T-tubule remodelling and the extra cellular matrix in heart failure

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Abstract

Transverse(t)-tubules are invaginations of the plasma membrane that form a complex network of ducts, 200-400 nm in diameter depending on animal species, that penetrates deep within the cardiac myocyte where they facilitate a fast and synchronous contraction across the entire cell volume. There is now a large body of evidence in animal models and humans that demonstrate pathological distortion of normal t-tubule structure has a causative role in the loss of myocyte contractility that underpins many forms of heart failure. Investigations into the molecular mechanisms of pathological t-tubule remodelling to date have focused on proteins that reside in the intracellular aspect of t-tubules membrane that form linkages between the membrane and myocyte cytoskeleton. In this review, we argue that the mechanisms of t-tubule remodelling are not limited to the intracellular side of the membrane. Our recent data has demonstrated that collagen is an integral part of the t-tubule network and that it increases within the tubules in heart failure suggesting a fibrotic mechanism could drive cardiac junctional remodelling. We examine the evidence that the linkages between the extracellular matrix, t-tubule membrane, and the cellular cytoskeleton should be considered as a whole when investigating the mechanisms of t-tubule pathology in the failing heart.

To appreciate how t-tubules aid a synchronous contraction and how their spatial remodelling can cause a loss of function necessitates a biophysical understanding of the excitation-contraction coupling process that governs contraction. Myocyte contraction is initiated by electrical depolarisation of the plasma membrane (or sarcolemma) in the form of an action potential which allows an influx of extracellular Ca^{2+} via voltage gated L-type Ca^{2+} channels (LTCCs) to trigger intimately arranged ryanodine receptors (RyRs) of the sarcoplasmic reticulum (SR). In a process called calcium-induced-calcium-release (CICR)[1], this evokes the synchronised and rapid release of Ca^{2+} at the flanks of every sarcomere of the cell. As free Ca^{2+} rises in the cytosol it binds to troponin C, on the contractile apparatus, causing a conformational shift that initiates cross bridge cycling and drives contraction. Contraction is subsequently terminated by a decrease in cytosolic Ca^{2+} concentration back to resting levels, predominately due to the action of SR calcium transport ATPase (SERCA) which pumps Ca^{2+} back into the SR and to a lesser extent the sodium calcium exchanger (NCX) extruding Ca^{2+} back into the extracellular space. NCX is crucial to ensuring that the initial trans-sarcolemmal Ca^{2+} influx through L-type Ca^{2+} channels is transported back into the extracellular space to avoid cellular Ca^{2+} accumulation over the contractile cycle. The t-tubules are an extension of the sarcolemma[2] and provide a signalling pathway for the rapid propagation of the action potential deep within the myocyte interior, within milliseconds, facilitating a synchronous cell wide Ca^{2+} release that is much faster and larger than if the cell relied on Ca^{2+} diffusion from initiation events at the cell surface[3][4][5][6][7][8]. For example, a typical Ca^{2+} transient in rat myocytes with intact t-tubules reaches its peak Ca^{2+} level within ~60 milliseconds; if the t-tubules are removed by osmotic shock induced detubulation with formamide peak $Ca^{2+}$is reached much later, in ~120 milliseconds[9].

T-tubules, junctions, and nanoscale organisation

A key structure of the CICR machinery is the cardiac junction or dyad where the plasma membrane (either at the sarcolemma surface or t-tubules) comes into close opposition, ~15 nm, with the sarcoplasmic reticulum[10] [11]. These structures form a coupling (couplon) between LTCC and RyR into cardiac "synapses" creating individual islands of Ca^{2+} release. The couplons are essential for the graded response to increasing levels of trigger current. In other words, increased activation of LTCC increases the number of active couplons, increasing SR calcium release, and increasing the force of contraction. Whereas decreased activation of LTCC, decreases the number of active couplons, decreasing Ca^{2+} release, and contractile force. This presents a paradox: 80% or more of the Ca^{2+} transient is released from the SR and it might be expected that this Ca^{2+} would induce further release from the RyR leading to total depletion of the SR Ca^{2+}. A solution to this paradox is the local control model of Ca^{2+} release that was first demonstrated computationally by Stern[12] and subsequently observed experimentally in confocal-line scan data as microscopic calcium release events called sparks[13]. The spatial separation of discrete Ca^{2+} release sites throughout the cells prevents a positive feedback loop that would lead to an all or nothing release. As diffusional distances from release site increases, Ca^{2+} levels rapidly declines below the threshold required to initiate RyR opening preventing activation of adjacent release sites. For example, average cytosolic free Ca^{2+} ranges from 0.1 µm at diastole to 1 µm at systole, well below the >10 µM free Ca^{2+} that is required to initiate RyR opening in single channel recording experiments[14]. Then how does the much smaller Ca^{2+} current stimulate release? This is thought to be due to restricted space of the junction concentrating Ca^{2+} from the LTCC to levels required to open the RyR. This trigger Ca^{2+}, called a sparklet, has been observed in confocal line-scan data, directly preceding the larger Ca^{2+} spark [15]. Spatial organisation of key players, particularly LTCC and RyR, is thought to be critical to the fidelity of CIRC. For example we have previously demonstrated in a modelling study that lateral displacement of these proteins within the junction on the order of tens of nanometres is enough to diminish Ca^{2+} release [16].

