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The role of Tenascin C in cardiovascular disease

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Abstract

The extracellular matrix protein tenascin C (TnC) is expressed in a variety of embryonic tissues but its expression in adult arteries is co-incident with sites of vascular disease. TnC expression has been linked to the development and complications of intimal hyperplasia, pulmonary artery hypertension, atherosclerosis, myocardial infarction and heart failure. This review identifies the growing collection of evidence linking TnC with cardiovascular disease development. The transient upregulation of this extracellular matrix protein at sites of vascular disease could provide a means to target TnC in the development of diagnostics and new therapies. Studies in TnC deficient mice have implicated this protein in the development of intimal hyperplasia. Further animal and human studies are required to thoroughly assess the role of TnC in some of the other pathologies it has been linked with, such as atherosclerosis and pulmonary hypertension. Large population studies are also warranted to clarify the diagnostic value of this extracellular matrix protein in cardiovascular disease, for example by targeting its expression using radiolabelled antibodies or measuring circulating concentrations of TnC.

Introduction

Tenascin C (TnC) is a large extracellular matrix glycoprotein and was the first member identified of a family of 4 structurally similar proteins including tenascin R, W and X [1-3]. During early development TnC is transiently expressed at a number of sites throughout the embryo, such as neural crest, central nervous system, lungs and cardiovascular system [1]. Despite this implied function during embryogenesis, knock out mice models of TnC grow to maturity without any overt signs of abnormalities [4]. In normal adult tissue only low levels of TnC are found. Higher levels of TnC expression have been reported in areas of wound healing, cancer development and cardiovascular disease [1]. Given this localisation of TnC to sites of pathology, there has been increasing interest in assessing the role of this glycoprotein in disease development and targeting the protein in both diagnosis and therapy for a variety of pathologies [1]. In this review we summarise previous studies which have examined the expression and potential influence of TnC in cardiovascular disease.

Structure of TnC

The structure of tenascin C is relevant to its functions in health and disease and has been described in detail in previous reviews [1-3]. TnC polypeptides are made up of a number of domains (Figure 1a) and include:

a) An amino-terminal Tn assembly domain (TA) which is responsible for interactions between TnC polypeptides important in assembly of the multimeric protein;

b) A contiguous group of repeats of epidermal growth factor-like domains;

c) A series of fibronectin type III domains;

d) A distal globular fibrinogen-homology domain.

While TnC is encoded by a single gene located at 9q33 in man, alternative splicing of mRNA can result in a large number of different isoforms with between 1 and 6 extra fibronectin type III domains (A1, A2, A4, B, C and D) (Figure 1b). Ultimately six TnC polypeptides can be assembled

into a six armed structure referred to as a hexabrachion via interaction at the TA domains. This form of TnC has been identified within the extracellular matrix such as that present during embryonic development. The relative expression of different forms of TnC present in diseased adult tissue and circulating in the blood has been poorly described.

TnC interactions and signalling pathways

Associated with its complex structure TnC has the capacity to interact with several different cell surface receptors. Different parts of the TnC protein have been ascribed to binding different receptors [1,3]. The epidermal growth factor-like domains can bind the epidermal growth factor receptor. The third fibronectin type III repeat binds αvβ3 and other integrins promoting adhesion. The variable spliced A-D fibronectin type III repeats bind annexin II thereby inhibiting adhesion. The variable splice region has also been shown to interact with F3/contactin and α7β1 integrin [3]. Thus different isoforms of TnC would be expected to have different functional effects although this has not been clearly defined.

***In vitro* studies assessing the determinants of TnC production and the interaction of TnC with vascular cells**

The determinants of TnC expression have been examined *in vitro* in a variety of cells relevant to vascular disease, including vascular smooth muscle cells (VSMCs), endothelial cells and monocyte-macrophages (Table 1) [11-32]. Overall, a range of factors implicated in cardiovascular disease, including cytokines, angiotensin II and haemodynamic forces appear to be able to upregulate TnC expression *in vitro*. A number of medications have been reported to reduce TnC expression including steroids, cilostazol and non steroidal anti-inflammatory drugs [18,21,30]. Identified intra-cellular regulators of TnC expression in vascular cells include homeobox transcription factor Prx1, Rho and extracellular signal-regulated kinases [17,24-26]. TnC expression has been shown to be under

post-transcription control in non-vascular sites, such as within breast cancer metastases, where micro RNAs, including miR-355, have been shown to control TnC expression [33].

