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The microtubule cytoskeleton in cardiac mechanics and heart failure

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Abstract

The microtubule network of cardiac muscle cells has unique architectural and biophysical features to accommodate the demands of the working heart. Advances in live-cell imaging and in deciphering the ‘tubulin code’ have shone new light on this cytoskeletal network and its role in heart failure. Microtubule-based transport orchestrates the growth and maintenance of the contractile apparatus spatiotemporal control of translation, while also organizing the specialized membrane systems required for excitation–contraction coupling. To withstand the high mechanical loads of the working heart, microtubules are post-translationally modified and physically reinforced. In response to stress to the myocardium, the microtubule network remodels, typically through densification, post-translational modification and stabilization. Under these conditions, physically reinforced microtubules resist the motion of the cardiomyocyte and increase myocardial stiffness. Accordingly, modified microtubules have emerged as a therapeutic target for reducing stiffness in heart failure. In this Review, we discuss the latest evidence on the contribution of microtubules to cardiac mechanics, the drivers of microtubule network remodelling in cardiac pathologies and the therapeutic potential of targeting cardiac microtubules in acquired heart diseases.

Eukaryotic cells rely on cytoskeletal polymers to perform essential, evolutionarily conserved tasks, including to divide, move, and maintain their shape and structure. However, the architecture and function of the cytoskeleton (comprising actin, microtubules and intermediate filaments) differ considerably across tissue and cell types. For example, actin is the canonical driver of cell motility, whereas microtubules orchestrate chromosome segregation during cell division, but mature heart muscle cells (cardiomyocytes) are neither motile nor proliferative. Instead, their cytoskeleton is uniquely adapted to meet the needs

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Author contributions

Both authors contributed substantially to all aspects of the article.

Competing interests

B.L.P. is an inventor on a pending patent application that is relevant to this Review: US Patent Application no. 15/959,181 for “Compositions and Methods for Improving Heart Function and Treating Heart Failure”. M.A.C. declares no competing interests.

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of a long-lived, constantly working muscle cell. To this end, most actin is organized into sarcomeric units that participate in the sliding filament model of contraction, but the adopted roles of microtubules have remained relatively obscure.

In this Review, we focus on the microtubule network (MTN) of cardiomyocytes. Advances in our understanding of the cardiac MTN have revealed its organization, mechanics and essential functions. Accumulating evidence has identified extensive MTN remodelling in response to various cardiac stressors, the consequential effects on cardiac mechanics and the therapeutic potential of reversing MTN remodelling in heart disease. We summarize the evidence to support these claims, and offer our perspective on the most intriguing aspects for further research.

Microtubule fundamentals

Microtubule structure and dynamism.

Microtubules are long, rigid tubes with a diameter of 25 nm that are both polarized and highly dynamic (FIG. 1a). Microtubule dynamics are visualized in Supplementary Video 1. Microtubules nucleate from their ‘minus’ end, which is stabilized at γ-tubulin-containing microtubule-organizing centres, and grow at their ‘plus’ end by the addition of GTP-bound α/β-tubulin dimers[1]. The GTP–tubulin ‘cap’ recruits a variety of microtubule-associated proteins (MAPs) and tubulin plus-end-interacting proteins (+TIPs) that collectively determine whether a microtubule grows, shrinks or stabilizes[2]. If the cap is lost, a microtubule shrinks rapidly from its plus end. The cell tunes these dynamics to create discrete microtubule populations, with lifetimes lasting from seconds to many hours[3], and the control mechanisms are adjusted to remodel the MTN during adaptive and maladaptive processes in the heart.

The tubulin code.

Functionally distinct subsets of microtubules are also created through the tubulin code — the many permutations of tubulin isotypes and post-translational modifications (PTMs)[4] (FIG. 1b). In humans, at least nine α-tubulin and nine β-tubulin isotypes are encoded by unique genes, which primarily diverge in the composition of their C-terminal tails. The C-terminal tail of tubulin is highly charged, exposed to the cytosol and a hotspot for enzymatic PTMs. Abundant PTMs include glutamylation, glycylation, acetylation and detyrosination, the last two being the most studied in muscle (FIG. 1c). Tubulin PTMs act via two overarching mechanisms: intrinsic modification of the biophysical properties of the microtubule (for example, acetylation to increase microtubule flexibility[5]) or modulation of interactions with effector proteins (for example, detyrosination to increase microtubule lifetime by protecting the microtubule from destabilizing factors[6,7]).

Microtubule-associated proteins.

Although the microtubule interactome is vast, the canonical group of effector proteins are termed MAPs, which can be separated into structural MAPs and motors (FIG. 1a). Structural MAPs, of which MAP4 is the most abundant in the heart[8,9], bind along the length of the microtubule and influence its dynamics. The microtubule motors kinesin

and dynein, respectively, drive plus-end (anterograde) and minus-end (retrograde) transport of cargoes along the microtubule tracks[10,11]. Motors can also crosslink microtubules to other cytoskeletal polymers[12,13] or directly stabilize or destabilize bound microtubules[6]. The binding of MAPs is tuned by both the tubulin code and PTMs to the MAP itself[4]. In summary, the intrinsic dynamism of microtubules, coupled with multiscale regulation by tubulin isotype composition, tubulin PTMs and MAPs, bestows flexibility and diversity on microtubule populations.

Microtubule network organization.

The organization of the cardiomyocyte MTN evolves considerably during postnatal development[14]. In embryonic cardiomyocytes, as in most proliferating cells, the perinuclear centrosome is the primary microtubule-organizing centre from which microtubules nucleate, extending their plus ends radially outwards towards the periphery[14]. In the early postnatal period, the centrosomes dissolve. Although many microtubule-organizing centres remain associated with the nuclear envelope and the proximal Golgi apparatus, they are also prominently found at Golgi apparatus outposts dispersed throughout muscle cells[15]. As such, the radial, ‘plus-end out’ organization of the network is replaced by a mixed polarity, predominantly longitudinal MTN organization (FIG. 2a).

Cardiomyocytes must maintain their structure and function for the lifetime of the organism, an incredible 2.5 billion beats for an average human. Accordingly, the cardiomyocyte cytoskeleton is more long-lived, stabilized and heavily crosslinked than its embryonic counterpart[16]. Microtubules use a ‘search and capture’ process, whereby growing, dynamic microtubules stabilize their plus ends by favourable interactions with cellular structures[17]. For example, +TIPs bind to various tethers to stabilize microtubule interactions at the transverse tubule (T-tubule), Z-disc and intercalated disc[17]. When coupled with the distributed nucleation of microtubules, these search and capture mechanisms help to form the unique architecture of the cardiac MTN and to reinforce discrete microtubule populations at specialized domains.

Cardiac microtubule-based transport

Motor proteins transport essential cargo along microtubule tracks, including membrane proteins, mRNA, ribosomes and entire organelles. Through the search and capture process, microtubules are stabilized at specific locations to produce preferred delivery routes. Microtubule PTMs can further bias transport by increasing the binding or motility of particular motors[13,18–20]. This tuneable delivery system helps to establish, support and remodel specialized domains in cardiomyocytes. Below, we focus on three of these domains: the dyad of excitation–contraction coupling, the intercalated disc and the newly discovered ‘translational hubs’ that facilitate local protein synthesis.

Organization of the dyad.