T-tubule remodelling, local Ca^{2+} release and heart failure

Evidence of defective CICR at the local scale in heart failure was initially observed in confocal line scan data from myocytes isolated from the salt sensitive hypertensive rat [17]. The myocytes from these animals had reduced numbers of calcium spark events despite a normal calcium current, indicating defective communication between LTCC and RyR channels. Loss of t-tubules have been documented in dog model of tachycardia induced heart failure[17][18]. Loss of both t-tubules and defective local Ca^{2+} release (sparks) in heart failure was subsequently reported in the spontaneous hypertensive rat [19] and myocardial infarction induce heart failure in mice[20] and sheep[21]. A consistent feature in these studies is the change of t-tubule orientation with loss of the transverse elements but increase in longitudinal elements. Both loss of t-tubule length and orientation could lead to a reduced density of couplons as RyR clusters are predominantly transversely aligned with the Z-line in myocytes. In the spontaneous hypertensive rat myocytes, confocal microscopy demonstrated a loss of co-localisation of LTCC with RyR suggesting distorted dyad microarchitecture may also result in reduced local Ca^{2+} release[19]. Similar loss of colocalisation has been observed in other animal models[22] and human heart failure[23]. Recently, development of 2-photon random access microscopy has allowed simultaneously imaging of action potential and Ca^{2+} release at t-tubules. This work has demonstrated in myocytes from the ischaemic heart failure rat that there can

be regions of apparently intact t-tubules that fail to propagate the action potential resulting in delayed local Ca^{2+} transient. Furthermore, in other t-tubular regions spontaneous Ca^{2+} release was found to trigger a local action potential (delayed after-depolarisation) that then in turn trigged a larger Ca^{2+} release in the same region, indicating a role of t-tubules in arrhythmic events common in heart failure[9]. To what extent these different mechanisms arising from t-tubule remodelling contribute to the development of heart failure remains to be elucidated. However, in general t-tubule remodelling appears to contribute mechanistically to loss of contractility found in heart failure[24]. For example, confocal imaging of the intact living hearts in thoracic aortic banded rats (model of pressure overload) demonstrated a progressive remodelling and loss of t-tubules as animal's transition from hypertrophy to chronic heart failure that was strongly correlated to loss of ejection fraction[25].

Disrupted local Ca^{2+} signalling has been previously documented in the myocytes from the failing human heart[26][27][8]. However, there have been conflicting reports on t-tubule remodelling in the failing human heart. An early electron microscopy (EM) study of hypertrophic cardiomyopathy tissue found loss or absence of t-tubules, which were irregularly shaped and often dilated[28]. However, subsequent studies (including EM and confocal) showed proliferation and dilation of t-tubules in both dilated and ischaemic cardiomyopathy tissue [29] [30]. Whereas a subsequent confocal study showed a loss and dilation of t-tubules in dilated cardiomyopathy tissue[31]. A study using isolated myocytes and 2-photon microscopy found no change in t-tubular volume in failing hearts of different aetiologies; dilated, ischemic, and familial hypertrophic cardiomyopathy [32]. In contrast a study of isolated myocytes analysed with ion-scanning conductance microscopy demonstrated a loss of t-tubule openings on the cell surface in similar aetiologies; ischemic, dilated, and hypertrophic cardiomyopathy [33]. In our confocal analysis of dilated cardiomyopathy tissue, in agreement with previous reports, t-tubule diameter was substantially increased in heart failure. However, we found a wide variability of t-tubule structure between myocytes: some cells had near normal t-tubule abundance and morphology, some cells largely lacked t-tubules, others cells were dominated by longitudinal t-tubule elements, and yet other cells were dominated by oblique running t-tubules that would cross several sarcomeres. Analysis of t-tubule angle showed a shift from dominance of transversely oriented tubules in non-failing hearts to shift to oblique and longitudinal angles in failing hearts [23], similar to changes observed in animal models. Suspecting that regional differences in cardiac function previously documented in DCM[34] may explain variability in t-tubule structure we undertook a regional cardiac MRI strain analysis of DCM patients. These tissue regions were subsequently biopsied at transplant and analysed by confocal. This analysis demonstrated a strong correlation between t-tubule structure and contractility, with regions having near normal contractility having largely intact t-tubules whereas regions with poor contractility had lost much of the transverse elements[35]. This data offers possible explanation to conflicting reports on t-tubule remodelling in human heart failure that the variability was due to unknowingly sampling regions of differing contractile function. Interestingly a recent EM study showed a marked remodelling of junctional structure in both dilated cardiomyopathy and ischaemic heart failure[36]. Junctional structure will likely also be impacted by structural remodelling of the SR in heart failure [37]. The consensus opinion based on the above studies and those on animal models is that remodelling of t-tubules is important feature in the pathology of many forms of heart failure[38].