The actions of TnC have also been examined *in vitro* employing a range of cell types and TnC fragments (Table 2) [16,26,27,34-56]. TnC has been reported to promote angiogenesis and release of pro-inflammatory cytokines and MMPs. TnC has also been reported to inhibit T cell proliferation and activation *in vitro*. The effects of TnC within *in vitro* studies seem to vary according to the fragment of TnC employed and the cell type studied. The region of TnC which contains the fibronectin type III repeats, and which varies by isoform type (Figure 1), appears to control the ability of TnC to influence cell adhesion [27,46,48,52,53]. The epidermal growth factor-like domains of TnC have been suggested to control cell survival, while the distal globular fibrinogen-homology domain has been associated with stimulating cytokine production [8,37].

Animal studies examining the expression and role of TnC in cardiovascular disease

The association of TnC with a range of cardiovascular pathologies has been examined in murine, lapine, porcine, bovine and canine models of human cardiovascular diseases (Tables 3-5) [11,16,21,23,27,29,39,40,57-76]. The most common pathology studied has been intimal hyperplasia (Table 3) [11,21,29,39,57-63]. TnC has been implicated in the development of intimal hyperplasia following angioplasty, stenting, arteriotomy and bypass grafting in animal species as diverse as mice and pigs [11,21,29,39,57-63]. TnC is expressed very rapidly following arterial injury in these models and its expression is reduced in situations where intimal hyperplasia is inhibited such as prostaglandin E2 deficiency or treatment with a nitric oxide donor [11,63]. Importantly, intimal hyperplasia has been reported to be reduced in two distinct mouse models of TnC deficiency suggesting that this protein plays an active role in this pathology [57,58]. Indeed in one study that employed arterial grafts placed in the carotid artery, a reduced proliferation of neointimal cells was demonstrated in TnC deficient

by comparison to wild type mice [57]. This same research group reported a similar finding of reduced number and proliferation of neointimal cells after aortotomy in TnC deficient mice [58].

Studies in rodent, pig and dog models of myocardial infarction have demonstrated that TnC is highly expressed from approximately day 1 to day 14 within the peri-infarct area. This has promoted interest in developing diagnostic aids that incorporate antibodies targeting this protein (Table 4) [40,64-69]. The TnC expression has been linked to an exaggerated repair process after myocardial infarction with reduced interstitial fibrosis reported in TnC deficient mice following coronary artery ligation [64]. TnC deficient mice also have reduced myocardial stiffness on echocardiography after myocardial infarction [64]. TnC expression has also been positively linked to a range of other cardiovascular pathologies, including atherosclerosis; pulmonary artery hypertension; neovascularisation; the peri-infarct repair process following stroke; angiotensin II induced cardiac fibrosis; vasospasm following subarachnoid hemorrhage and vascular calcification (Table 5). [16,23,27,29,64,70-76]. In keeping with *in vitro* findings noted earlier, neovascularisation has been reported to be reduced in TnC deficient mice, suggesting TnC promotes angiogenesis [16].

To summarise, studies in animal models most clearly support a role of TnC in intimal hyperplasia although the exact mechanisms for this are unclear. Although TnC is associated with many other cardiovascular pathologies in animal models clear evidence that links TnC with their development and outcomes is currently lacking.

Human studies examining the expression of TnC in relation to cardiovascular disease

A large number of studies have examined the expression of TnC in biopsies removed from patients with a variety of cardiac and other cardiovascular diseases (Tables 6 and 7) [15,16,26,37,40,44,77-89]. TnC expression within athero-thrombosis has been associated with acute coronary syndrome [16,44,77]. TnC staining was localised in areas of plaque rupture and macrophage

