Summary. The heart remodels myocardial tissue in physiological and pathological response. The cell-extracellular matrix (ECM) interaction provides not only structural and mechanical support but also important biological signaling during tissue remodeling. Among various ECM molecules, tenascin-C (TNC) is well known as a regulator of multiple cellular functions during embryogenesis, wound healing or cancer progression. In the heart, TNC appears in several important steps of embryonic development such as the initial differentiation of cardiomyocytes or coronary vasculo/angiogenesis, but it is not detected in a normal adult myocardium. However, TNC is found to re-express after myocardial injury and may regulate cellular behavior during tissue remodeling by modulating the attachment of cardiomyocytes to connective tissue, by enhancing migration and differentiation of myofibroblasts, and by inducing matrix metalloproteinases. TNC also interacts with other ECM molecules and may modulate progression of fibrosis. Furthermore, transient and site specific expression of TNC closely associated with myocardial injury and inflammation suggests not only its key roles during tissue remodeling but also that TNC can be a marker for myocardial disease activity.

Key words: Tenascin-C, Extracellular matrix, Remodeling, Fibrosis, Myocarditis, Myocardial infarction

Introduction

Living tissue is composed of heterogeneous cells and extracellular matrix (ECM) which is synthesized by those cells. Living tissues can dynamically remodel their structure by changing and rearranging their cellular and matrix components. Tissue remodeling is a widespread phenomenon which is essential for morphogenesis during embryonic development, physiological/pathological response to extrinsic stimuli, or tissue repair after injury. It is well recognized that, during this dynamic cellular activity, the extracellular matrix provides not only structural and mechanical support but also important biological signaling which influences cell motility, proliferation, differentiation, survival or apoptosis via cell-ECM interaction.

Among various ECM molecules, a category, matricellular proteins, has been introduced to emphasize their role as regulators of cellular functions (Bornstein, 1995; Bornstein and Sage, 2002). The group includes thrombospondin-1 and 2, SPARC (secreted proteins acid rich in cysteine; osteonectin), tenasin-C, X, osteopontin, and CCN (cyr-62,CTGF). Matricellular proteins are generally expressed at high levels during embryonic development and in response to injury. They do not contribute directly to the formation of structural elements such as fibrils or basement membrane but serve to modulate cell-matrix interaction and function as bioactive molecules especially during tissue remodeling. In this review, we will discuss about the interaction between cardiomyocytes and ECM in the myocardium, while focusing on the role of tenasin-C during tissue remodeling.

Linkage between cardiomyocytes and ECM in the normal heart

In myocardium, the major ECM components are type I and type III collagen, which form a continuous hierarchical fibrillar collagen network extending from the epicardium to endomysium, surrounding individual cardiomyocytes (Katz, 1989). In the normal heart, cardiomyocytes are firmly anchored to this extracellular framework with ‘costamere’ adhesion complexes which...
Cardiac remodeling

Cardiac remodeling consists physiologic and pathologic responses that may occur after myocardial infarction, inflammation, pressure overload (aortic stenosis, hypertension) and volume overload (Pfeffer and Braunwald, 1990; Braunwald and Bristow, 2000; Cohn et al., 2000). In cardiology, the term, cardiac remodeling is often manifested clinically as changes in left ventricular chamber volume associated with progressive heart failure after cardiac damage. Biologically, it involves structural alternation and rearrangement of cardiomyocytes, vascular cells, fibroblasts and interstitial connective tissue, followed by functional changes. Indeed, the cardiomyocyte is the major cell participating in the remodeling response (Cohn et al., 2000). Myocardial injury results in necrosis or apoptosis of cardiomyocytes, and surviving cardiomyocytes change connections among the cells and between the cells and the ECM, and become hypertrophied (Lorell and Carabello, 2000; Sutton and Sharpe, 2000). Necrosis of cardiomyocytes also elicits an inflammatory reaction, fibroblasts activation, and degradation and synthesis of ECM (Frangogiannis et al., 2002b). Consequently, the stroma in the lesions become fibrotic. The primary players in this fibroproliferative response are interstitial fibroblasts (reviewed in Manabe et al., 2002). Therefore, in ventricular remodeling, hypertrophy of cardiomyocytes and myocardial fibrosis occur usually simultaneously.

