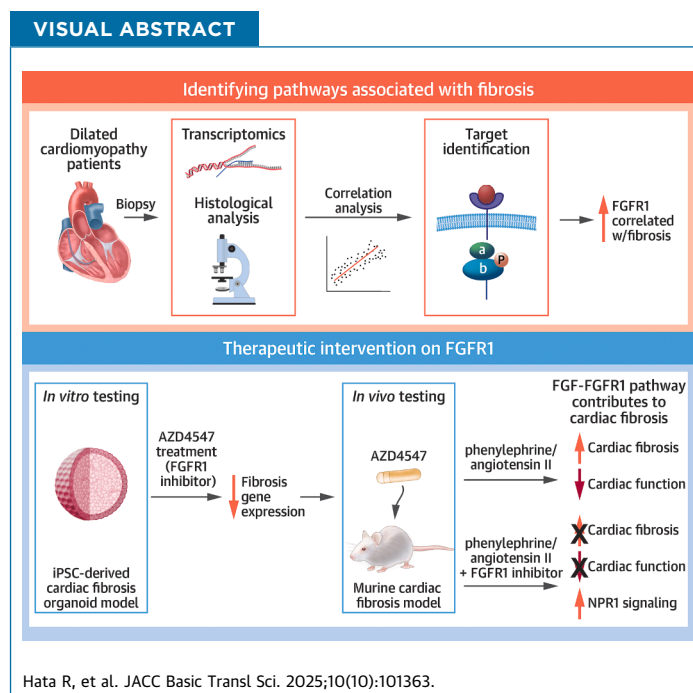


ORIGINAL RESEARCH - CLINICAL

Integrative Transcriptomic-Histological Analysis in Dilated Cardiomyopathy Unveils FGFR1 Inhibition as Anti-Cardiac Fibrotic and Cardioprotective Therapy



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HIGHLIGHTS

- Cardiac fibrosis is a key driver of dysfunction in dilated cardiomyopathy, with limited targeted therapies.
- FGFR1 expression correlates with fibrosis severity and emerges as a promising therapeutic target.
- FGFR1 inhibition reduces fibrosis and improves cardiac function in organoid and murine heart models.
- Future research should focus on clinical trials of FGFR1 inhibitors to advance fibrotic heart disease treatment.

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ABBREVIATIONS
AND ACRONYMS**DCM** = dilated cardiomyopathy**FGFR1** = fibroblast growth factor receptor 1**FSTL3** = follistatin like 3**GO** = gene ontology**HF** = heart failure**LVEF** = left ventricular ejection fraction**MMP2** = matrix metalloproteinase 2**RNA-seq** = RNA sequencing

SUMMARY

Cardiac fibrosis drives dysfunction in dilated cardiomyopathy (DCM); yet, effective therapies are limited. This study identifies FGFR1 as a critical target in cardiac fibrosis using transcriptomic and histological analyses of 58 human DCM biopsies. *FGFR1* expression correlated with fibrosis severity, and inhibition by AZD4547 reduced fibrosis and improved cardiac function in organoid and murine models. These findings validate FGFR1 inhibition as a promising therapeutic strategy for mitigating fibrosis and improving outcomes in heart failure associated with DCM. (JACC Basic Transl Sci. 2025;10:101363) © 2025 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Cardiac fibrosis, marked by excessive accumulation of extracellular matrix proteins in the myocardium, contributes significantly to various forms of cardiac dysfunction.¹ In cardiomyopathy, this fibrosis often leads to irreversible cardiac impairment. Dilated cardiomyopathy (DCM) is characterized by the enlargement and impaired function of the left ventricle, a condition that develops independently of coronary artery disease.²⁻⁴ Notably, approximately one-third of patients with advanced DCM experience interstitial fibrosis predominantly in the mid-wall.⁵ The fibrotic change forms a substrate for ventricular re-entrant arrhythmia and is independently associated with an increased risk of mortality and heart failure (HF)-related morbidity.⁶ Consequently, identifying key therapeutic targets pivotal to cardiac fibrosis and developing potent antifibrotic therapies against these targets are critical priorities in research.

Advanced transcriptome methodologies have led to numerous discoveries in cardiac fibrotic diseases, uncovering previously unattainable insights. Various research groups have established patient registries for DCM or HF patients to understand the heterogeneity through genetic and cardiac transcriptome profiling.⁷⁻¹² These studies have delineated distinct patient subgroups based on transcriptomic signatures, significantly enriching our understanding of the pathomechanisms underlying cardiac fibrosis. Although these advances have substantially contributed to the field, a substantial gap persists when translating research findings from bench to bedside, with notably low transferability for cardiac fibrosis research.

To address these challenges, we established a prospective case registry for patients with cardiac dysfunction eligible for myocardial biopsy, also known as the “Myocardial Biopsy Registry.” A key feature of our study using this registry is its integrated methodology, combining transcriptomic profiling of cardiac tissue with histopathological image analysis, further strengthened by in vitro and in vivo disease modeling for comprehensive validation. Specifically, we investigated the antifibrotic efficacy of target-specific inhibitors using in vitro human induced pluripotent stem cell (iPSC)-derived cardiac organoid models and assessed a selective inhibitor’s capability to reduce fibrosis and improve cardiac function in an in vivo murine model of cardiac dysfunction.

METHODS

Detailed materials and methods are described in the [Supplemental Material](#). This research conforms to the Declaration of Helsinki and adheres to the Ethical Guidelines for Life Sciences and Medical Research Involving Human Subjects, as well as the guidelines for Medical Research and Human Genome and Genetic Analysis Research established by the Ministry of Health, Labor, and Welfare. The Ethics Committees of Kyoto University Graduate School and the Faculty of Medicine reviewed and approved this research (Approval No. R1713-4). Written informed consent was obtained from all participants. In conducting animal experiments, this study strictly followed the Regulations Concerning the Conduct of Animal Experiments at Kyoto University, established by the Minister of Education, Culture, Sports, Science

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the [Author Center](#).

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and Technology. All procedures conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Ethics Committee of Kyoto University approved all experimental protocols. During heart excision and euthanasia, the animals were anesthetized using inhaled isoflurane (Abbott Japan). Initially, anesthesia was induced by allowing the animals to inhale a sustained concentration of 2.5% isoflurane through a cage connected to an anesthesia machine, ensuring loss of consciousness. During heart excision, continuous inhalation anesthesia was maintained using a mask-type inhalation system delivering isoflurane. The procedures were carried out without causing pain. The method of euthanasia employed was cervical dislocation under anesthesia, following institutional and international ethical guidelines.

DCM STUDY DESIGN AND POPULATION. The Myocardial Biopsy Registry, a physician-initiated, prospective, observational cohort study at Kyoto University Hospital, enrolled eligible patients for myocardial biopsy from December 2018 to December 2022.

PATIENT SELECTION AND EVALUATION. All patients underwent clinical evaluation, blood sampling, echocardiography, and genetic testing. Indications for myocardial biopsy included patients with non-ischemic cardiac structural abnormalities of unknown etiology,¹³ requiring thorough examination by a cardiologist. The diagnosis of DCM was based on the current guidelines to include the following: left ventricular ejection fraction (LVEF) <50% by echocardiography within the previous 6 months and excluding secondary cardiomyopathy, such as cardiac amyloidosis, sarcoidosis, infiltrative myocarditis, mitochondrial cardiomyopathy, drug-induced cardiomyopathy, hemochromatosis, takotsubo cardiomyopathy, pulmonary hypertension, and antimitochondrial-associated myopathy.¹⁴

STATISTICAL ANALYSIS. Categorical variables were compared using the chi-square or Fisher's exact test and presented as numbers and percentages. Continuous variables are expressed as the mean $\pm$ SD or the median with 25th-75th percentiles (Q1-Q3) or range. Continuous variables between 2 groups were compared using unpaired Student's *t*-test or Mann-Whitney *U* test, depending on data distributions. The significance threshold was determined by a 5% false discovery rate adjusted for multiple comparisons by the Benjamini-Hochberg method. Variance-stabilized raw reads underwent agnostic clustering using principal components analysis and unsupervised hierarchical methods. Clinical features linked to each gene cluster were ascertained via linear

regression and Pearson's correlation coefficient (*r*). Data normality was assessed using the Shapiro-Wilk test. One-way analysis of variance was used to compare >2 groups with Tukey's post hoc test for multiple pairwise comparisons. All statistical analyses were performed using R version 4.3.3 (2024-02-29 ucrt) (R Core Team, 2024) and GraphPad Prism version 10.3.1 (GraphPad Software).

RESULTS

BASELINE CHARACTERISTICS OF STUDY PATIENTS.

Among 171 patients documented at Kyoto University Hospital, 58 diagnosed with DCM were chosen for detailed analysis, as described in [Supplemental Table 1](#). Considering the heterogeneity of DCM patients, an overview of clinical demographics, echocardiographic findings, and blood draw data are shown in [Table 1](#) and [Supplemental Figure S1](#). The mean age was 63.3 ± 13.4 years. Among them, 37.9% were women. They exhibited a significant comorbidity burden, comprising hypertension (39.7%), diabetes (19.0%), and atrial fibrillation (20.7%). The median brain natriuretic peptide (BNP) concentration was 238.6 pg/mL (Q1-Q3: 114.1-421.1). Echocardiography revealed a mean LVEF of $28.8 \pm 9.1\%$, a mean left ventricular end-diastolic diameter of 59.5 ± 8.7 (mm), a mean left ventricular systolic diameter of 51.2 ± 10.4 mm, and a mean left atrial diameter of 42.9 ± 7.0 mm. Cardiac magnetic resonance imaging was conducted for 52 of the 58 patients. The findings revealed a mean LVEF of $28.0 \pm 12.1\%$, end-diastolic volume of 213.1 ± 85.1 mL, end-systolic volume of 161.2 ± 89.2 mL, and stroke volume of 53.7 ± 20.0 mL. Among the 42 patients who underwent contrast-enhanced cardiovascular magnetic resonance (CMR) imaging, 25 (59.5%) exhibited left ventricular late gadolinium enhancement (LGE).