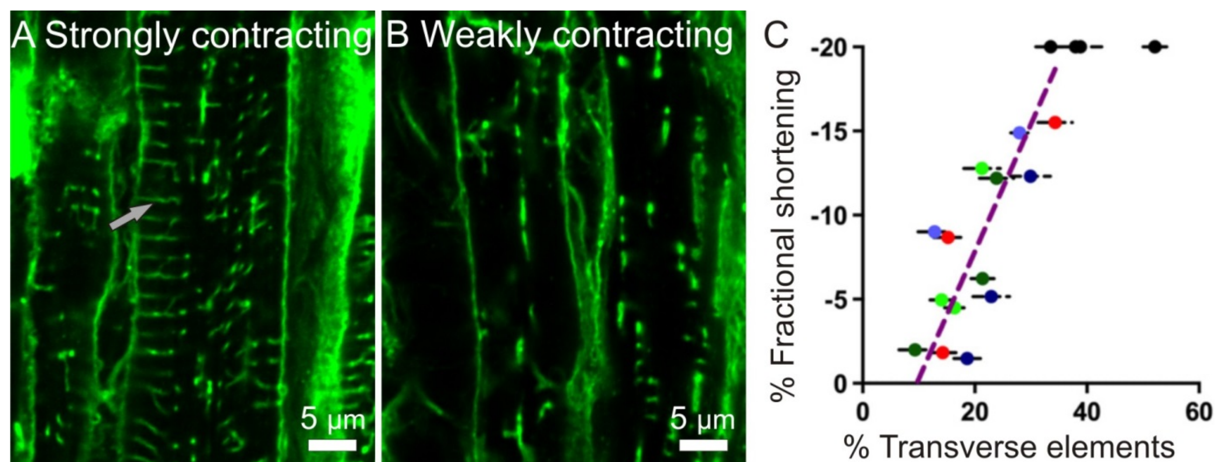

Fig 1. Loss of contractility in human heart failure is strongly correlated to loss of the transverse elements of the t-system. *Panels A and B show exemplar confocal micrographs of WGA stained t-tubules from strongly contracting region (-12% fractional shortening) and weakly contracting region (-2% fractional shortening) in a failing heart. Grey arrow in A indicates a t-tubule. Note loss of the transverse elements of the t-system in the poorly contracting tissue region. Panel C demonstrates the strong correlation (p<0.001) between the percent transverse elements (of the t-system) and fractional shortening of failing heart tissue regions. Five failing hearts were analysed with coloured circles of the same colour indicating different regions from the same heart. Note how the trend is consistent within the individual failing hearts. Black circles are normal donor hearts shown for comparison and are not part of the regression analysis. Figure adapted from Crossman et al (2015).*[35]