infiltration. Similar to animal studies, TnC expression has also been localised within areas of intimal hyperplasia (at sites of coronary restenosis or in saphenous vein coronary artery bypass grafts); myocardial infarction; cardiomyopathy; and coronary valve calcification [40,44,78-81]. High tissue levels of TnC have also been reported within a range of other cardiovascular pathologies including carotid atherosclerosis, pulmonary artery hypertension, abdominal aortic aneurysm, renal access graft intimal hyperplasia, renal transplant vasculopathy and varicose veins [15,26,37,82-89]. In contrast to the large number of studies examining the expression of TnC in tissue biopsies, there have been fewer investigations of the association of circulating concentrations of TnC with cardiovascular disease [75,90-96]. The serum or plasma concentration of TnC has been reported to be increased in patients with a range of cardiac problems, including acute myocardial infarction, pulmonary thromboembolism, pulmonary artery hypertension, left ventricular hypertrophy and dilated cardiomyopathy compared to controls in cross-sectional studies (Table 8) [90-96]. Overall, the number of subjects included in these studies has been small however with a total of only 408 cases and 136 controls included in the independent cross-sectional studies identified in this systematic review (Table 8). The TnC isoform measured in these studies has varied but in most instances appears to have been the high molecular weight isoform containing the fibronectin type III C domain. Assays have been performed using commercial enzyme linked immunoassays from two different companies [92-94]. The circulating TnC concentration has not only been reported to be increased in patients with cardiac disease but also related to specific clinical findings, imaging results and subsequent outcomes in these patients [92-98]. Serum TnC concentration has, for example, been correlated with New York Heart Association functional class and left ventricular ejection fraction in patients with heart failure [93,95]. Serum TnC has also been reported to predict the prospective incidence of cardiovascular events in patients who have recently had a myocardial infarction, have heart failure or chronic kidney disease [94,97,98]. The reported area under the curves of receiver operator characteristic (ROC) curves in these studies were between 0.77 and 0.79 [94, 98]. These findings suggest that most likely serum TnC would need to be combined with other clinical

and biomarker predictors to be of clinical value. In summary data from human association studies fit with animal data linking TnC with a range of cardiovascular diseases although the therapeutic and diagnostic value of these associations has little examined.

Association of genetic polymorphisms in the gene encoding TnC and cardiovascular disease

TnC is encoded by a large gene composed of 28 exons spanning nearly 100kB on Chromosome 9 (NCBI Nucleotide database Ref Seq NC_000009). Its transcription is directed by a single promoter and regulated by both positive and negative elements located in the first untranslated exon [99], which is separated from the translation initiation site in exon 2 by a large intron of approximately 18kb [100]. There are 1167 polymorphisms located in and around the gene, 67 of which affect the coding region (NCBI dbSNP), however, their functional consequences and association with cardiovascular disease has not been thoroughly investigated. It is tempting to speculate that inheritance of particular polymorphic variants could influence expression levels of TnC and account for some of the individual variation in risk of cardiovascular disease. A genome-wide association study of genes for biomarkers of cardiovascular disease identified rs17819305, located in intron 15 of the TnC gene, as being associated with gammaglutamyl transferase levels in 1955 hypertensive subjects [101]. Otherwise, there has only been a single published study specifically examining the association of genetic polymorphisms in *TNC* and cardiovascular disease, and this did not include rs17819305 [102]. Minear and colleagues genotyped a total of 35 single nucleotide polymorphisms (SNPs), including 21 haplotype tagging SNPs, in a range of subjects that had been assessed for different measures of atherosclerosis. The subjects examined included 205 heart transplant donors who had provided ascending aortic samples; 1325 patients who had undergone coronary angiography to assess severity of coronary atherosclerosis; and 879 families with a history of coronary heart disease. Three SNPs, rs3789875, rs12347433 and rs4552883, representing a block of linkage disequilibrium were significantly associated with aortic atherosclerosis plaque presence in the heart transplant donors and coronary heart disease in the two large subject groups. One of these SNPs, rs12347433,

is a synonymous polymorphism causing a change in the mRNA without affecting the amino acid sequence of the TnC protein. This type of synonymous polymorphism has been suggested to alter mRNA function or stability which could alter translation and thus TnC expression. However, none of these SNPs were associated with TnC expression measured by microarrays within the 104 patients in which aortic RNA was available, suggesting these polymorphisms may be acting via mechanisms unrelated to aortic concentration of TnC mRNA.