PATHOGENIC OR LIKELY PATHOGENIC VARIANTS. We generated high-coverage whole-exome sequencing (WES) data, which covered 99% of the 51 known DCM-associated genes with more than 10 reads. The detailed gene list is in [Supplemental Table 2](#). Of the 58 probands analyzed, 4 (6.9%) carry genetic variants classified as pathogenic (*n* = 3) or likely pathogenic (*n* = 1), according to ClinVar ([Table 2](#) and [Supplemental Table 3](#)). The specifics of the ACMG criteria used for classification are provided in [Supplemental Tables 3 and 4](#). Notably, the pathogenic and likely pathogenic variants were identified in *TNNT2*, *TTN*, *FLNC*, *MYBPC3*, and *RBM20* genes. Subjects carrying these pathogenic sequence variant were categorized into the pathogenic variant positive group (*n* = 10), while those without such sequence

TABLE 1 Patient Characteristics (N = 58)

Age, y	63.3 ± 13.4
Male	36 (62.1)
Medical history	
Smoking	29 (50.0)
Atrial fibrillation/flutter	12 (20.7)
Hypertension	23 (39.7)
Dyslipidemia	7 (12.1)
Diabetes mellitus	11 (19.0)
Chronic obstructive pulmonary disease	2 (3.4)
Stroke	3 (5.1)
Ventricular tachycardia/ventricular fibrillation	7 (12.1)
Chronic kidney disease	29 (50.0)
NYHA functional class	
II	23 (39.7)
III	27 (46.6)
IV	3 (5.1)
Laboratory values on admission	
Serum creatinine, mg/dL	1.10 ± 0.44
Uric acid, mg/dL	6.53 ± 1.76
Median brain natriuretic peptide, pg/mL	238.6 (IQR [Q1-Q3]: 114.1–421.1)
Echocardiographic parameters	
Left ventricular ejection, %	28.8 ± 9.1
Left ventricular end-diastolic diameter, mm	59.5 ± 8.7
Left ventricular end-systolic diameter, mm	51.2 ± 10.4
Intraventricular septal thickness in diastole, mm	9.7 ± 2.1
Posterior wall thickness in diastole, mm	9.7 ± 1.8
Left atrial diameter, mm	42.3 ± 7.0
Mitral valve regurgitation (≥moderate)	12 (20.7)
Cardiac magnetic resonance imaging parameters (n = 52)	
Left ventricular ejection, %	28.0 ± 12.1
Left ventricular end-diastolic volume mL	213.1 ± 85.1
Left ventricular end-systolic volume, mL	161.2 ± 89.2
Stroke volume, mL	53.7 ± 20.0
Left ventricular mass, g	161.0 ± 45.7
Left atrial diameter, mm	42.3 ± 7.0
Late gadolinium enhancement (n = 42)	25 (59.5)
Oral medications at discharge	
Beta-blockers	55 (94.8)
ACE-I or ARB	40 (69.0)
Aldosterone antagonists	39 (67.2)
SGLT2 inhibitors	13 (22.4)
Tolvaptan	4 (6.8)
Pimobendan	1 (1.7)
Amiodaron	4 (6.8)
Ivabradine	4 (6.8)
Warfarin	1 (1.7)
Direct oral anticoagulants	17 (29.3)

Values are mean ± SD or n (%) unless otherwise specified.

variant were categorized into the pathogenic variant negative group (n = 48). A subanalysis examining LVEF, left ventricular end-diastolic diameter, BNP, age, and percentage fibrosis between the 2 groups did not reveal any significant differences (Supplemental Figure 2A).

NETWORK ANALYSIS: IDENTIFYING HUB GENES IN THE DCM-RELATED MODULE. RNA sequencing (RNA-seq) data for 58 DCM samples underwent quality control to ensure reliability. The collection of healthy control samples was not feasible because of ethical limitations associated with cardiac biopsy registry studies. Consequently, publicly available data sets were utilized as controls for this exploratory analysis. Principal component analysis and heatmap visualization are presented in Supplemental Figures 3A to 3C. Using a weighted gene coexpression network analysis approach, the transcriptome was segregated into 16 distinct modules with a soft-thresholding power of 3 (Supplemental Figures 3D and 4). The module-trait relationship was subsequently analyzed to identify transcriptomic clusters corresponding with specific clinical phenotypic correlations. Four modules were positively correlated with the DCM trait (Figure 1A). Among these DCM-associated modules, the purple eigengene not only exhibited the strongest positive correlation with DCM status but also showed positive correlations with BNP and left ventricular dimensions and a pronounced negative correlation with LVEF, indicating that this composite “clinical fingerprint” most coherently reflects the core pathophysiology of DCM. To more clearly elucidate the distinct features within each module, we employed gene ontology (GO) analysis for detailed annotation (Supplemental Figures 5A to 5D). The purple module primarily captured processes related to muscle system activity, muscle contraction, and muscle tissue development. DisGeNet analysis highlighted a significant enrichment of terms related to DCM, including dilated cardiomyopathy, hypertrophic cardiomyopathy, and endomyocardial fibrosis, within the purple module (Supplemental Figure 6). Consequently, the purple module was identified as the central DCM-associated component. Furthermore, this analysis showed the enrichment of 9 fibrosis-associated genes in the purple module (*ACE*, *AERB1*, *CDON*, *FSTL3*, *HBEGF*, *MYLK3*, *PAK1*, *PRMT7*, *XIRP1*; detailed in Table 3), indicating a potential association between fibrosis and DCM pathology.

To identify hub genes within the purple module, we performed a network analysis focused on intra-modular connectivity. As illustrated in Figure 1B, this analysis revealed several hub genes unique to this module, including *HSPA4*, *RAB15*, *ANKRD1*, *DNAJB5*, and *FSTL3* (Intramodular connectivity: 9.93, 8.93, 7.91, 7.79, 7.20, respectively). The association of some of these genes with cardiac fibrosis reported in

TABLE 2 Distribution of Clinically Significant DCM Variants					
Gene	Chromosome	Allele Description	Protein	Patient	ClinVar Variation ID
TNNT2	1	NM_001001430.2:c.422G>A	R141Q	1 (DCM_39)	RCV000159297.1
TTN	2	NM_001267550.2:c.83416C>T	Stop gained	1 (DCM_21)	RCV001072067.3
FLNC	7	NM_001458.5:c.5478T>G	Stop gained (Y1826*)	1 (DCM_3)	RCV001388295.2
MYBPC3	11	NM_000256.3 (MYBPC3):c.2285T>A	V762D	1 (DCM_42)	RCV001038117.5

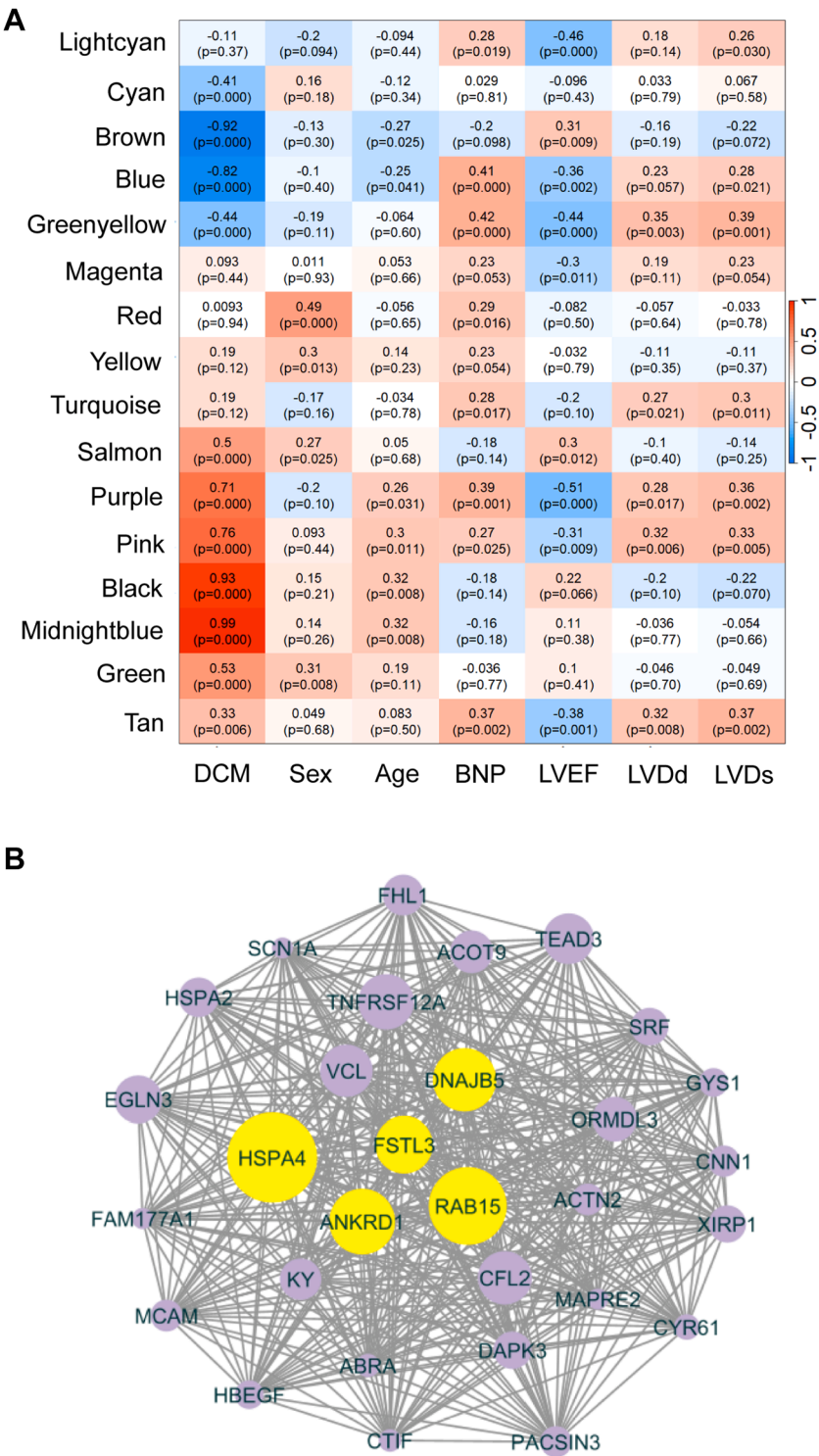
previous studies¹⁵⁻¹⁷ reinforces fibrosis as a primary pathology in DCM-affected hearts.

QUANTITATIVE ASSESSMENTS OF CARDIAC FIBROTIC REGIONS AND ASSOCIATED GENE EXPRESSION ANALYSIS. To assess histological images with greater precision and without bias, we have uniquely developed a machine learning-based evaluation method named “HALO AI.” HALO AI consists of 3 steps: first, defining the tissue classification; second, training based on this classification; and third, applying the classifier to new, unclassified data (Figure 2A). The validation of HALO-AI is supported by multiple studies across different medical domains.¹⁸⁻²⁰ An example of tissue stained by Elastica Masson is shown in Figure 2B. Manual measurement of interstitial fibrosis, entwined among muscle fibers, is labor-intensive and often inaccurate caused by observer variability. In contrast, the HALO AI method enables reliable and reproducible fibrosis quantification in our samples. As a result, across 58 DCM cases, we found a fibrotic area of $16.3 \pm 7.8\%$ (Figure 2C). Notably, these results align closely with those previously reported in DCM samples, further validating our methodology and findings.²¹ To identify transcriptomic associations with fibrosis, we first calculated correlation coefficients between fibrotic area (%) and the expression levels of all genes. These results are presented in Supplemental Figure 7, where genes are plotted based on their correlation coefficients and average expression levels. Among these genes, we subsequently focused on the 114 fibrosis-related genes referenced on the FibroAtlas website. This analysis revealed a significant positive correlation between the percentage of fibrotic area and the median expression of fibrosis-related genes ($r = 0.46$; $P < 0.001$), suggesting the influential role these genes may have on cardiac fibrosis progression (Figure 2D). Notably, among genes with an average expression exceeding 300, *MMP2* displayed the highest correlation coefficient ($r = 0.47$; $P < 0.001$), pointing to a potentially crucial role in cardiac fibrosis (Figures 2E and 2F). We further highlighted another set of top 10 genes strongly linked with cardiac fibrosis, comprising *SPRY1*, *SORBS3*, *SMAD3*,

RHOA, *HRH2*, *VIM*, *FGFR1*, *CYP2J2*, and *CTNNA1* (Figure 2E). To further investigate the relationship between *MMP2* expression and cardiac fibrosis, we examined 42 samples from patients who underwent CMR imaging. Among these, samples positive for LGE (LGE+) displayed markedly elevated expression of *MMP2* compared with the LGE-negative (LGE-) group (Figure 2G). This finding underscores the association of myocardial *MMP2* expression with cardiac fibrosis, as identified through both endomyocardial biopsy (EMB)- and CMR-based techniques.