Tenascin-C in the heart

Tenascin-C (TNC) is a prototype member of a matricellular protein. It is specifically expressed during

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Fig. 1. Diagram of adhesion linkage between cardiomyocytes and ECM in the normal heart. Individual Z-lines of myofibrils are anchored to connective tissue by costamere adhesion complexes which include vinculin, talin, integrin α6β1 and laminin. When the myofibrils contract, the forces are transmitted to extracellular connective tissue through these adhesion complexes. Other molecules such as dystrophin, dystroglycan, and desmin might be also involved in forming the costamere adhesion complex.
embryonic development or early stages of tissue remodeling during inflammation, wound healing or cancer progression (for review, see Jones and Jones, 2000a,b; Chiquet-Ehrismann and Chiquet, 2003). TNC is a hexameric glycoprotein, each subunit consisting of an assembly domain; epidermal growth factor (EGF)-like repeats, a variable number of fibronectin type III (FN III) repeats containing alternative spliced domains, and a C-terminal fibrinogen-related domain. Alternative spliced domains of FN III repeats are known to have the ability to promote cell proliferation, migration or regulate cells attachment (Chung and Erickson, 1994; Chung et al., 1996; Fischer et al., 1997; Tsunoda et al., 2003). TNC is barely detected in the normal adult heart but transiently expressed at distinct sites closely associated with active tissue remodeling during embryonic development and a heart affected by a pathologic condition.

Possible roles during cardiac development

TNC appears in several important steps during the very early stage of cardiogenesis. At first, TNC is expressed by precardiac mesodermal cells when they differentiate into cardiomyocytes. Once the cells become differentiated and express sarcomeric proteins, they stop expressing TNC (Imanaka-Yoshida et al., 2003). It is also worth noting that TNC can be involved in coronary vasculo/angiogenesis and recruitment of fibroblasts. In adult tissues, several lines of evidence have suggested its important role in neovascularization in wound healing or cancer stroma (Zagzag et al., 2002). The origin of coronary vascular cells and interstitial fibroblasts differs from that of cardiomyocytes. While the primitive heart tube is formed from the lateral plate mesoderm, mesenchymal cells of the transverse septum form a cauliflower-like structure called the proepicardial organ (PEO) between the primitive heart and the liver bud (Fig. 2). When the primitive heart tube starts looping, aggregate cells detach from the PEO, transfer and spread over the heart, and give rise to coronary vessels and fibroblasts (Mikawa et al., 1992; Poelmann et al., 1993; Mikawa and Gourdie, 1996). When the cells start to migrate from the PEO toward the heart, they express TNC. Interestingly, once the cells are transferred onto the heart, the expression of TNC quickly diminishes (Imanaka-Yoshida et al., 2003). It appears there may exist a mechanism which inhibits TNC expression in the normal myocardium. The restricted expression of TNC strongly suggests its significant roles in cell differentiation or coronary vasculogenesis, however, hearts develop normally in TNC knockout mice (Saga et al., 1992; Forsberg et al., 1996; Imanaka-Yoshida et al., 2003). Obviously, there should exist a compensatory mechanism, although the evidence of upregulation of other genes to substitute the loss of TNC has not been identified yet (Mackie and Tucker, 1999). Alternatively, quick clearance of TNC at appropriate time points might be more critical for heart development.

TNC is re-expressed in pathological conditions

In the normal adult heart, TNC is not detected in the myocardium except at the chorda tendinae of papillary muscles (Sato and Shimada, 2001) and base of valve leaflets (unpublished data). However, TNC reappears under various pathologic conditions such as myocardial infarction (Willems et al., 1996; Imanaka-Yoshida et al., 2001a), myocarditis (Imanaka-Yoshida et al., 2002; Sato et al., 2002), hibernating myocardium (Frangogiannis et al., 2002a) and some cases of dilated cardiomyopathy (Tamura et al., 1996). A prominent feature is its expression is inevitably associated with cell injury and active inflammation. In the mouse autoimmune myocarditis model, immunoreactivity of TNC appears at the initial stage of myocytolysis, persists during the active stage, and disappears in the healed stage. TNC is always observed in the vicinity of damaged cardiomyocytes in foci of inflammation, and the level of expression reflects the extent of inflammation (Imanaka-Yoshida et al., 2002).