Electrically triggered Ca^{2+} release from intracellular stores activates actomyosin contraction during systole. Proper control of Ca^{2+} release requires two specialized membrane systems, together termed the dyad. Dyads consist of invaginations of the plasma membrane (T-

tubules) that harbour voltage-gated Ca^{2+} channels in proximity (~15 nm) to Ca^{2+}-release channels (ryanodine receptors) in specialized regions of the junctional sarcoplasmic reticulum (SR) (FIG. 2d). Dyads allow Ca^{2+} influx from the T-tubule, which rapidly triggers Ca^{2+} release from nearby junctional SR stores, ensuring efficient excitation–contraction coupling. Microtubule transport facilitates the biogenesis, linking and stabilization of dyads and their maladaptive remodelling in response to cardiac stress.

Although T-tubule biogenesis is incompletely understood, live imaging data[21] support an endocytic capture model in which tubules grow inwards from the sarcolemma and are stabilized as tubes instead of completing endocytosis. Nascent T-tubules can be formed in developing muscle by the membrane-shaping protein BIN1 (also known as Myc box-dependent-interacting protein 1)[22], which promotes actin-dependent T-tubule folding and stabilization in the mature heart[23]. BIN1 also anchors microtubules at T-tubules[24] and directly interacts with the +TIP CLIP170 (also known as CAP-Gly domain-containing linker protein 1), and disrupting CLIP170 blocks BIN1-dependent T-tubule formation[25]. These findings implicate microtubule–CLIP170–BIN1 interactions in regulation of the T-tubule architecture, but the precise mechanisms of cardiac T-tubule formation remain unresolved.

When formed, T-tubules must be properly tethered to the junctional SR to stabilize the dyad. The SR is dynamic, with membrane tubulation driven by kinesin and dynein motility[26,27]. A physical link between the SR and microtubules is proposed to shape the specialized ends of the junctional SR, with the transmembrane protein CLIMP63 (also known as cytoskeleton-associated protein 4) thought to bridge microtubules and the junctional SR protein triadin[28] (FIG. 2d). In a sequential model, microtubule-dependent motility of junctional SR and T-tubule membranes might be an early step in dyad biogenesis, bringing the membranes in close apposition to facilitate stabilization by structural proteins such as junctophilin 2. Junctophilin 2 localizes to the junctional SR and bridges T-tubules and associated Ca^{2+} channels to structurally stabilize dyads[29,30] (FIG. 2d). The disruption of dyads is a common feature of heart failure (HF)[31] and seems to be at least partly driven by pathological remodelling of the MTN and subsequent loss of junctophilin 2 (discussed further below).

Organization of the intercalated disc.

The intercalated disc is another intriguing region with established clinical importance and emerging dependence on microtubules. The intercalated disc is a specialized junction between the ends of cardiomyocytes that facilitates their physical and electrical coupling. The disc consists of three distinct transmembrane complexes (FIG. 2c). Desmosomes connect to desmin intermediate filaments, adherens junctions connect to the actin cytoskeleton, and gap junctions provide electrical continuity through connexin 43 proteins that form low-resistance channels between neighbouring cells. Consistent with their essential role in conduction, variation in genes encoding proteins that form the intercalated disc is the leading cause of arrhythmogenic cardiomyopathy[32].

Microtubules control the trafficking of major components of the intercalated disc (FIG. 2c). Preferential trafficking is enabled by multiple tethering proteins that capture the +TIP EB1 (also known as MAP RP/ED family member 1) at the intercalated disc. At desmosomes, desmoplakin interacts with EB1 to stabilize microtubules, and variants in the EB1-binding

region of desmoplakin can impair connexin 43 trafficking and cause arrhythmogenic cardiomyopathy[33]. EB1 also anchors to adherens junctions through an interaction mediated by cadherin and the dynein motor complex[34]. Disrupting this interaction also abolishes connexin 43 accumulation at the intercalated disc, which occurs in response to oxidative stress and, again, gives rise to lethal ventricular arrhythmias[35]. Oxidative stress can act via various microtubule-dependent mechanisms to compromise intercalated disc function, which are discussed further below.

Furthermore, connexin 43 itself might capture microtubule tips to positively reinforce trafficking to the intercalated disc. A C-terminal region of connexin 43 is not necessary for its own localization, but is required for stabilizing EB1 and for trafficking of the essential voltage-gated Na^+ channel ($Na_v1.5$) to the intercalated disc[34]. Interaction between the cytoplasmic linker-associated protein 2 (CLASP2; also known as CLIP-associating protein 2) and EB1 has also been shown to be crucial for the enrichment of $Na_v1.5$ at the intercalated disc[36]. This feedforward model, in which trafficking of one protein promotes the delivery of another, might facilitate the assembly and stabilization of multi-protein complexes at the intercalated disc.

The intercalated disc is also a putative site for the addition of new sarcomeres, which occurs during cardiac hypertrophy[37]. Whether, how and where microtubules might be involved in new sarcomere addition are fascinating unanswered questions. Researchers have found that an activated form of AMP-activated protein kinase (AMPK) localizes to the intercalated disc in response to mechanical stress and phosphorylates the +TIP CLIP170 to promote microtubule dynamics[38]. Blocking CLIP170 phosphorylation forces microtubule hyperstabilization at the intercalated disc, leading to microtubule accumulation, elongation of cardiomyocytes and dilatation of the heart[38]. These findings are consistent with the observation that deletion of *Ampk* in mice drives MTN stabilization, pathological hypertrophy and impaired cardiac function in response to mechanical stress[39].

Organization of local translation.

The causal relationship between MTN stability and hypertrophy is well established[40–43], but the underlying mechanisms remain obscure. In 2021, our understanding advanced when the MTN was shown to support growth by controlling the location of new protein synthesis. Multiple independent reports have indicated that the MTN is the master regulator of mRNA distribution and localized translation in cardiac[44,45] and skeletal[46,47] muscle. Contrary to the traditional view of the protein secretory machinery, mRNA, ribosomes, the Golgi apparatus and the endoplasmic reticulum are not restricted to the perinuclear space in cardiomyocytes, but are instead distributed throughout the cell in a striated pattern, suggesting the presence of local, sarcomere-associated protein translation domains[45,48] (FIG. 2b). Active, microtubule-based transport is essential for distributing mRNAs and ribosomes to these domains[44,46], which is at least partly mediated by kinesin 1 (REF.[44]). Intriguingly, microtubules distribute mRNAs encoding either cytosolic or membrane proteins[45], and specific mRNAs usually (but do not always[46]) enrich at regions that correspond to the site of function of the encoded protein[44,48]. Furthermore, two distinct translational ‘hot spots’ are indicated: mRNAs and

ribosomes are strongly enriched on the flanking sides of the Z-disc and just proximal to the intercalated disc[44,48] (FIG. 2b,c).

In response to hypertrophic stimulation, the global rates of protein translation in cardiomyocytes increase. Intriguingly, however, mRNAs and ribosomes can also be redistributed to the ends of cardiomyocytes, concomitant with densification of the MTN and increased expression of kinesin 1 (REF.[44]). If microtubule transport is disrupted, global synthesis rates still increase, but the synthesis of new protein is mislocalized, and the cardiomyocyte fails to grow[44]. This finding indicates that the MTN-dependent localization of protein synthesis is a crucial determinant of the addition of new sarcomeres and cardiac hypertrophy.