VALIDATION OF POTENTIAL BIOMARKERS AND TREATMENT TARGETS FOR CARDIAC FIBROSIS USING IN VITRO HUMAN ORGANOID MODELS. We recently developed a mature cardiac organoid system using human iPSC-derived ventricular cardiomyocytes and epicardial cells. With appropriately differentiated cells and unique maturation methods, we successfully recapitulated cardiac development and diseases, including cardiac fibrosis, using this system as previously reported.²² The method for generating cardiac organoids, enhancing their maturation through a metabolic process, and inducing cardiac injury is detailed in Figure 3A. Under conditions that promote fibrosis, we noted a marked increase in the expression of fibrosis-related genes, such as *FN1*, *TGFB1*, *TNC*, *COL1A1*, *COL3A1*, and *FAP* in cardiac organoids (Supplemental Figure 8A). Immunohistochemical analysis revealed a significant accumulation of FN1 and COL1 in organoids subjected to fibrosis induction (Figures 3B and 3C, Supplemental Figures 8B and 8C, Videos 1 and 2), validating the effectiveness of our cardiac organoid models in simulating cardiac fibrosis pathology. We then probed the potential of *MMP2* as a biomarker of cardiac fibrosis. With fibrosis induction, *MMP2* mRNA expression significantly increased (Figure 3D). The expression of *MMP2* increased in response to varying TGFB1 concentrations along with endogenous *TGFB1*, *FN1*, and *COL1A1*, suggesting *MMP2* could serve as an indicator of fibrosis severity (Figures 3E, Supplemental Figure 8D). To validate the potential of *MMP2* as a serum marker, we measured *MMP2* protein levels in

FIGURE 1 Transcriptomic Analysis and Network Modeling in DCM and Controls



(A) Heatmap of Pearson's correlation coefficients (upper values) and corresponding *P* values (lower values) illustrating the module-trait relationships between gene coexpression modules (y-axis) and clinical traits (x-axis). (B) A display of the 30 foremost genes based on intramodular connectivity within the purple module. Genes highlighted in yellow represent the top 5 hub genes, and circle size correlates with the intramodular connectivity. (*HSPA4*: 9.93, *RAB15*: 8.93, *ANKRD1*: 7.91, *DNAJB5*: 7.79, and *FSTL3*: 7.20). BNP = brain natriuretic peptide; DCM = dilated cardiomyopathy; LVDd = left ventricular diastolic diameter; LVDs = left ventricular systolic diameter; LVEF = left ventricular ejection fraction.

TABLE 3 List of Fibrosis-Related Genes in the Purple Module				
Gene	Entrez Gene Name	Location	Type(s)	Entrez Gene ID (Human)
ACE2	Angiotensin converting enzyme 2	Plasma membrane	Peptidase	59,272
AERB1	Adrenoceptor beta 1	Plasma membrane	G-protein coupled receptor	153
CDON	Cell adhesion associated, oncogene regulated	Plasma membrane	Other	50,937
FSTL3	Follistatin like 3	Extracellular space	Other	10,272
HBEGF	Heparin binding EGF like growth factor	Extracellular space	Growth Factor	1,839
MYLK3	Myosin light chain kinase 3	Cytoplasm	Kinase	91,807
PAK1	p21 (RAC1) activated kinase 1	Cytoplasm	Kinase	5,058
PRMT7	Protein arginine methyltransferase 7	Cytoplasm	Enzyme	54,496
XIRP1	Xin actin binding repeat containing 1	Plasma membrane	Other	165,904

the culture supernatant by ELISA, revealing a significant increase in response to fibrotic stimuli (Figure 3F). To identify putative treatment targets for cardiac fibrosis, we selected several candidate genes, including *MMP2*, *FGFR1*, and *HRH2*, from 10 genes correlated with fibrosis severity listed in Figure 2E for further analysis. We examined the effects of inhibiting their gene products on cardiac fibrosis using organoid models and observed a significant reduction of *FN1* expression in organoids treated with AZD4547, an FGFR inhibitor. However, neither ARP100 (*MMP2* inhibitor) nor Famotidine (*HRH2* inhibitor) had any impact on *FN1* expression, thus specifically highlighting the potential of FGFR1 as a treatment target for cardiac fibrosis (Figure 3G).

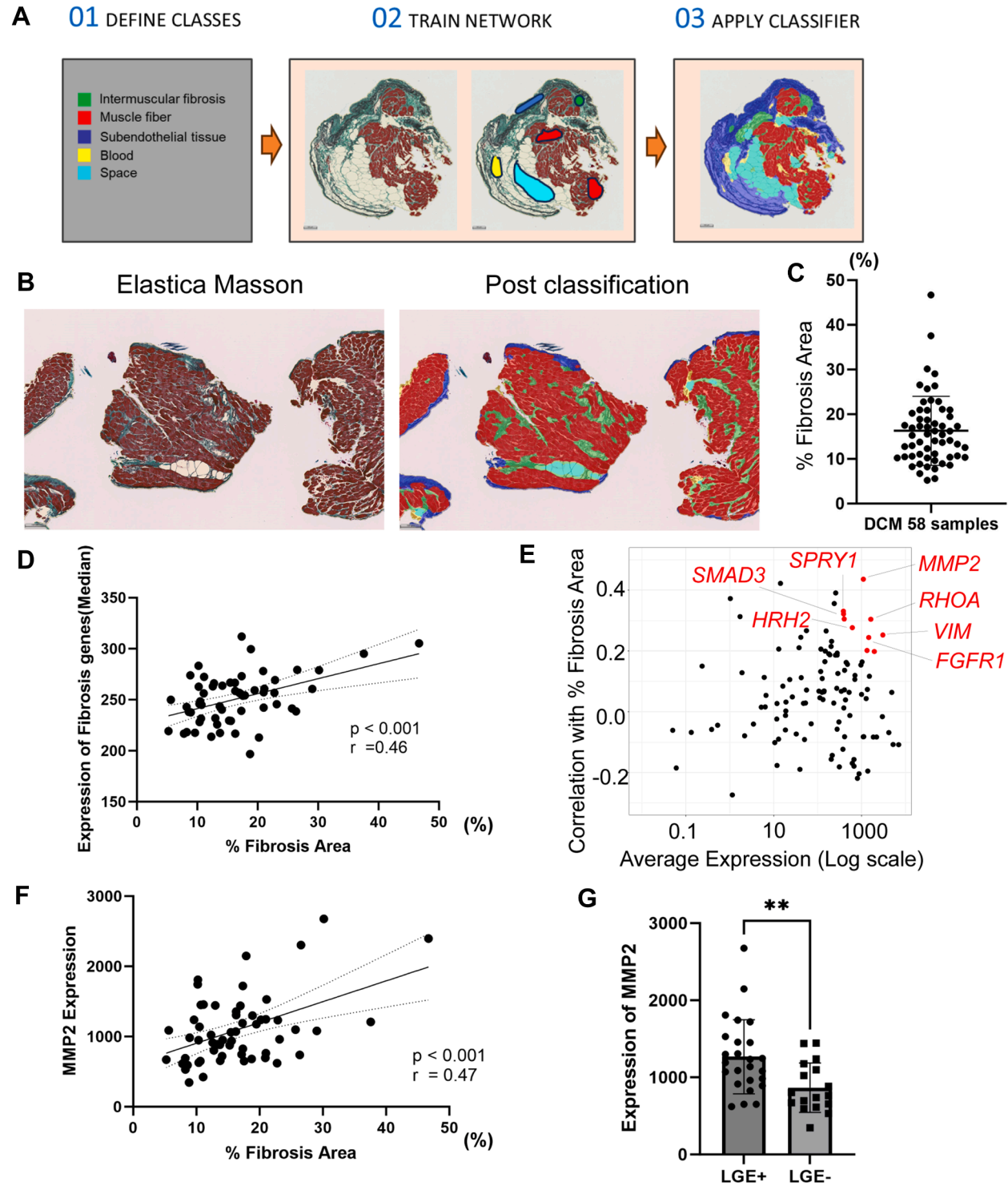
POTENTIAL THERAPEUTIC ROLE OF FGFR1 INHIBITOR IN CARDIAC FIBROSIS. As FGFR1 phosphorylation (pFGFR) plays an important role in this signaling pathway,²³ we first performed immunohistochemistry for both pFGFR1 and total FGFR in DCM patient biopsies (Figure 4A). Quantification confirmed a significant increase in the p-FGFR1/FGFR1 ratio in samples with high fibrosis (Figure 4B). To determine which cell types maintain activated FGFR1 in human myocardium, we performed coimmunofluorescence staining for pFGFR together with cardiac troponin T (cTNT) (cardiomyocytes) and vimentin (VIM) (fibroblast/interstitial cells). As shown in Figure 4C, the p-FGFR1 signal does not overlap with cTnT⁺ cardiomyocytes but colocalizes exclusively with VIM⁺ stromal cells. A similar pattern was observed for *MMP2*, which also colocalized with VIM⁺ fibroblast/interstitial cells but was absent from cTnT⁺ cardiomyocytes (Supplemental Figures 9A and 9B).

Next, we tested the impact of FGFR1 inhibition using a mature cardiac fibrosis organoid model (Figure 4D). Quantitative reverse transcription polymerase chain reaction analysis revealed that AZD4547 significantly suppressed multiple fibrosis-

associated transcripts (*TGFB1*, *TNC*, *COL1A1*, *FAP*, and *POSTN*) relative to DMSO-treated fibrotic organoids (Figure 4E). Immunofluorescence imaging further demonstrated a marked reduction in FN1 and COL1A1 deposition within epicardium-derived (GFP⁺) cells after AZD4547 treatment (Figure 4F, Supplemental Figure 9C), and quantification of mean FN1 and COL1A1 intensity confirmed this decrease (Figures 4G and 4H). Consistent with these findings, *MMP2* released into organoid supernatants was significantly lower following AZD4547 treatment (Figure 4I). To verify that FGF signaling in the organoid system parallels that observed in human tissues, we costained fibrotic organoids for p-FGFR1, VIM, and cTNT. In DMSO-treated fibrotic organoids, p-FGFR1 was markedly enriched in VIM⁺ fibroblast/interstitial regions but showed only low-level overlap with the densely packed cTnT⁺ cardiomyocyte areas. This activation was effectively abolished by AZD4547 (Figures 4J and 4K). We further confirmed that *MMP2* displayed the same cell type specificity in organoids, colocalizing with VIM⁺ stromal cells and showing minimal overlay with cTnT⁺ cardiomyocytes (Supplemental Figures 9D and 9E). We note, however, that the high cellular density in this organoid model may limit the spatial separation of cardiomyocyte and fibroblast compartments.

