrosis, however, remains controversial. Early investigation using cell lineage tracing with *Tie1* Cre-lox showed that about one-third of fibroblasts might arise from endothelial cells and promote cardiac fibrosis.[98] This view has been recently challenged. Using a dual recombinase EndoMTracer system that enables continuous tracing of endothelial cell fate even during transient marker expression, it has been shown that adult endothelial cells did not transdifferentiate into myofibroblasts nor transiently expressed mesenchymal markers (including αSMA) after cardiac injury in both TAC and MI models.[99–101] In addition, studies identified transient endothelial-mesenchymal activation, which is a reversible state in which endothelial cells briefly express mesenchymal genes post-MI, before returning to their endothelial identity, rather than fully transitioning to fibroblasts.[102] Therefore, the current evidence suggests that complete endothelial-to-mesenchymal

transition may be limited in adult cardiac injury, but rather partial or transient endothelial plasticity may contribute to fibrotic remodeling.

Although activated fibroblasts are the primary mediators of cardiac fibrosis, transcriptomics revealed that other cell types contribute to the fibrotic landscape (cellular plasticity) through lineage transitions and phenotypic changes. Pericytes are specialized mural cells critical for vascular stability and blood flow regulation.[103,104] Recent studies have shown that a subset of these cells can acquire a fibroblast-like phenotype.[105–107] These pericyte-derived fibroblasts are unique in that they maintain expression of pericyte markers while acquiring fibroblast identity genes such as *PDGFRA* (marker of resident fibroblasts) and *TCF21* (TF essential for fibroblast development) after MI.[108] Pseudotemporal trajectory analysis reveals their progressive adoption of a pro-fibrotic program, marked by increased expression of growth factors (TGFB1, PDGFA, and VEGFA), matrix proteins (COL1A2 and COL3A1), matrix-remodeling enzymes (MMP2 and TIMP3), and fibrogenic integrins (ITGA1, ITGA2, ITGAV, and ITGB1). While lineage tracing in human MI specimens shows that only about 4.3% of infarct fibroblasts originate from NG2$^+$ pericytes post-MI, their enhanced fibrogenic activity suggests an outsized role in promoting fibrogenesis and scar maturation. The dynamic nature of cellular transitions in cardiac fibrosis has been further investigated through spatial multi-omic mapping of human MI tissue. This approach identified distinct fibroblast states along a differentiation trajectory, with SCARA5$^+$ fibroblasts (scavenger receptor class A member 5) serving as progenitors that differentiate into *POSTN*$^+$*COL1A1*$^+$ myofibroblasts. Pseudotime analysis and lineage tracing confirmed the transition from progenitor to myofibroblast states (fibroblast-to-myofibroblast differentiation), suggesting a dynamic nature of fibroblast activation after injury.[109]

Macrophages have been recognized as contributors to cardiac remodeling and fibrosis through the process of macrophage-to-myofibroblast transition in a zebrafish model.[110] ScRNA-seq and lineage tracing in angiotensin II-induced cardiac fibrosis mouse model revealed that this transition is orchestrated by *ALKBH5* (an N6-methyladenosine [m6A] RNA demethylase)[111] (Figure 3). *ALKBH5* promotes *IL-11* expression by m6A demethylation of *IL-11* mRNA, leading to its stabilization under hypertensive stress. Without *ALKBH5*, IL-11 mRNA undergoes faster degradation, reducing IL-11 protein levels. The resulting activation of the IL-11/IL11RA1 signaling axis drives cardiac macrophage transdifferentiation into myofibroblast. Macrophage-specific knockout of *ALKBH5* not only reduces myocardial fibrotic burden (*COL1* and *COL3* expression) but also improves diastolic function. Nonetheless, it is increasingly recognized, through lineage tracing and immunophenotypic marker detection, that macrophages can influence fibroblast activation and ECM production largely via paracrine mechanisms rather than direct lineage conversion. Most studies of myocardial injury or chronic inflammation demonstrate that macrophages secrete pro-fibrotic growth factors, chemokines, and other soluble mediators (eg, TGF-β, IL-1β, and IL-6) that stimulate resident CFs to differentiate into myofibroblasts.[112–114]

Signaling Pathways, TFs, and Intercellular Communication

Transcriptomic studies have revealed the complex signaling networks implicated in modulating fibrosis, from master regulatory pathways to cell-type-specific signals. At the center of this network, the TGF-β signaling pathway is a well-established mediator, whose dysregulation can drive pathological fibrosis through multiple mechanisms.[2,115–117] Recent single-cell and spatial transcriptomics identified *HTRA3* as a key brake on this pathway. *HTRA3* directly degrades TGF-β protein, and *HTRA3* knockout in pressure-overload mouse hearts leads to exacerbated cardiac fibrosis with increased expression of matrix genes *COL1A1*, *COL3A1*, and *POSTN*. This protective role of *HTRA3* appears clinically relevant, as failing human hearts show significant *HTRA3* downregulation, correlating with increased TGF-β signaling and elevated *IGFBP7*, a cytokine strongly associated with advanced HF.[117] The coordinated dysregulation of TGF-β signaling extends to the β-adrenergic pathway by being significantly activated in CFs from genetically distinct mouse lines with differential fibrotic responses.[118] *LTBP2*, a regulator of TGF-β bioavailability and activation in the ECM, was identified as a consistently upregulated gene that could serve as a biomarker for fibrosis severity. The genetic background of HF can significantly influence these fibrotic responses. A genotype-stratified transcriptional atlas of failing human hearts using snRNA-seq revealed that pathogenic rare variants in cardiomyopathy genes influence cellular composition and gene expression profiles.[119] Mutations in *RBM20* increase pro-fibrotic gene expression, while *TTN* mutations deplete antiinflammatory fibroblast subpopulations. Genotype-specific activation patterns of TGF-β, IGF, and BMP signaling pathways are also seen.

Genome-wide studies have revealed how regulatory elements control fibrotic responses in HF. Cap analysis of gene expression identified over 17 000 promoters and 14 000 enhancers in human hearts, with promoter switching events in failing hearts leading to altered expression of proinflammatory chemokines *CXCL1*, *CXCL3*, and *CXCL8*. These chemokines promote fibroblast activation and ECM production through inflammatory cascades.[120,121]

Cell-type-specific transcriptional programs add another layer of regulation to cardiac fibrosis. ScRNA-seq revealed that the *PDGFRA* and *EGFR* signaling

pathways coordinate complex interactions between endothelial cells, macrophages, and epicardial cells to drive fibrosis in diabetic cardiomyopathy.[122] Inhibition of the *PDGFRA* axis reduces collagen deposition and improves cardiac function in diabetic mice, demonstrating the therapeutic potential of targeting these pathways. Additionally, members of the *SOX* family of TFs, *SOX4* and *SOX8*, are significantly upregulated in failing hearts and strongly correlated with the fibrosis-related genes *CTGF* (mediator of ECM production), *POSTN*, *COL1A1*, and *LOX*.[123] Overexpression of these TFs increases fibrosis markers in vitro. *MEOX1* is upregulated in activated CFs after pressure overload and represents a transcriptional switch that modulates chromatin accessibility.[124] BET protein inhibition with JQ1 reverses fibroblast activation through *MEOX1* suppression. Single-cell dual-omics combining transcriptomic and epigenomic profiling has identified 3 distinct fibroblast subpopulations, each with unique epigenomic signatures regulated by specific TFs including *TCF21* (a determinant of CF fate), *TWIST1* (an epithelial-to-mesenchymal transition regulator), and *bHLH*.[125] These subpopulations dynamically transition after MI, demonstrating epigenetic plasticity in response to cardiac injury. When activated under hypertensive stress, the stress-induced TF *ATF3* suppresses fibrosis through direct binding to the Map2K3 promoter.[81] This binding recruits HDAC1, establishing a repressive chromatin state that inhibits MAP2K3-p38 signaling and reduces TGF-β-dependent gene expression. Similarly, the YAP/TAZ transcriptional coactivators are established mediators of organ fibrosis[126] that are also involved in cardiac remodeling. Mechanistically, these Hippo pathway effectors regulate fibroblast-to-myofibroblast differentiation through TEAD-dependent transcriptional activation, modulated by cytoskeletal dynamics and matrix mechanotransduction, to control pro-fibrotic gene expression.[127] RNA-seq revealed that genetic ablation of fibroblast *YAP* attenuates cardiac fibrosis and improves cardiac function through *MRTF-A* inhibition[128]; furthermore, pharmacological YAP-TEAD interference in a mouse model using verteporfin reduces myocardial fibrosis and remodeling after injury.[129]

A systems-level analysis of LV transcriptomes in mice identified the procollagen N-proteinase *ADAMTS2* as a driver of isoproterenol-induced cardiac remodeling in mice through regulation of hypertrophic and pro-fibrotic genes (*NPPA*, *NPPB*, *TNC*, and *MFAP2*).[130] *ADAMTS2* acts as a negative regulator of TGF-β signaling, as demonstrated by increased fibrosis in *ADAMTS2*-knockout mice and diminished fibrosis in *ADAMTS2* overexpression.[131]