These studies raise many new questions. For example, what rules govern where specific transcripts are localized and which motors or RNA-binding proteins are involved in this localization? Furthermore, is mRNA actively translated during transport or is translation activated when a target destination is reached? What is the composition of translational hubs that surround the Z-disc and intercalated disc, and what role does non-sarcomeric actin have in establishing these domains? Finally, what mechanisms remodel the MTN to augment local translation during hypertrophy, and could redirecting trafficking differentially control eccentric (lengthening) versus concentric (thickening) remodelling of the heart? These questions should be addressed in future studies.

Microtubules in cardiomyocyte mechanics

As detailed above, an extensive, interconnected transport network is required to support cardiomyocytes. The MTN also densifies and adapts to help to change the size and shape of a myocyte during cardiac remodelling. However, this dense, interconnected railway imparts a mechanical cost. In HF, remodelling of multiple cytoskeletal networks can increase myocardial stiffness[49–51]. Different pathological processes in dilated, ischaemic and hypertrophic cardiomyopathies seem to converge on alterations to the MTN that augment its contribution to myocardial stiffness. Below, we dissect the contribution of microtubules to cardiac mechanics, scaling up from the level of individual cytoskeletal filaments, to the level of cytoskeletal networks, and to the levels of cells and tissues.

Mechanics of cytoskeletal filaments.

Cytoskeletal polymers are orders of magnitude stiffer and thicker than most proteins (FIG. 3a), and the cytoskeleton collectively determines the mechanical properties of the cytoplasm by its density, crosslinking and orientation. Despite the fact that the same three filaments comprise the cytoskeletons of all mammalian cells, organizational differences give rise to stiffnesses that span orders of magnitude from that of neurons (tens of pascals) to that of muscle (tens of kilopascals)[52]. Although microtubules are by far the stiffest cytoskeletal filament, they are not the primary determinant of stiffness in most cells, which speaks to the importance of filament organization and crosslinking in determining mechanical properties.

Mechanics of cytoskeletal networks.

The capacity for rod-like filaments to support a cell requires the filaments to be held together by crosslinking interactions (FIG. 3b). Otherwise, isolated filaments rearrange and slide past each other in response to deformation, rendering the cytoskeleton unable to support cell shape or to deform reversibly. Crosslinkers also function as high-gain mechanical amplifiers; in a network of purified cytoskeletal polymers, the addition of a crosslinker at fractions of the polymer concentration increases the network stiffness by orders of magnitude[53]. Cardiomyocyte stiffness is determined by a highly crosslinked cytoarchitecture and, in HF, small changes in crosslinking can have large mechanical consequences.

Crosslinking also imparts two fundamental properties to cytoskeletal networks in the myocardium: non-linear stiffening and viscosity[54,55]. In non-linear stiffening, the density of crosslinks and their spacing determines the length scale by which a cytoskeletal network can deform via rearrangement before crosslinks or filaments need to slip or break. When filaments become increasingly aligned through strain, the stiffness of the material increases dramatically[55] (FIG. 3b). In cardiomyocytes, an example is the giant protein spring formed by titin, which crosslinks the Z-disc to the myofilaments along its length (FIG. 3c). When a cardiomyocyte is stretched to a sarcomere length of >2.1 μm, the unfolding and alignment of titin drives non-linear stiffening that becomes a dominant determinant of myocyte stiffness[49,50,56].

Viscosity, the stiffness of a material depending on the rate of strain, arises when cytoskeletal crosslinks have a dynamic nature[57]. This principle is central to the contribution of microtubules to cardiomyocyte mechanics[58,59]. Cardiomyocyte viscoelasticity arises in part from crosslinking between microtubules and other cytoskeletal elements that is sufficiently weak that the crosslinks break and rapidly reform under mechanical stress (FIG. 3c, right). This phenomenon creates a drag-like, viscous interaction that generally impedes cardiomyocyte motion[60]. As crosslinks break and reform on rapid timescales, they dissipate rather than store energy, and viscous networks do not therefore provide a restoring force like an elastic spring. The viscous impediment increases with velocity (FIG. 3c, right), again in contrast to an elastic connection, which has the same stiffness irrespective of rate. Therefore, a viscous network such as the cardiomyocyte MTN demonstrates resistance to motion regardless of direction and which is steeply dependent on the rate of motion and the strength of crosslinking.

Mechanics of the cardiomyocyte MTN.

In 1997, clever mechanical tests first demonstrated the primarily viscous contribution of the MTN to cardiomyocyte stiffness[61], with subsequent confirmation by orthogonal approaches[58,62,63]. In 2016, live imaging of deforming microtubules in beating cardiomyocytes illuminated the molecular interactions and mechanical behaviours that produce this viscoelasticity[62]. Cardiomyocyte contraction causes the buckling of microtubules at short wavelengths that match sarcomeric periodicity (Supplementary Video 2). Buckling arises from longitudinal microtubules crosslinking to transverse desmin intermediate filaments at the sarcomere Z-disc. This lateral reinforcement at sarcomeric spacing forces compressed microtubules to adopt a high-energy, buckled conformation[62,64]

and enables them to bear compressive and tensile loads in systole and diastole (FIG. 3c). On the basis of the number of microtubules in a healthy cardiomyocyte and their high inherent stiffness, microtubule buckling is estimated to provide resistance equal to about 20% of the cardiomyocyte contractile force[62].

Microtubule crosslinking to intermediate filaments at the Z-disc requires post-translational detyrosination of microtubules[62]. Reducing detyrosination leads to a switch from microtubules buckling to sliding past contracting sarcomeres and a loss of viscous resistance[62]. Strikingly, the viscoelastic contribution of the MTN to stiffness is eliminated by either depolymerizing the MTN or by tyrosinating an intact network[58]. This finding indicates that mechanical coupling between microtubules, intermediate filaments and the contractile machinery requires detyrosination and is necessary for the contribution of the MTN to mechanics. Consistently, microtubule depolymerization or tyrosination increases the rate of contraction and relaxation independent of Ca^{2+} signalling changes, and measurements of cardiomyocyte and myocardial viscoelasticity support a direct mechanical effect of microtubule detyrosination viscously resisting changes in cardiomyocyte length[8,51,58,65].

This viscoelastic nature suggests that the crosslinking interaction between microtubules, intermediate filaments and myofilaments is limited in strength and is short-range. The interaction can be facilitated by various microtubule–intermediate filament crosslinkers such as plectin and kinesin[66,67], whose strength of interaction can be tuned by detyrosination[12,68], but can also be explained by direct interactions. A study using reconstituted microtubules and intermediate filaments in an elegant dual optical trap has demonstrated that intermediate filaments directly stabilize microtubules through electrostatic and hydrophobic interactions[69]. Detyrosination removes a hydrophobic tyrosine to expose charged glutamic acid residues on the microtubule surface that might strengthen this interaction, but this hypothesis remains to be formally tested. Regardless, in response to the depletion of desmin, both microtubule stabilization at the Z-disc[70] and the microtubule contribution to viscoelasticity are substantially blunted[62], supporting microtubule–intermediate filament crosslinking as an important determinant of their mechanical contribution.