To validate the effect of FGFR inhibition on cardiac fibrosis, we treated fibrotic organoids with another compound, PD166866, a selective FGFR1 tyrosine kinase inhibitor, following the same protocol as in Figure 4C, which resulted in similar suppression of fibrosis-related genes (Supplemental Figure 10). These results together support FGFR1 inhibition as a potential strategy to attenuate cardiac fibrosis. To examine global gene expression changes in organoids following AZD4547 treatment, RNA-seq analysis was performed to compare the 3 groups: healthy, fibrotic condition, and fibrotic condition with AZD4547 treatment. The gene expression

FIGURE 2 Comprehensive Analysis of Fibrosis and Gene Expression in DCM



(A) HALO AI workflow. (B) Representative images of an Elastica Masson-stained heart biopsy sample (left) and postclassification by HALO AI (right). Classified regions: "fibrotic area" (green), "cardiac muscle area" (red), "pericardial tissue area" (dark blue), "blood area" (yellow), and "empty area" (light blue). (C) Percentage distribution of fibrotic area in dilated cardiomyopathy (DCM) samples. N = 58. (D) Linear regression shows the relationship between fibrosis rate (%fib) and fibrosis score (median expression of 114 fibrosis-related genes) with 95% CIs (dotted lines). (E) Scatterplot of gene expression vs correlation with fibrotic area. The x-axis (log scale) shows average gene expression; the y-axis shows correlation coefficients. The top 10 genes with average expression >300 are highlighted in red. (F) Linear regression of fibrosis rate (%fib) vs MMP2 expression with 95% CIs (dotted lines). (G) Bar plot comparing late gadolinium enhancement (LGE)+ and LGE- groups. ** $P < 0.01$. n = 42. Error bars: SD. Analyses: Pearson's correlation (D, E, F); unpaired Student's t -test (G). Scale bar: 100 μ m.

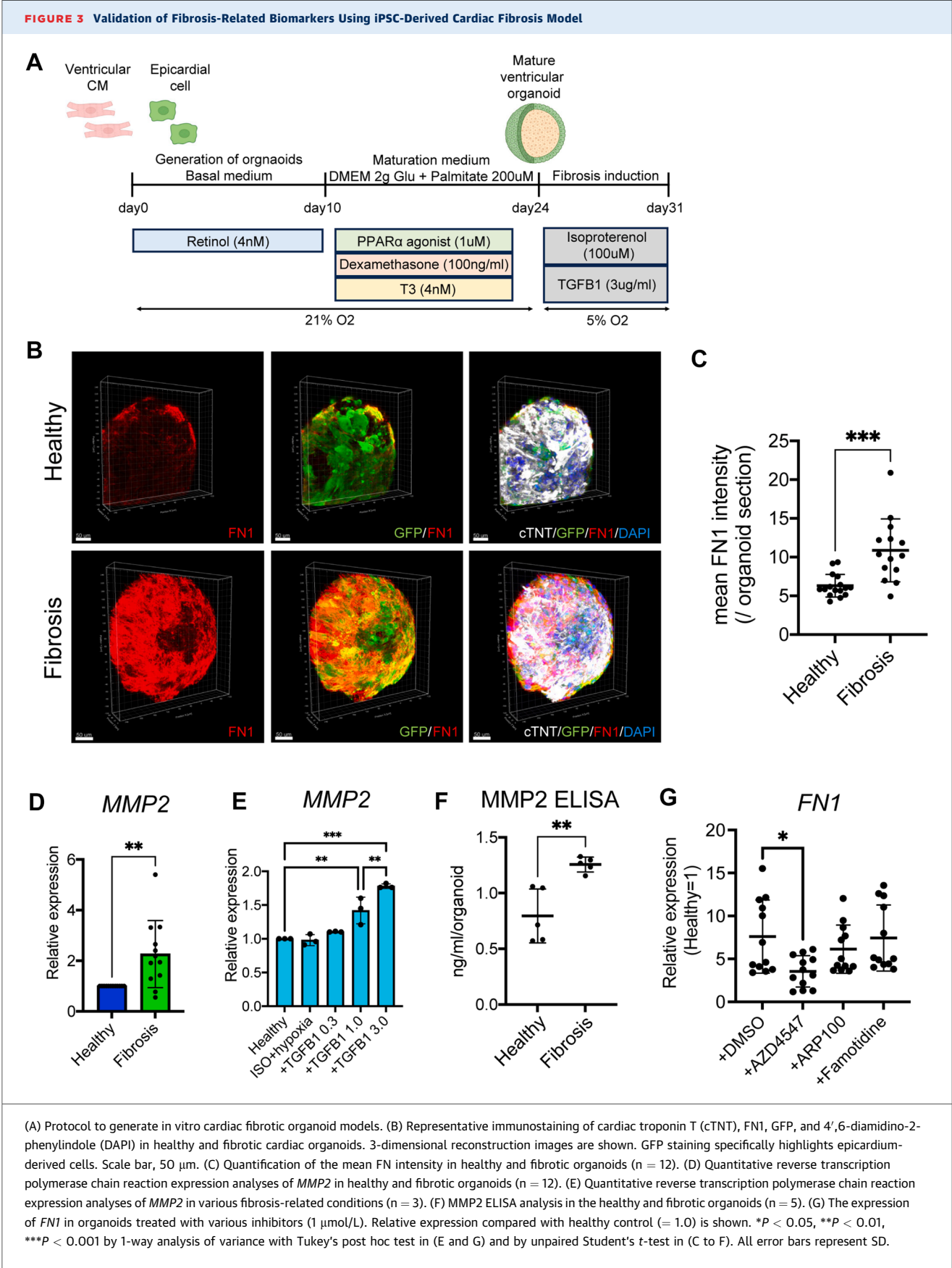
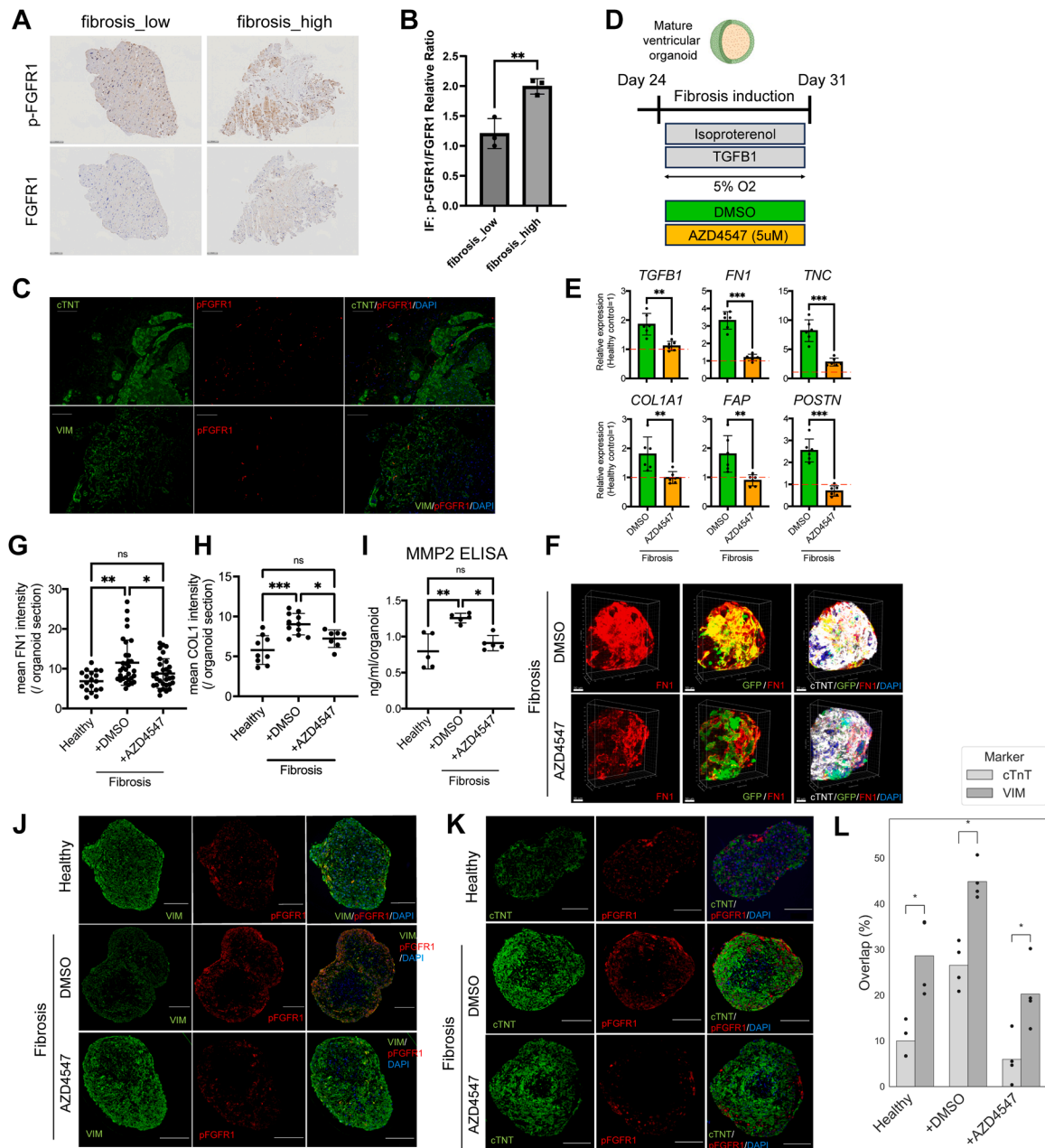


FIGURE 4 Inhibition of FGFR1 Signaling Pathway Attenuates Cardiac Fibrosis

(A) Representative immunostaining of pFGFR1 and FGFR1 in biopsy samples with larger and smaller fibrotic areas. Scale bar: 100 μ m. (B) Quantification of the pFGFR1/FGFR1 ratio in immunostained samples of the indicated conditions (n = 3). (C) Representative coimmunofluorescence of human cardiac biopsy sections showing pFGFR1 (red) with (top) cardiac troponin T (cTNT) (green) or (bottom) vimentin (VIM) (green) and DAPI (blue). Scale bar: 100 μ m. (D) Protocol to test the effects of FGFR1 inhibition by AZD4547. (E) Quantitative reverse transcription polymerase chain reaction expression analyses of indicated genes in the DMSO- and AZD4547-treated fibrotic organoids (n = 6). (F) Representative immunostaining of cTNT (white), FN1 (red), GFP (green), and DAPI (blue) in the DMSO- and AZD4547-treated fibrotic organoids. GFP staining specifically highlights epicardium-derived cells. Three-dimensional reconstruction images are shown. Scale bar: 50 μ m. (G, H) Quantification of the mean FN1 (G) and COL1A1 (H) immunofluorescence intensity in the indicated conditions (FN1, n = 19 for healthy; n = 32 for fibrotic organoids treated with DMSO or AZD4547, COL1A1, n = 9 for healthy; n = 11 for fibrotic organoids; n = 7 for AZD4547-treated fibrotic organoids). (I) MMP2 ELISA analysis in the indicated conditions (n = 5). (J, K) Representative coimmunofluorescence of fibrotic cardiac organoids treated with healthy, DMSO, or AZD4547, showing (J) VIM (green), pFGFR1 (red), and DAPI (blue); (K) cTNT, (green), pFGFR1 (red), and DAPI (blue). Scale bar: 100 μ m. (L) Quantification of FGFR1 colocalization with cardiomyocyte and fibroblast markers. Percentage overlap of FGFR1-positive area within (left) cTNT-positive cardiomyocyte regions and (right) VIM-positive fibroblast regions in control, fibrosis, and fibrosis + AZD4547 organoids. * P < 0.05, ** P < 0.01, *** P < 0.001 by 1-way analysis of variance with Tukey's post hoc test in (G and H) and by unpaired Student's t -test in (B and E). All error bars represent SD. Abbreviations as in Figure 3.

