Tenascin-C (TN-C) is a matricellular protein expressed during embryonic development, as well as in wound healing and cancer invasion in various tissues, and may regulate cell behavior and matrix organization during tissue remodeling. In the cardiovascular system, TN-C is transiently expressed at several important steps of embryonic development, playing important roles in the differentiation of cardiomyocytes and in coronary vasculo/angiogenesis. TN-C is sparse in normal adults, but upregulated under pathological conditions such as myocarditis, myocardial infarction, cardiac fibrosis, atherosclerosis, stenotic neointimal hyperplasia, and aneurysm, and is closely associated with tissue injury and inflammation. In view of its specific expression, TN-C could be a realistic and promising biomarker and a target for molecular imaging for the diagnosis of various cardiovascular diseases. TN-C also has diverse functions, including weakening of cell adhesion, up-regulating the expression and activity of matrix metalloproteinases, modulating inflammatory responses, promoting recruitment of myofibroblasts, and enhancing fibrosis. TN-C could exert both harmful and protective effects and might be a therapeutic target as a key molecule in the control of the balance of beneficial and undesirable cellular responses during tissue remodeling. (Circ J 2012; 76: 2513–2520)

**Key Words:** Biomarker; Inflammation; Molecular imaging; Remodeling; Tenascin-C

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**TN-C**

TN-C is a huge glycoprotein of approximately 300 kDa as an intact monomer, which is assembled into a hexamer. Multiple cell-surface receptors, including integrins α9β1, αvβ3, and αvβ6, and toll-like receptor 4 (TLR-4), bind to the respective domains of TN-C and transmit multiple signals that could control the balance of cell adhesion and de-adhesion, cell motility, proliferation, differentiation, and survival.

TN-C is expressed transiently at specific sites during embryonic development, wound healing, cancer invasion, and regeneration, at locations where the tissue structure is being dynamically remodeled. The expression of TN-C can be activated by a number of different stimuli, including cytokine growth factors and mechanical strain through multiple signaling pathways including nuclear factor κ B (NF-κB) and mitogen-activated protein kinase. Several factors including miRNA-335 and miRNA-145 may directly suppress its gene activation, which should be important for the strict regulation of its expression.

TN-C knockout (TN-C KO) mice were initially reported to undergo normal development and have a normal life span and fertility without distinct phenotypes. Recently, more detailed investigations have shown several differences in lung and prostate development in TN-C KO. Furthermore, evident differences have been reported in various disease models using TN-C KO, for example, attenuation of fibrotic/inflammatory lesions of various organs, including the cardiovascular system.

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**Extracellular matrix (ECM) components are important for mechanical support and effective functioning of the cardiovascular system. Therefore, alteration of the ECM may directly result in changes of mechanical properties and functional impairment. The ECM also plays significant roles in tissue remodeling in stress responses. Among the ECM components, increased attention has been focused on the matricellular proteins, which are a growing group of non-structural ECM proteins with different structures but common unique properties:**

1. High levels of expression during embryonic development and in response to injury.
2. Binding to many cell-surface receptors, components of ECM, growth factors, cytokines, and proteases.
3. Induction of de-adhesion or counter-adhesion.

This group originally included thrombospondin-1, osteonectin/SPARC, tenascin-C (TN-C), tenascin-X, osteopontin, and CCN (CY61/CTGF/NOV). Recently, new members such as perostin and galectin have joined the group. Matricellular proteins play a significant role in cardiovascular disease. This review will focus on the role of TN-C, a typical matricellular protein, in cardiovascular development and disease.
TN-C in the Heart

Heart Development and TN-C
During heart development, TN-C is transiently expressed at restricted sites at several important steps of heart morphogenesis: 27 (1) differentiation of cardiomyocytes, (2) cushion tissue and valve formation, and (3) coronary vessel formation.

It is particularly intriguing that the expression of TN-C appears to be closely related to cardiomyocyte differentiation. In the case of mice, the mesodermal cells from the cardiac crescent, called primary heart field or first heart field (FHF), differentiate to cardiomyocytes and endocardial cells at embryonic day 7.5. TN-C is expressed by the differentiating precardiac cells. Once the cells differentiate to cardiomyocytes, they rapidly stop expressing TN-C.