The analysis of transcriptomic responses in human hypertrophic hearts also revealed dysregulation of intercellular communication. SnRNA-seq of pressure-overloaded hearts revealed dramatic downregulation of *EPHB1*, a member of the Eph receptor family. The interaction between *EPHB1* and its ligand *EFNB2* on endothelial cells provides protection against pathological remodeling. *EFNB2* modulates cardiac fibrosis via the Stat3 and TGF-β/Smad3 signaling pathways, and its supplementation can prevent adverse cardiac remodeling.[132,133]

Translational and Posttranscriptional Regulation

In addition to the widespread transcriptional control of fibrosis-related genes, translational and posttranscriptional regulatory mechanisms drive the activated fibroblast phenotype. RBPs (RNA-binding proteins) mediate widespread posttranscriptional control of fibrosis by selectively controlling the translation of mRNAs encoding pro-fibrotic proteins. Through bulk RNA-sequencing and ribosome profiling in HCFs, 1 study showed that approximately one-third of genes involved in the TGF-β1-driven fibrotic response are regulated at the translational level by RBPs such as PUM2 and QKI.[134] Knockdown of these RBPs in fibroblasts reduced α-SMA (ACTA2) expression and *MMP2* secretion. Similarly, the role of the EPRS (enzyme glutamyl-prolyl-tRNA synthetase) has been elucidated in regulating proline-rich collagen synthesis. EPRS expression is significantly elevated in failing hearts, and its genetic ablation selectively reduces the translation efficiency of proline-rich collagens and other pro-fibrotic genes, attenuating fibrosis.[135]

Beyond translational control, posttranscriptional regulation of RNA can also affect fibroblast activation. The N4-acetylcytidine (ac4C) RNA modification enzyme NAT10, for instance, enhances the stability and translation efficiency of specific mRNAs, including *CD47* (an integrin-associated protein) and *ROCK2* (a regulator of actin cytoskeleton), promoting fibrosis and hypertrophy. Elevated ac4C levels contribute to hypertrophy and fibrosis, while *NAT10* inhibition by remodelin attenuates these effects.[89] The RNA methyltransferase *METTL3* catalyzes m6A RNA methylation and enhances *IGFBP3* (insulin-like growth factor binding protein-3) expression in CFs, which promotes fibrosis.[136] In atrial fibrillation patients, elevated *IGFBP3* and m6A levels correlate with increased fibrosis. The therapeutic potential is demonstrated by *METTL3* silencing, which reduces both *IGFBP3* levels and fibrotic markers.[136] In addition to RNA modifications, alternative splicing and polyadenylation drive cardiac fibrosis progression. Recent RNA-sequencing revealed over 3200 differentially spliced isoforms in HF, nearly half reverting to fetal-specific splicing patterns, indicating widespread dysregulation of splicing machinery during cardiac fibrogenesis.[137] RBPs such as RBFOX2, QKI, and PTBP1 modulate this process through regulated exon inclusion/exclusion, affecting genes involved in ECM production and fibroblast activation.[137,138] Similarly, alternative polyadenylation influences transcript stability and translation efficiency and ultimately fibroblast phenotypes. One such example is the

global shortening of 3' untranslated regions in activated CFs as early as day 1 post-MI, which enhances their proliferative capacity and promotes transdifferentiation into myofibroblasts.[138] Current evidence demonstrates the influence of alternative polyadenylation on the expression of fibrotic markers (α-SMA, collagen I, and fibronectin) in cardiac fibrosis.[139] However, further investigation is needed in this expanding research area to fully understand its mechanistic roles.

Long noncoding RNAs represent another crucial regulatory axis in cardiac fibrosis. Long noncoding RNAs are a diverse class of regulatory transcripts exceeding 200 nucleotides without protein-coding potential. These molecular scaffolds function through recruitment of chromatin-modifying complexes and regulation of gene expression (Figure 2). The CF-specific long noncoding RNA, CFIRL (cardiac fibroblast-specific long noncoding RNA), facilitates fibroblast proliferation and transdifferentiation in patients with DCM. CFIRL recruits *ENO1*, a multifunctional glycolytic enzyme, to the IL-6 promoter, thereby enhancing IL-6 secretion and triggering cardiomyocyte hypertrophy through JAK/STAT signaling.[140]

A unique class of regulatory RNAs, circular RNAs, are covalently closed RNA molecules formed by back-splicing. They regulate gene expression predominantly through miRNA sequestration and protein interactions. The circular RNA mmu_circ_0005019 demonstrates antifibrotic properties by acting as a molecular sponge for miR-499-5p, thereby regulating *KCNN3*, a calcium-activated potassium channel.[141] Overexpression of mmu_circ_0005019 increases expression of cardiac repolarization ion channels, potentially influencing arrhythmogenic susceptibility in fibrotic hearts.

MiRNAs constitute perhaps the most extensively studied class of noncoding RNAs in cardiac fibrosis. These small (≈22 nucleotides long) regulators fine-tune cardiac gene expression through posttranscriptional repression of target mRNAs. Several miRNAs demonstrate direct involvement in fibrotic pathways: miR-101 suppresses postinfarct fibrosis by targeting c-FOS, a TF that promotes TGF-β1 signaling[142]; miR-122 aggravates angiotensin II-induced apoptosis and autophagy imbalance by targeting the SIRT6-Elabela-ACE2 axis[143]; and miR-214 promotes CF proliferation and collagen synthesis by targeting MFN2, a mitochondrial fusion protein, leading to ERK1/2-MAPK (mitogen-activated protein kinase) pathway activation.[144] Therapeutic inhibition of miR-208a in Dahl salt-sensitive rats with hypertensive HF improves cardiac function and survival in HF by reversing pathological myosin isoform switching and reducing fibrosis.[145]

PROTEOMIC CHARACTERIZATION OF CARDIAC FIBROSIS

Cardiac fibrosis involves networks of dynamic protein interactions. Recently, high-throughput proteomic profiling, which assesses the full array of proteins in tissue or blood, has allowed us to identify a plethora of proteins and protein signatures that can act either as biomarkers or therapeutic targets for fibrosis (Table). Proteome-wide techniques such as data-independent acquisition mass spectrometry, proximity extension assay, spatial proteomics, and quantitative phosphoproteomics have mapped the interactome of important fibrogenic mediators from cell surface receptors to intracellular mechanosensory complexes. This systems-level protein analysis has been facilitated through advanced bioinformatics and large-scale proteomic platforms, including slow off-rate modified aptamer scan (SOMAscan), Olink, and tandem mass tag labeling, that can measure thousands of proteins simultaneously.

Fibrosis-Related Proteomic Biomarkers in HF

In a proteomic analysis of 4877 circulating plasma proteins in HF-free cohorts, 37 proteins demonstrated independent associations with HF risk, including SVEP1 (sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1), SPON1 (spondin 1), FSTL3 (follistatin like 3), IGFBP7, and MFAP4 (microfibril associated protein 4).[171] These proteins are enriched in pathways central to fibrotic remodeling, including PI3K/AKT signaling, STAT3 activation, and ECM organization. Mendelian randomization validated the causal relationships for 10 of these proteins, such as SVEP1, SPON1, and CCL15, with both HF risk and LV structure. Similarly, thousands of proteins and phosphorylation sites were enriched in fibrosis-related signaling cascades including TGF-β, WNT/β-catenin, PI3K-AKT, MAPK, and SMAD signaling pathways in a mass spectrometry-based phosphoproteomic study using human cardiac tissue samples, TAC mouse, and fibrosis-on-a-chip models.[174]

Examination of 4210 proteins across health-to-HF progression identified distinct proteomic signatures, including 52 differentially expressed proteins (DEPs) specific to HFpEF and 2122 DEPs in HFrEF, demonstrating a broader, possibly systemic, involvement in reduced EF.[162] Parallel investigation using aptamer-based proteomics targeting 1305 proteins revealed temporal evolution of protein signatures across HF stages.[176] Early stage disease was characterized by elevated levels of inflammatory and immune-related proteins, including NT-proBNP, C-reactive protein, IL-1RA, CXCL13, C5a, and TSP2, whereas advanced HF involved upregulation of ECM remodeling proteins. Notably, TSP2, a glycoprotein that regulates collagen assembly and fibroblast function, was consistently elevated across HF stages. This molecular complexity extends to aging-related cardiac dysfunction, where high-throughput profiling identified 286 proteins associated with HF, 48 of which were concurrently associated with frailty, including the fibrosis-related proteins COL28A1 and TGFBI.[163] These proteins