pattern of fibrosis-related genes in AZD-treated organoids closely resembled that of healthy organoids compared with fibrotic organoids (**Figure 5A**, **Supplemental Figures 11A and 11B**). Subsequently, differential gene expression assessments were performed to distinguish between fibrotic organoids with and without AZD4547 treatment. Strikingly, genes such as *POSTN*, *MMP14*, *COL8A1*, and *COL5A1* were substantially downregulated in AZD4547-treated organoids. GO analysis further showed an enrichment of genes associated with extracellular matrix organization among those down-regulated by AZD4547 (**Figures 5B and 5C**), implying that it broadly reduced the expression of genes linked to fibrosis. Conversely, genes up-regulated in AZD4547-treated organoids included those related to cardiac muscle, cardiac conduction, and cardiac action potential, suggesting a potential therapeutic benefit of AZD4547 in attenuating cardiac dysfunction (**Figure 5D**). In addition, we evaluated the expression levels of the top 10 fibrosis-related genes identified by bulk RNA-seq (**Figure 2E**). Additionally, we evaluated the expression levels of the top 10 fibrosis-related genes identified in bulk RNA-seq (**Figure 2E**). Although the absolute changes in this assay were modest, *MMP2* expression was significantly elevated in fibrotic (normal saline [NS]-treated) organoids relative to control and trended back toward baseline levels upon AZD4547 treatment (**Supplemental Figure 11C**). Collectively, these findings implicate FGFR1 activation and *MMP2* up-regulation in fibroblast-driven cardiac fibrosis and support FGFR1 inhibition as a potential antifibrotic strategy.

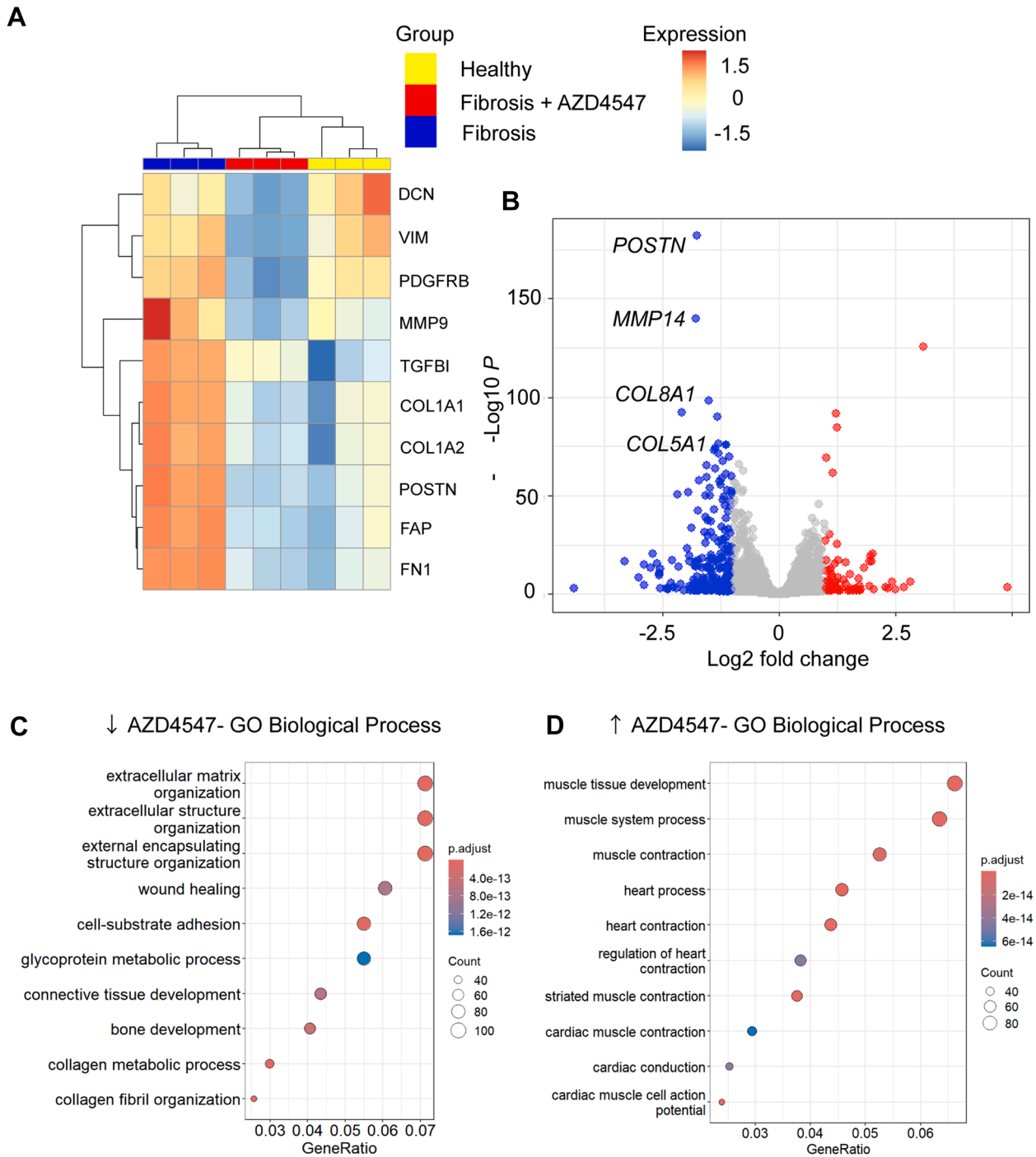
EFFICACY OF AZD4547 IN AMELIORATING CARDIAC FIBROSIS AND DYSFUNCTION IN A MOUSE MODEL. To evaluate the anticardiac fibrosis efficacy of AZD4547 in vivo, we utilized a well-validated mouse model of cardiac dysfunction with fibrosis involving a chronic infusion of angiotensin II and phenylephrine via implanted osmotic pumps for 1 month.²⁴ The mice exhibited significant cardiac fibrosis and signs of cardiac dysfunction. **Figure 6A** outlines the experimental timeline, illustrating the osmotic pump implantation and the subsequent administration of AZD4547 or normal saline over a period culminating in analysis at day 35. Cardiac function, assessed by echocardiography in PE/Ang II-treated mice, was significantly improved by AZD4547 administration compared with normal saline, as indicated by fractional shortening and ejection fraction measurements (**Figures 6B and 6C**). Pharmacokinetic and pharmacodynamic properties of AZD4547 have been previously characterized, demonstrating dose-

dependent inhibition of phospho-FGFR in preclinical models.²⁵ Microscopic examination of cardiac histology in ventricular tissue sections revealed increased fibrosis in the untreated PE/Ang II group relative to control subjects, consistent with the reported pathology of this model (**Figure 6D**). However, mice administered with AZD4547 exhibited substantially less fibrosis and were quantitatively similar to the control group, as evidenced by the significant reduction in fibrotic area shown in **Figure 6E**.

CHARACTERIZATION OF FIBROBLAST AND CARDIOMYOCYTE SUBSETS IN PE/ANG II-TREATED MICE.

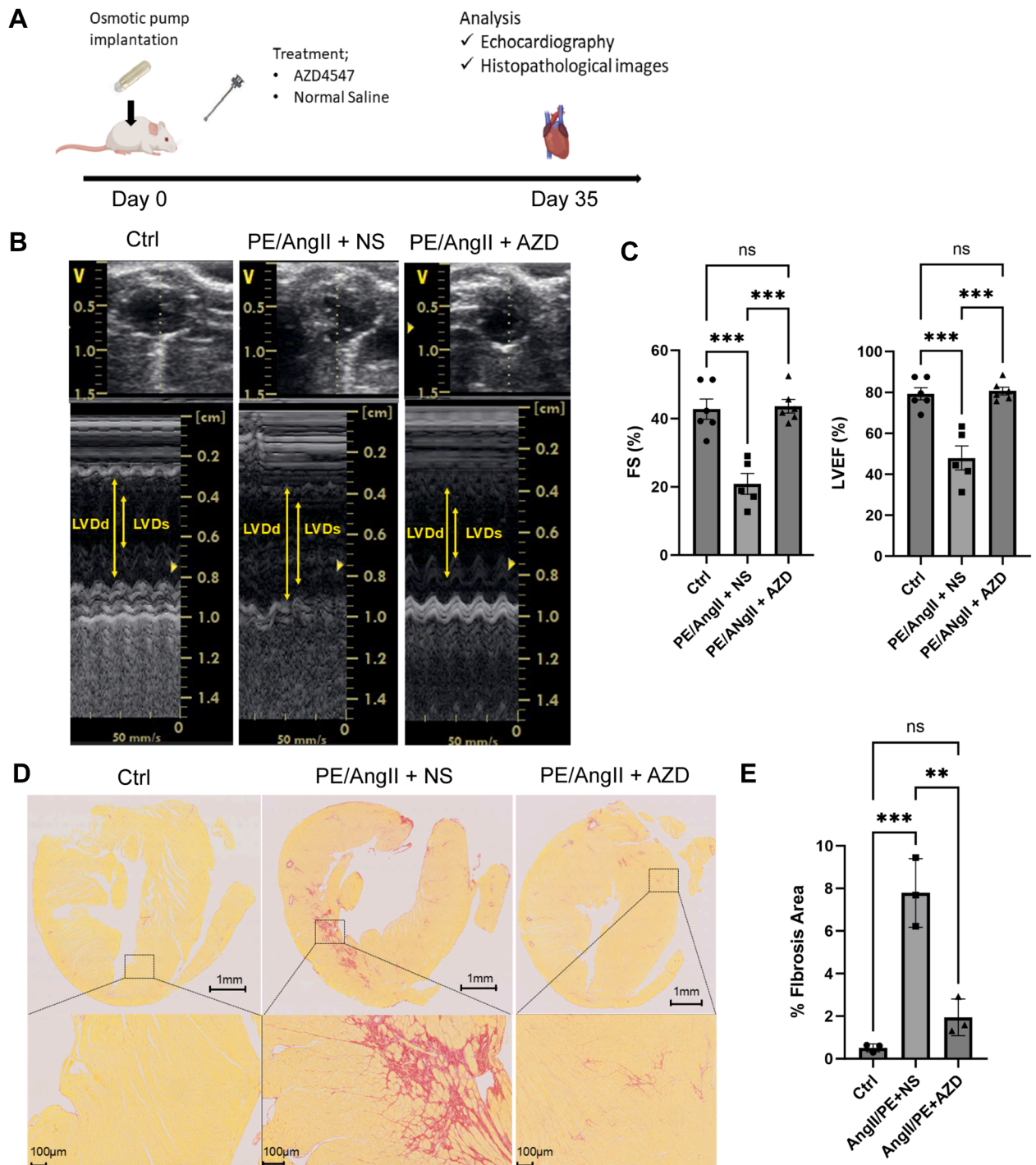
Single-cell analyses were conducted to assess further the antifibrotic and cardioprotective effects of AZD4547 and the impact on cell-cell interactions. Analyses were performed on left ventricular heart samples from control and PE/Ang II-treated mice with or without AZD4547 administration at 7 days after PE/Ang II treatment to validate these effects. Quality control measures ensured the cells were well integrated and distributed within clusters (**Supplemental Figure 12A**). Analysis of total transcripts per cell and identification of possible doublets confirmed the high quality of both data sets and successful integration (**Supplemental Figures 12B and 12C**). A total of 7 major cell types were annotated based on established cell type-specific gene markers: cardiomyocytes (CM) (3 clusters), endothelial cells (EC) (7 clusters), fibroblasts (FB) (2 clusters), pericytes (1 cluster), smooth muscle cells (1 cluster), and immune cells (lymphocyte [4 clusters], and macrophages [3 clusters]) (**Figure 7A**, **Supplemental Figures 12D**). Expression of characteristic marker genes allowed us to identify cell populations, such as cardiomyocytes expressing *Myh6*, *Myl2*, and *Tnni3* (**Supplemental Figure 12E**) and fibroblasts expressing *Col1a1*, *Col1a2*, and *Dcn* (**Supplemental Figure 12F**). Because 3 clusters expressed well-established markers but coexpressed markers of other cell types, they were therefore annotated as EC-FB (endothelial-fibroblast-like cluster), CM-FB (cardiomyocyte-fibroblast-like cluster), and CM-Peri (cardiomyocyte-pericyte-like cluster). Two clusters (lowQC-CM1 and lowQC-CM2, respectively, clusters 8 and 12) exhibited extremely high transcript levels and were deemed low quality and thus excluded from downstream analysis. We conducted a detailed subclustering analysis to elucidate the effects of AZD4547 treatment on fibroblasts, identifying 8 distinct clusters (**Figure 7B**). PE/Ang II + NS induced a remarkable profibrotic shift: clusters 5 and 6—both enriched for classic fibrosis markers *Col1a1* and *Col1a2* (**Supplemental Figure 13A**)—expanded from