Mechanisms of t-tubules remodelling

The mechanisms that drive t-tubule remodelling are poorly understood and are an area of active research. It has been argued that the local mechanical environment or mechanical stress that myocytes experience plays an important role in t-tubule dynamics[39]. For example, isolated rat cardiac myocytes, which are digested from the extracellular matrix and are mechanically unloaded, lose their t-tubules within 24 to 48 hours in culture [8]. Conversely too much stress as experienced in heart failure appears to be a driver for pathological remodelling[40]. Direct evidence for this mechanism was demonstrated by mechanically unloading of the ischaemic failing heart in the rat by heterotopic abdominal heart transplantation, this procedure reversed t-tubule remodelling and normalised local Ca^{2+} release[41]. The same procedure used for prolonged mechanical unloading of normal hearts led to loss of t-tubule structure and impaired Ca^{2+} signalling[42], indicating there is "Goldilocks zone" of mechanical load. The synchronisation between myocytes also appears important as dyssynchronous heart failure in the dog is characterised by loss of t-tubule structure and impaired calcium release that can be reversed by cardiac resynchronisation therapy [43][44]. So how can organ level load effect remodelling of t-tubules at the sub-cellular scale. For the heart to function it is necessary for individual myocytes to work together as a syncytium. In other words millions of individual myocytes are connected together in manner that the contractile forces generated by each individual cell are coordinated into a tightly synchronised organ level contraction required for effective mechanical pump. When the heart experiences mechanical overload or dyssynchrony these forces are presumably transmitted back to the individual cells. Consistent with this proposition is that mechanical strain applied to isolated living rabbit myocytes leads to dynamic changes in t-tubule shape [45]. Furthermore, the stretch sensitive Z-disc telethonin (Tcap) appears to help regulate t-tubule morphology. Knockout of this protein leads t-tubule remodelling and disrupted Ca^{2+} release which is exacerbated by overload induced by thoracic aortic banding model of

heart failure[46]. It is conceivable then that aberrant chronic load leads to pathological distortion of t-tubules and junctional structure leading to miss-communication between LTCC and RYR receptors.

At the molecular scale several candidate proteins have been identified that are required for normal junctional structure and appear to be involved in t-tubule remodelling in heart failure [47–52]. The first of these proteins identified was junctophilin-2 (JPH2) a protein that forms connection between the plasma and SR membranes. Knockout of this protein in mice was found to be embryonically lethal with hearts having abnormal junctional structure, diminished and irregular Ca^{2+} transients[11]. A later studied found mutations in JPH2 were associated with hypertrophic cardiomyopathy in humans [53]. Subsequent inducible and cardiac specific knock-down of JPH2 in adult mice resulted in loss of contractility, heart failure and increased mortality that was associated with disrupted Ca^{2+} release and reduced LTCC and RyR colocalisation[50]. Another protein found to be critical to junctional structure is the membrane scaffolding protein BIN-1. Knockout of BIN-1 is prenatally lethal with embryos displaying severe cardiomyopathy in electron microscopy [54]. Subsequent investigation in adult mice with immunocytochemistry, electron microscopy and immunoprecipitation revealed that BIN-1 was required for trafficking LTCC into t-tubules via the cellular microtubule network. Transient knockdown in mice cardiac myocytes reduced surface levels of LTCC and delayed the Ca^{2+} transient [55]. Furthermore, cardiac specific BIN-1 knockout leads to loss of dense membrane folds in the mouse promoting susceptibility to ventricular arrhythmia[52]. Microtubule densification, a known feature of heart failure, was later linked to defective JPH-2 trafficking and t-tubule remodelling[47]. Loss of JHP2 and t-tubule remodelling has also been linked to over activation of heterotrimeric G protein Gαq that occurs in cardiac hypertrophy [48][56]. Recently a novel junctional protein Striated Muscle Preferentially Expressed Protein Kinase (SPEG) has been identified and its knock out in mice leading to t-tubule remodelling, aberrant local Ca+ handling and heart failure[57]. Yet another protein linked to t-tubule morphology is caveolin-3 (cav-3) a protein involved in the formation of caveole, small (50-100nm) membrane invaginations[58]. Knock out of cav-3 in mice leads to abnormalities in skeletal muscle t-tubule structure and exclusion of the dystrophin –glycoprotein-complex (DGC) from lipid raft domains. Mutations in Cav-3 was are also associated with a form of limb-girdle muscular dystrophy[59]. Several of these proteins (e.g. JPH2[36], BIN1[60], SPEG[57]) can be down regulated in human heart failure but their relative contribution has yet to be determined. It is also likely that there is a complex interaction between these proteins and placing these interactions into a broader cellular and tissue context will be required to understand process of t-tubule remodelling.

Costameres, collagen, and t-tubule remodelling

The finding of interaction between proteins involved in t-tubule remodelling and the cytoskeleton, particularly the DGC, brings us to the next topic the costamere complex and its interaction with the extra-cellular-matrix (ECM). The costamere is a Z-disk associated sub-plasma membrane complex that physically couples the force-generating sarcomeres to the sarcolemma and ECM[61]. This complex has been described as the Achilles heel of striated muscle due to its involvement in muscular dystrophies and cardiomyopathies [30]. Two major costamere protein assemblies have been identified the dystrophin-glycoprotein complex and the vinculin-talin-integrin system. These complexes are found within the cardiac t-tubules (see Fig 2) which are located at Z-disk in cardiac muscle, and are thought to provide mechanical stability to the t-tubules during contraction[31]. In addition to the costameres role in muscle force production they are also signalling domains that

convert mechanical stimuli to biochemical signals[54][62]. Given this mechanotransduction role the costamere is a potential source of aberrant signalling that drives t-tubule remodelling in heart failure.

