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## Cadherin-11 and Cardiac Fibrosis: A Common Target for a Common Pathology

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### Abstract

Cardiac fibrosis represents an enormous health concern as it is prevalent in nearly every form of cardiovascular disease, the leading cause of death worldwide. Fibrosis is characterized by the activation of fibroblasts into myofibroblasts, a contractile cell type that secretes significant amounts of extracellular matrix components; however, the onset of this condition is also due to persistent inflammation and the cellular responses to a changing mechanical environment. In this review, we provide an overview of the pro-fibrotic, pro-inflammatory, and biomechanical mechanisms that lead to cardiac fibrosis in cardiovascular diseases. We then discuss cadherin-11, an intercellular adhesion protein present on both myofibroblasts and inflammatory cells, as a potential link for all three of the fibrotic mechanisms. Since experimentally blocking cadherin-11 dimerization prevents fibrotic diseases including cardiac fibrosis, understanding how this protein can be targeted for therapeutic use could lead to better treatments for patients with heart disease.

### Keywords

Cadherin-11; Cardiac Fibrosis; Myofibroblast; Extracellular Matrix; Inflammation

### I. Introduction

The cardiac extracellular matrix (ECM) is the structural scaffold in which cardiomyocytes, fibroblasts, inflammatory cells, and endothelial cells (among others) reside [1]. This fibrillar network is composed of a complex arrangement of structural and signaling proteins that regulate, in part, the biochemical and biomechanical functions of the cardiac tissue. Further, through cell-matrix interactions, the ECM plays a critical role in force transmission such that the contractile force of a single cardiomyocyte is transmitted throughout the entire heart.

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Fibrosis, the excessive deposition of ECM components, occurs in response to tissue injury or stress and is a well-recognized cause of morbidity and mortality [2]. In response to cardiac injury (e.g., myocardial infarction) or chronic stress (e.g., hypertension, atherosclerosis, and aortic stenosis), fibrotic remodeling of the ECM provides the necessary structural components and growth factors needed for healthy cardiac tissue to regrow [3]. Prolonged and/or unrestrained fibrosis can lead to subdued regeneration and eventual loss of tissue function through a variety of mechanisms. In the heart, excessive fibrotic remodeling can disrupt excitation-contraction coupling increasing the risk of arrhythmias and increase tissue stiffness leading to systolic, diastolic, and valvular dysfunction [4–8]. However, if insufficient fibrotic remodeling occurs, defective scar tissue may form resulting in ventricular dilation and, in the most adverse instances, tissue rupture [9,10]. Given that alterations in the ECM alter the active and passive mechanical properties of the heart tissue as well as the cellular signaling that occurs within the tissue, a thorough understanding of the biochemical and biomechanical underpinnings of cardiac fibrosis is necessary in order to find cardiovascular disease therapeutics.

In this review, we broadly consider the biochemical and biomechanical mechanisms that lead to cardiac fibrosis in cardiovascular diseases by discussing the cells involved in the fibrotic response. We then focus on the importance of cadherin-11, an intercellular adhesion protein that is required for myofibroblast activation and implicated in inflammatory remodeling of cardiac tissue. We propose that cadherin-11 is a therapeutic target for treating cardiac fibrosis, as it represents a common feature of the biochemical and biomechanical underpinnings of fibrosis.

## II. Biochemical Causes of Cardiac Fibrosis

In healthy cardiac tissue, resident fibroblasts maintain the ECM through the synthesis of ECM proteins (e.g., fibrillar collagens I and III, non-fibrillar collagens, elastin, and laminin) as well as their degradation through production of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) (Figure 1) [11]. The balance of this synthesis and degradation allows for ECM homeostasis. Recent work has shown that resident cardiac fibroblasts are not a homogenous group of cells but rather two fibroblast sub-populations defined by the high (F-SH) or low (F-SL) expression of *Scal* [12]. Both of these resident populations express canonical fibroblast markers, however, F-SH cells have a distinct cell adhesion phenotype while F-SL cells exhibit a secretory phenotype. F-SH fibroblasts seem to preferentially differentiate into activated fibroblasts and myofibroblasts while F-SL fibroblasts preferentially differentiate into the newly described *Wif1*-expressing fibroblasts that act to inhibit fibrosis through expression of Wnt and TGF- $\beta$  signaling inhibitors. This suggests that a balance of these resident fibroblast populations may also be needed to maintain a healthy cardiac ECM, though more work is needed to describe the function of these novel cell types.

Cardiac tissue injury during myocardial infarction [13,14], aortic valve disease [15], hypertension [16], and atherosclerosis [17] results in injured and necrotizing cells releasing damage-associated molecular patterns (DAMPs) as the first stage of the healing process. Leukocytes sense this damage and produce pro-inflammatory cytokines leading to

inflammatory cell recruitment to the site of damage [18]. The resultant increase in inflammatory cells leads to clearance of the damaged tissue and the release of pro-fibrotic signals that increase proliferation, recruitment, and activation of fibroblasts into secretory, contractile myofibroblasts. Myofibroblasts are required for the injury response since collagen fibrillogenesis and scar formation are necessary to stabilize the tissue architecture due to the limited regenerative capacity of cardiac tissues [11,19–21]. In healthy repair, clearance of inflammatory cells occurs after the heart's structure is stabilized leaving behind a stable fibrotic scar and healthy cardiomyocytes. In most tissues, myofibroblasts are also cleared at this point; however, a significant number of quiescent myofibroblasts in the heart are found long after a stable scar has formed (>60 days post myocardial infarction) [22]. Recent lineage tracing studies have shown that these cells are matrifibrocytes, differentiated myofibroblast-lineage cells that express bone-cartilage markers, lose their proliferative ability, and no longer express  $\alpha$ -SMA [23]. While these cells were described in the context of post-myocardial infarct fibrosis, matrifibrocytes have also been shown to be similar to fibroblast sub-populations in Ang-II induced cardiac fibrosis [24]. Thus, these cells potentially arise following multiple types of cardiac injury to stabilize fibrotic scar, but more work is needed to understand the role of these cells in this broader context. If clearance of inflammatory cells and differentiation of myofibroblasts into matrifibrocytes does not occur, feedforward signaling begins whereby inflammatory cells and myofibroblasts continue to promote fibrosis through chronically activated proinflammatory and profibrotic signaling pathways.