Another important possibility is that TN-C may play a role in coronary vessel development. The progenitor of the coronary vascular system comes from the proepicardial organ (PEO), a cauliflower-like projection of transverse septum between the primitive heart and the liver bud. Mesenchymal cells from the PEO migrate to the primitive heart at embryonic day 9.5. They eventually form epicardium, undergo epithelial-mesenchymal transition (EMT), and give rise to vascular endothelial cells, smooth muscle cells, and cardiac fibroblasts. 28,29 TN-C is expressed in the PEO before the cells start to migrate and at epicardial EMT (Figure 1). TN-C is also expressed upon the maturation of coronary vessels and may promote the recruitment of α-smooth muscle actin (SMA)-positive mural cells to the primitive endothelial tubes by facilitating platelet-derived growth factor (PDGF)-BB/PDGFβR signaling via integrin αvβ3. 30,31

Regardless of the potential roles during heart development predicted on the basis of spatiotemporal-restricted expression, hearts develop normally in TN-C knockout mice. 16,17,27 Furthermore, our recent preliminary data suggested that overexpression of TN-C in heart may not cause a distinct phenotype, either (unpublished data). Evidently, a compensatory mechanism should be present, which has not been identified yet.

Myocardial Injury, Inflammation in the Adult Heart, and TN-C
TN-C is sparsely detected in the normal adult myocardium, but reappears when the heart remodels its structure in response to pathologic insults, such as acute myocardial infarction (MI), 32–35 myocarditis, 36–38 hibernation, 39 ischemia-reperfusion, 40 hypertensive cardiac fibrosis, 41 chronic cardiac rejection 42 and some cases of dilated cardiomyopathy (DCM) 43,44 closely associated with inflammation.

Under these pathological conditions, myocardial cell death caused by destructive stress triggers inflammation, which is an important process for proper tissue repair. Accumulating evidence suggests that TN-C may enhance inflammatory responses. 25 For example, TN-C may activate innate immunity as DAMP (damage-associated molecular patterns) to stimulate the production of the proinflammatory cytokines in macrophages and fibroblasts via a TLR-4-mediated signaling pathway. 25,45 It also modulates adaptive immunity via the integrin α9 pathway 13,46 and cytokine upregulation 24 with the activation of NF-κβ. 23

Tissue Repair After Acute MI
Tissue repair after MI is a typical example of wound healing followed by scar formation. Necrosis of myocytes elicits acute inflammation to remove dead cells and matrix debris. Then, activated interstitial cells form capillary-rich granulation tissue, remodel ECM, and finally replace myocardial dropout.
Tenascin-C is expressed during inflammation and granulation formation. It is striking that TN-C is exclusively localized at the border zone between residual myocardium and infarcted lesion. Several roles of TN-C during tissue repair after MI have been proposed. First, TN-C could help tissue reconstruction of the edge of the residual myocardium as a “de-adhesion” protein. TN-C could loosen strong adhesion of cardiomyocytes and up-regulate the expression and activity of matrix metalloproteinases (MMPs), so that it may release surviving cardiomyocytes to reorganize their shape and arrangement. Concomitantly, TN-C may maintain weak attachment of the cardiomyocytes and protect against anoxia. The elastic property of the TN-C molecule also suggests that it may act as a molecular damper to protect the cells from destructive mechanical stress. Moreover, a recent paper reported a significant role of TN-C in the regeneration of cardiomyocytes in zebra fish. In mammals, a major player in myocardial repair is the interstitial cells, because of the limited ability for the regeneration of cardiomyocytes. It is considered that fibroblastic cells recruited from different sources become protomyofibroblasts, move from the peri-infarcted area into the damaged lesion, and differentiate to α-SMA-positive myofibroblasts. Myofibroblasts play a central role in wound healing by synthesizing collagens and exerting strong contractile forces to promote wound healing.

During myocardial tissue repair, the major source of TN-C is the interstitial fibroblasts in the vicinity of the injured cardiomyocytes, but cardiomyocytes themselves do not synthesize TN-C. Initially, α-SMA-negative interstitial cells in the border zones express TN-C, and then α-SMA-positive myofibroblasts appear in the TN-C-positive areas. In vitro, TN-C facilitates the migration and expression of α-SMA of cardiac fibroblasts. Furthermore, the appearance of myofibroblasts in the injured sites is delayed in TN-C KO. Therefore, it is suggested that TN-C synthesized by interstitial cells at an early phase induces differentiation to myofibroblasts and promotes migration into damaged areas in an autocrine and paracrine fashion.

Moreover, TN-C may regulate angiogenesis, another important element of myocardial repair. In addition to the potential to facilitate coronary vasculogenesis in the embryo, it may promote postnatal neovascularization by regulating bone marrow-derived endothelial progenitor cell homing and/or incorporation at sites of angiogenic induction, as well as local endothelial function.