The specific expression suggests that TNC can be a marker for myocardial disease activity. Recently, we evaluated the diagnostic value of TNC expression in biopsy specimens from patients with myocarditis, and confirmed that immunoreactivity reflects clinical disease
activity as observed in animal models, which suggests its practical usefulness. Furthermore, using anti-TNC antibody labeled with $^{111}$Indium, we succeeded imaging the inflammatory lesion in a rat autoimmune myocarditis model in vivo (Sato et al., 2002). Immunoscintigraphic imaging or measuring serum TNC level by ELISA may provide a new noninvasive and accurate approach for the diagnosis of myocardial disease activity including myocarditis or rejection of the transplanted heart.

**TNC inducing factors**

During myocardial tissue remodeling under pathological conditions, cardiomyocytes never express TNC, but interstitial fibroblasts are the major source of TNC (Imanaka-Yoshida et al., 2001a, 2002). Various factors upregulate TNC expression. In general, inflammatory cytokines or growth factors such as TNF-$\alpha$, IFN-$\gamma$, TGF-$\beta$, CTGF, $\beta$FGF, IL-1, IL-4 can upregulate the expression of TNC (Rettig et al., 1994; Harkonen et al., 1995; Sakai et al., 1995; Makhluf et al., 1996; Latijnhouwers et al., 1998; Noda et al., 2000; Gore-Hyer et al., 2002). Angiotensin II, a key regulator in cardiovascular tissue remodeling, is also known to stimulate TNC synthesis (Mackie et al., 1992; Sharifi et al., 1992). Furthermore, oxidative stress (Aziz et al., 1997; Yamamoto et al., 1999, 2001) and hypoxia (Lal et al., 2001) induce TNC expression. Recently, we found that extracellular acidosis upregulates the expression of TNC in cardiac fibroblasts (Fig. 3). These in vitro data suggest that ischemia and reperfusion could induce TNC expression in the heart.

Another important TNC inducing factor is mechanical stress. TNC has been known to be present in musculoskeletal regions in which high mechanical forces are transmitted (Jarvinen et al., 2000). Several lines of evidence demonstrate that mechanical stress upregulates TNC expression in vitro. (Chiquet-Ehrismann et al., 1994; Chiquet, 1999, 2003; Yamamoto et al., 1999, 2001) In fact, the TNC gene promoter contains a mechano-responsive element which contains a GAGACC sequence motif like ‘shear stress response element’ in the PDGF-B gene (Chiquet, 1999).

**Roles of TNC during tissue remodeling after myocardial infarction**

The most well-known function of TNC would be anti-adhesion (for review, see Orend and Chiquet-Ehrismann, 2000). Earlier work demonstrated that TNC disrupts the interaction of cells with fibronectin (Chiquet-Ehrismann et al., 1986) and causes down-regulation of focal adhesion (Murphy-Ullrich et al., 1991). Recently, the effects of TNC on cell adhesion is understood to be rather more complex than simple disruption or inhibition of adhesion, and the term, “de-adhesion”, is preferentially used.

Murphy-Ullrich and co-workers classified strength of cell-ECM adhesion into three levels: (1) weak adherence (attached but not spread), (2) intermediate adherence (spreading cells without focal adhesion and stress fibers) and (3) strong adherence (with stress fibers and focal adhesion). De-adhesion was defined as the transition from strong adherence to intermediate adherence, and it was characterized by the disassembly of focal adhesion plaques and stress fibers (Greenwood and Murphy-Ullrich, 1998; Murphy-Ullrich, 2001).

Tissue remodeling after myocardial infarction is a good example to discuss the role of TNC in myocardium. During tissue repair after infarction, the most intriguing point is its exclusive localization at the border zone between the infarcted lesion and intact myocardium (Fig.4). Since the edge of the residual myocardium is the most active site of tissue remodeling, this characteristic localization suggests its particular

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**Fig. 3.** Effect of acidosis on expression of tenascin-C in rat cardiac fibroblasts. RNA was isolated from cells cultured in control (pH 7.4) and at 6, 12, and 24 hours after medium replacement at pH 6.6. The membrane was subjected to nothernblotting using a digoxigenin labeled cRNA probe. As a loading control, 28 S and 18 S rRNA were used.