### Inflammatory Signaling in Cardiac Fibrosis

Cardiovascular diseases such as heart failure, aortic valve disease, and atherosclerosis are often initiated by acute inflammatory reactions caused by stressed and necrotic cardiomyocytes as a protective response to infection, injuries such as myocardial infarction, or stress conditions such as hypertension [16,25,26]. As mentioned previously, stressed cardiac cells release DAMPs such as heat-shock proteins, degraded ECM molecules, and DNA fragments. These signals are recognized by toll-like receptors (TLRs) 2 and 4 in the damaged cell as well as in surrounding, healthy cells promoting the production of proinflammatory cytokines (i.e., TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , and IL-6) and fibroblast growth factors through activation of NF- $\kappa$ B and AP-1 signaling cascades [27,28]. The first cells to respond to these signals are neutrophils that are recruited to the injured site to engulf damaged cells and tissue debris. Neutrophils also release proinflammatory cytokines, MMPs, reactive oxygen species, and serine proteases leading to both the remodeling of the ECM and further recruitment of inflammatory cells [29]. While this clearance is initially beneficial for the tissue, the neutrophil-mediated response lacks specificity and can result in further damage to the healthy cardiac tissue.

Macrophages that reside within the myocardium and those that are recruited via TLR signaling are the next cells to arrive at the injury site [30]. These cells initially take on an M1-like phenotype due to activation of IFN- $\gamma$  and TNF- $\alpha$  signaling pathways activated following cardiac injury and secrete large amounts of pro-inflammatory mediators and MMPs [18]. M1-like macrophages respond to the localized upregulation and secretion of MCP-1/CCL2 through their expression of C-C chemokine receptor 2 (CCR2) and infiltrate

the damaged tissue [31]. Once at the injury site, M1-like macrophages promote further increases to the inflammatory response by producing proinflammatory cytokines (e.g., CCL2, IL-1 $\beta$ , TNF- $\alpha$ , IL-23, IL-12) that both recruit other inflammatory cells and promote a proinflammatory phenotype in cardiomyocytes and resident fibroblasts [32]. Macrophages display marked heterogeneity and change their phenotypes based on their microenvironment [30,33,34]. Thus, during the reparative phase of the fibrotic response, macrophages can take on an anti-inflammatory, M2-like phenotype and lead to inflammatory resolution [34]. These cells secrete IL-10, arginase, and TGF- $\beta$ , cytokines that act to inhibit M1 macrophage polarization and Th<sub>1</sub> lymphocyte activity. M2-like macrophages also produce a variety of pro-fibrotic cytokines, MMPs, and TIMPs that will be discussed in more depth in a later section. The complexity of microenvironments can result in the generation of multiple subpopulations of macrophages leading to a complex balance pro- and anti-inflammatory signaling.

During subsequent stages of cardiac inflammation, T lymphocytes are recruited to the injury site and differentiated by IL-12 into Th<sub>1</sub> cells, IL-6 and TGF- $\beta$  into Th<sub>17</sub> cells, TGF- $\beta$  alone into T<sub>reg</sub> cells, and IL-4, IL-25, and IL-33 into Th<sub>2</sub> cells [35]. T lymphocytes can help to sustain and promote (Th<sub>1</sub> and Th<sub>17</sub> cells) or resolve (T<sub>reg</sub> and Th<sub>2</sub> cells) inflammation [36]. Th<sub>1</sub> cells secrete IFN- $\gamma$ , TNF- $\alpha$ , and IL-2, cytokines that lead to positive feedback to maintain the proinflammatory phenotypes of M1-like macrophages, neutrophils, cardiomyocytes, and fibroblasts. Th<sub>17</sub> cells produce the cytokine IL-17A, which is associated with increased and sustained neutrophil activation [37]. These pro-inflammatory T-cells are counteracted as T<sub>reg</sub> and Th<sub>2</sub> cells are recruited. These anti-inflammatory T-cells secrete TGF- $\beta$ , IL-4, and IL-13, pro-fibrotic cytokines that inhibit Th<sub>1</sub> and Th<sub>17</sub> cell differentiation and promote polarization of macrophages toward an M2-like phenotype [38]. The recruitment of these anti-inflammatory cells is essential to resolving the inflammatory cascade and preventing further fibrosis. For a more in-depth review of inflammatory signaling in cardiac fibrosis, please refer to Smolgovsky et al [39].

### Fibrotic Signaling in Cardiac Fibrosis

In addition to their roles regulating the inflammatory status of injured cardiac tissues, each of these cell types signal to fibroblasts to migrate to the site of injury and transdifferentiate into secretory, contractile cells termed myofibroblasts that become the primary drivers of cardiac fibrosis. Myofibroblasts are defined by their expression of contractile stress fibers, expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), and secretion of an abundance of ECM components [20]. These cells, though predominantly from resident, interstitial fibroblasts, originate from a variety of sources including circulating fibrocytes, endothelial cells, epithelial cells, and pericytes [40–42]. Regardless of their origin, the differentiation of cells into myofibroblasts in the injured heart requires several key factors [20]. First, TGF- $\beta$  signaling through the Smad2/3 signaling cascades promotes  $\alpha$ -SMA transcription. Second, expression of cell surface receptors that can promote growth factor signaling cascades leads to increased myofibroblast activation. Next, alterations to the mechanical properties and composition of the ECM mediates differentiation of fibroblasts into myofibroblasts by modulating their responses to mechanical and growth factor signaling. Last, direct mechanical stimulation of myofibroblasts results in the maintenance of the myofibroblast

phenotype through RhoA signaling pathways. The first two of these requirements originate from signals from the cell types previously mentioned as they take on a second, pro-fibrotic role.

While initially recruited to the injury site to remove damaged cells and cellular debris, neutrophils directly and indirectly promote fibrosis. As previously mentioned, neutrophils release MMPs, elastase, and cathepsins that initially help to breakdown and reorganize the ECM so it can be replaced [29]. This breakdown can release latent fibroblast growth factors sequestered in the ECM and directly activate fibrotic pathways. Further, neutrophils release large amounts of reactive oxygen species through NADPH oxidase in a process referred to as the respiratory burst [43]. Reactive oxygen species directly aid in myofibroblast differentiation through the Smad2/3, MAPK, JNK, and Src pathways to activate signaling intermediates within proto-myofibroblasts, myofibroblasts who have formed stress fibers but do not yet express  $\alpha$ -SMA [44]. All of these signaling cascades induce the transcription of target genes (i.e.,  $\alpha$ -SMA, fibrillar collagens, and fibronectin) that are necessary for ECM deposition and myofibroblast activation. Recent work from Daseke et al. also suggests that neutrophils alter their signaling pathways during myocardial infarction such that they also secrete fibronectin and fibrinogen at later stages of injury and directly aid in ECM deposition [45]. Neutrophils also play an indirect role in inducing a fibrotic response by further activating the macrophage response and inducing a switch from pro-inflammatory to pro-fibrotic macrophages when they are phagocytized by M1-like macrophages [46].