Summary and future directions

A large number of studies suggest that TnC is transiently expressed in association with a range of cardiovascular diseases in both animal models and patients. Whether this association is part of the repair process or pathological is not completely resolved in most instances. Studies from TnC deficient mice suggest that in the case of intimal hyperplasia (perhaps the best studied example) that TnC plays a pathological role, most likely because of the ability of TnC to promote MMP production, and VSMC proliferation and chemotaxis [26,40,43,50,57,58]. The role of TnC in atherosclerosis is less clear cut although a number of findings (such as its expression at sites of plaque rupture, its involvement in neovascularisation and its ability to influence VSMC phenotype and pro-inflammatory cytokine/ MMP production) would suggest it may play a role in promoting the development and complications of this pathology [8,16,42,44,51,77]. We identified no studies examining TnC deficiency, overexpression or inhibition on atherosclerosis progression in animal models. Studies of this type are required to provide further insight on the role of this extracellular matrix protein in cardiovascular disease. The rapid upregulation of TnC following ischemia events, such as myocardial infarction, suggests the possibility of targeting TnC as a diagnostic or prognostic aid in patients with cardiovascular disease, e.g. as a circulating or tissue biomarker [81,94]. Further studies in larger populations are however required to assess the feasibility and clinical value of such an approach.

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Legend to the figure

Figure 1: Structure of Tenascin C.

(a) This diagram has been adapted from previous work using predicted domain boundaries to determine the overall structure of the protein. A recent study suggested the earlier delineations derived from reverse transcription polymerase chain reaction and Western blotting ((b) shown below the main structure) had several flaws and did not correlate to the natural domain boundaries particularly in the A1-4 region [5]. The N-terminal domain is called the tenascin assembly domain (TA) and is involved in the formation of the quarternary hexabrachion structure. Within this region there is a heat shock protein 33 motif probably responsible for TnC aggregation within the cell [5]. The next region includes 14 epidermal growth factor (EGF) like repeats which are quite consistent. The EGF-like repeat domain modulates cell adhesion and cell motility [6]. This region is considered to be counter adhesive for fibroblasts, neurons and glia and may be involved in neuronal migration and axon path finding during development [1]. The following region contains the fibronectin (FN) III like repeats. The FN-III repeats vary considerably in amino acid sequence and have a variety of ligands [7]. The final C-terminal domain is the fibrinogen (FG)-like domain. This domain is the region of the protein that binds to toll-like receptor (TLR)-4 as an endogenous ligand [8]. Due to alternative splicing of pre mRNA of the FN III-like repeats, 6-12, TnC exists as a number of isoforms with varying functions and sizes. The smallest isoform has a predicted molecular weight of 171.3 kDa and is missing repeats 6-12. The largest isoform with a predicted molecular mass of 240.8 kDa has all the FN III-like repeats included [9]. TnC is also glycosylated [10] giving rise to the range of sizes reported for the various isoforms e.g. the large isoform has a reported size range of 280-350kDa.

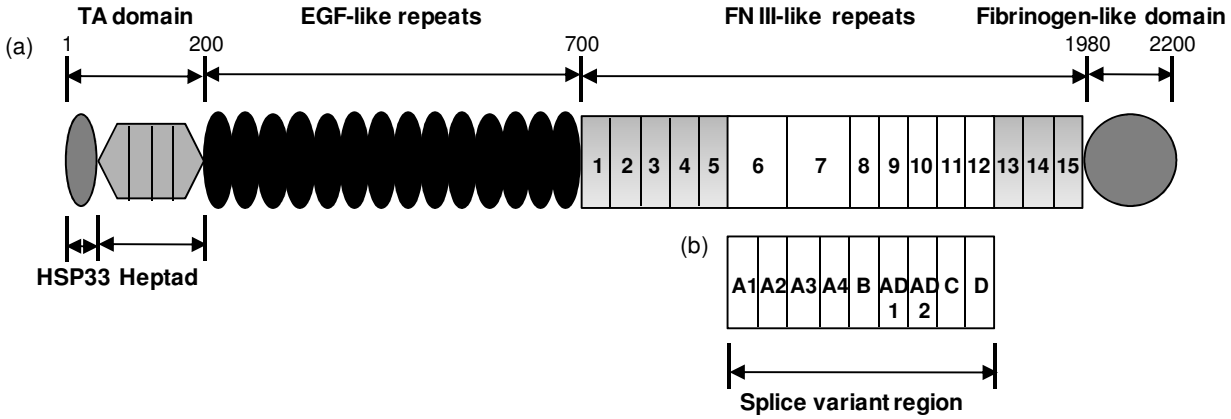

Table 1: Determinants of TnC expression *in vitro* in a variety of cells relevant to cardiovascular disease

