



IDENTIFICATION OF CENTRAL REGULATORY HUBS IN THE MOLECULAR INTERACTION NETWORK UNDERLYING CARDIAC FIBROBLAST-TO-MYOFIBROBLAST TRANSITION

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Abstract

Cardiac fibrosis is a pathological process characterized by excessive accumulation of extracellular matrix components, leading to structural and functional impairment of the heart. A key event in this process is the transition of cardiac fibroblasts into myofibroblasts, which are responsible for increased extracellular matrix production and tissue remodeling. The aim of this study was to identify molecular mechanisms involved in fibroblast-to-myofibroblast transition using bioinformatic analysis. A protein–protein interaction network was constructed based on genes associated with cardiac fibrosis and functional enrichment analysis was performed. The results identified TGF- β signaling, extracellular matrix remodeling, inflammatory response and mechanotransduction as the major biological processes involved in fibroblast activation and fibrosis progression. These findings highlight the complex molecular network regulating cardiac fibrosis and indicate potential targets for future therapeutic strategies.

Running title: Regulatory hubs in cardiac fibroblast transition

Keywords: cardiac fibrosis, fibroblast-to-myofibroblast transition, TGF- β , extracellular matrix, protein–protein interaction network, bioinformatics

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Introduction

Fibroblasts are the primary cells of connective tissue responsible for producing and maintaining the extracellular matrix, which provides tissues with strength, elasticity and proper organization [1]. In this way, they give mechanical support to organs and create the environment necessary for the proper functioning of neighboring cells. They secrete numerous signaling molecules, such as cytokines and growth factors, which regulate repair and regeneration processes [2,3]. Furthermore, fibroblasts act as progenitor cells capable of differentiating into other mesenchymal cells, such as osteoblasts that form bone or adipocytes that store fat [4,5].

Although fibroblasts share many common characteristics and respond to similar biological signals, they differ depending on the tissue in which they are found [6]. Individual fibroblast populations are specialized and adapted to the needs of a specific organ, supporting its development, maintenance and regeneration following injury. They arise during embryonic development from various cell lines and colonize organs derived from all three germ layers, such as the skin and mammary gland, skeletal muscles and the heart, and the lungs. As a result, fibroblasts in different organs perform specific functions corresponding to the structure and requirements of the given tissue [1,6].

The heart muscle is a complex organ responsible for contracting and pumping blood. Its main cell population consists of cardiomyocytes, which connect with one another to form a functional network that enables the conduction of electrical impulses and the coordination of the heart's activity [1,7]. In addition to cardiomyocytes, the heart contains numerous fibroblasts, which are primarily responsible for the production and remodeling of the extracellular matrix, essential for maintaining the organ's structure and proper conduction of electrical signals [7]. With age, increased stress and strain, and in the case of a history of heart disease, the balance between the synthesis and degradation of extracellular matrix components is disrupted, leading to impaired fibroblast function and, consequently, the activation of fibroblasts and their differentiation into myofibroblasts [6–8].

The fibroblast-to-myofibroblast transition (FMT) is a process in which fibroblasts are activated by biochemical and mechanical signals and transform into myofibroblasts [9]. These cells are characterized by increased contractility, the presence of smooth muscle α -actin (α -SMA) filaments and enhanced production of extracellular matrix components such as type

I collagen, fibronectin, cadherin-2 and vimentin [10,11]. Myofibroblasts themselves produce and secrete a range of signaling mediators that are crucial for maintaining the inflammatory response to cardiac injury. These include: chemokines, such as interleukins and TNF- α (tumor necrosis factor); cytokines, including IL-8, MCP-1 (monocyte chemotactic protein), and MIP-1 α (macrophage-derived protein); growth factors: TGF- β (transforming growth factor beta), HGF (hepatocyte growth factor), IGF (insulin-like growth factor), and inflammatory mediators, which include, among others: phospholipase A2-activating protein and prostacyclin [6].

Prolonged activation of myofibroblasts and sustained secretion of profibrotic factors lead to the development of myocardial fibrosis, a pathological process characterized by excessive accumulation of extracellular matrix components, primarily type I and III collagen, in the myocardial interstitium. This process develops in response to various forms of cardiac injury and overload, such as myocardial infarction, hypertension, diabetes or cardiomyopathies [12–14].

The aim of this study was to identify and characterize key genes and protein-protein interaction (PPI) networks involved in the transition of cardiac fibroblasts into myofibroblasts, which is one of the main mechanisms leading to cardiac fibrosis. Based on a systematic literature review and data from public biological databases, genes associated with fibroblast activation, cytoskeletal reorganization, extracellular matrix remodeling, TGF- β signaling and mechanotransduction were selected. Subsequently, an analysis of molecular interaction networks was conducted to identify the most important regulatory nodes and signaling pathways involved in FMT and the development of cardiac fibrosis.

Materials and methods

Prediction of protein-protein interactions

Physical and functional interactions between the protein products of selected genes were retrieved from the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins, version 12.0). A confidence score threshold of ≥ 0.700 (High Confidence) was applied. The interactome network was constructed as a graph in which nodes represent proteins and edges represent documented interactions.