Macrophages are functionally plastic, thus their role in regulating fibrosis is complex and dependent on the microenvironment where they reside. These cells are the primary source of several MMPs and TIMPs [47]. As previously mentioned, the MMPs and TIMPs that are most directly related to the pro-fibrotic response tend to be produced by M2-like macrophages. The pro-fibrotic MMP-9 is of particular importance in cardiac fibrosis as its inhibition reduces fibrosis in dilated cardiomyopathy and myocardial infarction, seemingly by inhibiting MMP-9's activation of TGF- $\beta$ 1 [48]. TGF- $\beta$  is the best characterized pro-fibrotic growth factor in chronic fibrotic diseases [49]. Of the three TGF- $\beta$  isoforms, TGF- $\beta$ 1 is the most relevant and abundant in cardiac injury [34,50]. TGF- $\beta$  neutralization prevents cardiac fibrosis and improves diastolic dysfunction in pressure-overloaded rats [51]. Further, TGF- $\beta$ 1 knockout mice, though exhibiting other disease phenotypes, present with markedly reduced collagen deposition [52]. M2-like macrophages produce large amounts of TGF- $\beta$  that then binds to TGF- $\beta$  receptors on fibroblasts leading to the potent activation of myofibroblast differentiation and enhanced synthesis of ECM proteins through the Smad family of transcription factors. Further, TGF- $\beta$ 1 induces the expression of protease inhibitors in myofibroblasts and macrophages resulting in the preservation of the remaining ECM. TGF- $\beta$ 1 can also promote collagen synthesis through Smad-independent pathways through its activation of JNK/MAPK protein kinase cascades [53]. M2-like macrophages in later stages of the fibrotic response produce other pro-fibrotic cytokines such as IL-4, IL-13, and TNF- $\alpha$  [54]. IL-4 and IL-13 lead to further activation of myofibroblasts via STAT6, AKT, and MAPK pathways through which they promote collagen production [55]. These cytokines also potently induce several chemokines (e.g., CCL2/MCP-1 and CCL3) that can recruit fibroblasts to the site of injury [56]. TNF- $\alpha$  secretion by macrophages can promote

expression of TGF- $\beta$ 1 and recruit more inflammatory cells resulting in an increased and sustained fibrotic response [57].

Much like macrophages, the pro-fibrotic role of T cells in cardiovascular disease is context-dependent. Th<sub>2</sub> cells are characterized by the secretion of IL-4, IL-5, and IL-13, pro-fibrotic cytokines that lead to the phenotypic shift of fibroblasts to myofibroblasts [38]. IL-13 also induces TGF- $\beta$  expression in macrophages and inhibits MMP synthesis by fibroblasts leading to reduced matrix degradation and excessive collagen deposition [58]. The role of Th<sub>1</sub> cells in promoting fibrosis seems to be through IFN- $\gamma$  recruitment of other inflammatory cells, though IFN- $\gamma$  can also increase expression TNF- $\alpha$  which promotes maturation of pro-fibrotic, M2-like macrophages [59,60]. Th<sub>17</sub> cells are essential for the development and progression of cardiac fibrosis [61]. Their expression of IL-17 causes the proliferation of cardiac fibroblasts and, in parallel, induces expression of MMP-1 and MMP-2, both of which cleave fibrillar collagens and aid in turnover of collagens I and III [62]. T<sub>reg</sub> cells release IL-10, which can have a dual role in the development of fibrosis. IL-10 inhibits collagen synthesis in cardiac fibrosis via a reduction in STAT3 signaling; however, prolonged expression of IL-10 can promote fibrocyte recruitment and M2-like macrophage activation [63,64].

B-cells, while not as well studied in the context of cardiac fibrosis, produce pro-fibrotic cytokines IL-6, CCL2, TNF- $\alpha$ , and TGF- $\beta$  in addition to B-cell-activating factor (BAFF) [18]. IL-6 is predominantly known for its pro-inflammatory role as it promotes the infiltration, migration, and polarization of macrophages. However, IL-6 also induces the conversion of fibroblasts into myofibroblasts suggesting that this cytokine can directly result in fibrosis [65]. BAFF induces the production of collagens, TIMPs, MMP-9, and  $\alpha$ -SMA in fibroblasts [66]. While B-cells seem to play a role in promoting cardiac fibrosis, the extent of their importance in this pathology requires further study.

In addition to the effects of the aforementioned cell types on myofibroblast differentiation, myofibroblasts also secrete cytokines that act in a paracrine manner to augment the fibrotic process. Activated myofibroblasts secrete IL-6, IL-1 $\beta$ , IL-10, TNF- $\alpha$ , CCL2, and TGF $\beta$ , all of which directly promote the fibrotic response by promoting myofibroblast proliferation and activation [67]. Further, these cells secrete cytokines ROS and TNF- $\alpha$  that can maintain the inflammatory response and prolong the injury response through the previously discussed mechanisms.

### Cellular Senescence in Cardiac Fibrosis

The aforementioned pro-inflammatory and pro-fibrotic mechanisms are complicated when considered in the context of aging and cellular senescence. While not specifically discussed here, the signaling mechanisms related to fibrosis in the aging heart are largely similar to those in cardiovascular diseases though they are complicated with the addition of cellular senescence. For detailed reviews on cardiac fibrosis in aging, please refer to Shimizu and Minamino [68], Lu et al. [69], and Biernacka and Frangiannis [70].

Cellular senescence is the stable state of cell cycle arrest resulting in cellular resistance to growth stimuli-induced proliferation that is associated with age. Senescence can be caused



by either telomere attrition during cell division leading to accumulated DNA damage or through various external and internal stress signals including oxidative stress, metabolic stress, and constitutive activation of mitogenic stimuli [68]. These signals trigger activation of signaling pathways, primarily the p53/p21 and p16 signaling pathways, and the expression of other senescence-associated markers such as the pro-inflammatory senescence-associated secretory phenotype and MAPK signaling activation. The p53 and p16 signaling pathways are broadly responsible for cell cycle arrest, DNA damage repair, and apoptosis [71]. Senescent cells produce a variety of pro-inflammatory cytokines (e.g., IL-6, IL-1 $\beta$ , IL-13), chemokines (e.g., CCL8 and CCL13), MMPs/TIMPs, reactive oxygen species, and ECM components (e.g., collagens, fibronectin, laminin) as part of the senescence-associated secretory phenotype [71]. Because these components are involved in both the pro-inflammatory and pro-fibrotic pathways described above, the role of cellular senescence in the progression of cardiac fibrosis is complex.

While we know cellular senescence is broadly associated with cardiac disease and cardiac fibrosis, the relationship does not seem to be simple. Humans with end-stage heart failure have increased p53 levels and markers of apoptosis in the myocardium [72]. Further, p53 expression is also elevated in patients with hypertrophic cardiomyopathy and dilated cardiomyopathy compared to the non-failing heart [73,74]. Elevated p53 in the myocardium has also been associated with the suppression of angiogenesis, tissue hypoxia, and cardiac dysfunction [75]. However, studies in cardiac fibroblasts have shown that increased p16 suppresses age-induced cardiac fibrosis [76]. The reduction of senescence-associated markers ataxia telangiectasia mutated, p53, and p21 enhances fibrosis in the non-infarct area following myocardial infarction [77]. These studies suggest that while cardiomyocyte senescence may result in increased fibrosis through increasing pro-inflammatory and pro-fibrotic signaling in other cell types, cardiac fibroblast senescence may actually be beneficial.