Table. Proteomic Biomarkers Profiling in Cardiac Fibrosis

Protein biomarkers	Proteomic detection platform	Molecular mechanism	Disease association (clinical relevance)	Pathway	References
ADAMTS5	Label-free LC-MS/MS	ECM proteoglycans degradation (ECM turnover modulation)	HF	…	146
Adiponectin (ADIPOQ)	LC-MS/MS	Myofibroblast differentiation inhibition and ECM production inhibition	PPCM	AMPK and PI3K/AKT signaling pathways	147
Angiopoietin-2 (ANGPT2)	SOMAscan v4	Angiogenesis and vascular remodeling (endothelial permeability enhancement)	HFrEF, HFpEF, post-MI HF, LV dysfunction, and LA dysfunction	Tie2 receptor signaling pathway and VEGF signaling pathway	148–151
ANGPTL2	SOMAscan	Chronic inflammation, angiogenesis, and fibroblast activation	HCM and vascular remodeling	NF-κB signaling pathway	152
Asporin (ASPN)	Label-free LC-MS/MS	TGF-β signaling modulation, collagen mineralization inhibition, and ECM assembly regulation	Ischemic HF	TGF-β signaling pathway	146
Biglycan (BGN)	Label-free LC-MS/MS	Collagen fibrillogenesis and ECM structure stabilization	HF and ischemic HF	TLR2/4-NF-κB signaling pathway	146
BMP1	SOMAscan	Procollagen processing (collagen maturation and ECM accumulation)	HCM and MACE prediction	…	153
Cathepsin D (CTSD)	Olink cardiovascular III	ECM degradation	Interstitial fibrosis	…	154
Cathepsin L (CTSL)	Olink PEA	ECM degradation	Cardiac remodeling	…	155
Caveolin-1 (CAV1)	Phosphoproteomics (MS-based)	TGF-β signaling modulation, fibroblast activation regulation, and membrane stability	HF and LVAD responders	TGF-β signaling pathway	156
CD44	Olink PEA	Cell-ECM adhesion, fibroblast migration, and proliferation	Cardiac remodeling	MAPK/ERK pathway, PI3K/AKT pathway, and Src signaling pathway	155
CHI3L1 (YKL-40)	Olink cardiovascular III	Fibroblast proliferation, collagen production, and inflammatory marker	DCM-HF	MAPK/ERK and PI3K/AKT pathways	157
CITP	AlphaLISA	Collagen type I cross-linking degradation indicator	HF and fibrosis	…	158
COL1A1	CE-MS, SILAC-AHA LC-MS/MS, LC-MS/MS, and phosphoproteomics (MS-based)	ECM structural integrity, tissue stiffness, and fibrosis progression	HF, fibrosis, MI, COVID-19 heart injury, and LVAD responders	…	156,159–161
COL28A1	SOMAscan v4	Collagen fibril organization and ECM structural integrity	Ischemic HFrEF, HF, and frailty	…	162,163
COL3A1	CE-MS, SILAC-AHA LC-MS/MS	ECM structural integrity and fibrosis progression	HF, fibrosis, and MI	…	159,160
COL4A1	CE-MS	Basement membrane structure	HF	…	159
COL5A2	CE-MS	Collagen fibril assembly regulation and ECM structural integrity	HF	…	159
COL6A1	CE-MS	ECM organization	HF	…	159
Cystatin C (CST3)	SOMAscan v4	Cysteine protease inhibition and ECM degradation	HFpEF, HFrEF, HFmrEF, frailty, and renal function	…	150,162–164
Decorin (DCN)	Label-free LC-MS/MS	Collagen fibrillogenesis and ECM assembly	HF	TGF-β and EGFR signaling pathways	146
E-Selectin (SELE)	Olink cardiovascular III	Leukocyte recruitment and endothelial adhesion	Interstitial fibrosis	…	154

(*Continued*)

Table. Continued

Protein biomarkers	Proteomic detection platform	Molecular mechanism	Disease association (clinical relevance)	Pathway	References
EGFR	SOMAscan v4	Fibroblast proliferation and ECM synthesis	Cardiac remodeling	EGFR signaling pathway, MAPK/ERK pathway, and PI3K/AKT pathway	162
Endoglin (ENG)	Olink cardiometabolic	TGF-β signaling enhancement and fibroblast activation	HFpEF	TGF-β signaling pathway	165
Fibronectin (FN1)	SOMAscan, LC-MS/MS, label-free LC-MS/MS, SILAC-AHA LC-MS/MS, and TMT-labeled LC-MS/MS	Fibroblast attachment and ECM deposition	High-risk HCM subtype, fibrosis, DCM, ICM, PPCM, MI, and COVID-19 heart injury	Integrin signaling pathway and focal adhesion pathway	147,160,161,166–170
Fibulin-3 (EFEMP1)	SOMAscan v4	Elastic fiber organization and ECM stabilization	HF, HFpEF, frailty, mortality, and fibrosis	…	149,163,164
Fibulin-5 (FBLN5)	SOMAscan	Elastic fiber assembly and ECM stabilization	Elevated LAP	…	148
FSTL3	SOMAscan v4	Fibroblast proliferation, ECM production, and hypertrophy regulation	Cardiac fibrosis, HF, frailty, mortality, and post-MI HF	Activin signaling pathway antagonism	151,163,164,171
Galectin-3 (Gal-3)	SOMAscan	Fibroblast activation, collagen synthesis, and inflammation	HCM and MACE prediction	TGF-β signaling pathway, Wnt/β-catenin pathway, and NF-κB signaling pathway	152,153
Galectin-9 (Gal-9)	Olink cardiovascular II	Immune response modulation	HFpEF and incident HF hospitalization	TIM-3and NF-κB signaling pathways	172
GDF-15	SOMAscan v4 and olink cardiovascular II	Fibroblast proliferation, ECM deposition, and inflammatory response	HTN/DM, HF, frailty, HFrEF, HFmrEF, HCM, disease severity, and MACE prediction	TGF-β signaling pathway	150,152,153,163,164,173
GSK3	TMT-labeled LC-MS/MS	Wnt/β-catenin signaling regulation and fibrotic gene expression	Cardiac fibrosis and HCM	Wnt/β-catenin signaling pathway	174
GSK3β	TMT-labeled LC-MS/MS	Phosphorylation and cytoskeletal modulation	ICM	Wnt/β-catenin signaling pathway	170
HGF	SOMAscan	Antifibrotic effects (fibroblast activation reduction) and TGF-β signaling inhibition	HCM and MACE prediction	HGF/c-Met signaling pathway, MAPK/ERK pathway, and PI3K/AKT pathway	153
ICAM-1	Olink cardiovascular II	Leukocyte adhesion and transmigration promotion	HTN and myocardial fibrosis	…	173
IGFBP-1	Olink cardiovascular III and olink explore 1536	IGF signaling modulation	Interstitial fibrosis and HF	IGF signaling pathway	154,175
IGFBP2	Olink cardiovascular III	IGF signaling modulation	Interstitial fibrosis	IGF signaling pathway	154
IGFBP3	SOMAscan v4	IGF signaling modulation	HFpEF and renal function	IGF signaling pathway	162
IGFBP4	Olink explore 1536	Oxidative stress reduction	HF	IGF signaling pathway	175
IGFBP7	SOMAscan v4, olink cardiometabolic, and olink cardiovascular III	IGF signaling modulation and LVEF reduction	HF, HFpEF, LV remodeling, LV dysfunction, HCM, DCM-HF, MACE prediction, and high-risk HCM subtype	IGF signaling pathway	148,150,152,153,157, 165,168,171
IL-1RA	Olink cardiovascular II	IL-1β signaling inhibition and antiinflammatory	HFpEF	IL-1 signaling pathway	172
IL-6	SOMAscan, olink PEA, olink cardiovascular II	Fibroblast proliferation and collagen production stimulation (proinflammatory cytokine)	HF, HTN/DM, myocardial fibrosis, and high-risk HCM subtype	JAK/STAT3 signaling pathway	155,168,173
KLF4	Phosphoproteomics (MS-based)	Myofibroblast differentiation inhibition and antihyptrophic effect	HF and LVAD responders	…	156

(*Continued*)