FIGURE 5 Transcriptomic and Functional Analysis of Gene Response to AZD4547

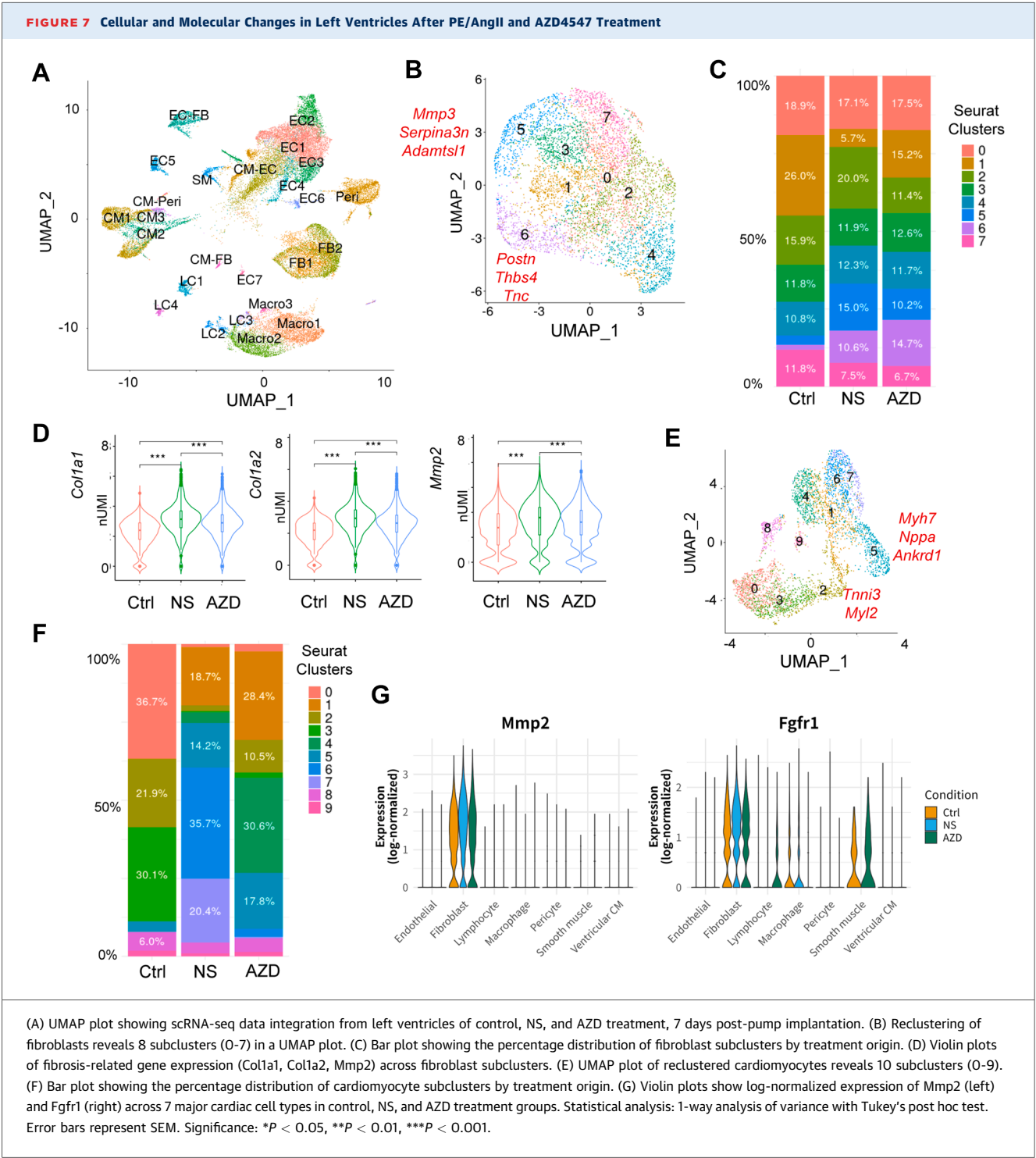


(A) Heatmap representation of fibrosis-related gene expression in control organoids (healthy; n = 3, shown in yellow), fibrotic organoids (fibrosis; n = 3, shown in blue), and fibrotic organoids treated with AZD4547 (fibrosis+AZD4547; n = 3, shown in red). Expression values have been log2-transformed for visualization. (B) Volcano plot comparing gene expression between fibrosis and fibrosis+AZD4547. Red and blue dots represent significantly up- and down-regulated genes by fibrosis +AZD4547, respectively, and gray dots signify genes with no significant change. The x-axis represents Log2 fold change, and the y-axis represents -log₁₀ (P) - adjusted P values. (C, D) GO enrichment analysis, emphasizing biological processes, based on differentially expressed genes in fibrosis+AZD4547. Circle size signifies the gene ratio, which indicates the proportion of genes in a specified pathway relative to all differentially expressed genes. The color gradient indicates Fisher exact P value, adjusted by the Benjamini-Hochberg method for multiple comparisons: (C) for genes down-regulated by AZD4547 and (D) for genes up-regulated by AZD4547.

FIGURE 6 AZD4547 Treatment Enhances Cardiac Function in Mouse Cardiac Injury Model



(A) Study design: Adult C57BL/6 mice were treated with saline or Ang II/PE via an osmotic minipump for 35 days. AZD4547 treatment commenced the day after implantation with daily oral gavage. (B) M-mode echocardiographic images showing LVDd and LVDs. (C) Echocardiographic analysis of fractional shortening (FS) and left ventricular ejection fraction (LVEF), showing improvement in AZD4547-treated mice. $n = 6$, $n = 5$, $n = 6$ biologically independent mice per group. (D) Picosirius red staining of cardiac sections showing collagen (pink). Reduced fibrosis was observed in PE/Ang II + AZD4547 compared to PE/Ang II + saline. Insets: magnified LV myocardium view. Scale bar: 100 μ m. (E) Quantitative fibrosis analysis (% of the ventricular area) from (D). $n = 3$ biologically independent mice per condition. Statistical analysis: 1-way analysis of variance with Tukey's post hoc test. Error bars denote the SEM. Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations as in Figure 1.



3.0% and 1.7% in control subjects to 15.0% and 10.6%, respectively. Cluster 5 was characterized by elevated *Mmp3*, *Serpina3n*, and *Adamtsl1*, whereas cluster 6 showed the highest expression of *Postn*, *Thbs4*, and *Tnc* (Supplemental Table 5), confirming both as activated, ECM-producing fibroblasts. Supplemental Figure 14 provides GO Biological Process enrichment for all 8 fibroblast subclusters (Supplemental Figures 14A to 14H), with clusters 2 (Supplemental Figure 14C), 5 (Supplemental Figure 14F), and 6

(Supplemental Figure 14G) showing strong enrichment for extracellular matrix organization and collagen-related pathways. By contrast, cluster 1—marked by *Cdh5*, *Cav1*, *Aqp1*, and *Kdr*—represents a quiescent, restorative fibroblast population (Supplementary Table 5) that decreased from 26.0% to 5.7% under PE/Ang II. When clusters 2, 5, and 6 are considered together as pathogenic fibroblasts, they comprise 46.1% of all fibroblasts under PE/Ang II + NS but fall to 36.3% with AZD4547 treatment, while the quiescent/restorative cluster 1 increases correspondingly. AZD4547 thus partially reverses this remodeling—cluster 1 rebounded to 15.2%, and clusters 5 and 6 reset to 10.2% and 14.7%, respectively—restoring overall subcluster balance. Consistent with these compositional shifts, fibrosis-associated genes *Col1a1*, *Col1a2*, and *Mmp2* were up-regulated by PE/Ang II and robustly suppressed by AZD4547 (Figure 7D), demonstrating both a reduction in pathogenic fibroblast burden and the attenuation of profibrotic gene programs.