Network topology analysis

The structural analysis of the network was performed using the NetworkX library in a Python 3.14 environment. For each node, key topological parameters were calculated: Degree Centrality to identify so-called hub genes

(nodes with the highest number of connections), which constitute the regulatory centers of the network and Betweenness Centrality to identify proteins acting as communication bridges (bottlenecks) between individual functional groups.

Gene set enrichment analysis (GSEA)

To confirm the biological significance of the identified clusters, a functional enrichment analysis was performed using the GSEAPy library and the Enrichr platform. Mapping was performed against the KEGG (2021 Human) and GO Biological Process (2023) databases. The statistical significance of the enrichment was determined based on an adjusted p-value (FDR – False Discovery Rate) < 0.05. The results were visualized using dot plots and bar charts.

Identification of functional hubs

To integrate topological data with functional data, “functional hubs” were defined. These were identified by calculating the frequency of

occurrence of individual genes in statistically significant signaling pathways (KEGG/GO). This approach allowed for the identification of proteins with the highest pleiotropic potential in the process of cardiac fibrosis.

Results

Gene selection and functional classification

Based on a systematic review of the scientific literature in the PubMed database, a set of genes was selected whose involvement in the fibroblast-to-myofibroblast transition and in the development of myocardial fibrosis has been experimentally confirmed.

The selected genes were classified into four main functional groups reflecting the key mechanisms regulating the FMT process. The first group consisted of genes associated with cardiac fibroblast activation and cytoskeletal reorganization, including *ACTA2*, *TAGLN*, *VIM*, *POSTN*, *FAP*, *S100A4*, *DDR2*, *PDGFRA*, *PDGFRB* and *THY1*. The second group consisted of genes responsi-