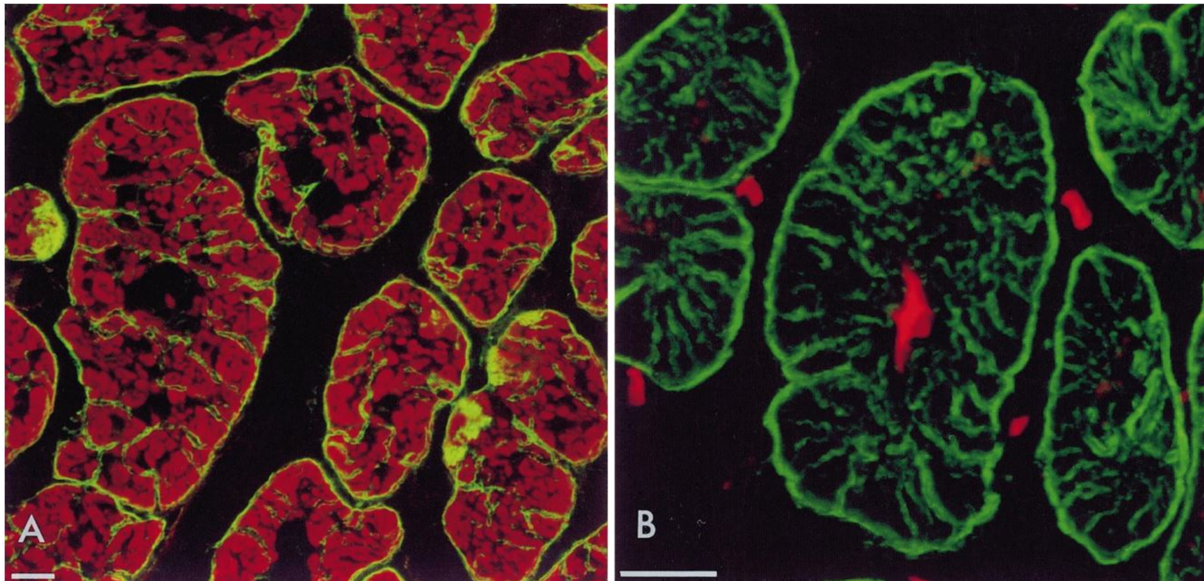

Fig 2. Costamere complexes are present within the t-tubules of the human heart. A Vinculin (green) labels both the surface and t-tubule sarcolemma seen as radial finger like projections situated between myofibrils, labelled with TRITC-conjugated phalloidin (red). B Dystrophin (green) labels both the surface and t-tubule sarcolemma. Nuclei are stained red with 7-AAD. Scale bars 10 µm. Figure adapted from Kostin et al. 1998[31].

The initial clue that led us to consider the costamere complexes and its interaction with the ECM in t-tubule remodelling came from our finding of enlargement and disarrangement of t-tubules in the failing human heart using confocal imaging of tissue sections labelled with wheat germ agglutinin (WGA) [23, 35]. WGA is a plant lectin that selectively binds to N-acetylglucosamine and N-acetylneuraminic acid (sialic acid) residues of glycolconjugates of protein and lipid [63]. This label is commonly used in fluorescence microscopy to visualise the cell membrane including the visualising of t-tubules in cardiac myocytes[18, 44, 64–68]. In addition to its membrane association it also has extensive ECM labelling in human heart tissue [23, 35], see figure 1, and appears to bind to collagen in histological sections of skin and heart [69] [70]. Furthermore, WGA chromatography has been used to isolate the dystrophin glycoprotein complex from muscle[71]. This complex is critical to normal muscle function with mutations in the associated genes commonly leading to muscular dystrophy and to heart failure in older patients[72, 73]. Moreover changes in dystrophin labelling have been observed in end-stage human HF[74] and dystrophin remodelling is associated with hypertrophied t-tubules in the failing human heart[30]. This led us to hypothesize that increases in one or more members of DGC could be responsible for the observed increased WGA labelling and dilation of t-tubules in heart failure.