Upregulators of TnC	Cell type studied	TnC form induced
Prostaglandin E2	Mouse VSMCs [11]	mRNA
LPS and other TLR ligands	Monocyte derived cells such as macrophages [12]	mRNA and protein
Wnt pathway	Mouse pulmonary artery VSMCs [13]	mRNA and protein
CD137 ligation	RAW264.7 (murine myeloid cell line) [14]	mRNA
ERK 1/2 mitogen-activated protein kinases	Human pulmonary artery VSMCs [15]	mRNA and protein
RhoA and Rho kinase ROCK	Rat pulmonary artery endothelial cells [16,17]	mRNA
Interleukin-4	Human peripheral blood derived macrophages [18]	mRNA
Cyclic stretch	Human aortic VSMCs Rat aortic VSMCs [19,20]	mRNA and protein
Platelet derived growth factor	Rat aortic VSMC [21-23]	mRNA and protein including three isoforms (210, 220 and 250 kDa)
Prx1 (homeobox transcription factor)	VSMC cell line [24]	mRNA
Denatured collagen (via β3 integrin and ERK 1/2)	VSMC cell line [25,26]	mRNA and protein
Angiotensin II	Human aortic VSMC	mRNA and protein

	Rat aortic VSMC Human aortic endothelial cells [22,23,27,28]	
Transforming growth factor beta	Human aortic VSMC Rat aortic VSMC Human aortic endothelial cells [23,27]	mRNA and protein
Downregulators of TnC	**Cell type studied**	**TnC form downregulated**
Shear stress mimicking atheroprone flow	Human iliac vein endothelial and VSMC co-culture [29]	mRNA
Dexamethasone	Human peripheral blood derived macrophages [18]	mRNA
Cilostazol	Rat aortic VSMC [21]	mRNA
Glafenine hydrochloride (NSAID)	Human aortic VSMCs [30]	Protein
9-cis retinoid acid	Human aortic VSMCs [31]	Protein
Polymerised (compared to monomer) type 1 collagen	Human umbilical artery VSMCs [32]	mRNA

TnC, Tenascin C; LPS, Lipopolysaccharide; TLR, Toll-like receptor; Wnt, Wingless; VSMC, Vascular smooth muscle cell; NSAID, Non-steroidal anti-inflammatory drugs.

Table 2: Reports of the effects of TnC on cells relevant to cardiovascular disease *in vitro*

TnC form or intervention	Cell type	Effects
TnC fragment (A2 isoform)	Human dermal microvascular endothelial cells	Proliferation inhibited [36]
TnC from commercial company (Chemicon)	Rat cardiac microvascular endothelial cells	Promotes response to angiogenic signals such as PDGF and VEGF [16]
TnC from commercial company (Chemicon)	Bovine and human retinal endothelial cells	Promotes endothelial cell tube formation and branching [38]
Recombinant chick TnC	Bovine aortic endothelial cells	Stimulates actin cytoskeletal reorganisation typical of sprouting endothelial cells [45]
Large and small splice variants of TnC	Bovine aortic endothelial cells	TnC fragment containing Fn A-D induces loss of focal adhesion by binding annexin II [52,53]
TnC blocking antibody	Bovine aortic endothelial cells	Inhibits signs of angiogenesis such as sprouting cells [54]
TnC from a cell line	Human umbilical endothelial cells	Binds to α2β1 and αvβ3 integrins [55]
Large isoform of TnC	Rat and Human VSMC	Upregulates MMP-2 which cleaves TnC [37]
EGF-like TnC domain	Rat and Human VSMC	Induces apoptosis [37]
Recombinant A1A2 isoform	Rat VSMC	Promotes VSMC chemotaxis