Table. Continued

Protein biomarkers	Proteomic detection platform	Molecular mechanism	Disease association (clinical relevance)	Pathway	References
LOXL3	SILAC-AHA LC-MS/MS	Collagen cross-linking facilitation and ECM stiffness	MI	…	160
LTBP4	SOMAscan v4	Latent TGF-β complex binding (TGF-β activation pathway activation)	HFpEF and post-MI HF	TGF-β signaling pathway	149,151
Lumican (LUM)	SOMAscan, label-free LC-MS/MS, and LC-MS/MS	Collagen fibril organization and ECM stabilization	HCM, COVID-19 heart injury, LV dysfunction, and HF	…	146,148,161,166
MFAP4	SOMAscan v4	Elastic fiber assembly and hyptrophic effect	LV remodeling and hypertrophy	…	171
MMP1	AlphaLISA	Collagen cross-linking degradation	HF and fibrosis	…	158
MMP2	SOMAscan	ECM degradation	HFrEF and LV dysfunction	…	148,150
MMP3	Olink cardiovascular II	ECM degradation	HTN/DM	…	173
MMP7	Olink PEA	ECM degradation	HF	…	155
MMP9	SOMAscan	ECM degradation	HCM	…	152
Notch-3	SOMAscan	Myofibroblast differentiation	LA function	Notch signaling pathway	148
NT-proBNP	SOMAscan v4, olink PEA, olink cardiovascular II, and olink cardiovascular III	Cardiac stress biomarker	HF, advanced HF, HFrEF (stages C and D), HFpEF, ischemic HFrEF, DCM-HF, HCM, HTN, myocardial fibrosis, interstitial fibrosis, post-MI HF, and MACE prediction	Natriuretic peptide signaling pathway	151–155,157,162,173,176
Osteopontin (SPP1)	Olink PEA and olink cardiovascular III	Inflammatory response, fibroblast activation, and collagen deposition	DCM-HF, cardiac remodeling, and HF severity	Integrin and NF-κB signaling pathways	155,157
PAI-1	Olink cardiovascular III	Fibrinolysis inhibition (ECM degradation reduction)	Interstitial fibrosis	TGF-β signaling pathway	154
PCSK6	SILAC-AHA LC-MS/MS	Latent TGF-β1 activation and ECM synthesis	MI	TGF-β signaling pathway	160
PDGF	SOMAscan	Fibroblast proliferation and migration	HCM and hypertrophy	PDGF signaling pathway, PI3K/AKT pathway, and MAPK/ERK pathway	152
PDGFR	SOMAscan	PDGF receptor activation	High-risk HCM subtype	PDGF signaling pathway, PI3K/AKT pathway, and MAPK/ERK pathway	168
Periostin (POSTN)	LC-MS/MS and label-free LC-MS/MS	Fibroblast activation and ECM stabilization	HCM and HF	Integrin and TGF-β signaling pathways	146,166,167
PRELP	Olink cardiovascular II	Collagen fibrillogenesis and ECM organization	HFpEF	…	172
RARRES2	Olink cardiovascular III	Fibroblast chemotaxis and activation	Interstitial fibrosis	Chemokine signaling pathway (CMKLR1)	154
ROCK1	TMT-labeled LC-MS/MS	Cytoskeletal dynamics regulation and myofibroblast differentiation	DCM	RhoA/ROCK and ROCK1-vimentin signaling pathways	170
Serpin H1 (SERPINH1 and HSP47)	SILAC-AHA LC-MS/MS	Collagen-specific chaperone and proper collagen folding (ECM accumulation)	MI	…	160
SMAD3	SILAC-AHA LC-MS/MS	Signal transduction (TGF-β signaling mediator)	MI	TGF-β/SMAD signaling pathway	160
Spondin 1 (SPON1)	SOMAscan v4	Fibroblast adhesion to ECM	HF and LV remodeling	PI3K/AKT	171

(*Continued*)

Table. Continued

Protein biomarkers	Proteomic detection platform	Molecular mechanism	Disease association (clinical relevance)	Pathway	References
ST2	SOMAscan	Inflammatory response modulation	HCM and MACE prediction	IL-33/ST2 signaling pathway	152,153
STAT3	SOMAscan	Signal transduction	High-risk HCM subtype	JAK/STAT3 signaling pathway	168
SVEP1	SOMAscan v4	ECM protein and cell adhesion	HF, HFpEF, frailty, mortality, inflammation, and fibrosis	…	149,164,171
TGF-β1	SOMAscan, SILAC-AHA LC-MS/MS, olink cardiovascular II, label-free LC-MS/MS	Central fibrosis mediation and pro-fibrotic signaling	High-risk HCM subtype, MI, HTN/DM, and fibrosis	TGF-β signaling pathway	160,166,168,173
TGFBI	Olink cardiometabolic	TGF-β pathway mediation	HFpEF	TGF-β signaling pathway	165
Thrombospondin-1 (THBS1)	SOMAscan, LC-MS/MS, label-free LC-MS/MS	Latent TGF-β1 activation (fibroblast activation and ECM deposition)	HCM, COVID-19 heart injury, and HF	TGF-β signaling pathway	146,152,161
Thrombospondin-2 (THBS2)	SOMAscan v4	ECM deposition	Fibrosis, hypertrophy, HFpEF, HFrEF, high-risk HCM subtype, and post-MI HF	TGF-β signaling pathway	149–151,168,171
Thrombospondin-4 (THBS4)	LC-MS/MS	ECM assembly	HCM	FAK/PI3K/AKT pathway	167
TIMP1	Olink cardiovascular II and olink PEA	MMP inhibition (ECM degradation reduction)	HTN/DM, HF, and HFpEF	…	155,172,173
Tubulin (detyrosinated tubulin)	LC-MS/MS	Microtubule stabilization and cell stiffness	Sarcomere mutation-positive HCM	…	167
Tubulin (α-tubulin)	LC-MS/MS	Cytoskeletal structure	Sarcomere mutation-positive HCM	…	167
Versican (VCAN)	Label-free LC-MS/MS	ECM proteoglycan accumulation	HF, ischemic HF, and HCM	…	146,166
Vimentin (VIM)	TMT-labeled LC-MS/MS	Intermediate filament organization	DCM	TGF-β and ROCK1-vimentin signaling pathways	170

AlphaLISA indicates amplified luminescent proximity homogeneous assay; AMPK, AMP-activated protein kinase; CE-MS, capillary electrophoresis-mass spectrometry; DCM, dilated cardiomyopathy; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; HCM, hypertrophic cardiomyopathy; HF, heart failure; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HGF, hepatocyte growth factor; HTN/DM, hypertension with diabetes mellitus; ICM, ischemic cardiomyopathy; IGF, insulin like growth factor 1; LA, left atrial; LAP, left atrial pressure; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LV, left ventricular; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; MACE, major adverse cardiovascular events; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase; MI, myocardial infarction; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PEA, proximity extension assay; PI3K/AKT, phosphoinositide-3-kinase/protein kinase B; PPCM, peripartum cardiomyopathy; SILAC-AHA LC-MS/MS, stable isotope labeling by amino acids in cell culture using azidohomoalanine combined with LC-MS/MS; SOMAscan, slow off-rate modified aptamer scan; TGF-β, transforming growth factor-beta; TLR2, toll like receptor 2; TMT-labeled, tandem mass tag-labeled; and VEGF, vascular endothelial growth factor.

were enriched in pathways related to fibrosis, inflammation, and ECM organization, suggesting common pathobiological mechanisms in aging-related cardiovascular decline and development of HF and cardiomyopathy.

A study on participants from the MESA cohort using a proximity extension assay targeting 92 cardiovascular-related proteins revealed 17 proteins significantly associated with increased ECV on cardiac MRI, including increased NT-proBNP, IGFBP1/2, and PON3.[154] Conversely, lower levels of plasminogen activator inhibitor-1 were seen in advanced fibrosis, suggesting its role in maintaining proteolytic balance in ECM turnover. Serial proteomic profiling during HF treatment can capture molecular adaptations in response to therapy. An evaluation of plasma proteomics in patients with chronic HFrEF, in relation to cardiac function parameters, identified 723 proteins associated with reduced LVEF, 249 with reduced global longitudinal strain, and 792 with impaired left atrial reservoir strain.[148] Strong associations with ECM component proteins, such as MFAP4, IGFBP7, and MMP2, were noted across the parameters. Additionally, longitudinal plasma proteomic analysis of patients with HF receiving either left ventricular assist device (LVAD) or angiotensin receptor-neprilysin inhibitor therapy identified 5 core proteins—NT-proBNP, ESM-1, cathepsin L1, osteopontin, and MCSF-1—that remained consistently elevated regardless of treatment type.[155] Interestingly, post-LVAD, stage D patients with HF show

proteomic shifts toward profiles resembling less severe HF. Similarly, patients who show significant improvement in cardiac function after LVAD implantation (termed LVAD responders) show unique proteomic profiles involving cell cycle and ECM regulation, with 29 DEPs and 93 phosphopeptides distinguishing them from nonresponders.[156]

Recent studies have revealed distinct proteomic signatures of antifibrotic therapies through large-scale analyses. Patients treated with spironolactone showed distinct urinary proteomic signatures compared with those on standard care, with significant modulation of 27 collagen fragments and reduced procollagen type I carboxy-terminal propeptide to carboxy-terminal telopeptide of collagen type I (PICP/CITP) ratio, indicating decreased collagen synthesis relative to degradation.[159] More detailed proteomic analysis using the SOMAscan assay revealed significant changes in 7 proteins, notably CARD18, an antiinflammatory, antiapoptotic caspase-1 inhibitor, showing the most significant upregulation.[164] There was also downregulation of proteins linked to cardiac hypertrophy and fibrosis, such as HGF and IGF2R. The top canonical pathways significantly modulated by spironolactone included apelin signaling, stellate cell activation, glycoprotein 6 signaling, and LXR (liver X receptor)/RXR (retinoid X receptor) activation. Multiple collagens increased in patients receiving placebo but decreased in those randomized to spironolactone. Similarly, SGLT2 (sodium-glucose cotransporter 2) inhibitor therapy induced significant modulation of 32 DEPs involved in autophagic flux promotion, with the largest effects seen in IGFBP1 and TfR1, along with consistent downregulation of TGF-β signaling pathways.[175] Angiotensin receptor-neprilysin inhibitor therapy showed perhaps the most significant proteomic reductions in fibrosis markers and ECM metabolism, characterized by decreased TGF-β signaling, modulation of NT-proBNP-associated proteins involved in myocardial remodeling, and distinct changes in JAK-STAT, PI3K-Akt, and chemokine signaling pathways.[165] Despite their different primary pharmacological targets, the convergence of these treatments on TGF-β pathway suppression and ECM protein reduction suggests a common mechanism in attenuating cardiac fibrosis.