### III. Biomechanical Mechanisms of Cardiac Fibrosis

The fibrotic response is regulated at the site of cardiac injury through the complex signaling within and between the previously discussed cell types. These biochemical mechanisms that promote fibrosis are further complicated when considered with the mechanical environment in which the cells reside. The pumping function of the heart requires that cardiac tissue maintain enough stiffness to resist its mechanical load yet be compliant enough to contract [78]. To balance these mechanical needs, fibroblasts must respond to changes in load by increasing production of ECM components, MMPs, and TIMPs as necessary. In cardiac disease, the previously mentioned biochemical signals induce tissue remodeling and ECM degradation. This loss of ECM, as well as pressure overload, results in an increase in diastolic strain [9]. In response to pathological strains, aortic valve interstitial cells and cardiac fibroblasts proliferate and increase expression of MMPs and  $\alpha$ -SMA [11,79–81]. The expression of these proteins allows for increased cardiac fibroblast migration into the injured region and contraction of 2D and 3D substrates *in vitro* that contributes to the fibrotic response [82,83]. Cardiac fibroblasts subjected to increased cyclic strain also increase production of ECM components and TGF- $\beta$  which, as previously discussed, strongly promotes further pro-fibrotic signaling [84].

In later stages of cardiac fibrosis, fibroblasts experience increased substrate stiffness due to the deposition and cross-linking of ECM components. In 2D culture systems, stress fiber formation in fibroblasts is prevented on very soft (1–3 kPa) substrates [85]. Progressively increasing substrate stiffness promotes myofibroblast differentiation such that  $\alpha$ -SMA negative stress fibers form in fibroblasts on stiffer (3 kPa) substrates and very stiff (>20 kPa) substrates are required for  $\alpha$ -SMA positive myofibroblasts to completely differentiate [86]. In addition to  $\alpha$ -SMA, cardiac myofibroblasts increase expression of TGF- $\beta$ 1 and collagen I on stiff substrates demonstrating that myofibroblasts respond to increased substrate stiffness by exacerbating the fibrotic phenotype [87,88]. Further, myofibroblasts increase internal cellular tension by contracting to match the increased extracellular tension of the ECM [89]. This contraction results in a pulling force on both the ECM and surrounding cells because myofibroblasts are bound to both the ECM, cardiomyocytes, and other myofibroblasts. Thus, myofibroblast contraction can increase the substrate stiffness of nearby cells leading to a progressive and pathological fibrotic phenotype.

Tissue mechanics are sensed via mechanosensitive adhesion proteins that are incorporated into focal adhesions and adherens junctions, the physical tethers that, respectively, link cells to the ECM and to other cells. These mechanosensors include the integrin and cadherin protein families with the specific isoform of each family's members being associated with specific cellular and disease phenotypes. Integrins are transmembrane glycoproteins consisting of  $\alpha$ - and  $\beta$ -subunits that bind to the ECM and recruit other focal adhesion proteins that bind to the actin cytoskeleton, providing a direct mechanical link between stress fibers and ECM proteins [90]. Through focal adhesions, fibroblasts periodically pull on the ECM to sample the stiffness of their microenvironment via actomyosin contractions [91]. On soft matrices, the matrix resistance is not sufficient to deform focal adhesion proteins, thus fibroblasts remain in a quiescent state. Stiff matrices, however, resist this pulling force and allow for mechanical deformation of focal adhesion proteins resulting in activation of stretch-dependent signaling pathways.

The intracellular tails of integrin subunits bind to signaling proteins such that the combination of integrin subunits determines both ECM binding specificity and downstream intracellular signaling [92]. Thus, the precise pathways activated during cardiac fibrosis is a product of which integrin subunits are present. Others have provided detailed reviews on the role of specific integrins in regulating cardiac fibrosis, thus we will focus on the signaling mediators activated downstream of focal adhesions [92–94]. One signaling mediator of particular importance in cardiac fibrosis is focal adhesion kinase (FAK), a tyrosine kinase that colocalizes with integrins through its association with other focal adhesion proteins [80,95]. This protein is activated by cyclic stretch and activates the AKT and MAPK pathways to promote myofibroblast differentiation [80,96]. However, FAK's role in cardiac fibrosis is complicated as activation of FAK through FGF-2 signaling prevents myofibroblast differentiation and reverses TGF- $\beta$  induced  $\alpha$ -SMA expression [95,97]. Silencing of FAK by siRNA or pharmacologically blocking this protein attenuates fibrosis, collagen content, and MMP-2 activity in pressure overload cardiac hypertrophy, thus FAK can broadly be considered as a pro-fibrotic mediator though this activity is context-dependent [98].



An additional pathway activated downstream of focal adhesions is the RhoA small GTPase activation of SRF/MRTF-A transcription factors. In healthy conditions, G-actin binds to and sequesters MRTF-A in the cytoplasm [99]. RhoA activation promotes actin stress fiber formation and stabilization leading to the release of MRTF-A as G-actin is recruited to form these filaments [100]. Once released, MRTF-A translocates to the nucleus where it pairs with SRF and transcriptionally activates genes needed for myofibroblast differentiation and migration to further the fibrotic response. MRTF-A deficient mice with isoproterenol-induced myocardial stress or myocardial infarction develop reduced fibrosis and a smaller scar signifying the importance of this transcriptional activation for the development of cardiac fibrosis [101].

As previously mentioned, TGF- $\beta$  is a primary mediator of fibrotic disease and can be sequestered in a latent form in the ECM. TGF- $\beta$  is non-covalently linked to the ECM through a protein complex recognized by  $\alpha$ v integrins referred to as the large latent complex [102–104]. With increasing traction forces as myofibroblasts become contractile in the context of a stiff ECM, a conformational change occurs in the latency associated peptide within the latent complex leading to the release of active TGF- $\beta$  that promotes myofibroblast differentiation and ECM deposition. Blocking  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5 integrins in cardiac fibroblasts prevents activation of latent TGF- $\beta$  and myofibroblast differentiation *in vitro* suggesting this pool of TGF- $\beta$  is important for the fibrotic response.