**Fig. 4.** Immunostaining for tenascin-C in a rat myocardial section obtained 5 days after coronary ligation. Tenascin-C molecule deposits in the border zone between infarcted and intact areas.
Tenascin-C in the heart

role. In the normal heart, as we discussed above, cardiomyocytes join each other by intercalated disks, and are also linked laterally to the ECM with costamere adhesion. After myocardial infarction, borderline cardiomyocytes which lose neighboring cells must extensively rearrange cytoskeletons, cell shape, and form new cell-ECM attachments (Matsushita et al., 1999). For this active structural change, the residual cardiomyocytes must not be stably fixed. Therefore, it is fascinating to think that the de-adhesion molecule TNC deposited in the lesion may unfasten cardiomyocytes from their linkage to connective tissue. Our recent in vitro data suggest the mechanism to modulate adhesion of cardiomyocytes by TNC can be complex (Imanaka-Yoshida et al., 2001a). When cardiomyocytes isolated from an adult rat are plated on laminin coated glasses, the presence of TNC in the substratum increases the number of attached cells. Under interference reflection microscopy (IRM), these cell attachment sites mostly appear as gray contacts but do not show the striated distribution of dark contact corresponding to costameric adhesion. Under IRM, dark areas of the images indicate the distance between the ventral surface and the substratum is very close, about 10 to 15 nm, while gray areas indicate a separation of about 30 nm (Izzard and Lochner, 1980). This finding means that TNC shifts strong costameric adhesion to loose gray contacts. However, addition of exogenous TNC does not cause disruption of the stable costameric adhesion. Instead, TNC can upregulate transcription and activity of metalloproteinases (MMPs) in some types of cells (Tremble et al., 1994; Jian et al., 2001b; Kalembeyi et al., 2003). Cardiac fibroblasts are stimulated to upregulate MMPs (unpublished data).

Taken together, it seems likely that, during myocardial tissue repair, TNC at the border zone induces MMPs which degrade the linkage between residual cardiomyocytes and the surrounding matrix, leaving cardiomyocytes free to move. Simultaneously, TNC itself weakly adheres to cardiomyocytes. Adhering to the matrix is essential for most cells, because various important outside-in / inside-out signals, including survival signals, are transduced through the cell adhesion sites. Thus, even during tissue remodeling, cardiomyocytes need to somehow keep themselves attached under continuous mechanical stress during the contraction/relaxation cycle. TNC may tentatively attach the cells just like a ‘Post-it’ tape until they adapt to a new environment.

Furthermore, the TNC molecule possesses an elastic property (Oberhauser et al., 1998) and is suggested to act as a ‘shock absorber’ for mechanical stress (Chiquet et al., 2003; Jarvinen et al., 2003). This is another attractive possibility because borderzone myocardium is subjected to most heavy mechanical loading.

TNC may regulate recruitment of myofibroblasts

During wound repair of various tissues, residential fibroblasts at the edge of the injured tissue transform to myofibroblasts which migrate into the damaged area and produce procollagen and other ECM proteins as well as ECM-degrading enzymes (Gabbiani, 1998, 2003; Serini and Gabbiani, 1999; Powell, 2000). Myofibroblasts share characteristics with both fibroblasts and smooth muscle cells. They express smooth muscle specific proteins such as α-smooth muscle actin, have a well-developed contractile apparatus and exert mechanical forces on the matrix that minimize the wound area (Clark, 1996).

A close relationship between TNC and myofibroblasts in cancer stroma or repertories of myofibroblasts in normal tissue have been reported (Hanamura et al., 1997; Kalembey et al., 1997; Yoshida et al., 1997). Recently, we carefully compared the spatiotemporal distribution of myofibroblasts and TNC molecules during myocardial tissue remodeling, and found that TNC deposition precedes the appearance of myofibroblasts, and that myofibroblasts appear in the area where TNC molecules are deposited (Imanaka-Yoshida et al., 2001a). This observation indicates that TNC may regulate the recruitment of myofibroblasts. Previous reports have shown that TNC stimulates the migration and proliferation of cancer cells (Yoshida et al., 1999; Tsunoda et al., 2003). In culture, TNC enhances migration and expression of α-smooth muscle actin of cardiac fibroblasts (unpublished data). TNC could be a major modulator of recruitment of myofibroblasts at an early stage of myocardial tissue repair.