		(unlike other TnC isoforms) [39]
TnC isolated from a glioma cell line	Adult rat cardiomyocytes	Promotes cardiomyocyte attachment to laminin [40]
TnC antisense oligonucleotide	Rat pulmonary arteries in organ culture	Promotes VSMC apoptosis and upregulates osteopontin expression [43]
Human TnC from commercial company (Chemicon)	Rat pulmonary artery VSMCs	Stimulates proliferation and survival via αVβ3 integrin [26,50]
TnC fragment containing Fn A-D	Human aortic VSMC Human aortic endothelial cells	Reduces focal adhesion VSMC> endothelial cells [27]
TnC deficient mouse	Mouse macrophages	Behaved as wild type macrophages in response to TGFβ1 [34]
Human recombinant TnC (fibrinogen-like globe)	Human macrophages	Stimulated TNFα, IL-6 and IL-8 production [8]
TnC extracted from chick embryo brains	Human polymorphonuclear leukocytes and monocytes	Inhibited chemotaxis via α5β1 integrin [42]
TnC from commercial company (Chemicon)	Human monocyte-macrophages	Stimulates MMP-9 secretion [44]
TnC from commercial company (Life Technologies)	Mouse macrophage cell line (RAW264.7)	Stimulates MMP-9 expression [51]
TnC from chick embryo fibroblast cultures	Human monocytes and T lymphocytes	Inhibited monocyte adhesion to fibronectin and T cell

		activation by alloantigens not anti-CD3 antibody [56]
TnC isolated from U251 glioma cell line and recombinant fragments	Human T lymphocytes	TnFnIII A1A2 inhibits T cell activation [41]
Recombinant TnC fragments	Human T lymphocytes	TnfnIII 1-5 inhibits αVβ1 and α4β1 mediated adhesion to fibronectin [46]
TnC isolated from U251 glioma cell line (Chemicon)	Human T lymphocytes	Inhibited anti CD3 induced cell proliferation [47]
Recombinant TnC fragments	Human T lymphocytes	Supports tethering and rolling via binding to the terminal fibrinogen like domain of TnC in a parallel-plate flow chamber [48]
Plasmin cleaved TnC	Human T lymphocytes	Plasmin cleavage of TnC converts it from a nonadhesive to an adhesive substrate for T cells [49]
TnC isolated from U251 glioma cell line	Human platelets	Platelets adhere to and are activated by TnC [35]

TnC, Tenascin C; PDGF, Platelet derived growth factor; VEGF, Vascular endothelial growth factor; MMP, matrix metalloproteinase; TGF, Transforming growth factor; TNF, Tumour necrosis factor; IL, Interleukin; TnfnIII, Tenascin C fibronectin type III repeat domain; VSMC, Vascular smooth muscle cells.

Table 3: Association of TnC expression with intimal hyperplasia in animal models

Intimal hyperplasia model and species	Findings at site of intimal hyperplasia
Wire femoral artery injury; mouse	Reduced intimal hyperplasia in PGE_2 deficient mouse associated with reduced TnC mRNA expression [11]
Abdominal aorta-to-carotid artery interposition grafting; mouse	Reduced neointimal hyperplasia in TnC deficient mice [57]
Longitudinal aortotomy; mouse	TnC deficient mice have reduced intimal hyperplasia [58]
Balloon aortic injury; rat	Increased TnC protein expression first within the media and later the neointima [29]
Arterial graft; rat	Topical cilostazol inhibits intimal hyperplasia associated with decreased TnC protein expression [21]
Balloon carotid injury; rat	Increased AIA2 TnC isoform mRNA and protein expression associated with intimal hyperplasia [39]
Balloon carotid injury; rat	TnC protein expression increased within the intima after balloon injury [59]
Balloon carotid injury; rat and pig	Increased TnC mRNA and protein expression in adventitial myofibroblasts early and intima late after injury [60]
Left coronary artery stenting; miniature pig	TnC mRNA and protein expression associated with the severity of intimal hyperplasia [61]
Coronary artery angioplasty; pig	Upregulation of TnC mRNA within 2 h of injury [62]
Jugular vein grafts implanted in carotid artery; hypercholesterolemic rabbit	Topical nitric oxide donor reduces intimal hyperplasia and TnC protein expression within graft [63]

TnC, Tenascin C; PGE_2, Prostaglandin E2.

Table 4: Association of TnC expression with post myocardial infarction changes in animal models

Myocardial infarction model and species	**Findings**
Coronary artery ligation; mouse	TnC deficient mice had less interstitial fibrosis in peri-infarct areas [64]
Temporary left coronary occlusion; mouse	Smad 3 deficient mice had reduced myocardial TnC protein expression post infarction [65]
Temporary left coronary occlusion; rat	(125)I-labeled anti-TnC antibody uptake at 1-3 days after infarction; reduced by 7 days [66]
Left coronary ligation; rat	111In anti-TnC antibody uptake increased day 1-5 following infarction [67]
Coronary artery ligation: rat	TnC mRNA and protein expression in fibroblasts in the area of infarction within 24 hours which disappears by 14 days- involvement in the phases of MI healing [40]
Left anterior descending artery occlusion; pig	Mesenchymal stem cell injection association with cardiac TnC protein upregulation and increased cardiac nerve density (a possible source of arrhythmia) [68]
Temporary coronary occlusion; dog	Cardiac TnC protein upregulated [69]

TnC, Tenascin C; MI, Myocardial infarction.