The precise roles of the many known microtubule crosslinking proteins expressed in the heart remain ambiguous, perhaps in part owing to the complexity of their interactions (TABLE 1). MAPs and other crosslinkers regulate the connectivity of microtubules with themselves[71,72], intermediate filaments[66,68], actin filaments[71,73,74], T-tubules[24,40], costameres[75,76] and the nuclear envelope[77–79], to name a few. Each of these proteins has some role in regulating cardiomyocyte mechanics through cytoskeletal interconnectivity (TABLE 1). Accordingly, the MTN bridges connections from the extracellular matrix through the contractile machinery to the nucleus, also facilitating its role in mechanotransduction[80–82]. Further dissection of the molecular interactions, biophysical properties and disease implications of crosslinkers is an open area for future research.

Mechanics of the myocardium.

Myocardial mechanics are regulated at the myofilament, cytoskeletal and extracellular levels, with the contribution of different elements depending on the length, speed

and direction of deformation[56,58,63,83]. Contraction is driven by actomyosin crossbridge interactions that can be tuned by PTMs and changes in sarcomere stoichiometry. Titin functions as a giant crosslinked spring that preserves sarcomere integrity and establishes myofilament resting tension, and PTMs can increase or decrease the compliance of titin in disease[84]. Similarly, the extracellular matrix forms an aligned, crosslinked network that holds the myocardium together. The organization of titin and extracellular matrix crosslinking means that they show non-linear stiffening at the upper limits of typical working sarcomere lengths[56,85]. Consequently, titin and collagen become dominant factors in determining passive stiffness with large extensions beyond resting length and prevent over-stretching of the myocardium[56].

At a typical working range during the cardiac cycle (sarcomere length of 1.8–2.1 μm), the MTN contributes substantially to the stiffness of the myocardium[51,58]. The viscoelastic connectivity of the MTN means that its contribution to stiffness is largely unchanged with increasing length and, therefore, is likely to be overwhelmed at longer lengths by elements that non-linearly stiffen. Instead, the viscoelastic nature of the MTN means that it depends strongly on the rate of lengthening or shortening, which might have important implications for cardiac performance during exercise. During exercise, the rate of diastolic filling increases[86] to maintain stroke volume when the heart rate is elevated[87,88]. This situation creates a condition in which viscoelastic forces might limit cardiac stretch and stroke volume[50,89] and, consistently, patients with diastolic HF often have reduced end-diastolic volumes despite increased diastolic pressures during exercise[88,90].

Microtubule remodelling in HF

The cytoarchitecture of cardiomyocytes is tuned to meet the workload of the heart and must adapt to stressors, including exercise, hypertension and ischaemic injury. Sustained stress can drive substantial hypertrophy of the myocardium, which is accompanied by remodelling of the microtubule, intermediate filament and actomyosin networks[8,9,91–93]. MTN remodelling enables hypertrophic growth, but also imparts a mechanical load that stiffens the myocardium in HF. A deeper understanding of the mechanisms of MTN remodelling might, therefore, identify new targets for therapeutic intervention.

Two proteomic assessments have defined the landscape of cytoskeletal remodelling in advanced HF in humans[89]. In myocardium from patients with dilated, ischaemic or hypertrophic cardiomyopathy[8], or obstructive hypertrophic cardiomyopathy[9], several findings are consistently observed: an (often striking) increase in total tubulin protein levels, an increase in MTN density, increased expression of stabilizing MAPs, increased microtubule detyrosination and acetylation, and a stark upregulation of intermediate filaments. These changes are particularly pronounced in patients with advanced, decompensated hypertrophic cardiomyopathy[8] and in patients harbouring disease-causing variants in genes encoding sarcomeric proteins[9]. Concomitant with the microtubule and intermediate filament densification, a loss of actomyosin content and organization often occurs in advanced HF[8,94,95].

How does the cytoskeleton get to this point? Important questions remain, but new work has provided information on the trajectory and mechanisms of MTN remodelling in response to common cardiac stressors, including adrenergic stress, haemodynamic overload and ischaemic injury.

Transcriptional and post-transcriptional (auto)regulation of tubulin.

In certain forms of HF, such as hypertrophic cardiomyopathy, the myocardial levels of tubulin protein increase dramatically (approximately fivefold)[9]. However, in these same patients, the levels of tubulin mRNA are unchanged or downregulated[9]. To understand this discordance, we must consider the mechanisms that control tubulin mRNA stability and the balance between soluble (free) tubulin and polymerized microtubules. The growth rate of microtubules is determined by the concentration of free tubulin available for polymerization[1]; therefore, an increase in free tubulin levels will inherently densify the MTN. However, cells maintain a brake on this feedforward mechanism through tubulin autoregulation, a rheostat by which elevated free tubulin levels trigger the degradation of tubulin mRNA[96]. Conversely, if free tubulin levels decrease, autoregulation is relaxed to increase mRNA stability and restore free tubulin levels. Autoregulation has been shown to operate on all detectable tubulin isoforms in cardiomyocytes[97].

A consequence of this autoregulation is that shifting the dynamics between free and polymerized tubulin feeds back to regulate tubulin transcripts. A stabilizing factor that pushes tubulin into the microtubule fraction can transiently reduce free tubulin, in turn releasing autoregulation to increase tubulin mRNA and protein levels[97]. This feedforward loop allows sustained stabilization to promote both densification of the MTN and increased total tubulin content. Prominent stabilizing factors in the heart include detyrosination[7,70], acetylation[98,99] and MAP4 binding[100,101], and stabilization is sufficient to drive pathological remodelling and impair cardiac function[100].

Microtubule stabilization in response to cardiac stress.

Within hours of adrenergic stress or pressure overload, microtubule detyrosination[97] and upregulation of mRNA encoding specific tubulin isoforms are rapidly induced[97,102,103]. These changes precede detectable alterations in tubulin protein level, other tubulin PTMs or indicators of stability, indicating that transcriptional induction of tubulin and microtubule detyrosination additively and rapidly prime the system for densification. The primed system then manifests as a denser MTN with released autoregulation and increased free and polymerized tubulin content within days of sustained stress[97].

The signalling cascades and transcription factors that increase the levels of mRNA encoding tubulin isoforms are poorly understood, as are the mechanisms that might regulate detyrosinase activity in response to mechanical stress. STAT3, JAK and other kinases are implicated in the upregulation of tubulin mRNA levels[104–106], and vasohibin 2 (VASH2; also known as tubulinyl-Tyr carboxypeptidase 2) also seems to be transcriptionally induced by adrenergic stress[97]. Post-translational mechanisms probably also tune the activity of detyrosinating enzymes, but this regulation is only beginning to be explored[107].

When pressure overload is sustained (FIG. 4a,b), both MAP4 abundance and dephosphorylation increase. MAP4 dephosphorylation promotes microtubule binding that stabilizes the MTN for subsequent densification[101,103]. Increased microtubule lifetime then facilitates detyrosination and acetylation by increasing the available substrate for enzymatic modification. With chronic overload and advanced HF, a marked upregulation of intermediate filaments occurs that further stabilizes the MTN via crosslinking and protection from depolymerization[8,62,69,93]. Therefore, sustained haemodynamic overload drives multiple mechanisms that produce a dense, stable, and heavily detyrosinated and crosslinked MTN. Intriguingly, this effect seems to be at least partly reversible, given that mechanically unloading failing hearts in rats[108] and patients[62] restored levels of detyrosination towards those in healthy control hearts.