Cardiac Fibrosis
Fibrosis is defined as the increase of fibrillar collagen in interstitial spaces. Increased fibrosis is recognized as enhancing myocardial stiffness and causing heart failure.

Fibrosis is classified into replacement (secondary) and reactive (primary) types. Scar formation after MI is a typical example of replacement fibrosis. Therefore, fibrosis is often considered to be the end inflammatory reaction. The fibrotic lesions form through multiple steps of synthesis and degradation of various matrix proteins. TN-C is expressed at an acute stage of the cascade, preceding collagen fibril formation.

In the case of reactive fibrosis, collagen fibers increase in perivascular regions without loss of cells and eventually extend among individual cardiomyocytes. It is recognized that inflammation mediated by the renin–angiotensin II–aldosterone system plays a significant role in the progression of this type of cardiac fibrosis. TN-C is expressed at perivascular inflammatory lesions associated with macrophage infiltration.

Although it is unclear whether reactive fibrosis is formed through the same molecular mechanism as that of replacement fibrosis, in either case, it is evident that fibrosis and inflammation are closely related, and that TN-C could be involved in the fibrotic process. In fact, deletion of TN-C was shown to attenuate interstitial fibrosis after MI and angiotensin II-induced cardiac fibrosis.

Several studies have demonstrated the interaction of TN-C with ECM molecules, including fibronectin, proteoglycans, and peristin; however, the precise role of TN-C in the molecular pathways of collagen fibrillogenesis remains to be determined.

Ventricular Remodeling and TN-C
Ventricular remodeling is clinically manifested as changes in left ventricular chamber volume associated with progressive heart failure after cardiac damage. Biologically, it consists of physiologic and pathologic responses involving structural alternation, characterized by hypertrophy of cardiomyocytes and fibrosis. Recently, the significance of chronic inflammation in
Ventricular remodeling has been clarified.\textsuperscript{57} Various remodeling mediators, including proinflammatory cytokines, TGF-\(\beta\), PDGF, angiotensin II, endothelin-I, hypoxia, and reactive oxygen species (ROS), increase the synthesis of TN-C in vitro.\textsuperscript{13,49} In addition to the potential of TN-C enhancing inflammatory responses as discussed earlier, recent studies suggest that TN-C may enhance several signaling pathways, such as endothelin-1/endothelin receptor-A,\textsuperscript{58} PDGF/PDGFR\(\beta\),\textsuperscript{31} and transforming growth factor (TGF)-\(\beta\),\textsuperscript{59} in several types of cell. TN-C induced upon inflammation in the heart may modulate ventricular remodeling by controlling these signaling pathways.

This raises the following question: Is TN-C harmful or beneficial for ventricular remodeling? It is difficult to determine this logically, because TN-C has conflicting effects (Figure 4). TN-C may loosen cell adhesion, upregulate MMPs, and enhance inflammatory responses. Although these functions help cell rearrangement and allow myofibroblasts and capillary vessels to spread into the tissue under restoration, they might cause tissue vulnerability, resulting in ventricular dilatation. Furthermore, an increase of myofibroblasts and accelerated fibrosis should prevent ventricular dilatation by generating traction forces. On the other hand, excessive fibrosis would lead to stiffer and less compliant ventricles. Moreover, a compensatory system for a lack of TN-C evidently exists. A recent study demonstrated that deletion of TN-C significantly reduced ventricular remodeling and improved cardiac function at day 28 after permanent coronary ligation in mice.\textsuperscript{26} Therefore, it appears that TN-C exerts harmful effects for tissue remodeling after infarction, at least in the chronic stages.

**Clinical Application**

Given its specific expression, TN-C could be a useful marker for evaluating myocardial disease activity. Immunostaining for TN-C significantly improves the sensitivity of a histological diagnosis of myocarditis.\textsuperscript{30} The serum level of TN-C measured by enzyme-linked immunosorbent assay may reflect its local expression in the myocardium. For example, serum TN-C in patients with acute MI is significantly elevated, peaks at day 5, and then gradually decreases. Interestingly, patients with high peak levels of TN-C have a greater incidence of ventricular remodeling after 6 months\textsuperscript{34} and major adverse cardiac events and poor survival during 5-year follow-up.\textsuperscript{60}

Likewise, elevated serum TN-C could be a marker for LV remodeling and a predictor of cardiac events in heart failure in patients with DCM, which is comparable to plasma B-type natriuretic peptide (BNP).\textsuperscript{61,62} Particularly interesting is that combining the serum TN-C level with the plasma BNP level is a stronger predictor than either single biomarker alone in

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**Figure 3.** Representative photographs of reactive fibrosis in angiotensin II (AngII)-induced cardiac fibrosis mouse model. (Adapted from Nishioka et al.\textsuperscript{41})

**Figure 4.** Possible roles of tenascin-C (TN-C) in myocardial tissue repair. ECM, extracellular matrix; MMP, matrix metalloproteinase.
Tenascin-C in Cardiovascular System

both acute MI and DCM. The combination of BNP secreted from cardiomyocytes and TN-C synthesized in interstitial fibroblasts could enable more precise assessment of the whole heart by reflecting both cardiomyocytes and interstitial cells.