Combining traditional clinical markers with novel biomarker panels demonstrates enhanced predictive power. A 13-protein model that included KIM1, Gal-9, NGAL, and NEMO improved the area under the curve (AUC) for HFpEF discrimination from 0.82 to 0.92 when added to NT-proBNP.[172] Integration of NT-proBNP-associated proteins in HFpEF revealed strong correlations with SVEP1, ANGPT2, and EFEMP1, which supports their roles in fibrosis and endothelial dysfunction.[149] Furthermore, right ventricular dysfunction is a marker of poor prognosis in HFrEF. One study identified FGF-23 as a novel marker showing a >2.5-fold increase in worsening right ventricular dysfunction independent of LVEF or renal function.[177] Combining FGF-23 with BNP significantly improved the prediction of severe right ventricular dysfunction. Similarly, baseline PICP levels below 108.1 ng/mL predict improved LV reverse remodeling and reduce the risk of cardiovascular death or HF hospitalization in patients with HFrEF and mid-range ejection fraction.[158]

HF Subtype-Specific Fibrosis-Related Proteomic Signatures

Distinct proteomic signatures have emerged that define the molecular basis of cardiac fibrosis across HF subtypes. Plasma protein analysis revealed 120 DEPs between patients with HFrEF and HFpEF, demonstrating divergent fibrotic mechanisms.[150] The proinflammatory mediators IL-6, TNF-α (tumor necrosis factor-α), and VEGFA were significantly upregulated in HFrEF, driving inflammatory fibrotic responses. In contrast, VEGFC was increased in HFpEF, suggesting distinct angiogenic-fibrotic crosstalk. Interestingly, heart failure with mid-range ejection fraction showed an intermediate molecular profile but with unique ECM remodeling enrichment in MMPs and IGF/IGFBP pathways.[150]

Cardiomyopathy demonstrates unique proteomic signatures that drive its characteristic fibrosis. Plasma profiling of 1681 proteins in patients with HCM compared with hypertensive LV hypertrophy controls identified a 30-protein signature (AUC, 0.89) that distinguishes HCM from LV hypertrophy.[152] This signature revealed significant upregulation of Ras-MAPK pathway proteins and activation of complement and coagulation cascades, both implicated in promoting fibroblast activation and ECM production. Building on these findings, a subsequent study by the same group prospectively identified a 20-protein predictive model for major adverse cardiovascular events in HCM (AUC, 0.81), with increased major adverse cardiovascular event risk linked to sustained activation of pro-fibrotic Ras-MAPK and TGF-β signaling pathways.[153] At the tissue level, proteo-metabolomic analysis of HCM septal tissue revealed marked downregulation of key mitochondrial proteins involved in fatty acid oxidation and ATP production, specifically ACADVL (acyl-coA dehydrogenase very long chain) and HADHA (hydroxyacyl-coA dehydrogenase trifunctional multienzyme complex subunit alpha), accompanied by a 70% to 95% decrease in long-chain acylcarnitines.[166] This metabolic rewiring may promote the pro-fibrotic phenotype. While increased levels of ECM proteins such as fibronectin, thrombospondin-4, and periostin were observed across all patients with HCM, proteome analysis identified genotype-specific cytoskeletal alterations in sarcomere mutation-positive HCM.[167] Specifically, detyrosinated α-tubulin was upregulated, leading to cardiomyocyte stiffness. Machine learning analysis of plasma proteomics identified 4 molecular HCM subtypes.[168] The high-risk subtype D showed significantly increased major adverse cardiovascular event risk (hazard ratio, 3.41 [95% CI, 1.54–7.55]) and distinctive upregulation of 948

proteins linked to fibrosis, hypertrophy, and inflammation pathways.

In DCM, liquid chromatography-mass spectrometry-based proteomics of failing LV tissue identified 757 DEPs and significant phosphorylation of CTNNA3 (catenin alpha 3), a protein essential for cell-cell adhesion at the intercalated disc.[169] Overexpression of a phosphonull CTNNA3 mutant in mice induced LV dilation and reduced LVEF. Comparative proteomic and phosphoproteomic analyses between DCM and ICM revealed DCM-specific upregulation of cytoskeletal proteins and ECM components like THBS1 and COL1A1, coupled with extensive downregulation of mitochondrial proteins.[170] Further analysis identified 13 DCM-specific DEPs in patients with HF, including highly upregulated SPP1 and IGFBP7—both established mediators of fibrotic responses.[157] A panel of the 5 most upregulated proteins achieved remarkable diagnostic accuracy (AUC, 0.96) for distinguishing patients with DCM with HF from controls, shedding light on its clinical utility. In peripartum cardiomyopathy, data-independent acquisition mass spectrometry identified 15 DEPs, with a multi-marker panel comprising NT-proBNP, QSOX1 (quiescin sulfhydryl oxidase 1), ADIPOQ (adiponectin), and ITIH3 (inter-alpha-trypsin inhibitor heavy chain 3) achieving high diagnostic accuracy (AUC, 0.90).[147] In ICM, proteomic analysis revealed a significant accumulation (>2.5-fold) of ECM proteoglycans, particularly versican, resulting from impaired cleavage by ADAMTS5.[146] This ECM dysregulation leads to structural disarray and compromised cardiac function, though interestingly, β-blocker therapy was associated with reduced versican levels.

Beyond primary cardiomyopathies, unique signatures were also identified across other cardiac conditions. In MI, hypoxic cardiomyocytes significantly upregulate PCSK6, a protein that activates TGF-β signaling leading to increased fibrosis and impaired LV function.[160] Moreover, proteomic analysis of post-MI patients identified 36 plasma proteins from an initial set of 212 DEPs associated with HF development.[151] NT-proBNP, TNNT2 (troponin T2, cardiac type), ANGPT2 (angiopoietin-2), THBS2 (thrombospondin-2), LTBP4 (latent transforming growth factor beta binding protein 4), and FSTL3 showed a strong correlation with reduced LVEF at 4 months post-MI. ANGPT2 and THBS2 emerged as promising biomarkers complementing traditional markers. COVID-19-associated cardiac injury demonstrates unique regional proteomic changes. Spatial proteomics identified 228 DEPs in the left atrial and 347 in the LV, particularly affecting inflammatory markers while downregulating mitochondrial and ECM proteins.[161] In patients with hypertension, the presence of diabetes significantly alters the myocardial fibroproteomic landscape.[173] Patients with both conditions had elevated GDF-15, strongly associated with replacement fibrosis and increased ECV.

METABOLOMIC SIGNATURES IN CARDIAC FIBROSIS

Metabolomics and lipidomics represent a powerful systems biology approach that provides a clear snapshot of cellular metabolism and lipid biology. High-resolution metabolomic profiling captures the functional end points of molecular cascades and reveals metabolic reprogramming and substrate use patterns in disease and during disease progression. Mass spectrometry, nuclear magnetic resonance spectroscopy, stable isotope tracing, and sophisticated bioinformatics now enable us to characterize both static metabolite concentrations and dynamic metabolic flux in cardiovascular disease. These techniques also allow us to gain insight into metabolic crosstalk between CFs and other cardiac cells during energy-intensive ECM synthesis and secretion. The development of targeted and untargeted metabolomic platforms and spatial metabolomics made it possible to map metabolic gradients within the injured myocardium, and advances in lipidomics allow the study of bioactive lipid mediators in fibroblast activation and matrix organization.