After acute ischaemic injury, an almost paradoxical paradigm seems to increase detyrosinated microtubules via decreased MAP4 decoration of microtubules (FIG. 4c). Myocardial infarction rapidly induces detyrosination by approximately fourfold in the surrounding myocardium, which is causally implicated in impaired contractile function[109]. In response to myocardial infarction, MAP–microtubule affinity-regulating kinase 4 (MARK4) phosphorylates MAP4 to weaken its association with cardiac microtubules[109]. This weakened association facilitates the binding of VASH2–small vasohibin-binding protein (SVBP) to the microtubule to increase detyrosination, which again promotes interaction with desmin and viscoelastic resistance to cardiomyocyte motion[109]. Importantly, cardiomyocyte-specific deletion of *Mark4* blocks the myocardial infarction-induced MAP4 phosphorylation and dissociation from the microtubule, protecting the heart from increased detyrosination and contractile dysfunction[109]. In the absence of MARK4, normal infarct size and pathological remodelling are still observed in the month after myocardial infarction, but cardiac function is markedly preserved[109]. These data support targeting the MARK4–MAP4–VASH2 axis for preservation of cardiac function after ischaemic injury.

Oxidative stress can also directly modify microtubules to drive network densification in HF (FIG. 4c). In HF, oxidative stress causes the oxidation of cysteine residues of microtubules, which leads to structural damage in the microtubule lattice[110]. Incorporation of new, GTP-bound tubulin into these oxidized regions then directly stabilizes microtubules by protecting them from depolymerization, which is sufficient to drive MTN densification[110].

In summary, multiple mechanisms can synergistically contribute to pathological remodelling of the MTN. These include: transcriptional induction of tubulin isoforms, MAPs and modifying enzymes; autoregulation of tubulin content; and microtubule stabilization, which occurs through intermediate filaments, MAPs, and enzymatic and oxidative modifications of tubulin. Despite these diverse routes, a consistent signature of increased microtubule density, stability and post-translational detyrosination is observed in advanced HF.

Targeting microtubules in heart disease

HF remains a leading cause of death and encompasses a wide array of pathologies arising from genetic variation, ischaemic injury, metabolic disease, hypertension and arrhythmia[111]. Despite this pathological diversity, the increased abundance of stable detyrosinated

microtubules is a recurrent feature[112]. Evidence from animal models indicates that tuning the MTN can increase cardiac power output (work per unit time)[113], blunt pathological remodelling[42,44,114], modulate general protein recycling[44,115] and control arrhythmias[116]. However, most studies rely on blunt tools whose clinical potential is limited, and more targeted manipulations are needed.

Clinical studies.

To date, clinical evidence for the efficacy of targeting microtubules for the treatment of cardiovascular disease has been limited to systemic colchicine treatment (TABLE 2). In some studies, long-term, low-dose colchicine has been associated with improved cardiovascular outcomes[117], but whether this efficacy is related to destabilization of cardiac microtubules remains unknown. At low doses, preferential destabilization of microtubules in immune and inflammatory cells might underlie an anti-inflammatory benefit of colchicine, as observed in the treatment of gout[118]. To destabilize cardiac microtubules, a minimum colchicine dosage of 0.4 mg/kg per day is required in mouse models[41,43,44]. This dosage is ~24-fold the tolerable dosage in humans (0.017 mg/kg per day for a 70-kg patient), which is limited by gastrointestinal adverse effects. At the mouse dose, plasma colchicine levels peak at ~2 μmol/l and rapidly drop to 0.25 μmol/l within an hour[119,120], which is already a submaximal dose for depolymerizing cardiac microtubules. Therefore, the development of more potent or targeted destabilizers of cardiac microtubules is needed.

Conversely, microtubule stabilizers (taxanes) are associated with the development of heart failure, ischaemia and conduction abnormalities when used as chemotherapeutic agents[121]. Although taxanes are associated with various forms of cardiomyopathy, arrhythmic phenotypes are the most prevalent, motivating a closer examination of the influence of the MTN on cardiac conduction.

Preclinical studies: targeting MTN stability.

Although high-dose colchicine is not tolerated in humans, numerous animal studies have indicated that high-dose colchicine can destabilize the MTN and improve HF outcomes either by blocking pathological remodelling or by restoring performance. Colchicine blunts cardiac hypertrophy in animal studies[40–42,44,113] and hypertrophy of isolated cardiomyocytes[44,122], and can improve systolic function[41,43,113]. Microtubules have been shown to densify in response to pressure overload in large-animal and small-animal studies[40,41,43,102,113,123,124], and colchicine treatment rescues myocardial performance after pressure overload of the right ventricle in cats[125,126] and the left ventricle in dogs[113,124]. Other studies have indicated that colchicine does not significantly improve ex vivo myocardial performance[127,128]; however, we note that these studies did not examine whether the MTN was destabilized by colchicine or was pathologically remodelled. Colchicine also protected the myocardium from adverse remodelling in a rat model of pulmonary hypertension[114] and after myocardial infarction in mice[129]. Colchicine significantly improves the speed and extent of contraction and relaxation of cardiomyocytes isolated from patients with dilated or hypertrophic cardiomyopathy[8] and reduces diastolic stiffness in human failing myocardium[51]. Taken together, these data indicate that partial destabilization

of microtubules can protect the heart from pathological remodelling and improve mechanical performance.

Preclinical studies: targeting detyrosination.

Detyrosinated microtubules impair cardiomyocyte motion and are inversely correlated with left ventricular function in human cardiomyopathy[62] and in mice after myocardial infarction[109]. Targeting this PTM might also allow a larger therapeutic index than gross MTN depolymerization. Pharmaceutically reducing detyrosination with parthenolide acutely reduces stiffness and improves contractile function of failing cardiomyocytes from patients with dilated or hypertrophic cardiomyopathy[8]. Parthenolide also reduced infarct size and tissue damage in a mouse model of ischaemic cardiomyopathy[130], and reduced stiffness[108] and improved contractile performance of mouse cardiomyocytes isolated from infarcted hearts[109] or those harbouring hypertrophic cardiomyopathy-causing genetic variants[9]. However, parthenolide has substantial off-target adverse effects, such as altered immunological[131] and oxidative[132] responses.

Overexpression of tubulin–tyrosine ligase (TTL) offers a more specific route to reduce detyrosination because tubulin is its only known target. TTL overexpression reduces viscoelasticity[51,58] and improves contractile performance in human failing cardiomyocytes[8] and cardiomyocytes from infarcted mice[109]. TTL overexpression can also destabilize microtubules by sequestering free tubulin[65], which might affect tubulin polymerization and autoregulation. Therefore, inhibiting the detyrosinase complex (VASH–SVBP) offers a more direct means to reduce detyrosination without sequestering tubulin, and also improves the mechanical properties and contractile performance of human failing cardiomyocytes[65]. VASH2 is expressed at lower levels than VASH1 in the heart, but might have a more important role in detyrosination in response to adrenergic stress[97] or myocardial infarction[109]. Small-molecule inhibitors of the VASH–SVBP complex are currently in development and offer intriguing but untested potential for modulating the course of HF.

Preclinical studies: targeting MAPs and kinase regulation.