In addition to the signaling pathways originating from outside the cell, integrins can participate in inside-out signaling whereby focal adhesion proteins activate integrins by modifying their intracellular domains resulting in strengthened ECM adhesions. Vinculin is a focal adhesion protein that strengthens and stabilizes focal adhesions under high tension [105–107]. Activated vinculin also recruits FAK to further activate fibrotic signaling [105]. Through these focal adhesion mediated pathways, myofibroblasts increase expression of  $\alpha$ -SMA positive stress fibers and become more contractile creating a positive feedback loop.

In addition to the cell-ECM mechanosensing pathways, cells also interact with each other through adherens junctions. The mechanosensitive protein mediating cell-cell interactions are cadherins, a family of transmembrane proteins linked to actin stress fibers that bind to cadherins of the same type on adjacent cells [108,109]. When myofibroblasts increase their intracellular forces, adherens junctions allow for the cells to mechanically activate signaling pathways in neighboring cells by physically pulling on homophilic cadherin dimers. Cadherins will be discussed in the next section with a special focus on cadherin-11, so the focus here is the signaling pathways downstream of cadherin activation.

Cadherins are linked to multiple intracellular pathways that promote myofibroblast differentiation and cardiac fibrosis [110–113].  $\beta$ -catenin is a dual-function protein that both links cadherins to the actin cytoskeleton and acts as a transcription factor when activated along with TGF- $\beta$  or Wnt signaling pathways to promote a mesenchymal cell phenotype [114].  $\beta$ -catenin binds to TGF- $\beta$  and Wnt signaling genes as well as ECM genes in cardiac fibroblasts, thus its activation through adherens junctions can promote an increased fibrotic response in a positive feedback loop [115]. In addition to activation of  $\beta$ -catenin signaling pathways, cadherins act through SRF/MRTF-A to increase expression of  $\alpha$ -SMA promoting

myofibroblast differentiation and through MAPK to induce expression of IL-6 and MMPs further promoting inflammation [114,116]. Cadherin engagement can also lead to vinculin recruitment to adherens junctions by  $\alpha$ -catenin [117–119]. This recruitment stabilizes the intercellular adhesion and increases cell contractility which can further activate adherens junction signaling. For more information on the role of mechanobiology in the development of cardiac fibrosis, please see the review by Herum et al [120].

#### IV. Cadherin-11 in Fibrosis

As previously mentioned, classical cadherins regulate calcium-dependent, cell-cell interactions at adherens junctions [108,109]. These proteins are most well known for their roles in cellular migration and adhesion in embryonic development as well as providing solid tissues with mechanical and functional integrity in adulthood. Classical cadherins share a common protein structure consisting of five extracellular domains, a single-pass transmembrane domain, and a cytoplasmic tail domain [121]. This family of cadherins is subdivided into two types based on the presence of a histidine-alanine-valine (HAV) tripeptide motif and one, conserved, N-terminal tryptophan (type I) or the lack of an HAV motif and two, conserved, N-terminal tryptophans (type II) [122]. The extracellular domain of type II classical cadherins dimerize in a homophilic manner between neighboring cells (in *trans*) via the two tryptophans (W2 and W4) in the EC1 domain. Intracellularly, the cytoplasmic tail binds to  $\beta$ -catenin, which in turn binds to  $\alpha$ -catenin (Figure 2) [123].  $\alpha$ -catenin interacts directly with the filamentous actin cytoskeleton, which helps to provide tension and stability for maintenance of these intercellular junctions.

Cadherin expression is differentially regulated during development and disease leading to strengthened or weakened cell-cell adhesion and differential activation of cadherin-associated signaling pathways [124]. The primary cadherin expressed in quiescent fibroblasts in the heart is cadherin-2 (CDH2 or N-Cadherin) whose expression is associated with increased  $\beta$ -catenin stability and low expression of  $\alpha$ -SMA in aortic valve interstitial cells and stromal cells in ischemic heart injury [125,126]. During myofibroblast differentiation, the predominant cadherin expressed switches from cadherin-2 to cadherin-11, which can withstand two-fold higher forces than N-cadherin allowing for stronger contractile forces and transmission of higher intracellular tension [127–130].

Cadherin-11 (CDH11 or OB-Cadherin) is a type II classical cadherin that was first identified in osteoblasts but has since been noted in other cells of mesenchymal origin [131]. While this protein was originally studied in the context of tissue development and morphogenesis, cadherin-11 has more recently been recognized as a driver of the myofibroblast phenotype and regulator of fibroblast inflammation [127,129,132–134]. These findings have uncovered this protein's regulatory role in fibroblast mediated diseases such as pulmonary fibrosis [130], rheumatoid arthritis [112], systemic sclerosis [135], metastatic cancer [136], and kidney fibrosis [137] as well as fibrotic diseases of the cardiovascular system, calcific aortic valve disease [128,138,139], and myocardial infarction [93] (Figure 2).

Cadherin-11 is upregulated during myofibroblast differentiation through TGF- $\beta$ 1 signaling concurrently with a decrease in expression of cadherin-2 [127]. This cadherin switch

promotes myofibroblast invasion [140]. Further, cadherin-11 expression has been observed on TGF- $\beta$  producing macrophages [113]. Since cadherin-11 is involved in cell migration, its expression in macrophages raises the possibility that this protein both recruits inflammatory cells to the injury site and maintains these cells in close proximity to promote the profibrotic niche. Cadherin-11 is also the only cadherin known to participate in focal adhesions where it promotes cell-substrate adhesion [141]. In addition to its mechanical role in strengthening adherens junctions and focal adhesions, cadherin-11 also activates intracellular signaling pathways to influence cellular behavior. Cadherin-11 engagement activates SRF through the RhoA pathway leading to increased expression of  $\alpha$ -SMA and cadherin-11 and also promotes differentiation of smooth muscle cells by upregulating expression of TGF- $\beta$ 1 [142]. Likewise, overexpression of cadherin-11 in fibroblasts increases expression of Wnt and  $\beta$ -catenin and extracellular matrix components [143,144]. Thus, cadherin-11 expression and engagement promotes differentiation of myofibroblasts and activates downstream pathways that further serve to strengthen and stabilize cadherin-11 containing adherens junctions. Additionally, cadherin-11 engagement promotes IL-6, MCP-1, and MMP-1 expression in synovial fibroblasts which could further promote inflammatory invasion in fibrotic diseases.[133,145] Altogether, cadherin-11 expression promotes the differentiation of myofibroblasts and modulates both the mechanical and inflammatory pathways associated with fibrotic diseases.