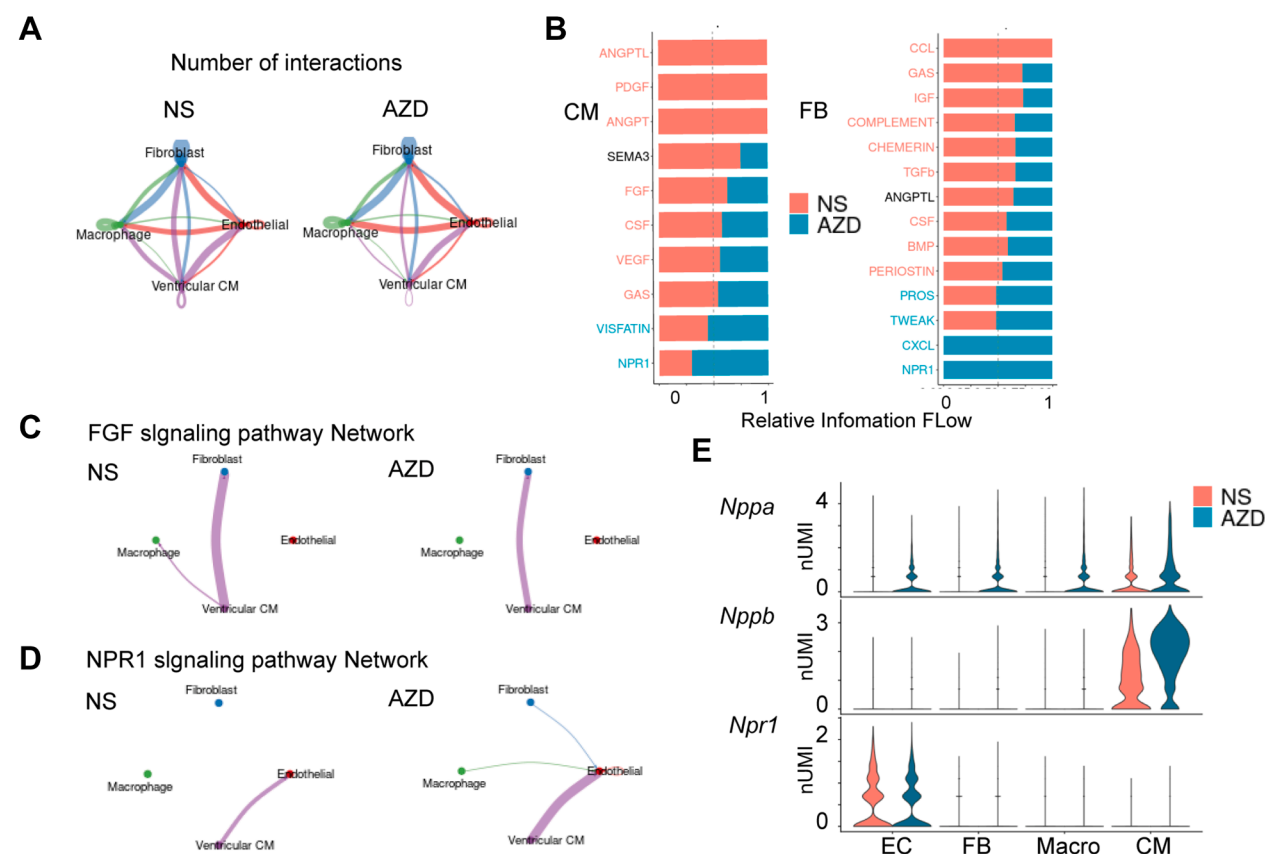
These results suggest that PE/AngII treatment induces cardiac fibrosis through the mobilization of activated fibroblasts and that AZD4547 treatment has an antifibrotic effect by reducing the expression of fibrosis-related genes. We next extended our subclustering to ventricular cardiomyocytes, revealing ten distinct clusters (Figure 7E). In particular, cluster 5, characterized by high expression of *Myh7*, *Nppa*, and *Ankrd1*, markers indicative of a cardiac muscle stress response and hypertrophic cardiomyopathy,^{26,27} increased in PE/Ang II-treated mice regardless of AZD4547 treatment (Figure 7F, Supplemental Figure 13B). In contrast, cluster 2, which exhibited elevated levels of cardiac muscle proteins such as *Myh2* and *Tnni3* was predominantly found in the control group and reduced in PE/Ang2-treated hearts. Its top marker genes—mitochondrial transcripts *mt-Cytb* and *mt-Nd6*, along with *Adgrf5*, *Nid1*, *Rgs5*, *Col6a2*, *Ets1*, *Cygb*, *Marcks*, and *Crip1* (Supplemental Table 6)—strongly suggest a high-oxidative capacity phenotype. GO Biological Process enrichment of these markers—shown specifically for cluster 2 in Supplemental Figure 15C (and for clusters 0-9 across S15A-J)—highlights pathways involved in oxidative phosphorylation, electron transport, aerobic respiration, and energy metabolism, defining cluster 2 as a metabolically optimized cardiomyocyte pool specialized for efficient ATP generation and sustained contractility. Notably, AZD4547 treatment enhanced the percentage of cluster 2 cells compared to untreated hearts, suggesting that FGFR inhibition potentially has beneficial effects on cardiomyocytes (Figure 7F). Finally, in Figure 7G, we highlight that

MMP2 and *FGFR1* expression is selectively elevated in fibroblasts following Ang/PE II and is reversed by AZD4547—mirroring our human cardiac biopsy and fibrotic organoid pFGFR1 immunostaining (Figures 4C, 4J, and 4K) and emphasizing FGFR signaling as a central regulator of fibroblast activation in cardiac fibrosis.

CELLULAR COMMUNICATION AND SIGNALING PATHWAYS IN AZD4547-TREATED HEARTS. Next, we evaluated cellular communication between cell types, particularly among cardiomyocytes, fibroblasts, and endothelial cells. Utilizing the bioinformatic tool CellChat,²⁸ we inferred the cell-cell communication network to analyze and visualize the impacts of PE/AngII-induced fibrosis and AZD4547 treatment. CellChat analysis revealed significant changes in intercellular interactions in PE/AngII-treated hearts with and without AZD4547 administration. Overall, hearts treated with AZD4547 displayed reduced signaling events by cardiomyocytes compared with untreated hearts. This reduction was particularly notable in the interactions visualized in circular plots, where thinner, purple-labeled connections indicated fewer cardiomyocyte interactions, primarily those with fibroblasts. In the FGF signaling network, there was a large decrease in signaling from cardiomyocytes to fibroblasts, followed by a reduction in signaling from cardiomyocytes to macrophages (Figure 8A). Detailed visualization of each signaling pathway or ligand-receptor pair between ventricular cardiomyocytes, fibroblasts, endothelial cells, and macrophages revealed the down-regulation of Fgf1-Fgfr1 signaling (Supplemental Figure 16). These findings suggest that AZD4547 treatment primarily affects cardiomyocyte-to-fibroblast signaling (Figure 8B).

Figure 8C shows the ranking of all significant signaling pathways based on their differences in overall information flow within the inferred networks between AZD4547 and NS treatments. These transcriptomic data suggest that NPR1 signaling may be enhanced in both cardiomyocytes and fibroblasts, with the most pronounced increase observed in cardiomyocyte-endothelial interactions (Figure 8D). In line with these findings, we observed increased expression of *Nppa* and *Nppb*, both known NPR1 ligands, in cardiomyocytes treated with AZD4547 relative to untreated hearts (Figure 8E). Although elevated *Nppa* and *Nppb* levels can be associated with protective or adaptive responses, they may also reflect ongoing cardiac remodeling rather than direct functional rescue. Furthermore, *Npr1*—predominantly expressed in endothelial cells—exhibited comparable expression between the 2 groups.

FIGURE 8 AZD4547 Modulates Ligand Receptor-Mediated Intercellular Communication and Target Gene Expression in PE/Ang II-Treated Hearts



(A) Network diagrams of total predicted ligand-receptor interactions among major cardiac cell types—fibroblasts (FB), endothelial cells (EC), ventricular cardiomyocytes (CM), and macrophages (Macro)—under PE/Ang II + Normal saline (NS) and PE/Ang II + AZD4547 (AZD) conditions. Edge thickness is proportional to the number of inferred interactions between each pair of cell types. (B) Relative information flow for the top 10 signaling ligand-receptor pairs in (left) the FGF pathway acting on cardiomyocytes (CM), and (right) the NPR1 pathway acting on fibroblasts (FB). Red bars denote NS, and teal bars AZD4547. Ligand names in bold indicate those most reduced by AZD4547. (C) FGF signaling networks plotted as circular chord diagrams for NS (left) and AZD (right). Chord width reflects interaction strength from the source (ligand-producing) to the target (receptor-expressing) cell types. (D) NPR1 signaling networks, displayed as in (C), highlight altered communication from fibroblasts and endothelial cells to ventricular CM under NS vs AZD. (E) Violin plots of normalized UMI counts for *Nppa*, *Nppb*, and *Npr1*, grouped by cell type (EC, FB, Macro, CM) and colored by condition (NS, red; AZD, teal).

Altogether, these observations raise the possibility that AZD4547 administration attenuates fibrosis by reducing FGF signaling between ventricular cardiomyocytes and fibroblasts while supporting a potential role for enhanced NPR1 signaling between cardiomyocytes and endothelial cells in mitigating cardiac dysfunction. Nonetheless, these findings require additional functional validation to confirm their therapeutic implications.

DISCUSSION

This is the first comprehensive study of the pathological mechanisms underlying cardiac fibrosis,

integrating both myocardial transcriptome analysis and histological assessments of fibrotic areas in myocardial tissues from the same patient, with validation via iPSC-derived cardiac organoid and mouse models. Using this comprehensive approach, we elucidated the following: First, by quantifying myocardial fibrotic regions and correlating these with gene expression analysis, we identified key candidate factors among cardiac fibrosis-related genes. Second, our study established a clear association between *FGFR1* expression and its phosphorylation status in DCM myocardial tissue and the severity of fibrosis. Notably, treatments with various inhibitors, particularly AZD4547, a selective FGFR inhibitor,

significantly reduced fibrotic markers in our human cardiac fibrotic organoid models. Additionally, echocardiographic assessments in a PE/Ang II-induced murine model of cardiac dysfunction showed that AZD4547 treatment led to substantial improvements in cardiac function and markedly reduced fibrosis compared with NS. Altogether, our data suggest that AZD4547 treatment attenuates fibrosis by inhibiting FGF signaling between ventricular cardiomyocytes and fibroblasts and potentially mitigates cardiac dysfunction via increased NPR1 signaling between cardiomyocytes and endothelial cells.

In the context of DCM, previous studies have reported functional enrichment analyses of cardiac tissue transcriptomes. For instance, Liu et al²⁹ proposed that up-regulated genes in DCM are implicated in extracellular matrix remodeling and collagen formation. Consistent with bulk observations, recent single-cell atlases show that such ECM pathways are driven almost exclusively by activated fibroblasts.^{10,11} An integrated bulk and single-cell RNA sequencing meta-analysis further demonstrated that expression of *MMP23B* is tightly cell state-dependent, increasing progressively with fibroblast activation.¹² However, these studies lacked the integration of transcriptomic data with detailed histological analysis, as demonstrated by our work here. Our study represents a significant breakthrough by merging transcriptome information with AI-assisted histological assessment. This innovative method has successfully identified a specific set of genes correlated with the severity of cardiac fibrosis, as observed by histological analysis of cardiac tissues. For top-ranked genes correlated with the severity of cardiac fibrosis, we utilized data from single-cell RNA-seq analysis of the left ventricle in a mouse model of cardiac injury to evaluate cell lineage-specific gene expression. The Ang/PEII exposure group exhibited elevated expression of *FGFR1* and *MMP2* specifically in fibroblasts, compared with control subjects and the AZD4547-treated Ang/PEII exposure group (Figure 7G, Supplemental Figure 17). This supports the view that *MMP2* expression is not simply a reflection of fibroblast quantity but represents disease-related activity.