Metabolic Substrate Alterations Drive Fibrotic Remodeling

Under physiological conditions, the heart demonstrates remarkable metabolic flexibility and is capable of utilizing various energy substrates, including fatty acids, glucose, lactate, and ketone bodies, to maintain continuous ATP production necessary for contractile function. While fatty acid oxidation accounts for most of the ATP production, this balance in substrate preference and use is disrupted in the failing heart (Figure 1). Both animal models and human studies reveal that the progression of cardiac fibrosis coincides with a fundamental shift from fatty acid oxidation toward increased glucose use and ketone metabolism.[178–181] Transcardiac liquid chromatography-mass spectrometry-based metabolomic profiling of patients with HFpEF has revealed significantly reduced medium- and long-chain acylcarnitines despite normal plasma levels.[180] This finding is consistent with impaired fatty acid uptake and oxidation that promotes fibroblast activation.[182] The metabolic disruption triggers compensatory increases in glucose uptake and glycolysis, though this adaptation may be insufficient for maintaining cellular energetics.[181]

The concept of metabolic inflexibility in HF (ie, primarily relying on glucose metabolism due to impaired fatty acid oxidation) has been challenged by recent findings. Studies of patients with nonischemic HFrEF demonstrate preserved substrate selection capacity, with the ability to increase both glucose and fatty acid utilization in response to workload.[183] Nevertheless, despite this preserved flexibility, these hearts show fundamental bioenergetic impairment, marked by reduced phosphocreatine/ATP ratio and total ATP content—changes that precede fibroblast activation.[184]

Altered amino acid metabolism, particularly of branched-chain amino acids (BCAAs), plays a significant role in cardiac remodeling and fibrosis progression. HFpEF myocardium demonstrates high levels of the BCAAs leucine, isoleucine, and valine alongside reduced levels of their downstream catabolites, suggesting impaired BCAA oxidation.[180] This metabolic pattern extends to HCM, where targeted proteo-metabolomics revealed marked downregulation of mitochondrial proteins that are fatty acid oxidation intermediates, such as ACADVL and HADHA, accompanied by accumulation of ketone bodies and BCAAs.[166]

Comparative metabolomic profiling has uncovered disease-specific metabolic signatures associated with fibrotic progression. In DCM, plasma acylcarnitines (particularly C16:0 and C18:1), sialic acid, and glutamic acid show the strongest associations with disease severity.[185] These metabolites reflect both impaired fatty acid metabolism and systemic inflammation driving fibrosis. Similarly, metabolomic profiles of DCM show enrichment in α-linolenic acid metabolism, while profiles of ICM show specific alterations in linoleic acid and arginine biosynthesis pathways.[186]

Mitochondrial Dysfunction and Fibrosis

Mitochondrial dysfunction is a central node in cardiac pathology characterized by impaired oxidative phosphorylation, reduced ATP production, and disrupted nicotinamide adenine dinucleotide (NAD^+) metabolism. Emerging evidence points to oxidative stress resulting from mitochondrial dysfunction as driving metabolic perturbation in HF. Elevated levels of reactive oxygen species and oxidative damage markers have been observed in various HF models.[187] NAD^+ is an important coenzyme in mitochondrial energy metabolism, and multiple studies demonstrated that HF is marked by significant NAD^+ depletion[188,189] that is closely linked with diastolic dysfunction and increased fibrosis. NAD^+ repletion through nicotinamide riboside (precursor) supplementation or salvage pathway enhancement can attenuate cardiac fibrosis in experimental models.[189,190]

As cardiac fibrosis progresses, the energy metabolism of the heart becomes increasingly compromised. This is evidenced by declining phosphocreatine-to-ATP (PCr/ATP) ratios and electron transport chain dysfunction.[191,192] These energetic deficits become particularly pronounced in pressure-overload HF. SGLT2 inhibitor therapy shows promise in this setting by promoting substrate oxidation while reducing glycolysis.[192]

Cell-Specific Metabolic Reprogramming in Fibrosis

Many of the metabolic shifts that accompany fibrotic remodeling originate from distinct cell-type-specific programs and do not necessarily reflect global myocardial changes. Targeted liquid chromatography-mass spectrometry-based metabolomics in human induced pluripotent stem cell-derived CFs demonstrated that the transition to an activated myofibroblast phenotype using TGF-β is driven by intrinsic metabolic shifts such as increased oxygen consumption and glycolysis, alongside increased collagen synthesis.[193] Importantly, inhibiting glutaminolysis reversed these effects, confirming a fibroblast-specific dependency on glutamine metabolism for myofibroblast activation. Similarly, choline supplementation in ex vivo fibroblast models exacerbated ECM deposition through the NLRP3-TGF-β axis.[179]

The cardiomyocyte, on the contrary, displays disrupted fatty acid oxidation as a driver of adverse remodeling. Studies examining hypertrophied cardiomyocytes noted suppressed expression of fatty acid oxidation genes (*PPARα*, *CD36*, and *CPT1A*) and diminished BCAA catabolism, all of which correlates with increased fibrotic burden.[180] In parallel, interfering with NAD^+ metabolism in cardiomyocytes (eg, through *KDM8* or *TBX15*) can trigger oxidative stress and indirectly stimulate pro-fibrotic signaling.[190] In addition to these parenchymal cell populations, isolated cardiac microvascular endothelial cells in both pressure-overloaded mouse hearts and human aortic stenosis biopsies showed elevated fatty acid oxidation and enhanced proline synthesis that promotes collagen deposition and fibrosis.[194]

Novel Metabolic Mechanisms in Cardiac Fibrosis

Recent investigations have uncovered novel metabolic regulators of cardiac remodeling. The gut microbiome modulates host metabolism, and alteration in the gut microbiome-heart axis has emerged in recent studies as an important mediator in cardiac remodeling.[195,196] Reduced abundance of short-chain fatty acid-producing bacteria like Ruminococcus was observed in patients with HFpEF, which correlates with lower dietary fiber intake.[196] The metabolomic profiles of these microbial changes are linked to pro-fibrotic inflammation and oxidative stress. Supplementation with indole-3-propionic acid, a microbiota-derived metabolite, protects against HFpEF through epigenetic modulation, and restores gut microbiota balance while improving heart function.[195]

Lipidomic analyses have revealed specific lipid signatures in fibrotic cardiac pathology. DCM exhibits disrupted phosphatidylcholine metabolism and elevated levels of the ceramide species CER 16:0, strongly linked to HF risk and fibrotic progression.[197] Conversely, certain long-chain sphingolipids (SM, 24:1) may protect against fibrosis progression.[197]

Large-scale metabolomic profiling in the Framingham Heart Study unveiled novel biomarkers of adverse cardiac remodeling and fibrosis. Elevated levels of kynurenine and aminoadipate significantly correlate with adverse

structural remodeling,[198] while increased myo-inositol serves as a specific marker for HFpEF and is linked to disease severity (high myo-inositol levels are associated with increased mortality/HF hospitalization).[199] These metabolites present promising opportunities for the early detection and ongoing monitoring of cardiac fibrosis.

Temporal metabolomic profiling after MI using untargeted liquid chromatography-mass spectrometry and bioinformatic pathway analysis in patients with MI with serial blood draws revealed phase-specific metabolic signatures in the development of cardiac fibrosis.[200] The glycerophospholipid metabolism is activated, and toll-like receptor and IL-17 signaling pathways are markedly upregulated during the acute phase (day 1). By the subacute phase (day 7), the metabolic profile shifts to reflect active fibrosis initiation with 229 differentially accumulated metabolites predominantly involved in glycerophospholipid and glycosylphosphatidylinositol-anchor biosynthesis pathways, increasing TGF-β signaling and ECM receptor interactions that drive CF activation and matrix production. By the chronic phase (3 months), metabolic profiles reflect predominant vascular regeneration and adaptation responses driven by VEGF signaling, marked by 370 differentially accumulated metabolites enriched in tryptophan metabolism and glycerophospholipid metabolism pathways.

Sex-specific metabolic variations in lipid metabolism and oxidative stress markers have been observed in human patients.[201] Additionally, females show lower phosphocholine levels (odds ratio, 0.59), indicating impaired phospholipid metabolism and potential membrane dysfunction. Further validation in large cohorts revealed that female patients exhibit distinct patterns in BCAA metabolism and ketone utilization, along with significant differences in oxidative stress markers and inflammatory markers.[202]

These metabolic effects appear to be complex and may vary between patient subgroups. The Bogalusa Heart Study identified through ultra-high-performance liquid chromatography-tandem mass spectroscopy 8 metabolites, including N-formylmethionine and butyrylcarnitine, that show stronger associations with diastolic dysfunction in Black participants.[203]

CLINICAL IMPLICATIONS

Clinical Assessment of Cardiac Fibrosis

Cardiac MRI-based native T1 mapping and ECV determination with late gadolinium enhancement imaging enable quantification of myocardial fibrosis. ECV percentages, for instance, correlate strongly with interstitial collagen burden.[204] Echocardiographic techniques, including global longitudinal myocardial strain, ultrasound elasticity imaging, and integrated backscatter analysis, may also reveal the burden of fibrosis but are generally less specific.[205,206] Novel fibrosis-specific molecular imaging that labels collagen peptides[207] and fibroblast activation protein[208] are promising newer techniques but require validation. Histological examination of endomyocardial biopsy samples (eg, via Masson's trichrome or picrosirius red staining) provides a more definitive but invasive measure of collagen volume fraction.[209] Biomarkers such as PICP, procollagen type III N-terminal propeptide, galectin-3, soluble ST2, and some miRNAs may reflect fibrosis burden, yet they lack the spatial resolution and anatomic specificity afforded by imaging.[210,211] Although these markers could serve as useful surrogate end points especially when testing the efficacy of anti-fibrosis therapy, they would not supplant the importance of clinical end points that are influenced by fibrosis, such as sudden cardiac death, ventricular arrhythmias, and HF hospitalizations.