Cadherin-11 expression is highly expressed in the endothelial (VECs) and interstitial cells (VICs) of diseased heart valves and in the non-cardiomyocyte cells of the infarcted heart [93,128,138,139]. In the diseased aortic valve, interstitial cells take on a myofibroblast phenotype and mediate the structural remodeling that leads to aortic stenosis [20,146–148]. In these cells, cadherin-11 is upregulated downstream of TGF- $\beta$  through both the Smad2/3 and MAPK signaling pathways and this upregulation occurs both during development then again in aortic valve pathogenesis [149]. Further, cadherin-11 expression regulates dystrophic calcific nodule formation in aortic valves, a process that follows an increase in myofibroblast contractility [128]. Our group has more recently shown that cadherin-11 knockout mice have reduced aortic valve calcification and decreased expression of myofibroblast and inflammatory markers including IL-6 [134]. Further, cadherin-11 was shown to regulate VIC contractility through its association with focal adhesions. Other groups have supported cadherin-11's role in promoting myofibroblast differentiation and subsequent calcification in valve disease [138,139,150]. Overexpressing cadherin-11 in adult mouse aortic valves leads to increased ECM components, numbers of focal adhesions, and valve calcification [150]. Further, cadherin-11 overexpression in VICs increases cell migration, adhesion, and stress fiber bundles suggesting that expression of this protein promotes the myofibroblast phenotype. Our group has also shown that these markers of aortic valve disease can be prevented through blocking cadherin-11 with a monoclonal antibody (SYN0012) in the *Notch1<sup>+/-</sup>* mouse model of this disease [151]. Along with cadherin-11's known role in strengthening adherens junctions, these findings suggest that cadherin-11 broadly regulates the fibrotic response in calcific aortic valve disease.

While less is known about cadherin-11's role in the myocardium, it is expressed in cardiac fibroblasts [152]. This expression and the cadherin-11's regulation of a broad range of fibrotic diseases led our group to recently study the role of this protein in the fibrotic

response to myocardial infarction [93]. Following myocardial infarction, cadherin-11 expression increased nearly 10-fold in non-cardiomyocyte cells of the heart and was particularly high among infiltrating M2-like macrophages. This increase following myocardial infarction is transient as cadherin-11 expression increases by day-3 post-infarct, stays elevated through 2-weeks post-infarct, but returns back to baseline expression when scar formation is stable 4-weeks post-infarct [23]. Importantly, outcomes following myocardial injury were improved when wild-type mice given cadherin-11 null bone marrow. Further, treating wild-type mice with SYN0012 also improved cardiac function following myocardial infarction by limiting transcription and secretion of IL-6. In angiotensin II-induced fibrosis, cadherin-11 expression seems to remain elevated in matrifibrocyte-like, fibrosis-associated cell populations suggesting that this protein could play a prolonged role in stress-associated cardiac fibrosis [24]. Cadherin-11 has also been described as senescence-responsive whereby its expression is suppressed in senescent endothelial cells and this suppression is greater when senescent cells are under shear stress [153]. p53 is also suppressed downstream of cadherin-11 in a STAT3-dependent manner [154]. This senescence responsiveness and downregulation of p53 downstream of cadherin-11 suggests that a reciprocal relationship exists between cadherin-11 and senescence-associated signaling pathways. Thus, cadherin-11 expression may prevent the beneficial effects of cardiac fibroblast senescence. Through these findings and the findings in the context of aortic valve disease, cadherin-11's role in regulating cardiac fibrosis through the biochemical and biomechanical signaling pathways that drive this pathology are becoming clear.

## V. Targeting Cadherin-11 in Disease Models

As we have discussed, fibrotic diseases originate as a consequence of chronic inflammation and biomechanical changes leading to prolonged activation of myofibroblasts, yet most available therapies have focused on targeting molecules with central roles in innate and/or adaptive immunity. While these therapies prevent chronic fibrosis with some efficacy, the clinical success of immunomodulatory therapies in cardiac disease is still limited as prolonged immune suppression leads to severe side effects and they fail to address the concurrent mechanical signals that aid in the initiation and progression of fibrotic disease [155]. Given that cadherin-11 is a known regulator of mechanical signaling pathways whose expression increases in fibrotic diseases and given this increased expression is associated with altered inflammatory pathways, this protein seemingly serves as a link between the biomechanical, pro-fibrotic, pro-inflammatory, and senescence signaling pathways. Therefore, cadherin-11 may serve as an ideal therapeutic target in fibrotic disease. This idea has gained support through experiments targeting cadherin-11 with monoclonal antibodies blocking cadherin-11 homotypic bond formation. Treatment with this antibody prevents disease phenotypes in a variety of fibrotic diseases. Specifically, reducing cadherin-11 activity either through genetic knockout or with an anti-cadherin-11 antibody is effective in reducing symptoms in animal models of systemic sclerosis [135], rheumatoid arthritis [112], pulmonary fibrosis [130], calcific aortic valve disease [128,138,139], and improving outcomes following myocardial infarction [93]. These findings were so compelling that the humanized form of this antibody underwent clinical trials for rheumatoid arthritis [156].

These trials were ended, however, due to the drug not providing an increased response compared to TNF- $\alpha$  inhibitors, the current standard of care. Further, the progressive nature of cardiac fibrosis makes administration of an anti-cadherin-11 antibody as a preventative measure difficult as it would require patients to receive monthly injections for an unspecified period of time. Nevertheless, preclinical data suggests that targeting cadherin-11 could be beneficial for diseases that are accompanied by cardiac fibrosis.

As a type II classical cadherin, the method of targeting cadherin-11 that seems effective for preventing fibrotic disease is to block homophilic bond formation via the EC1 domains of these proteins (Figure 3A). As previously mentioned, this binding event requires two tryptophans (W2 and W4) found within a hydrophobic pocket of this domain (Figure 3B and 3C), thus competitive inhibition of homophilic dimerization likely occurs through blocking the availability of these residues. SYN0012, the CDH11-blocking monoclonal antibody, acts through this mechanism as does celecoxib, a cyclooxygenase-2 (COX2) inhibitor and nonsteroidal anti-inflammatory drug used to treat pain and inflammation, and dimethyl celecoxib, an analog of celecoxib that does not inhibit COX2 [112,157]. Celecoxib and dimethyl celecoxib both have a benzene sulfonamide backbone that interact with tryptophan 4 and histidine 25 within this hydrophobic pocket through intermolecular hydrogen bonds [158]. The binding of small molecules to these residues prevents the dimerization of CDH11. The finding that these drugs binds to CDH11 seems to have clinical relevance as well since celecoxib is used to treat rheumatoid arthritis, though its translatability into cardiovascular diseases seems to be more complicated. Our group recently showed that celecoxib promotes aortic stenosis compared to ibuprofen and naproxen controls in human patients [159]. Further, celecoxib induces a myofibroblast phenotype in aortic valve interstitial cells *in vitro* and promotes dystrophic calcification; however, dimethyl celecoxib prevented the onset of these fibrotic events. Thus, dimethyl celecoxib may be better suited for cardiovascular fibrosis though further research is needed on this topic. Vaidya et al. extended upon these findings by showing that celecoxib and dimethyl celecoxib can both induce osteogenic aortic valve calcification in *ex vivo* porcine aortic valve leaflets in a glucocorticoid-dependent manner [160]. These findings suggest that any cadherin-11 binding small molecules may be effective at treating cardiac fibrosis, however, these molecules should be evaluated for off target risks in the presence of glucocorticoids prior to approval.