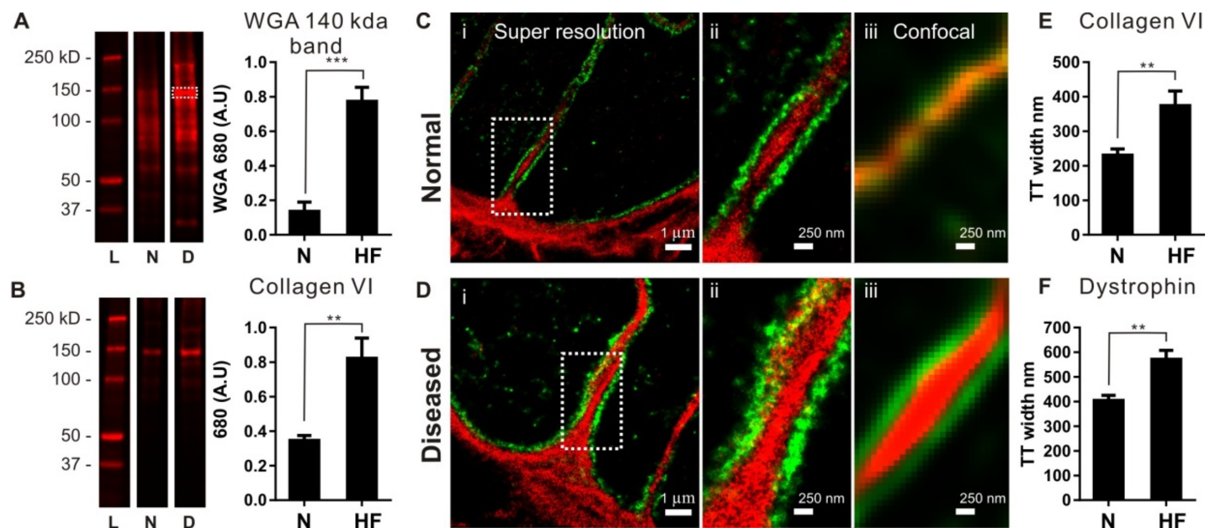

Fig 3. Increased WGA labelling of t-tubules is due to collagen VI in idiopathic dilated cardiomyopathy in humans. A**, WGA western identifies an increase in 140 kDa band in disease. **B**, Westerns (& mass spectrometry) identify this band is Collagen VI. **C&Di**, Super resolution of normal and diseased t-tubules labelled for Collagen VI (red) and dystrophin (green). **C&Dii**, Zoom of box in "i". **C&Diii** Confocal of equivalent t-tubules. **E&F** T-tubule diameter (dia) measured from ColVI and Dystrophin labelling. Symbols, L= ladder, N = normal, HF = heart failure. For westerns, n=7 normal and 11 diseased hearts. For super resolution n=15 t-tubules from 5 normal hearts, and 15 t-tubules from 5 diseased hearts. P<0.001. P<0.01**. Figure adapted from Crossman et al. (2017).*[75]

To identify which glycoproteins are bound by WGA, protein blots of human heart were probed with fluorescently labelled WGA[75] (fig 2). This analysis highlighted a 5.7 fold increase in a 140 kDa WGA positive band in heart failure. This band was subsequently analysed by protein fingerprinting using mass spectrometry and identified collagen VI as a likely candidate. Western blotting confirmed identity as collagen VI and demonstrated a 2.4 fold increase in heart failure. Pertinently, mutations in collagen VI can result in Ulrich congenital muscular dystrophy and Bethlam myopathy [73] suggesting important role of this collagen in muscle function. Confocal demonstrated colocalisation of collagen VI and dystrophin at t-tubules. However, a higher resolution method was required to resolve the distribution of these proteins around the small diameter t-tubules which can be small as ~50 nm in diameter[2]. This was achieved with a super resolution microscopy method that produces 30 nm resolution images using conventional fluorescent dyes[76][77][78]. This technique is a form of localisation microscopy where only a few but highly resolved fluorophores are imaged at any one time. These molecules can then be localised to a precision of ~15 nm. By controlling the photochemistry different and random subset of fluorophores are switched on and off and their position recorded. During an imaging run, tens of thousands of fluorophores are localised, from this data a high resolution fluorescent image is generated. Super resolution imaging of human heart tissue clearly revealed collagen VI was within the t-tubular lumen and that collagen bundle diameters increased in heart failure (Fig 2). Assessment of tissue levels of collagen VI showed a change of distribution from predominately basement membrane labelling pattern to one dominated by increased interstitial labelling reminiscent of fibrillar collagen fibrosis. Subsequent, confocal and super-resolution microscopy then identified increased fibrillar collagens, type I and III, within the t-tubular lumen in heart failure[75]. This data is highly suggestive that increases of collagen could be involved in the aberrant t-tubule remodelling that occurs in heart failure.