Clinical Application and Treatment Response

The clinical translation of multi-omics has begun to reveal promising therapeutic targets and strategies specifically targeting cardiac fibrosis. A schematic of these multi-omic interactions and their contributions to cardiac fibrosis is provided in Figure 4. Genomic analyses integrated with Mendelian randomization proteomics have identified several fibrosis-associated pathways amenable to therapeutic intervention. Combined GWAS and proteomic data revealed druggable proteins involved in fibrotic signaling, including CAMK2D (calcium/calmodulin dependent protein kinase II delta), PRKD1 (protein kinase D1), and MAPK3 (mitogen-activated protein kinase 3), which regulate stress responses and calcium signaling during CF activation.[58] Additionally, proteins like APOC3 (apolipoprotein C3) and TNFSF12 (TNF superfamily member 12) showed causal associations with HF and fibrotic remodeling, suggesting therapeutic potential in targeting lipid metabolism and inflammatory pathways.

Transcriptomic profiling, particularly at single-cell resolution, provides a deeper look into fibroblast activation and facilitates therapeutic targeting. Through integrated transcriptomic and proteomic profiling in failing hearts, CDR132L was developed as a locked nucleic acid-based antisense oligonucleotide inhibitor of miR-132. It demonstrated efficacy in reducing fibrotic remodeling in animals with ischemic and nonischemic HF[212–214] while modulating fibrotic and hypertrophic pathways involving *MEF2* (TF and mediator of stress-dependent fibrosis)[215] and *GATA3* (zincfinger TF regulator of fibrogenic macrophages).[216] This drug constitutes one of the first miRNA-directed therapies for HF to reach clinical testing in patients.[217] A phase 1b first-in-human study in individuals with stable chronic HF reported that CDR132L was safe, well-tolerated, and achieved not only a dose-dependent reduction in circulating miR-132 but also resulted in QRS narrowing and lower NT-proBNP levels.[218] Additionally, it

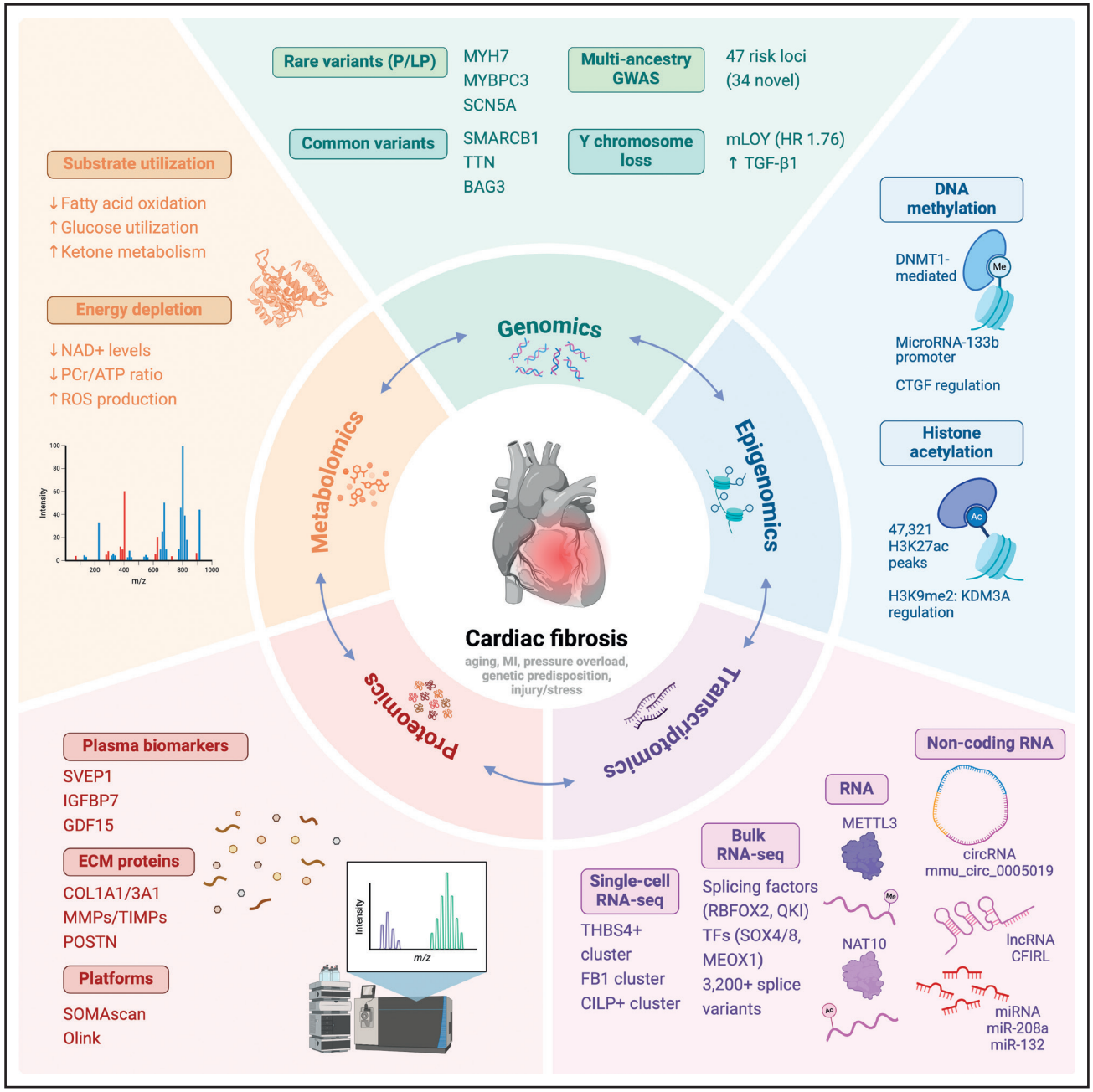

Figure 4. Multi-omic integration and systems-level understanding of cardiac fibrosis.
Multi-omics reveal cardiac fibrosis as a complex pathological process integrating multiple molecular layers. Genomics identified both rare variants (*MYH7* and *MYBPC3*) that predispose to early fibrotic remodeling even before clinical symptoms and common variants like *SMARCB1* that enhance TGF-β1-mediated fibrosis through chromatin remodeling. Mosaic Y chromosome loss (mLOY; hazard ratio, 1.76) promotes cardiac fibrosis through enhanced TGF-β1 (transforming growth factor-β1) signaling. The epigenomic layer includes DNA methylation changes, including DNA methyltransferase 1 (DNMT1)-mediated hypermethylation of miR-133b that leads to CTGF upregulation and enhanced fibrosis, as well as histone acetylation changes marked by 47 321 H3K27ac peaks and H3K9me2 regulation by KDM3A. Single-cell transcriptomics uncovered distinct fibroblast subpopulations such as the *THBS4*$^{+}$ cluster that drives late-stage fibrosis through enhanced extracellular matrix (ECM) production. Bulk RNA-sequencing identified over 3200 splice variants and regulatory factors like *RBFOX2* and *QKI*. Noncoding RNAs, including circRNA mmu_circ_0005019 and the cardiac fibroblast-specific lncRNA CFIRL, provide additional regulatory complexity. Proteomic profiling identified novel biomarkers like SVEP1 and IGFBP7 that show causal relationships with heart failure (HF) development and key ECM proteins (COL1A1/3A1, matrix metalloproteinases/tissue inhibitors of metalloproteinases [MMPs/TIMPs]) detectable through platforms like SOMAscan and Olink. Metabolomics revealed a shift from fatty acid oxidation to glucose utilization in failing hearts, increased ketone metabolism, and nicotinamide adenine dinucleotide (NAD^{+}) depletion as a driver of diastolic dysfunction and fibrosis, accompanied by reduced phosphocreatine-to-ATP (PCr/ATP) ratio and increased ROS (reactive oxygen species) production. This integrated molecular view explains why targeting single pathways often yields suboptimal clinical outcomes. GWAS indicates genome-wide association studies; MI, myocardial infarction; P/LP, pathogenic/likely pathogenic; POSTN, periostin; SOMAscan, slow off-rate modified aptamer scan; and TF, transcription factor. Adapted from Nanotherapeutics in Cancer, by BioRender.com (2025). Retrieved from https://app.biorender.com/biorender-templates.