Myocardial fibrosis, characterized by alterations in the ECM, is widely recognized as a defining feature of DCM.³⁰ Previous literature suggested a relationship between DCM progression and increased fibrosis,³¹ including markers linked to collagen synthesis and degradation, notably *PICP*, *PIIINP*, and *CTPI*.³¹⁻³⁴ In addition, ECM proteolytic enzymes, particularly matrix metalloproteinases (MMPs) and their tissue

inhibitors, also play a pivotal role.³² MMPs, a protease family specialized in ECM degradation, exhibit a highly conserved protein structure. They not only play a vital role in cardiac fibrosis by regulating ECM turnover and inflammatory signaling³⁵ but also promote ECM production by regulating the activity of fibroblasts.³⁶ MMP2 has been considered a potential biomarker for heart failure, with heightened plasma MMP2 levels observed in patients with congestive HF.³⁷ However, the extent of the correlation between *MMP2* mRNA expression in myocardial tissues and the degree of fibrosis progression was not fully understood. In this study, we confirmed the cardiac-specific role of MMP2 in fibrotic pathology by examining *MMP2* expression in cardiac tissues and its correlation with fibrosis based on both EMB and CMR in a distinct DCM patient cohort. In a subanalysis, we compared *MMP2* expression with and without pathogenic variants in DCM, but no significant differences were observed (Supplemental Figure 2B).

There have been several studies involving mouse models that have examined whether knockout or knockdown of *FSTL3* and *MMP2* can attenuate DCM phenotypes, particularly fibrosis. For example, research by Shinichiro Matsumura et al³⁸ demonstrated that *MMP2* activity is involved in left ventricular remodeling after acute myocardial infarction and that inhibiting *MMP2* activity reduces left ventricular remodeling. Specifically, 3 tagged single nucleotide polymorphisms in the promoter region of the *MMP2* gene (rs243865, rs2285052, and rs2285053) have been identified and linked to the development of DCM.³⁹ Regarding *FSTL3*, recent research has demonstrated that cardiomyocyte-specific ablation of *FSTL3* suppresses stress-induced myocardial hypertrophy, indicating that *FSTL3* is essential for the full development of the hypertrophic response.⁴⁰ This emphasizes the importance of *FSTL3* in cardiac remodeling. In our study, we focused on their potential as biomarkers. However, in our study, single-cell analysis of AngII/PE-exposed mice revealed a low detection rate of *FSTL3*-expressing cells, making it challenging to propose *FSTL3* as a reliable biomarker for cardiac fibrosis progression.

FGFR1 is expressed in cardiomyocytes and other cardiac cell types and has opposing protective and detrimental effects on the heart. FGFR1 signaling within cardiomyocytes can elevate contractility, potentially leading to hypertrophic cardiomyopathy.⁴¹ In contrast, chronic FGFR1 activation can provoke pathological remodeling and subsequent fibrosis.⁴² Our findings strongly indicate a complex relationship between FGFR1 expression, its phosphorylation, and the severity of fibrosis in DCM.

Additionally, they suggest that FGFR1 inhibition effectively suppresses the changes induced by fibrotic stimulation in the cardiac organoid model, positioning FGFR1 as a potential therapeutic target. Anti-FGFR drugs, primarily explored for cancer treatments, have gathered considerable attention recently. Indeed, several clinical trials for cancer patients are currently underway, testing different candidate drugs, including AZD4547,⁴³ rogaratinib (pan-FGFR inhibitor),⁴⁴ and erdafitinib (tyrosine kinase inhibitor targeting FGFR1-4).⁴⁵ As the landscape of clinical trials evolves, it is worth noting that specific anti-FGFR1 agents have not yet been investigated for their antifibrotic attributes. The results of these clinical trials should be closely monitored, not only for their primary effects on cancer but also for their potential impact on fibrosis. Although our study demonstrates the efficacy of pharmacological FGFR1 inhibition in attenuating cardiac fibrosis, it is important to note that genetic studies were not conducted. Conditional knockout models or CRISPR-based gene silencing approaches would provide more definitive mechanistic validation by distinguishing the direct effects of FGFR1 in different cardiac cell populations. Future studies incorporating genetic models will be crucial for further elucidating the specific role of FGFR1 in cardiac fibrosis and dysfunction.

This study revealed that while the antifibrotic effects of FGFR1 inhibition could be mediated by the reduction of FGF signaling between fibroblasts and cardiomyocytes, its potential cardioprotective effects might be related to increased NPR1 signaling mainly between cardiomyocytes and endothelial cells. Studies using *Npr1* knockout mice have demonstrated that the endogenous ANP/BNP-NPR1 system plays a protective role in the heart by suppressing the overproduction of plasma aldosterone and cardiac IL-6.⁴⁶ Furthermore, cardiac hypertrophy was enhanced in mice lacking *Npr1* in cardiomyocytes or endothelial cells, thus suggesting the cardioprotective effects of the ANP/BNP-NPR1 system were mediated by interactions between cardiomyocytes and endothelial cells. Most importantly, our findings indicate that the FGFR1 inhibitor increases *Nppa* and *Nppb* expression in ventricular cardiomyocytes under stress in a cardiac injury mouse model, which may contribute to cardioprotective effects. However, further mechanistic studies are needed to confirm this role. *NPPA* and *NPPB* expression during left ventricular remodeling after myocardial injury was reported to suppress remodeling itself, including myocardial structure, functional maintenance, and repair, and is associated with cardioprotection.^{47,48}

NPR1 signaling plays a crucial role in maintaining vascular endothelial homeostasis and modulating interactions between endothelial cells and other cell types. NPR1 signaling decreased the levels of intercellular adhesion molecule-1 to inhibit monocyte adhesion to endothelial cells and is a significant factor during inflammatory responses when tumor necrosis factor- α induces cell adhesion.⁴⁹ In the heart, NPR1 localizes to specific membrane invaginations called transverse tubules, which confines cGMP signals produced by NPR1.⁵⁰ cGMP signaling modulates cardiac contractility, hypertrophy, and remodeling and is essential for protecting against ischemic injury and cardiomyopathy.⁵¹ Our results revealed that AZD4547 treatment in cardiac stress increased the expression of *Nppa* and *Nppb* in ventricular cardiomyocytes, leading to cardioprotection through interactions with *Npr1* expressed by endothelial cells. The association between FGFR inhibitors and cardioprotective effects can be explained by the suppression of irreversible fibrotic replacement in a cardiac injury model, which prevents the reduction of myocardial tissue. Specifically, we interpret this as the apparent activation of a homeostatic maintenance mechanism in the heart, mediated by NPR1 signaling in response to pressure overload. This finding suggests that FGFR inhibitors could represent a promising therapeutic strategy to limit cardiac fibrosis, although further in-depth investigations are warranted.

In this study, we employed an in vitro human heart disease model derived from human iPSC-derived cardiac cells to validate several discoveries from our clinical research. Although iPSC technology offers a valuable platform for studying disease mechanisms in vitro, challenges remain caused by the immature features of iPSC-derived cells and the lack of a fully physiologic environment. Recently, we successfully established a 3-dimensional mature cardiac organoid platform by combining specialized cell differentiation methods, such as ventricular CMs and epicardial cells, with an efficient maturation protocol.^{22,52-54} Our results show that these in vitro organoids mirrored several molecular and phenotypic changes identified in animal models, suggesting they can serve as a relevant platform to investigate disease mechanisms and evaluate potential therapies. Although further refinements are needed to optimize maturity and functional fidelity, this approach provides a practical, human-relevant tool for examining cardiac fibrosis and screening novel treatments, complementing traditional in vivo models.

STUDY LIMITATIONS. Our investigation provides a foundational framework for analyzing cardiac fibrosis in DCM and deciphering mechanistic pathways. To encompass a broad spectrum of biopsied heart samples from DCM patients, we employed total myocardial RNA sequencing instead of single-cell RNA sequencing. Myocardial tissue samples were derived from the right ventricular septum, the conventional site for endomyocardial biopsy. It remains uncertain, however, whether gene expression in this area accurately reflects gene expression in the LV in DCM. Our control tissues were sourced exclusively from Caucasian donors; hence, genetic differences could have influenced the outcome of our analysis. Despite these limitations, by annotating the modules derived from weighted gene coexpression network analysis, we could select the module pertinent to DCM for further analysis.

CONCLUSIONS

This study enhances our comprehension of cardiac fibrosis in DCM through integrated analyses encompassing transcriptomic data, histological images, and in vitro organoid and in vivo murine models. This approach enabled the identification of crucial genes, including *FGFR1*, associated with cardiac fibrosis. AZD4547, an FGFR inhibitor, demonstrated the ability to reduce fibrosis and enhance cardiac function by enhancing NPR1 signaling between ventricular cardiomyocytes and endothelial cells, highlighting its potential as an effective treatment for cardiac fibrosis.

DATA AVAILABILITY. RNA sequencing data for this study were deposited in the Gene Expression Omnibus (GEO) under the reference series GSE246299. This SuperSeries consists of the following SubSeries: GSE245825: RNA-seq analysis of 58 DCM samples.

GSE246298: RNA-seq analysis of a cardiac organoid model using human induced pluripotent stem cell (hiPSC)-derived ventricular cardiomyocytes and epicardial cells, categorized as control ($n = 3$), fibrotic condition ($n = 3$), and fibrotic condition treated with AZD4547 ($n = 3$). Single-cell RNA sequencing data for

this study are deposited in the GEO under the GSE274292.

In addition, nonfailing left ventricular samples ($n = 12$) were sourced from previously published data sets, available publicly in the GEO database under GSE116250. All code used can be downloaded from https://github.com/Yoshida-Labs/JACC-BTS_figures.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study demonstrates that FGFR1 expression strongly associates with the severity of cardiac fibrosis in DCM, and FGFR1 inhibition suppresses fibrosis-related gene expression, alleviates interstitial fibrosis, and preserves cardiac function in both in vitro organoid and in vivo murine models. These findings suggest that targeting FGFR1 may provide a novel therapeutic strategy for managing advanced fibrotic heart conditions, particularly when standard heart failure treatments are insufficient.

TRANSLATIONAL OUTLOOK 1: The integration of transcriptomic and histological data with iPSC-derived

cardiac fibrosis organoid models has enabled the identification and validation of FGFR1 as a therapeutic target. This translational approach highlights the potential of using advanced organoid systems to bridge the gap between basic science discoveries and therapeutic development.

TRANSLATIONAL OUTLOOK 2: Future research should focus on optimizing FGFR1 inhibitors for clinical use, including ensuring safety and efficacy across diverse patient populations and conducting large-scale clinical trials. These efforts will be crucial for overcoming translational barriers and advancing personalized treatment options for fibrotic heart diseases.

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APPENDIX For supplemental tables and figures, please see the online version of this paper.