Collagens and nanoscale t-tubule remodelling

Collagen provides a unifying mechanistic link between organ level changes (increases in load or dysscyhronous contraction) that could drive t-tubule remodelling in heart failure, particularly as changes in mechanical load including cyclical load influences collagen synthesis [79][80][81]. We also note that the loss of t-tubules in culture cardiac myocytes could be related to loss of collagen as

collagenases are used to isolate cells from the heart before culturing. In heart failure fibroblasts are the source of increases of fibrillar collagen that characterises fibrosis [82] indicating fibroblasts could be involve in t-tubule remodelling. Fibroblasts are also the likely source of collagen VI as evidence by the increased numbers of collagen VI positive fibroblasts we found in human dilated heart failure[75] and is in agreement with a cell culture study of skeletal muscle that only found collagen VI protein and mRNA in fibroblasts and not in other cell types [83]. Particularly interesting is the identification of collagen VI positive fibroblast filopodia within the lumen of some of the enlarged t-tubules in heart failure, supporting a likely role of fibroblasts in either maintaining or remodelling t-tubules from within the tubule lumen.

The linkage between costamere complexes and the ECM, particular the collagens, indicate a mechanism, which by either direct mechanical interaction or signalling (mechanotransduction) under extremes of load (high or low) could lead to disrupted junctional complexes. For, example collagen VI is likely part of the dystrophin complex, because mutations in both proteins result in muscular dystrophy[73, 84], WGA binds collagen VI (Figure 2), and WGA chromatography has been used to isolate dystrophin through binding to unknown component of dystrophin glycoprotein complex[71] which we suggest is collagen VI. In addition the protein biglycan can bind to both collagen VI and the DGC[85–87]. Dystrophin connects the sarcolemma to cellular cytoskeleton components including actin filaments and microtubules [88, 89] which in turn link to the L-type Ca^{2+} channel[90]. Furthermore, microtubules have been implicated in trafficking both LTTC and RyR to cardiac junctions [47] [55]. Potentially the increased dilation of t-tubules on order of ~150 nm could lead to structurally remodelling of the cellular cytoskeleton leading to displacement of LTTC and RyR in cardiac junctions on the order 10-20 nm required to disrupt Ca^{2+} release. Consistent with this proposal, the MDX mouse that has no dystrophin has disrupted cellular cytoskeleton [90] and disrupted calcium release [91]. In addition, the vinculin-talin-integrin system could likely have role in t-tubule remodelling given its linkage to actin cytoskeleton[92] and the promiscuity of integrin (depending on receptor sub-type) to bind to different extracellular matrix components including laminin, fibronectin, and collagens including types I, III, IV, and VI [93][94], which have all been shown to be within the t-tubules[75][31]. These observations highlight that any number of ECM proteins could participate in either mechanical or chemical communications which could determine the local t-tubule structure.

Future directions:

The data linking collagen to t-tubule remodelling was obtained in samples of end-stage human heart failure. In order to establish collagen as a mechanism of t-tubule remodelling the time course of changes in collagens, t-tubular structure, Ca^{2+} handling and contractile function, in response to a suitable intervention would be required to thoroughly test the hypothesis. This would almost certainly require, in the medium term, the use of suitable animal model. This begs the question what animal model would be most appropriate. As noted above changes in t-tubular structure in animals models mimic the change in orientation from dominance in transverse direction in normal myocytes, to dominance of longitudinal direction in failing myocytes that is characteristic of the failing human heart. However a consistent feature of human heart failure is the increased dilation of t-tubules, which we suggest is a consequence of increased collagen deposition. The dilation of the t-tubules in in animal models of heart failure has remained hidden until relatively recently[95]. This likely reflects the smaller diameter of t-tubules in rodents that are commonly used to study t-tubule remodelling in heart failure. For example rodent t-tubules are ~200 nm in diameter where as in humans the diameter is ~400 nm. Critically, this difference in diameter is at the boundary of the optical resolution limit of ~300 nm for the confocal systems commonly used to image t-tubules and has likely led to obscuring of this feature of pathology in rodent models of heart failure.