displays minimal off-target effects because it undergoes selective uptake by cardiac tissue after administration. The ongoing HF-REVERT trial[219] is evaluating CDR132L in a phase 2 study to determine whether early miR-132 inhibition post-MI might prevent or reverse adverse LV remodeling in HFrEF. Single-cell multi-omics of myofibroblasts combining spatial transcriptomics and chromatin accessibility assays enabled identification of FAP as a therapeutic target, leading to the development of engineered chimeric antigen receptor T cells that effectively reduce CF activation in treated tissue.[220,221] Similarly, multimodal single-cell profiling (snRNA-seq, assay for transposase-accessible chromatin using sequencing, and CITE-seq [cellular indexing of transcriptomes and epitopes by sequencing]) on human LV samples identified IL-1β as a critical mediator of immune-fibroblast communication, leading to successful therapeutic targeting of the fibrotic response.[222] High-throughput screening coupled with multimodal molecular profiling (snRNA-seq and assay for transposase-accessible chromatin using sequencing) on cardiac tissue from pressure overload and ischemia-reperfusion HF models identified *MD2* as a promising target and artesunate as a potent antifibrotic compound through multi-omic validation of its mechanism.[223] Similarly, combined proteomic and transcriptomic profiling in PW1 reporter mice revealed αv-integrin on PW1$^+$ stromal cells as a therapeutic target, leading to successful testing of cilengitide in reducing post-MI fibrosis.[224]

In the clinical practice of precision medicine, proteomics can be used to inform risk stratification and guide therapeutic decision-making. A multimodal fibrosis assessment combining late gadolinium enhancement on cardiac MRI and PICP blood levels demonstrated superior prognostic value for fibrosis assessment in idiopathic DCM.[225] Patients with both positive late gadolinium enhancement and elevated PICP had worse outcomes, including HF hospitalization and transplantation, compared with those without these markers (adjusted hazard ratio for late gadolinium enhancement, 3.54 [95% CI, 1.90–6.60]). This combined profile significantly improved risk reclassification (NRI [net reclassification index], 0.28), aiding in identifying high-risk patients who may benefit from aggressive management. Proteomic analysis has facilitated the identification of novel therapeutic targets. Through Mendelian randomization, 8 proteins were identified as therapeutic targets and showed robust causal effects in HF.[226] Among these, CSF-1 (recruiter and activator of TGF-β-secreting macrophages) and Gal-3 (fibroblast activator and collagen deposition enhancer) were associated with increased fibrosis risk. Conversely, ADM demonstrated protective effects through inhibition of TGF-β and myofibroblast activation. These discoveries have already translated to clinical investigation, as ADM agonists and galectin-3 (Gal-3) antagonists are currently under clinical trial. As discussed above, trials assessing these therapeutic interventions should include clinical end points that reflect outcomes of the entire disease entity, such as ventricular arrhythmias, sudden death, and HF hospitalizations; however, adding fibrosis markers (imaging, blood) as surrogate end points may contribute to the understanding of the role of fibrosis in attenuating those clinical end points. Post-MI patients represent the most pragmatic initial target population, given the clear temporal association between injury and fibrosis development. Patients with hypertensive heart disease, cardiomyopathy, and patients with HFpEF with imaging evidence of fibrosis would also be important study subjects.

Identifying metabolic changes through metabolomics has paved the way for the therapeutic targeting of the metabolome. Although initially developed for the management of diabetes, SGLT2 inhibitors demonstrate cardioprotective benefits that extend beyond energy metabolism modulation to include antifibrotic functions.[191,192,227] However, despite these metabolic enhancements, the EMPA-VISION trial found no significant improvement in myocardial PCr/ATP ratio.[191] Therefore, the cardioprotective effects of SGLT2 inhibitors may involve mechanisms beyond simple energy charge restoration, potentially including antiinflammatory and antifibrotic functions and reduced oxidative stress. Nonetheless, their direct effects on fibrosis remain under investigation.[228,229] Intriguingly, metabolomic studies have revealed a complex relationship between ketone metabolism and fibrosis. While acute ketone supplementation may improve cardiac energetics,[230,231] prolonged adherence to ketogenic diets high in long-chain triglycerides may promote cardiac fibrosis by disrupting normal substrate utilization, leading to maladaptive metabolic remodeling.[232] Recent metabolomics-guided studies also identified metabolic signatures that may predict treatment response. For instance, baseline metabolomic profiles can distinguish responders from nonresponders to LVAD therapy,[190] and specific metabolite patterns may predict reverse remodeling following standard HF therapies.[233]

Reversibility of Cardiac Fibrosis

A growing body of evidence suggests that cardiac fibrosis is not simply a unidirectional process culminating in a permanently insoluble scar but can, under certain molecular and biomechanical conditions, undergo varying degrees of remodeling or reversal.[234,235] Recent studies have shown that even in mature fibrotic tissue, a significant fraction of newly deposited collagen remains dynamically cross-linked, and thus seemingly end-stage fibrosis may still be amenable to therapeutic intervention.[236] For instance, findings in preclinical models of pressure overload and ischemic injury have revealed that antifibrotic strategies targeting important regulators, such as TGF-β signaling,[235] MD2/TLR4 interactions,[223] and lysyl hydroxylation-based cross-link formation,[236]

are capable of attenuating or even reversing advanced fibrosis and improving myocardial function. Moreover, single-cell and multi-omics studies show that discrete subpopulations of myofibroblasts can transdifferentiate into less activated or quiescent phenotypes with reduced ECM secretion if, for example, specific epigenetic modulators[237] or reprogramming factors[234] are pharmacologically disrupted. Although there is general consensus that extensively cross-linked collagen may present a "point of greater difficulty," current data suggest that this threshold is fluid rather than absolute.[236] Beyond simply limiting new collagen synthesis, effective therapy appears to require a multifaceted approach involving the disruption of pro-fibrotic feedback loops (eg, reprogramming, cell-cycle reentry, or cross-link disruption), promoting matrix proteolysis, and harnessing fibroblast plasticity.[234,236,237] As a result, interventions such as direct antagonism of MD2/TLR4 signaling,[223] small-molecule inhibition of eicosanoid-degrading enzymes,[238] or epigenetic remodeling of myofibroblast states[237] have all shown promise in restoring a more compliant, less stabilized ECM and improving diastolic or systolic function in murine and human fibrotic models.

CURRENT CHALLENGES AND FUTURE DIRECTIONS

The explosive growth of omics data is faced with multifront challenges. Data processing and bioinformatics infrastructure have scaling limitations since unprecedented volumes of complex, heterogeneous data are being generated.[239] This becomes even more challenging given differing signal-to-noise ratios and varying levels of data completeness across omics platforms,[240] which warrants sophisticated data warehousing solutions (often at the terabyte scale) and semantic Web technologies that effectively integrate and analyze the diverse high-throughput platforms.[241] Recent accelerated developments in artificial intelligence and machine learning may assist in overcoming some of these computational difficulties by improving data processing efficiency and integration.[242] It is equally critical to develop quantitative methods, as current approaches struggle with missing data patterns across modalities and lack standardized statistical frameworks for experimental design and reproducibility.[240] This is particularly pertinent for mass spectrometry-based platforms like proteomics and metabolomics, where batch effects are often more pronounced and mechanisms for missing data can be more complex than those of sequencing-based approaches. In addition, it remains crucial to validate multi-omics discoveries functionally. While omics excel at generating candidate genomic regions and pathways, rigorous validation through mechanistic studies (in cellular or animal models) establishes the clinical relevance and therapeutic potential of the biological knowledge. Looking ahead, the field would benefit from large, well-designed cohort studies focusing on cardiac fibrosis as a primary end point (ie, integrating comprehensive multi-omics profiling with carefully phenotyped fibrosis measures, such as cardiac MRI-derived ECV). Success in addressing these pitfalls will require continued collaboration between computational biologists, statisticians, and basic/clinical investigators to translate multi-omics findings into mechanistic understanding and therapeutic advances that target pathological fibrosis.

CONCLUSIONS

The past decade has transformed our understanding of cardiac fibrosis from a disorder of excessive ECM deposition to an intricate pathological process integrating a myriad of cellular and molecular mechanisms. The simultaneous profiling of genomic variants, transcriptional networks, protein-protein interactions, and metabolic signatures has revealed previously unappreciated molecular connections: activated CFs undergo profound metabolic reprogramming while initiating pro-fibrotic transcriptional cascades, immune cells communicate with fibroblasts through specific cytokine networks to drive disease progression, and epigenetic modifications at enhancer regions control critical transcriptional programs of fibroblast activation. These discoveries explain why therapeutic targeting of canonical pathways, such as TGF-β or angiotensin signaling alone, often yields suboptimal clinical outcomes. The identification of molecularly distinct fibroblast populations with unique signatures challenges our traditional view of cardiac fibrosis and may explain the heterogeneity in therapeutic responses. Indeed, the intricate relationship between metabolic rewiring and epigenetic control of fibroblast state transitions suggests that effective therapies may need to target multiple pathways and signaling nodes simultaneously. Omics-informed precision medicine explains that the heterogeneity in disease susceptibility and treatment response observed across patient populations is due to genetic architecture associated with genetic variants that modulate downstream molecular cascades. This mechanistic understanding has already catalyzed the development of promising therapeutic modalities primarily directed at targets identified by agnostic omic approaches, including oligonucleotide inhibitors and engineered T cells. Success in explicating multi-omic discoveries into clinical practice will require addressing challenges in data integration and standardization and developing computational frameworks that can synthesize heterogeneous molecular data streams to inform precision therapeutic strategies.

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REVIEW

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