Kinase activity potently regulates microtubule stability through the phosphorylation of structural MAPs, which generally encourages their dissociation from microtubules. Hypophosphorylation of MAP4 in pressure overload promotes its binding to and stabilization of microtubules[102,133]. AMPK becomes inactivated in response to pressure overload, promoting MAP4 hypophosphorylation[39]. Drug-induced activation of AMPK is cardioprotective in conditions of pressure overload, concomitant with hyperphosphorylation of MAP4 and MTN destabilization[39]. Consistently, treatment with adenosine or analogues[43], or cardiomyocyte-specific deletion of adenosine kinase[122] (which converts adenosine to AMP), all protect against MTN stabilization, MTN detyrosination, pathological cardiac hypertrophy and cardiac contractile dysfunction in response to sustained pressure overload. Together, these studies indicate that adenosine metabolism and MAP4 hypophosphorylation are potential targets to mitigate pathological MTN remodelling in response to haemodynamic overload.

Nevertheless, balance and disease origin are important considerations when targeting MAP4. As detailed above, in the context of acute myocardial infarction, increased MARK4 activity hyperphosphorylates MAP4, leading to its dissociation from the microtubule, increased VASH binding, detyrosination and contractile dysfunction[109]. Furthermore, hyperphosphorylation of particular MAP4 residues is observed in the heart of patients with tetralogy of Fallot or myocardial infarction[101]. Genetic mouse models mimicking MAP4 hyperphosphorylation have progressive cardiac hypertrophy, cardiac fibrosis, and systolic and diastolic dysfunction[101]. Additional work is needed to examine the phosphorylation-dependent regulation of MAP4 (and lesser-studied MAPs, such as MAP tau) across different pathologies of HF.

Preclinical studies: targeting excitation–contraction coupling and arrhythmia.

Consistent with the role of the MTN in the organization of the dyad and intercalated disc, preclinical studies suggest that targeting the MTN might improve excitation–contraction coupling and conduction defects in cardiomyopathy. Dyad disruption is a hallmark of HF[31,134], and dyad restoration (for example, through BIN1 replacement[135]) can restore cardiac function in a mouse model of HF. Dyad disruption can be driven by both reduced BIN1 expression and redistribution of junctophilin 2 away from the dyad, with concomitant loss of T-tubules[40,135,136]. In mouse models of pathological remodelling, modest destabilization of microtubules or targeting of microtubule transport preserved junctophilin 2 localization, T-tubule organization and cardiac function[40,136]. A proteomic assessment of the microtubule interactome in rats with pulmonary arterial hypertension linked pathological MTN remodelling with metabolic dysregulation[106]. Activation of the inflammatory glycoprotein 130 (gp130; also known as IL-6 receptor subunit-β) drives MTN stabilization and pathological remodelling, whereas gp130 antagonism normalizes MTN and junctophilin 2 abnormalities and protects the right ventricle from T-tubule remodelling, hypertrophy and impaired function[106].

Although multiple reports link adverse MTN and T-tubule remodelling, key unanswered questions limit our precise targeting of this pathway. Which molecular mechanisms maintain BIN1 and/or junctophilin 2 localization at the dyad and give rise to junctophilin 2 redistribution in HF? What provides the driving force needed to form or remodel T-tubule membranes? Finally, what specific features of MTN remodelling (that is, the tubulin code, stabilization and transport bias) drive dyad disruption, and can they be specifically targeted?

Oxidative stress is also strongly linked with MTN remodelling, impaired excitation–contraction coupling and conduction defects. In dystrophic cardiomyopathy, pathological MTN remodelling contributes to elevated levels of oxidative stress, aberrant Ca^{2+} homeostasis and arrhythmogenesis via altered mechanotransduction (known as X-ROS signalling)[80,81,137]. Connexin 43 phosphorylation has been identified as a downstream target of this signalling, which promotes gap junction redistribution from the intercalated disc to the lateral cardiomyocyte membrane[138]. Reversing MTN remodelling with colchicine treatment corrects both the connexin 43 localization[138] and workload-induced arrhythmias in dystrophic mice[80].

Although disruption of the intercalated disc is an unsurprising cause of arrhythmic cardiomyopathy, causative variants in *LMNA*[32] are more challenging to reconcile. *LMNA* encodes the nuclear intermediate filament lamin A, which governs nuclear architecture. Interestingly, loss of MTN acetylation might contribute to mislocalized connexin 43 and conduction defects in murine laminopathy[139]. This finding is consistent with changes to the nucleoskeleton remodelling the cytoskeleton via 'inside-out' signalling[140]. Curiously, another study found that disrupting the nucleoskeleton–cytoskeleton interaction conferred robust cardio protection in a mouse model of severe laminopathy[141]. Whether these mechanisms are linked is unknown, but these studies highlight the MTN as a putative effector of *LMNA*-associated cardiomyopathy.

Evidence from human genetics.

Tubulinopathies, which arise from variations in the genes encoding tubulin isoforms, manifest primarily as neurological and blood disorders and are not an established cause of human cardiomyopathy. However, reports have now begun to link variation in MAP-encoding genes (such as *MTUS1* and *MAP7D1*) and motor-encoding genes (such as *KIF20A*) to congenital cardiomyopathy[142,143] and doxorubicin-induced cardiomyopathy and HF[144]. A more comprehensive assessment of the associations between variation in genes encoding MAPs, modifying enzymes and motor proteins and cardiovascular disease is needed.

Conclusions

Accumulating evidence implicates MTN remodelling in the progression of HF from various causes. The dynamicity and interconnectivity of cardiomyocyte microtubules enable their on-demand regulation of cardiomyocyte mechanics, excitation–contraction coupling, conduction and growth. The MTN maintains and remodels the sarcomere, dyad and intercalated disc, but diverse pathogenic mechanisms converge on hyperstabilization and detyrosination of microtubules, which promotes adverse remodelling and functional decline. The dense, detyrosinated MTN mechanically impedes cardiomyocyte motion and stiffens the myocardium. Microtubule destabilization can be cardioprotective and confer functional improvements in several animal models of cardiomyopathy, but has a limited therapeutic index owing to on-target toxicity. More granular, cardiac-specific approaches are attractive and will benefit from increased understanding of the multiple levels and functional effects of MTN regulation in heart disease.

Supplementary Material

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Key points

- Microtubule-based transport establishes, maintains and remodels important subcellular compartments in cardiomyocytes, including the intercalated disc, transverse tubule and sarcoplasmic reticulum membrane systems.
- Microtubules distribute mRAN and the translational machinery throughout cardiomyocytes to control local protein synthesis and cardiomyocyte hypertrophy.
- The microtubule network remodels in terms of its density, post-translational modifications, tubulin isoform composition, stability and crosslinking in various cardiac pathologies.
- A modified, dense and crosslinked microtubule network increases the viscoelastic resistance to cardiomyocyte motion in heart failure, which can contribute to elevated myocardial stiffness.
- Although gross microtubule disruption improves cardiac outcomes in certain large-animal and small-animal models of heart failure, more targeted therapeutic approaches are needed for clinical application.