## VI. Conclusions

A healthy heart requires maintenance of a healthy ECM to transmit force throughout the tissue and regulate biochemical and biomechanical signaling pathways. Most cardiovascular diseases are accompanied by cardiac fibrosis where ECM accumulation helps to stabilize the tissue while remodeling and/or scar formation occurs; however, the limited regenerative capacity of cardiac tissue often results in excessive ECM deposition leading to systolic dysfunction, diastolic dysfunction, and arrhythmias. Cardiac fibrosis results from a complex interplay of pro-inflammatory, pro-fibrotic, and biomechanical signaling; however, current therapies do not target each of these components of the fibrotic response. Cadherin-11 is a component of focal adhesions and adherens junctions in myofibroblasts and regulates fibroblast inflammation. Preventing homodimerization of this protein effectively prevents

fibrotic diseases, including calcific aortic valve disease, and leads to better outcomes following myocardial infarction. While more research is needed to uncover its importance in other types of cardiac fibrosis, cadherin-11 may provide a therapeutic target to effectively treat cardiac fibrosis as this protein acts a link between inflammatory, fibrotic, and biomechanical causes of fibrosis.

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## Abbreviations

<b>ECM</b>	Extracellular matrix
<b>CDH11</b>	cadherin-11
<b>CDH2</b>	cadherin-2
<b>MMP</b>	matrix metalloproteinase
<b>TIMP</b>	tissue inhibitors of metalloproteinase
<b>F-SH</b>	fibroblast <i>Scal</i> -high
<b>F-SL</b>	fibroblast <i>Scal</i> -low
<b>DAMP</b>	damage-associated molecular pattern
<b>TLR</b>	toll-like receptor
<b>TNF-<math>\alpha</math></b>	tissue necrosis factor $\alpha$
<b>IFN-<math>\gamma</math></b>	interferon $\gamma$
<b>IL</b>	interleukin
<b>NF-<math>\kappa</math>B</b>	nuclear factor kappa-light-chain-enhancer of activated B cells
<b>AP-1</b>	activator protein 1
<b>MCP-1/CCL2</b>	monocyte chemoattractant protein-1/chemokine ligand 2
<b>CCL8</b>	chemokine ligand 8
<b>CCL13</b>	chemokine ligand 13
<b>CCR2</b>	chemokine receptor 2
<b>TGF-<math>\beta</math></b>	transforming growth factor $\beta$
<b><math>\alpha</math>-SMA</b>	$\alpha$ smooth muscle actin
<b>Smad2/3</b>	Mothers against decapentaplegic homolog 2/3



<b>RhoA</b>	Ras homolog family member A
<b>MAPK</b>	mitogen-activated protein kinase
<b>JNK</b>	c-Jun N-terminal kinase
<b>Src</b>	proto-oncogene tyrosine-protein kinase Src
<b>STAT</b>	signal transducer and activator of transcription
<b>Akt</b>	protein kinase B alpha
<b>BAFF</b>	B-cell activating factor
<b>ROS</b>	reactive oxygen species
<b>FAK</b>	focal adhesion kinase
<b>FGF-2</b>	basic fibroblast growth factor
<b>SRF</b>	serum response factor
<b>MRTF-A</b>	myocardin-related transcription factor A
<b>VEC</b>	valve endothelial cells
<b>VIC</b>	valve interstitial cells
<b>COX2</b>	cyclooxygenase 2

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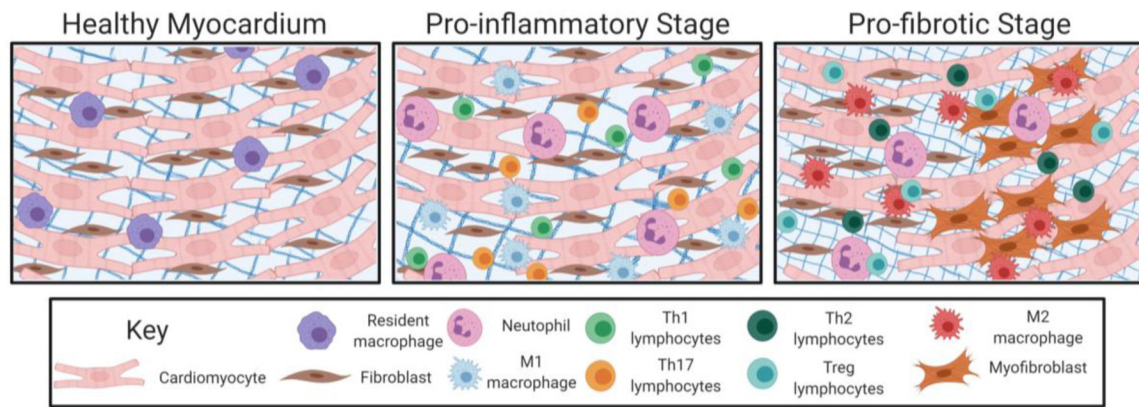
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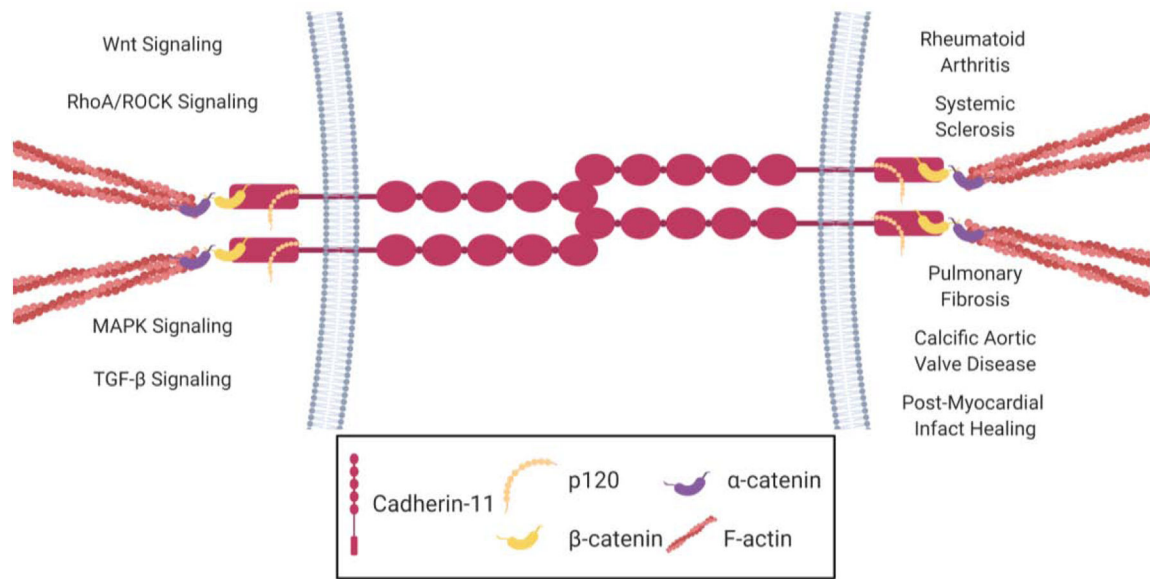
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**Highlights:**