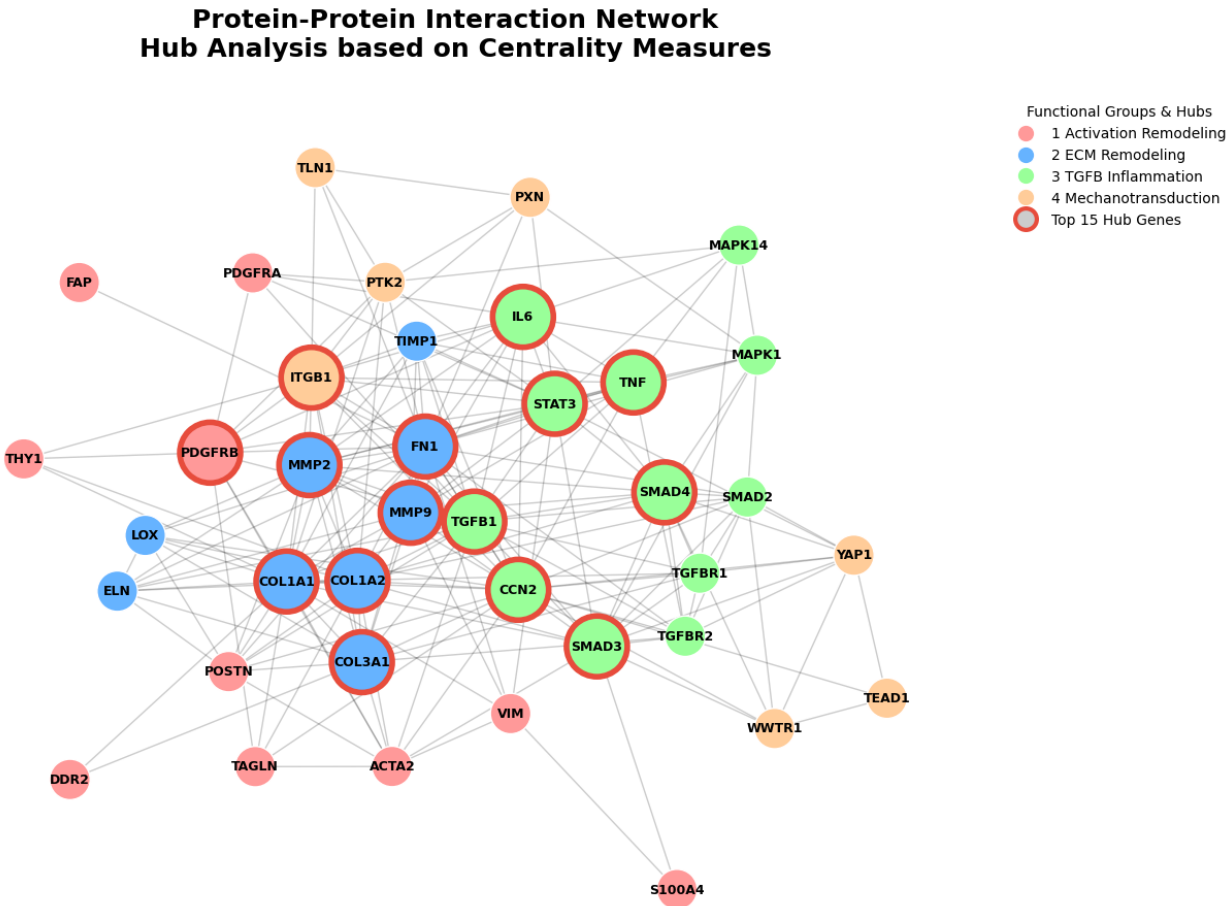


FIGURE 1 A network of protein-protein interactions involving genes associated with cardiac fibroblast activation, the fibroblast-to-myofibroblast transition and extracellular matrix remodeling in the process of myocardial fibrosis. Nodes represent proteins, while edges denote confirmed or predicted functional interactions between them. The colors of the nodes correspond to four functional groups: (1) fibroblast activation and cytoskeletal reorganization, (2) extracellular matrix remodeling, (3) TGF-β signaling and inflammatory regulation, and (4) mechanotransduction and the Hippo/YAP-TAZ pathway. The central position of the proteins TGFβ1, FN1, COL1A1, ACTA2 and SMAD3 indicates their key role in the regulation of FMT and the development of cardiac fibrosis. The 15 genes with the highest centrality, identified as the main regulatory nodes of the network, are highlighted with a red border. Network generated using the STRING database

ble for extracellular matrix remodeling and the fibrogenic response, including *COL1A1*, *COL1A2*, *COL3A1*, *FN1*, *ELN*, *LOX*, *TIMP1*, *MMP2* and *MMP9*. The third group included components of the TGF- β pathway and genes associated with the regulation of the inflammatory response, such as *TGFB1*, *TGFBR1*, *TGFBR2*, *SMAD2*, *SMAD3*, *SMAD4*, *CTGF* (*CCN2*), *IL6*, *TNF*, *STAT3*, *MAPK1* and *MAPK14*. The fourth group included genes associated with mechanotransduction and Hippo/YAP-TAZ signaling, including *YAP1*, *WWTR1*, *TEAD1*, *ITGB1*, *TLN1*, *PXN* and *PTK2*. It is worth noting that mechanotransduction is a process that plays a key role in the development of myocardial fibrosis, as it enables fibroblasts to detect and convert mechanical stimuli such as increased stretching, pressure or tissue stiffness into molecular signals that regulate gene expression. In pathological conditions, such as hypertension, myocardial infarction or heart failure, there is an increase in the mechanical load on the heart wall, which leads to the activation of fibroblasts [15].

The final set of genes was used to construct a protein-protein interaction network in the

STRING database and for further topological analysis aimed at identifying key regulatory nodes involved in the transition of cardiac fibroblasts into myofibroblasts and the development of myocardial fibrosis. The functional classification was performed based on data from the literature and annotations from the Gene Ontology and KEGG databases.

Identification of key regulatory nodes in the transition of cardiac fibroblasts into myofibroblasts

An analysis of the protein-protein interaction network conducted using the STRING database revealed the existence of a tightly interconnected molecular network comprising genes involved in cardiac fibroblast activation, the transition of fibroblasts into myofibroblasts and extracellular matrix remodeling. Based on topological network analysis and an assessment of centrality measures, 15 genes of the highest biological significance were identified and considered to be the main hub genes (Fig. 1).