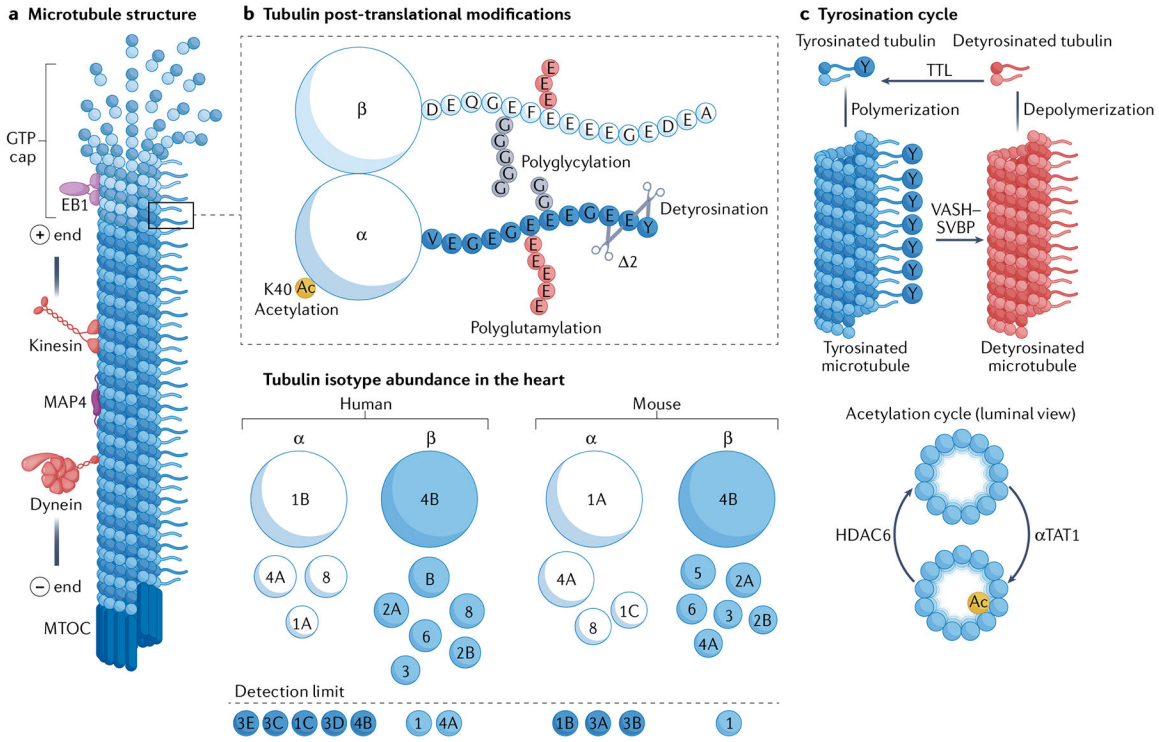

Fig. 1 |. The tubulin code in the heart.

Diverse isoforms and post-translational modifications of tubulin give rise to discrete microtubule populations. **a** | The microtubule minus end is stabilized and nucleated at the microtubule-organizing centre (MTOC), whereas the dynamic plus end undergoes cyclic rounds of polymerization and depolymerization. Kinesin and dynein power cargo transport, whereas microtubule-associated proteins, such as MAP4, bind to the microtubule, regulating its stability, transport and interactome. Tubulin plus-end-interacting proteins, such as microtubule-associated protein RP/ED family member 1 (EB1), specifically bind to the GTP–tubulin cap to regulate microtubule dynamic instability. **b** | The C-terminal tails of the α-tubulin and β-tubulin heterodimer are hot spots for post-translational modifications, such as detyrosination, Δ2 formation (removal of the next glutamine residue after detyrosination), glutamylation and glycylation. Tubulin can also be acetylated, for example, at lysine 40 (K40). In humans, at least nine α-tubulin and nine β-tubulin isoforms exist, further contributing to tubulin diversity. In the lower panel, the circles are scaled to show the relative abundances of different tubulin isoforms in the heart of humans (left) and mice (right)[7,8]. **c** | The most-studied post-translational modifications of microtubules in the heart are C-terminal detyrosination and luminal acetylation, both of which are induced in heart failure. The tyrosination cycle is regulated by the tyrosinating enzyme tubulin–tyrosine ligase (TTL) and the detyrosinating enzymatic complex formed by vasohibin (VASH) and small vasohibin-binding protein (SVBP). Acetylation is regulated by histone deacetylase 6 (HDAC6) and α-tubulin *N*-acetyltransferase 1 (αTAT1).

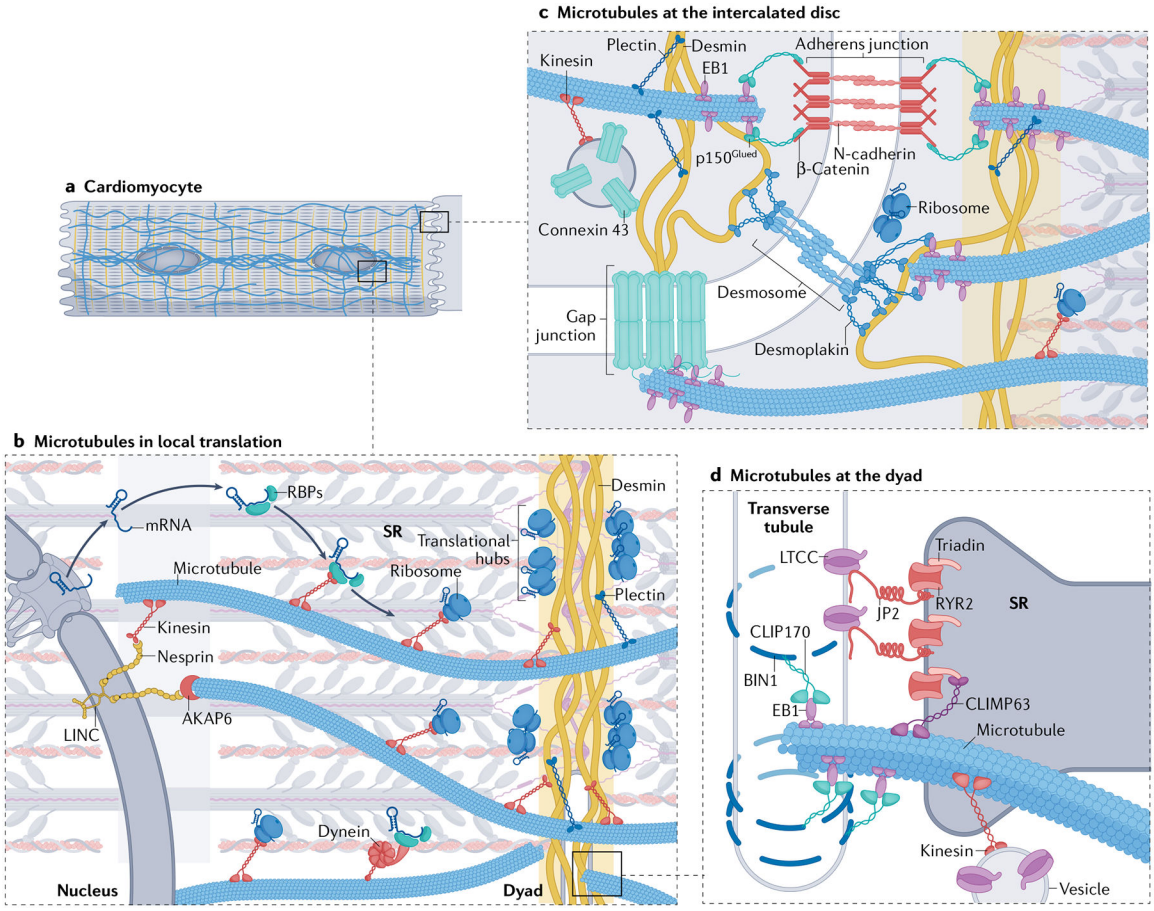

Fig. 2 |. Microtubules organize subcellular domains in cardiomyocytes.