- Fibrotic diseases are caused by persistent inflammatory, fibrotic, and mechanical signaling.
- While myofibroblasts are the effector cells that secrete extracellular matrix components, inflammatory cells are necessary for initiating and sustaining fibrotic remodeling.
- Biomechanical signaling is a primary factor generating cardiac fibrosis.
- Cadherin-11 engagement increases pro-inflammatory and pro-fibrotic mediators of fibrosis and results in extracellular matrix deposition.
- Blocking cadherin-11 dimerization holds potential as a therapeutic target for cardiac fibrosis.

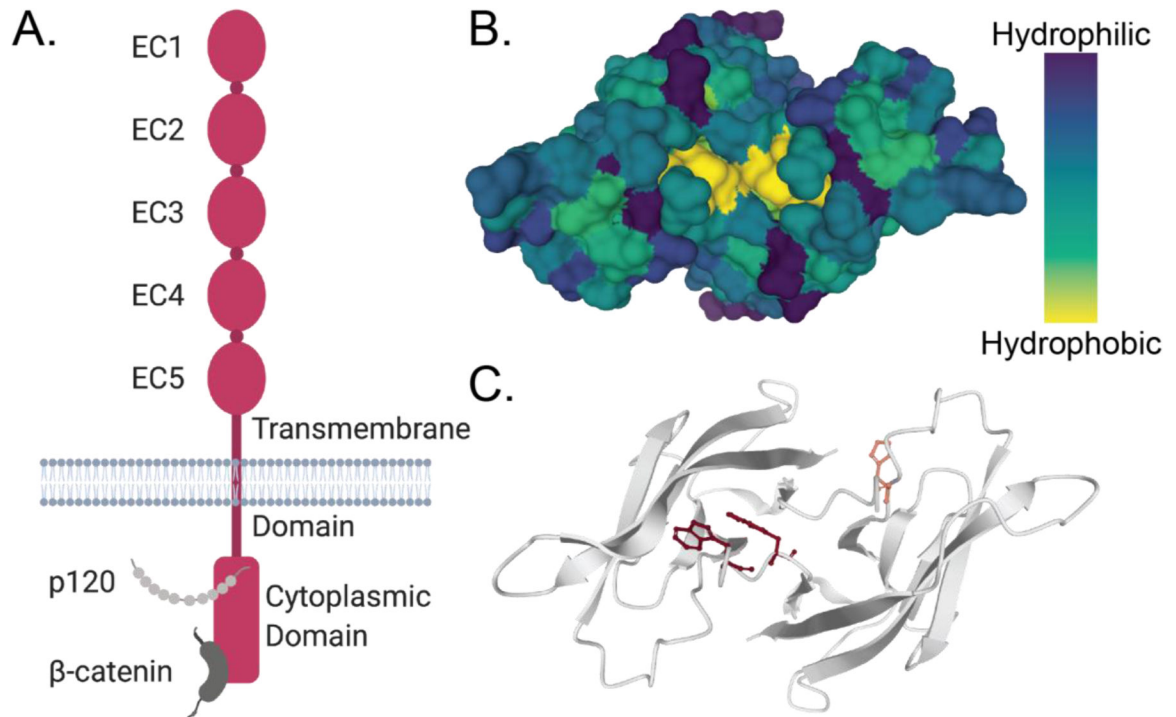
**Figure 1:**

Cell involvement in healthy cardiac tissue and during the cardiac fibrotic response. Left Panel: In the healthy myocardium, healthy cardiomyocytes (red), resident fibroblasts (brown), and resident immune cells (i.e., macrophages; purple) reside. In this state, resident fibroblasts help to maintain a normal ECM through the production of collagens, elastin, and laminin. These resident cells also produce a balance of MMPs and TIMPs to help maintain a normal ECM in which these cells can reside. Center Panel: In the pro-inflammatory stages of cardiac fibrosis, DAMPs secreted by stressed or necrotizing cells lead to neutrophil (pink) invasion. This sets off an inflammatory cascade whereby macrophages are recruited and activated so that these cells take on an M1 pro-inflammatory phenotype (blue). In later stages of inflammation, Th1 (green) and Th17 (orange) lymphocytes are recruited and help to maintain activation of other inflammatory cells. Right Panel: As inflammatory signaling persists, macrophages take on an M2, pro-fibrotic phenotype (red). Th2 (dark green) and Treg (teal) lymphocytes also secrete anti-inflammatory and pro-fibrotic mediators leading to this switch toward increased fibrosis. Neutrophils (pink) also persist at this stage and directly secrete ECM components. Signaling from each these cell types activates pathways leading to fibroblast (brown) proliferation (left) and activation into myofibroblasts (dark orange; right). Myofibroblasts are contractile and secretory and are the predominant cell type secreting extracellular matrix proteins. The activation and accumulation of these cells results in a dense ECM (blue grid behind cells). These cells eventually differentiate into matrifibrocytes (not shown) to maintain a stable fibrotic scar. Created with [BioRender.com](https://www.biorender.com)



**Figure 2:** Cadherin-11's role in signaling pathways and disease. Cadherin-11 forms homophilic dimers between neighboring cells. These intercellular linkages aid in transmitting mechanical signals between cells to their respective cytoskeletons by binding to p120 catenin and β-catenin. β-catenin binds to α-catenin which is linked to the actin cytoskeleton. Through these physical tethers, cadherin-11 transmits mechanical signals to molecular signaling pathways in the cells (left) that are associated with fibrotic diseases (right). Created with [BioRender.com](https://www.biorender.com)





**Figure 3:** Targeting cadherin-11 to prevent homodimer formation. A. Cadherin-11 is composed of five extracellular (EC) domains, a transmembrane domain, and a cytoplasmic domain. EC1 and EC2 allow cadherin-11 to dimerize while the rest of the protein transmits physical forces for cellular signaling. B. The EC1 domains of a cadherin-11 dimer contain a hydrophobic pocket that be targeted by small molecules to inhibit dimer formation. C. The amino acids that, when blocked, effectively inhibit cadherin-11 dimerization are part of this hydrophobic pocket. These residues should be considered when targeting cadherin-11 with drugs to prevent cadherin-11 mediated disease. Image created using Mol\*.[161]