An increasing number of studies have reported the utility as a biomarker of serum TN-C in patients with LV hypertrophy, supported by mechanical circulatory support devices, with cardiomyopathy, and with cardiovascular disease of chronic kidney disease. However, TN-C is not synthesized specifically in the myocardium. Normal liver and lung constitutively express TN-C. The elevated levels of circulating soluble inflammatory mediators in heart failure patients might further stimulate secretion of TN-C from the liver or lung. Therefore, it would be necessary to identify the origin of serum TN-C. Molecular imaging could be a promising approach for this. In vivo inflammatory lesions have been successfully imaged in rat models using In-labeled anti-TN-C Fab or single-chain Fv fragments of anti-TN-C.

A precise diagnosis of myocardial inflammation should be important for DCM patients because they may constitute a heterogeneous group, including cases of different phases of inflammatory cardiomyopathy. A recent analysis of myocardium obtained at left ventriculoplasty showed that approximately 50% of 64 DCM patients had significant active inflammation associated with expression of TN-C. A flow chart for risk stratification and determination of the appropriate therapy for DCM patients using TN-C as a marker is proposed (Figure 5). Distinguishing inflammatory cardiomyopathy from other types of DCM would improve the management of patients.

### TN-C in the Vascular System

Although the expression of TN-C in the vascular system appears a little more complex compared with that in the heart, the expression of TN-C in the normal vascular wall is generally low. High tissue levels of TN-C have been reported within a variety of vascular diseases, including intimal hyperplasia, atherosclerosis, pulmonary artery hypertension, and abdominal aortic aneurysm. A fascinating topic recently reported is that TN-C may be involved in cerebral vasospasm after subarachnoid hemorrhage.

Considerable attention has been directed to pulmonary hypertension (PAH). TN-C is upregulated in the vascular lesion and may promote proliferation and migration of vascular smooth muscle cells (SMCs), a main pathogenic component of PAH. The mutated bone morphogenetic protein type II receptor gene reported in familial PAH induces TN-C transcription, suggesting a direct contribution of TN-C to the progression of PAH.

A recent paper has reported the association of genetic polymorphisms in TN-C and atherosclerosis. The involvement of TN-C in the intimal hyperplasia of occlusive/stenotic vascular disease has also been extensively studied. Intimal hyperplasia is defined as excessive migration and proliferation of SMCs.

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**Figure 5.** Flow chart for risk stratification and determination of the appropriate therapy for dilated cardiomyopathy (DCM) patients using tenascin-C as a marker.
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with deposition of ECM proteins. TN-C is expressed in the neointima at an early stage and accelerates progression\(^{21,22}\) and deletion of TN-C reduces intimal lesions\(^{71-79}\). Therefore, TN-C may be a target molecule for controlling stenotic neointimal formation. Recent drug-eluting stents have remarkably reduced the rate of restenosis and, at the same time, have created a need for more precise evaluation of pathological change of the lesion combined with vascular imaging.\(^{95}\) It has been proposed that anti-TN-C molecular imaging of advanced atherosclerotic plaques may be useful\(^{96}\) for detecting unstable plaque.\(^{80,81}\) Conversely, the unfavorable function of TN-C to accelerate neointimal formation is applicable to improving the efficacy of endovascular treatment of aneurysms. In fact, treatment of a rat model with TN-C-coated coils remarkably accelerated organization of the cavity and reduction of the lumen size of the aneurysm (Figure 6).\(^{97}\)

**Conclusions**

TN-C has diverse functions, regulating cell behavior, matrix organization, and inflammation, and should play significant roles during tissue morphogenesis in the embryo as well as in reconstruction of adult tissue after injury. TN-C could exert both harmful and protective effects and might be a therapeutic target as a key molecule in the control of the balance of beneficial and undesirable cellular responses during tissue remodeling.

Furthermore, in view of its specific expression, it is evident that TN-C could be a realistic and promising biomarker and a target for molecular imaging for the diagnosis of various cardiovascular diseases.

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Tenascin-C in Cardiovascular System

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