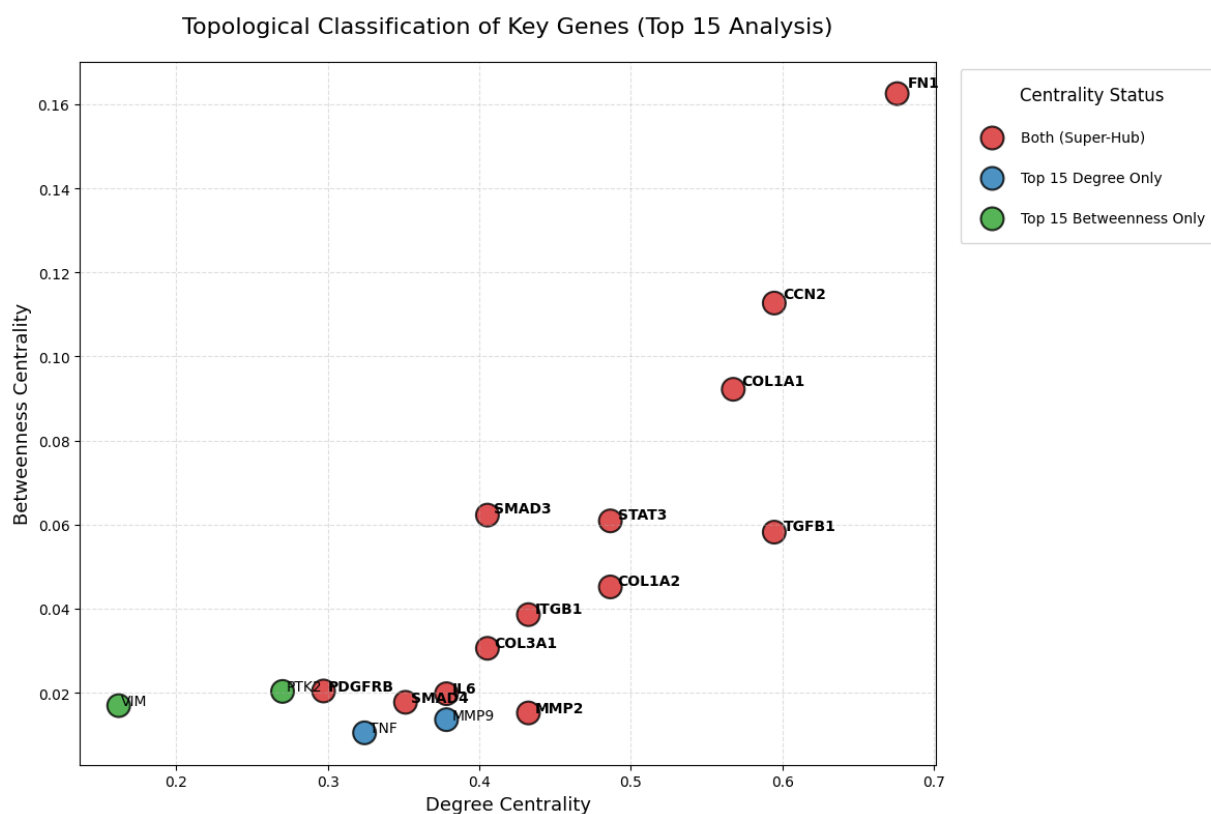


FIGURE 2 Topological classification of 15 key genes identified based on an analysis of degree centrality and betweenness centrality in the protein-protein interaction network. The X-axis represents degree centrality, reflecting the number of direct interactions of a given protein, while the Y-axis represents betweenness centrality, determining the protein's role in transmitting information between different functional modules of the network. Genes whose protein products were characterized by both a high number of connections and a high ability to control the flow of information in the network (so-called super-hubs) are marked in red. Genes with high degree centrality, functioning as classic hubs with numerous direct interactions, are marked in blue. Genes with high betweenness centrality, which despite having fewer connections play a significant role in maintaining the stability and integrity of the network, are marked in green. The graph was created using the NetworkX library

The most important nodes in the network included the following genes: *TGFB1*, *FN1*, *COL1A1*, *COL1A2*, *COL3A1*, *MMP2*, *MMP9*, *ITGB1*, *SMAD3*, *SMAD4*, *STAT3*, *IL6*, *TNF*, *CCN2* and *PDGFRB*. They were characterized by a high degree of connectivity with other network elements and formed a functional hub integrating signals responsible for fibroblast activation, cytoskeletal reorganization, regulation of the inflammatory response and extracellular matrix remodeling.

FN1, *TGFB1*, *CCN2*, *SMAD3* and *STAT3* occupied particularly significant positions, exhibiting high centrality and numerous connections with members of various functional groups. This indicates their role as key regulators coordinating communication between the TGF- β pathway, inflammatory processes and ECM remodeling. Genes encoding the main components of the extracellular matrix (*COL1A1*, *COL1A2* and *COL3A1*) also occupied prominent positions in the network, underscoring the importance of collagen synthesis in the development of myocardial fibrosis.

Among the hub genes, *IL6*, *TNF* and *STAT3* were also identified representing the inflammatory component of the network. The presence of these genes in the central part of the interaction network indicates a close link between inflammatory processes and fibroblast activation and fibrosis progression.

In turn, the presence of *ITGB1* and *PDGFRB* among the hub genes underscores the crucial role of mechanotransduction and receptor signaling in the regulation of FMT.

MMP2 and *MMP9* are also noteworthy, because despite their numerous connections to other network components, they primarily function as regulators of extracellular matrix remodeling. This suggests that the fibrotic process results not only from increased synthesis of ECM components but also from changes in the mechanisms governing their degradation and reorganization.

The results indicate that the transition of cardiac fibroblasts into myofibroblasts is a process regulated by an integrated network of molecular interactions in which the following proteins play a central role: *TGFB1*, *SMAD3/4*, *FN1*, *COL1A1* and *ACTA2*. These proteins constitute potential regulatory nodes and may be considered as important therapeutic targets for limiting myocardial fibrosis.

Identification of bottleneck genes based on betweenness centrality analysis

An analysis of betweenness centrality identified bottleneck genes whose protein products play a key role in maintaining the global cohesion of the protein-protein interaction

network (**Fig. 2**). These proteins act as connectors between distinct functional modules, enabling the integration of signals related to fibroblast activation, extracellular matrix remodeling, the inflammatory response and mechanotransduction.

FN1 exhibited the highest value for betweenness centrality, indicating its pivotal role in coordinating interactions between extracellular matrix structural proteins and signaling pathway regulators. High values of this parameter were also observed for *CCN2*, *COL1A1*, *TGFB1*, *STAT3* and *SMAD3*, confirming their importance as key mediators of profibrotic signaling. *COL1A2*, *ITGB1* and *COL3A1* also held significant positions, linking the processes of extracellular matrix synthesis and organization with mechanotransduction.

Most genes with high mediation centrality also belonged to the group of genes with the highest degree centrality, indicating their dual role as both local and global network regulators. This category included, among others, *FN1*, *TGFB1*, *SMAD3*, *STAT3*, *CCN2* and genes encoding type I and III collagens.

An interesting observation concerned the *TNF* and *MMP9* genes, which were characterized by relatively high degree centrality, but low betweenness centrality. This means that their protein products interact with many elements of the network but do not play a dominant role in transmitting information between individual functional modules. This result suggests that *TNF* and *MMP9* are primarily involved in the local regulation of inflammatory processes and extracellular matrix remodeling, rather than in the integration of signals between the main pathways involved in the transition of cardiac fibroblasts into myofibroblasts.