Interactions between TNC and other remodeling-related proteins during progression of fibrosis

Myocardial fibrosis as well as hypertrophy of cardiomyocytes is often observed in ventricular remodeling. Fibrosis is defined as the increase of fibrillar collagen in intermyocardial spaces. Myocardial fibrosis has been classified into two groups: replacement fibrosis and reactive fibrosis (Weber et al., 1989). In replacement fibrosis, myocytes necrosis elicits an inflammatory reaction and myocardial dropout is filled by collagen fibers. In contrast, in case of reactive fibrosis, fibrillar collagen surrounds individual cardiomyocytes increases without the loss of cardiomyocytes. Histologically, it seems that collagen fibrils start to increase in the perivascular region in the myocardium and eventually extend among individual myocytes. This type of fibrosis is often observed in the pressure-overloaded hypertrophied heart. Recently, low grade inflammation of small arteries, in part, initiated by local activation of the renin-angiotensin-aldosteron system, has received much attention as a trigger of reactive fibrosis (Stier et al., 2002; Virdis and Schiffrin, 2003).

Fibrotic lesions do not form by abrupt deposition of collagen molecules, but through multiple steps of synthesis and degradation of various matrix proteins.
which are the consequence of numerous cell-ECM and ECM-ECM interactions. TNC is one of the acute stage proteins of the cascade of progression of fibrosis and has been supposed to work as a scaffold for collagen deposition (Yamada et al., 1992; Imanaka-Yoshida et al., 2001a,b; 2002). TNC not only controls cellular activity but also interacts with other ECM molecules directly or indirectly. TNC is often co-localized with FN in cancer stroma (Hanamura et al., 1997; Yoshida et al., 1997), and binds to soluble FN, FN fibrils in vitro (Chiquet-Ehrismann et al., 1991; Chung et al., 1995). TNC also binds to several proteoglycans (Chiquet and Fambrough, 1984; Vaughan et al., 1987, 1994; Salmivirta et al., 1991; Barnea et al., 1994; Grumet et al., 1994; Milev et al., 1994). Binding to a heparansulphate proteoglycan, perlecain, mediates the incorporation of TNC into FN fibrils (Chung and Erickson, 1997). Furthermore, TNC causes a reduction in the assembly of an FN matrix by fibroblasts through regulation of Rho GTPase activity or FAK phosphorylation (Wenk et al., 2000; Midwood and Schwarzbauer, 2002). TNC also inhibits FN-induced integrin signaling by interfering with the binding of FN to syndecan-4 (Huang et al., 2001). Therefore, some functions of TNC such as down-regulation of focal adhesion may be due to attenuation of cellular signaling from FN.

TNC often co-expresses with MMPs in areas of active tissue remodeling in pathological conditions (Cowan et al., 1999; Streuli, 1999; Jian et al., 2001a). In addition to its potential to induce MMPs, TNC can enhance the effect of TGF-β on MMP-9 induction in breast cancer cells (Kalembeyi et al., 2003). Conversely, denaturation of native collagen type I by MMP upregulates TNC expression in smooth muscle cells (Jones et al., 1997). These findings indicate reciprocal regulation between TNC and MMPs which degrade ECM molecules.

Conclusion and future directions

Traditionally, “cardiac remodeling” has been considered as changes associated with progressive heart failure and is often regarded as a detrimental process almost equal to ventricular dilatation. However, the term itself is neutral and should describe both favorable and unfavorable changes. Recently, imbalance of MMPs and inhibitors of MMPs (TIMPs) have received much attention in progression of unfavorable cardiac remodeling (Mann and Spina, 1998; Mann, 2002; Spina, 2002; Heeneman et al., 2003). Conceptually, excessive activation of MMPs might cause progressive degradation of ECMs and slippage of myocytes within the LV wall, and resulting the LV wall thinning and dilatation.

Overall, is TNC harmful or beneficial for myocardial remodeling? Induction of MMPs and de-adhesion by TNC might cause LV dilatation. On the other hand, TNC enhances the recruitment of myofibroblasts and possibly supports collagen fibers deposition. Logically, an increase of myofibroblasts and acceleration of fibrosis generate traction forces that prevent ventricular dilatation. However, excessive fibrosis would lead to a stiffer and less compliant ventricle. Therefore understanding the function of TNC in myocardial remodeling may not be straightforward. Nevertheless, TNC can be a key molecule to explore and diagnose cardiac remodeling and might be a potential therapeutic target to control the balance of beneficial and undesirable cellular responses.

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