Table 5: Association of TnC expression with a range of cardiovascular pathologies in animal models

Cardiovascular pathology	Model and species	Findings
Atherosclerosis	Apolipoprotein E deficient; mouse	Increased TnC protein expression in areas of atheroma [29]
Atherosclerosis	Apolipoprotein E deficient; mouse	Increased TnC protein staining in areas of atheroma and activated macrophages [70]
Atherosclerosis	Spontaneously hypertensive; rats	Aortic TnC protein staining increased with age, hypertension, and at branch points [23,27]
Pulmonary artery hypertension	Chronic hypoxia induced PAH; rat and calf	Circulating monocyte/ macrophage precursors contribute to production of TnC [71]
Pulmonary artery hypertension	Monocrotaline induced PAH; rat	Upregulation of pulmonary artery TnC protein associated with PAH; Endothelin B deficiency promotes PAH and TnC protein expression [72]
Pulmonary artery hypertension	Pulmonary artery ligation; pig	Increased pulmonary TnC mRNA and protein expression [73]
Neovascularisation	Bone marrow transplant plus intramyocardial PDGF injection; mouse	Donor derived cells recruited to the heart within 24h of PDGF injection at sites of TnC protein expression [16]
Neovascularisation	Cardiac transplant; mouse	TnC deficient mice reduced neovascularisation [16]
Cerebral infarction	Middle cerebral artery occlusion; rat	Genomic study of peri infarct cortex showed upregulation of TnC mRNA [74]

Cardiac fibrosis	Angiotensin II infusion; mouse	Cardiac fibrosis associated with increased TnC mRNA and protein expression [64]
Sub-arachnoid hemorrhage	Cisternal injection of blood; rat	TnC protein staining increased at sites of artery vasospasm [75]
Vascular calcification	Subdermal injection of elastin; rat	Increased TnC protein staining along with MMPs at sites of calcification [76]

TnC, Tenascin C; PAH, Pulmonary artery hypertension; PDGF, Platelet derived growth factor; MMP, Matrix metalloproteinase.

Table 6: Studies examining the expression of TnC in biopsies taken from patients with cardiac disease

Number of cases and controls	Biopsies	Cases	Controls	Findings
4	Coronary artery thrombus	Acute coronary syndrome	None	TnC expressed within coronary artery thrombus and co-localises with EPC marker Tie-2 [16]
51	Coronary atheroma	Acute coronary syndrome	Stable angina	TnC staining area larger in atheroma from patients with ACS and correlated with thrombus, angiogenesis, intraplaque hemorrhage and macrophage/ lymphocyte infiltration [77]
15	Coronary atheroma	Patients having coronary bypass surgery	Internal thoracic artery from the same patients	TnC staining in areas of plaque rupture and correlated with macrophage infiltration [44]
20	Right atrial auricle	Valvular heart disease	Stable coronary heart disease	TnC expression increased in biopsies with more severe histological evidence of cardiac damage [78]

22	Aortic and pulmonary valves	Ischemic or dilated cardiomyopathy	PM cases with no history of cardiac disease	Cardiomyopathy cases increased TnC expression [79]
12	Aortic valve cusps	Valvular heart disease having valve replacement	PM cases with no history of cardiac disease	Increased TnC in calcified valves [80]
43	Coronary artery stenoses obtained by atherectomy	Restenosis after coronary angioplasty	Primary coronary stenoses	TnC expression increases transiently within 1 month of coronary angioplasty [40]
20	Coronary bypass grafts	Heart transplant recipients	Saphenous vein from patients undergoing coronary bypass	TnC protein expressed within the adventitia and media of patent vein grafts but not within occluded vein grafts or non-arterialised control saphenous veins [44]
40	Myocardial biopsies	Myocardial infarction	Normal myocardium	TnC expressed post MI up to 3 weeks after [81]

EPC, endothelial progenitor cells; ACS, acute coronary syndrome; TnC, tenascin C; PM, post mortem; MI, myocardial infarction.