Genes such as *VIM* and *PTK2* were also identified as genes exhibited high mediating centrality despite having a relatively small number of direct connections, suggesting a specialized role in signal transduction between individual functional modules. This means that the proteins encoded by these genes do not interact with a very large number of partners, but rather link key signaling pathways together, acting as “bridges” in the network. Due to their position at the intersection of mechanotransduction, cytoskeletal remodeling and profibrotic signaling, even with fewer direct interactions, these proteins play a crucial role in maintaining the integrity and functional cohesion of the entire network.

The results indicate that bottleneck proteins play a significant role in the integration of signaling pathways responsible for the transition of cardiac fibroblasts into myofibroblasts. Of

particular importance are FN1, TGFB1, CCN2, SMAD3, and STAT3, which may serve as key molecular regulators and potential therapeutic targets for limiting myocardial fibrosis.

Analysis of functional enrichment and identification of key biological processes
TGF-β signaling and biological processes associated with FMT

A Gene Ontology (GO) enrichment analysis was performed to determine which biological processes are most significantly represented among the key genes identified in the protein-protein interaction network. The figure shows the 10 biological processes with the highest

statistical significance (**Fig. 3**). The identified processes primarily include TGF-β signaling, extracellular matrix organization, regulation of cell proliferation and mechanisms associated with cellular phenotypic changes, such as the epithelial-mesenchymal transition. These results indicate that the transition of cardiac fibroblasts into myofibroblasts is controlled by an integrated network of processes responsible for cell activation, response to profibrotic stimuli and connective tissue remodeling.

The most significant biological process identified in the GO enrichment analysis was the Transforming Growth Factor Beta Receptor Signaling Pathway, which includes the following

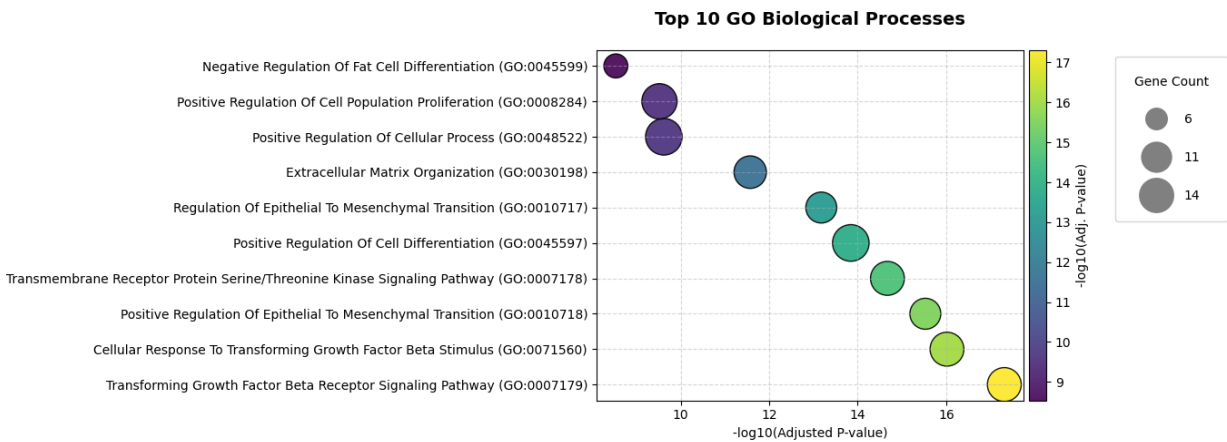


FIGURE 3 Gene Ontology (GO) enrichment analysis of key genes associated with the transition of cardiac fibroblasts into myofibroblasts and cardiac fibrosis. The figure shows the 10 biological processes with the highest statistical significance. The X-axis represents the $-\log_{10}$ value of the adjusted p-value, while the size of the points corresponds to the number of genes assigned to a given biological process. The color of the points reflects the level of enrichment significance. The graph was generated based on a functional enrichment analysis performed for the genes selected for PPI network analysis using the GSEApY library and the Enrichr platform

TABLE 1 Gene Ontology (GO) Biological Process enrichment analysis of the identified gene set. The top three significantly enriched terms from the GO Biological Process 2023 database are shown, ranked by adjusted p-value (Adj. P-value). Overlap indicates the ratio of query genes to the total genes in the GO term. Odds Ratio and Combined Score reflect the magnitude and statistical strength of the enrichment, respectively. Overlapping genes for each functional category are listed below each term

Gene set	Term	Overlap	Adj. P-value	Odds Ratio	Combined Score
GO_Biological_Process_2023	Transforming Growth Factor Beta Receptor Signaling Pathway (GO:0007179)	12/72	4.86699e-18	153.09	7171.19
		Genes	ITGB1;SMAD2; SMAD4; COL3A1; TGFB1; SMAD3; COL1A2; PXN; STAT3; TGFB1; PTK2; TGFB2		
	Cellular Response To Transforming Growth Factor Beta Stimulus (GO:0071560)	12/96	9.61405e-17	109.22	4714.56
		Genes	ITGB1; SMAD2; SMAD4; COL3A1; TGFB1; SMAD3; COL1A2; PXN; STAT3; TGFB1; PTK2; TGFB2		
	Positive Regulation Of Epithelial To Mesenchymal Transition (GO:0010718)	10/47	2.96385e-16	192.33	8007.40
		Genes	SMAD2; COL1A1; WWTR1; SMAD4; IL6; TGFB1; SMAD3; TGFB1; PTK2; TGFB2		