The advent of super-resolution microscopy is resolving this issue. For example, stimulated emission depletion microscopy, with ~50 nm resolution, has been developed for live cell imaging of isolated myocytes[95]. This study examined myocytes from coronary artery ligation mouse model of

ischaemic heart failure, demonstrating a significant increase in t-tubule diameter from 197 nm in sham animals to vs 216 nm in heart failure animals. Recently we have used Airyscan confocal microscopy[96], which has 1.7 fold improvements in resolution, to examine the WGA labelled t-tubules in coronary artery ligation rat model of ischaemic heart failure. This analysis, presented in figure 4, demonstrates not only a disrupted t-tubule organisation but significantly increased diameter of t-tubules in the failing heart consistent with findings in human and mouse heart failure. The rapid rise in popularity and availability of super-resolution instruments will open access to this technology to many cardiac researchers and will likely play a key role in the understanding the mechanisms of t-tubule remodelling in future heart failure studies. For detailed discussion of super-resolution instrumentation and how it relates to cardiac research the reader is referred to the following review articles[97][98][99].

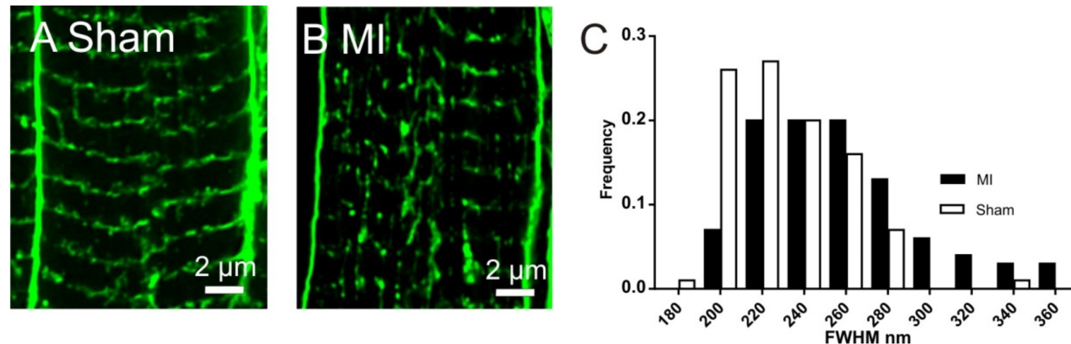

Figure 4. T-tubules are dilated in myocytes from the coronary artery ligation rat model of myocardial ischaemic heart failure. Sections of rat heart were labelled with WGA-488 and imaged on an Zeiss LSM880 Airyscan with 63x 1.4 NA oil lens. ***A**, Exemplar of t-tubule labelling of a myocyte from a sham operated animal.* ***B,** Exemplar of t-tubule labelling in myocyte from a ischaemic heart failure (MI) animal.* ***C,** The frequency distribution of the full width half maximum (FWHM) of the transverse component of t-tubules were measured in 150 sham t-tubules from 5 hearts and 150 MI t-tubules from 5 hearts. The mean diameter of WGA t-tubules in sham myocytes was 222 ± 3 nm and was significantly smaller compared to those in MI myocytes 253 ± 4 nm (p= 0.02* 2 factor nested ANOVA).*

Recent studies in rodents also indicate that collagen, particularly collagen VI, could have important role in pathogenesis of t-tubule remodelling in ischaemic heart failure. In mice with the knockout of the collagen VI gene, were paradoxically protected from the development of heart failure after coronary ligation induction of myocardial infarction. This was demonstrated by improved ejection fraction and reduced infarct size relative to wild type controls[100]. T-tubule structure was not assessed in this study but we suspect that this functional improvement, at least partially, could be due to the prevention of t-tubule remodelling. Furthermore, t-tubule remodelling is reduced using beta blockade in ischaemic heart failure in mice[101], a treatment that is known to mitigate fibrosis in ischaemic heart failure in rats[102]. In general there is paucity of data on the role of ECM and t-tubule dynamics. Future studies will need to determine which collagens (or other ECM proteins) are involved and the how the time frame of changes occur in relation to disease development to identify optimal times for therapeutic intervention. Ischaemic patients generally present early after a myocardial infarction, before progression to HF, offering a therapeutic window prior to development of chronic disease. For this reason, effective preventative therapies will likely have most impact for ischaemic heart failure. Targeted therapies could include some of the newer anti-fibrotic drugs such as pirfenidone that blocks collagens I synthesis in human lung fibroblasts [103] and can reduce fibrosis in ischaemic heart failure in rats [104]. Furthermore, the data in figure 3 indicate increased glycosylation of collagen VI in heart failure (due to the greater increase in WGA binding relative to the smaller increase in the amount of collagen VI protein). Importantly, glycosylation of hydroxylysines is essential for the function of collagen VI.[105] Although the significance of this increased WGA binding is not presently known, if it was linked to t-tubule remodelling, it could

potentially be targeted with anti-glycosylation drugs that have been developed for other human diseases[106].

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