Table 7: Studies examining the expression of TnC in biopsies taken from patients with a variety of cardiovascular diseases

Number of cases and controls	Biopsies	Cases	Controls	Findings
20	Carotid atheroma and control 'normal' iliac artery	Patients undergoing carotid endarterectomy	Patients having AAA repair	Staining for TnfnIII marked in atherosclerotic plaques and particularly macrophage rich areas [82]
16	Carotid atheroma	Patients undergoing carotid endarterectomy	None	Large (280kD) and small (220kD) TnC isoforms and 85 and 65kD EGF-like domain fragments detected [37]
10	Long saphenous vein	Patients undergoing varicose veins surgery	Patients undergoing coronary bypass surgery	Upregulation of TnC [83]
NS	Long saphenous vein	Patients undergoing varicose veins surgery	Patients undergoing coronary bypass surgery	Increased intimal TnC expression [84]
18	Pulmonary artery	Familial pulmonary artery hypertension	None	TnC highly expressed in all biopsies [15]
7	Pulmonary	Pulmonary artery	None	TnC staining correlates with

	artery	hypertension		grade of pulmonary artery pathology (Heath-Edwards grading) [26]
17	Infra-renal aorta	Patients undergoing AAA repair	Organ donors	TnC upregulated in AAA [85]
23	Infra-renal aorta	Patients undergoing AAA repair	Patients undergoing aortic bypass for occlusive disease	Increased staining for TnC in AAA samples association with adventitial inflammation and neovascularisation [86]
15	Thoracic aortic biopsies	Marfan syndrome and bicuspid aortic valve undergoing thoracic aortic aneurysm repair	NS	Reduced TnC expression by VSMCs from aneurysm biopsies [87]
12	Graft stenoses	Failed PTFE loop arterio-venous grafts	None	TnC staining marked in luminal layer of intimal hyperplasia at the site of cell proliferation based on proliferating cell nuclear antigen expression [88]
10	Renal arteries	Failed kidney transplants	None	Increased TnC expression observed in media early in rejection process [89]

EGF, Epidermal growth factor; TnC, Tenascin C; AAA, Abdominal aortic aneurysm; VSMC, Vascular smooth muscle cells; PTFE, Polytetraflurorethylene; NS, Not stated.

Table 8: Case-control studies examining the association of circulating TnC concentrations with cardiac disease

Cases			**controls**			**Sample**	**Other findings**
Diagnosis	**N**	**TnC (ng/ml)**		**N**	**TnC (ng/ml)**		
Pulmonary thromboembolism [90]	34	120±38*	Healthy volunteers	20	16±3	Plasma	
Pulmonary artery hypertension [91]	36	111±13*	Age and gender matched healthy volunteers	44	44±2	Plasma	AUC of ROC curve 0.87
Hypertensive heart disease [92]	95	1000 (700-1200)**†‡	Healthy volunteers	12	500 (400-600)†	Serum	TnFNIIIB higher in subjects with eccentric compared to concentric LV hypertrophy
Dilated cardiomyopathy [93]	107	73±35*	Healthy volunteers	20	31±9	Serum	TnC correlated with NYHA functional class and LV echographic parameters
Acute myocardial	105	83±43*	Healthy	20	27±12	Serum	Peak TnC

infarction (day 5) [94]			volunteers				predicted increase in LV end diastolic volume and MACE during follow-up
Dilated cardiomyopathy [95]	31	69±33*	Age and gender matched healthy volunteers	20	40±14	Serum	TnC correlated with NYHA functional class and LV echographic parameters
Hypertensive heart disease [96]	64	60±40	Patients responding to CRT	46	47±30*	Serum	TnC dropped in 72% of treated patients at 6 month follow-up

Comparisons of TnC between cases and controls: *p<0.01; **p<0.05. Shown are mean and standard deviation except † where median and inter-quartile range are shown. ‡In this study the lower molecular weight FNIIIB domain containing TnC isoform was measured while in other studies the higher molecular weight FNIIIC domain containing TnC isoform appears to have been measured. TnC, Tenascin C; AUC, Area under the curve; ROC, Receiver operator characteristic; LV, Left ventricular; NYHA, New York Heart Association; MACE, major adverse cardiovascular events; CRT, cardiac resynchronisation therapy.