genes: *ITGB1*, *SMAD2*, *SMAD4*, *COL3A1*, *TGFB1*, *SMAD3*, *COL1A2*, *PXN*, *STAT3*, *TGFBR1*, *PTK2* and *TGFBR2* (**Tab. 1**). This process reflects the key signaling pathway responsible for the activation of cardiac fibroblasts and their differentiation into myofibroblasts. Upon binding of the *TGFB1* ligand to the *TGFBR1* and *TGFBR2* receptors, *SMAD2* and *SMAD3* proteins are phosphorylated, which then form a complex with *SMAD4* and translocate to the cell nucleus, where they regulate the expression of genes associated with extracellular matrix synthesis and the acquisition of a contractile phenotype.

The presence of the *COL1A2* and *COL3A1* genes indicates that activation of this pathway directly leads to increased production of type I and III collagen, which are the main components of the fibrous extracellular matrix. In turn, *ITGB1*, *PXN* and *PTK2* (FAK) link TGF- β signaling with mechanotransduction, enabling the integration of mechanical and biochemical stimuli. The involvement of *STAT3* suggests additional interaction between inflammatory and profibrotic pathways, enhancing fibroblast activation. These results confirm that the TGF- β receptor pathway constitutes a central molecular mechanism regulating the transition of cardiac fibroblasts into myofibroblasts and the development of myocardial fibrosis.

Another significantly enriched process was Cellular Response to Transforming Growth Factor Beta Stimulus which included the genes *ITGB1*, *SMAD2*, *SMAD4*, *COL3A1*, *TGFB1*, *SMAD3*, *COL1A2*, *PXN*, *STAT3*, *TGFBR1*, *PTK2* and *TGFBR2* (**Tab. 1**). This process reflects changes occurring in the cell in response to TGF- β stimulation and involves the activation of pathways regulating cell proliferation, migration and differentiation. The presence of genes encoding TGF- β receptors (*TGFBR1*, *TGFBR2*), signaling proteins (*SMAD2*, *SMAD3*, *SMAD4*) and extracellular matrix components (*COL1A2*, *COL3A1*) indicates a close association of this process with fibroblast activation and ECM remodeling. Additionally, the involvement of the *ITGB1*, *PXN* and *PTK2* genes suggests that the response to TGF- β interacts with mechanotransduction mechanisms.

Another significantly enriched process was Positive Regulation of Epithelial-Mesenchymal Transition which includes the genes *SMAD2*, *SMAD3*, *SMAD4*, *TGFB1*, *TGFBR1*, *TGFBR2*, *IL6*, *PTK2*, *WWTR1* and *COL1A1* (**Tab. 1**). Although the analyzed cells are of mesenchymal origin, this process indicates the activation of molecular pathways responsible for phenotypic changes leading to increased migration, contractility and extracellular matrix production,

which are also characteristic of the differentiation of fibroblasts into myofibroblasts.

The *TGFB1*-*TGFBR1*/*TGFBR2*-*SMAD2/3/4* axis plays a central role in this process, serving as the primary mechanism that induces phenotypic changes in cells. Activation of this pathway leads to cytoskeletal remodeling, increased expression of genes associated with cell adhesion, and enhanced synthesis of extracellular matrix components. The presence of the *COL1A1* gene further indicates a close link between this process and collagen deposition and the development of fibrosis.

Functional analysis revealed the involvement of *WWTR1* (TAZ) and *PTK2* in processes related to mechanotransduction, the organization of cell adhesion and the response to mechanical stimuli. These genes were associated with the regulation of fibroblast activation and extracellular matrix remodeling, suggesting their potential role in the transition of cardiac fibroblasts into myofibroblasts.

Taken together, these results indicate that the regulation of EMT in this network reflects the activation of a molecular program responsible for the acquisition of myofibroblast characteristics by fibroblasts, involving the interaction of TGF- β signaling, mechanotransduction and the inflammatory response. These mechanisms lead to increased collagen production, extracellular matrix remodeling and the progression of myocardial fibrosis.

Although the TGF- β signaling pathway and processes related to EMT regulation showed the highest enrichment, the other identified biological processes also participate in the regulation of FMT and myocardial fibrosis. The results obtained highlight the multifactorial nature of this process and the close interconnection of profibrotic, inflammatory and mechanotransduction mechanisms.

Analysis of KEGG pathways for key regulators of the FMT network

An enrichment analysis of KEGG pathways revealed that the key genes identified in the PPI network are involved in numerous biological processes associated with myocardial fibrosis, including profibrotic signaling, the inflammatory response, mechanotransduction, cellular stress regulation and extracellular matrix remodeling. The top 10 most significant pathways included, among others, the AGE-RAGE signaling pathway in diabetic complications, the Hippo signaling pathway, the FoxO signaling pathway, the Relaxin signaling pathway, Diabetic cardiomyopathy and Th17 cell differentiation (**Fig. 4**).

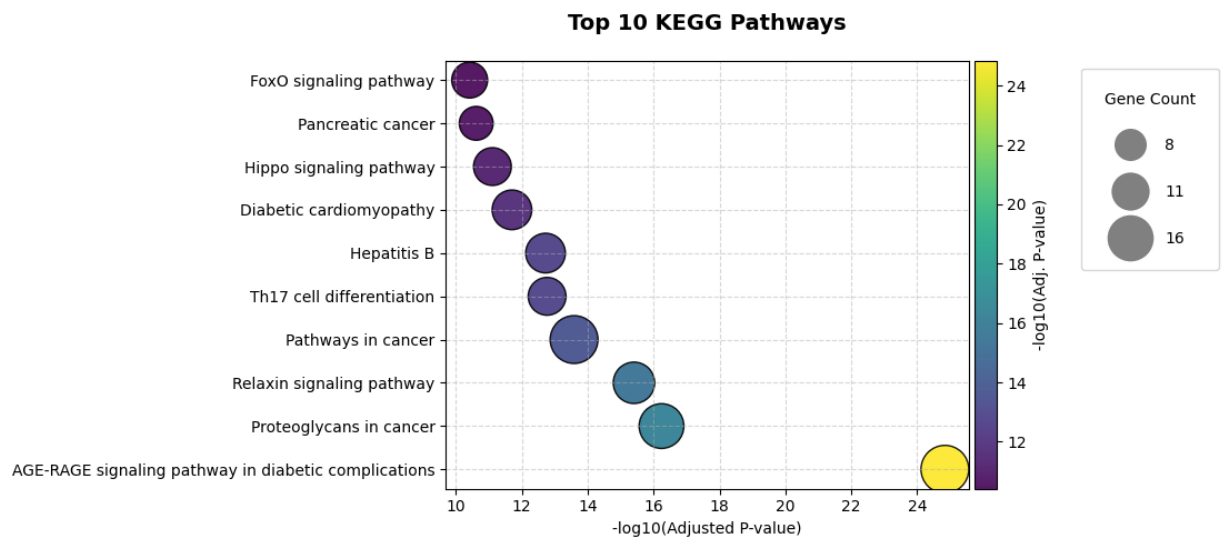


FIGURE 4 Analysis of KEGG pathway enrichment for key genes identified in the protein–protein interaction network associated with the transition of cardiac fibroblasts into myofibroblasts and cardiac fibrosis. The X-axis represents the value of $-\log_{10}(\text{adjusted p-value})$, reflecting the statistical significance of the enrichment, while the size of the points corresponds to the number of genes involved in a given pathway. The color of the points indicates the level of statistical significance. The plot was generated based on a functional enrichment analysis performed for genes selected for PPI network analysis using the GSEApY library and the Enrich platform

TABLE 2 KEGG pathway enrichment analysis of the identified gene set. The table displays the highly significant pathway from the KEGG 2021 Human database. Overlap indicates the number of query genes relative to the total number of genes annotated within the pathway. Odds Ratio and Combined Score quantify the strength and statistical magnitude of the enrichment, with contributing genes listed below

Gene set	Term	Overlap	Adj. P-value	Odds Ratio	Combined Score
KEGG_2021_Human	AGE-RAGE signaling pathway in diabetic compli-cations	16/100	1.44222e-25	172.10	10721.78
	Genes	SMAD2; SMAD4; TGFB1; SMAD3; MMP2; STAT3; FN1; MAPK14; TNF; TGFB1; TGFB2; COL1A1; COL3A1; IL6; COL1A2; MAPK1			

The AGE-RAGE signaling pathway in diabetic complications deserves special attention, as it exhibited by far the highest level of statistical significance, clearly surpassing the other pathways. This finding suggests a potentially dominant role for metabolic disorders in the activation of cardiac fibroblasts and the development of myocardial fibrosis.

The AGE-RAGE pathway under analysis involves a number of genes that can be divided into several functional groups (**Tab. 2**). *TGFB1* plays a central role, along with the *TGFB1* and *TGFB2* receptors and the *SMAD2*, *SMAD3*, and *SMAD4* proteins, forming the main profibrotic pathway responsible for fibroblast activation and the induction of gene expression encoding collagen and other components of the extracellular matrix. *SMAD3* is particularly important, as it directly regulates the transcription of genes associated with fibrosis, such as *COL1A1*, *COL1A2*, *COL3A1* and *FN1*.

The cytokines IL-6 and TNF, as well as the transcription factor STAT3, also play a significant role in maintaining chronic inflammation and enhancing profibrotic signaling.

MAPK1 (ERK2) and MAPK14 (p38 MAPK) also play a significant role in the AGE-RAGE pathway, transmitting signals related to oxidative stress and inflammatory stimuli. Their activation leads to increased expression of profibrotic genes, enhanced fibroblast proliferation and differentiation into myofibroblasts. MAPK14 plays a particularly important role in the response to chronic hyperglycemia and cellular stress.

The *FN1*, *COL1A1*, *COL1A2* and *COL3A1* genes encode the main components of the extracellular matrix, whose overexpression leads to increased myocardial stiffness. *MMP2*, on the other hand, participates in the degradation and remodeling of the matrix, regulating the balance between the deposition and removal of its

components. A disruption of this balance promotes the progression of fibrosis.

Collectively, the genes involved in the AGE-RAGE pathway integrate metabolic, inflammatory and mechanical signals, leading to fibroblast activation, excessive extracellular matrix production and the progression of myocardial fibrosis. Of particular importance are *TGFB1*, *SMAD3*, *STAT3*, *MAPK14* and *FN1*, which act as central regulators of this process.

Discussion

This study analyzed the protein-protein interaction network of genes associated with the transition of cardiac fibroblasts into myofibroblasts and the development of myocardial fibrosis. The results indicated that genes associated with TGF- β signaling, extracellular matrix remodeling, the inflammatory response and mechanotransduction play a central role in the studied network.

Network topology analysis revealed several hub genes with high centrality, including *TGFB1*, *FN1*, *COL1A1*, *COL1A2*, *COL3A1*, *SMAD3*, *STAT3* and *CCN2*, suggesting their pivotal role in fibroblast activation and fibrotic progression. Among these, TGF- β plays a particularly important role, as it regulates extracellular matrix remodeling, influences cell migration and modulates the immune response. This factor also participates in controlling cell proliferation and differentiation, which promotes the acquisition of myofibroblast characteristics by fibroblasts. The results of the analysis are also significant in the context of diabetes, in which chronic inflammation and increased activity of the TGF- β pathway promote fibroblast activation and the development of myocardial fibrosis. The presence of genes associated with TGF- β signaling, the inflammatory response and extracellular matrix remodeling confirms their potential role in the pathogenesis of diabetic cardiomyopathy [16].

A prominent feature of the network was the enrichment of genes associated with ECM organization, particularly collagens (*COL1A1*, *COL1A2*, *COL3A1*) and fibronectin (*FN1*). These molecules are essential structural components of the cardiac extracellular matrix. Their overexpression contributes to excessive collagen deposition, resulting in increased myocardial stiffness and impaired mechanical and electrical function of the heart.

FN1, a key ECM glycoprotein, facilitates interactions between collagen, integrins, and proteoglycans, thereby regulating cell adhesion, migration and signal transduction. Its central position in the network underscores its dual structural and signaling roles in fibrotic remodeling [17].

Inflammatory mediators, particularly IL6, TNF and STAT3, were also identified as key nodes within the network, underscoring the contribution of chronic inflammation to fibroblast activation and fibrosis progression. IL-6 and TNF act as upstream regulators that promote fibroblast proliferation and enhance ECM gene expression while also potentiating TGF- β signaling. STAT3, as a major downstream effector of IL-6 signaling, regulates genes involved in cell survival, proliferation and collagen synthesis. Together, these pathways establish a sustained pro-inflammatory and profibrotic feedback loop that amplifies myocardial remodeling.

Meléndez GC et al. report that elevated levels of circulating IL-6 in patients correlate with the severity of heart failure and are predictors of mortality [18]. Therefore, the cytokines IL-6 and TNF, as well as the transcription factors STAT3 and SMAD3/SMAD4, played a significant central role, indicating a close link between inflammatory signaling and the activation of profibrotic pathways. IL-6 and TNF are key mediators of the inflammatory response that stimulate fibroblast proliferation, enhance the expression of genes associated with extracellular matrix remodeling and potentiate the action of TGF- β . STAT3 acts as the primary effector of IL-6 signaling, regulating the expression of genes responsible for cell survival, proliferation and collagen production [19].

Genes associated with mechanotransduction, including *ITGB1*, *PTK2* and *WWTR1* (*TAZ*), further highlight the importance of biomechanical signaling in fibroblast-to-myofibroblast transition. TAZ, a key effector of the Hippo pathway, integrates mechanical cues from the extracellular environment and regulates the expression of profibrotic genes [20].

PTK2 (focal adhesion kinase) mediates signal transduction from integrins and focal adhesions, controlling cytoskeletal organization, cell migration and proliferation. These findings suggest that increased myocardial stiffness not only results from fibrosis but also actively promotes its progression through a positive feedback loop driven by mechanotransduction [21].

These findings suggest that mechanical signals generated by increased stress on the heart wall may play a role just as significant as that of classical cytokines and growth factors. Increased tissue stiffness leads to further activation of fibroblasts, creating a positive feedback loop that promotes the progression of fibrosis.

The identified pathways converge in the context of diabetic cardiomyopathy, where chronic hyperglycemia induces non-enzymatic glycation of ECM proteins, leading to the formation of advanced glycation end products (AGEs).

AGE accumulation in myocardial tissue activates the RAGE receptor on cardiac fibroblasts, cardiomyocytes and endothelial cells, triggering oxidative stress and downstream MAPK and JAK/STAT signaling pathways.

This signaling cascade enhances inflammatory responses and promotes fibroblast proliferation while stimulating TGF- β secretion and ECM deposition. Consequently, persistent activation of the AGE–RAGE axis contributes to interstitial fibrosis, increased myocardial stiffness and impaired cardiac function. These processes are further associated with the development of heart failure and arrhythmias [16,22–24].

Conclusions

In summary, this study demonstrates that cardiac fibroblast-to-myofibroblast transition is governed by an interconnected network involving TGF- β signaling, inflammatory pathways, ECM remodeling and mechanotransduction. The identified hub genes represent key regulatory nodes that integrate these processes and may serve as potential therapeutic targets for future strategies aimed at mitigating myocardial fibrosis, particularly in the context of diabetic cardiomyopathy.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest

The authors declare they have no conflict of interest.

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