a | Longitudinally oriented microtubules anchor at the nucleus, Z-disc and intercalated disc of cardiomyocytes. **b** | Detyrosinated microtubules anchor to the nucleus via A-kinase anchor protein 6 (AKAP6) binding to the LINC (linker of nucleoskeleton and cytoskeleton) complex. After export from the nuclear pore, mRNA binds to RNA-binding proteins (RBPs) or the ribosome, where it is transported on the microtubule motors kinesin and dynein to translational hubs flanking the sarcomeric Z-disc. **c** | The intercalated disc is a specialized region of mechanical and electrical connectivity consisting of two types of anchoring complex — adherens junctions and desmosomes — as well as low-resistance transmembrane channels at gap junctions. Microtubule-associated protein RP/ED family member 1 (EB1) anchors and stabilizes the microtubule plus tips at all three intercalated disc complexes by interfacing with N-cadherin via $p150^{Glued}$ and β-catenin at the adherens junction, desmoplakin at the desmosome and with the C-terminal tails of connexin 43 channel proteins at gap junctions. **d** | The dyad is the specialized region of excitation–contraction coupling in cardiomyocytes. Invaginations of the sarcolemma, called transverse tubules, are stabilized in close proximity to the sarcoplasmic reticulum (SR) calcium store. L-type calcium channels (LTCCs) initiate calcium-induced calcium-release from ryanodine receptor 2 channels (RYR2), which are held in close proximity by junctophilin 2 (JP2). Microtubule-dependent redistribution of JP2 is implicated in the loss of dyad organization in heart failure. The microtubule plus-end-interacting protein EB1 interacts

with CAP-Gly domain-containing linker protein 1 (CLIP170) to stabilize transverse tubules, whereas cytoskeleton-associated protein 4 (CLIMP63) spans the SR membrane to anchor microtubules to the transmembrane protein triadin. Myc box-dependent-interacting protein 1 (BIN1) binds to the transverse tubule membrane to support its curvature and interacts with the coiled-coil domain of CLIP170 to stabilize the microtubule tip.

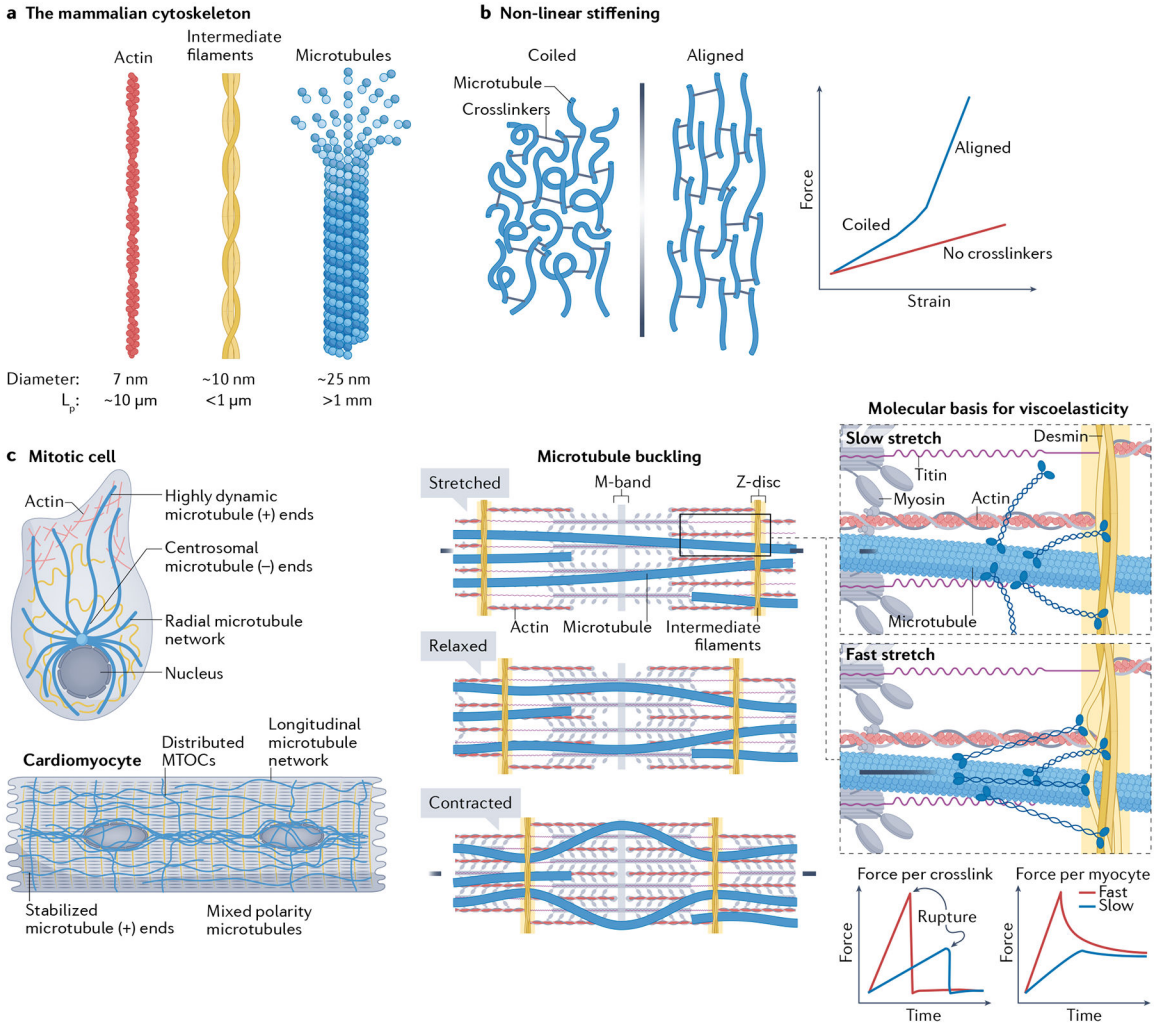

Fig. 3 |. The mechanical properties of the microtubule cytoskeleton.

Cytoskeletal filaments are rigid, interconnected polymers that bestow mechanical integrity on cells. **a** | The three cytoskeletal filaments of mammalian cells are actin, intermediate filaments and microtubules. Microtubules are the largest in diameter and the stiffest (as measured by persistence length, L_p). **b** | Crosslinked networks of filaments impart non-linear stiffening that is a hallmark of biological materials. In response to being stretched to a length at which the polymers align, crosslinkers engage and the network becomes harder to stretch. Non-crosslinked networks continue to stretch and do not stiffen in response to strain. **c** | The organization of filaments and their crosslinking dramatically influence their mechanical properties. Dynamic microtubules in mitotic cells have a minor role in cell mechanics, whereas the stabilized microtubules in cardiomyocytes resist contraction and stretch (left panel). Detyrosinated microtubules crosslink to desmin intermediate filaments at the Z-disc, where they buckle to resist contraction and slide to resist stretch (centre panel). Dynamic crosslinking between the microtubules and intermediate filaments imparts viscoelasticity to cardiomyocytes (right panel). Crosslinks break and reform when strained and, as the strain rate becomes faster, the breaking force increases, giving rise to microtubule-based

stiffness (viscoelasticity) that increases with the speed of stretch or contraction. MTOC, microtubule-organizing centre.

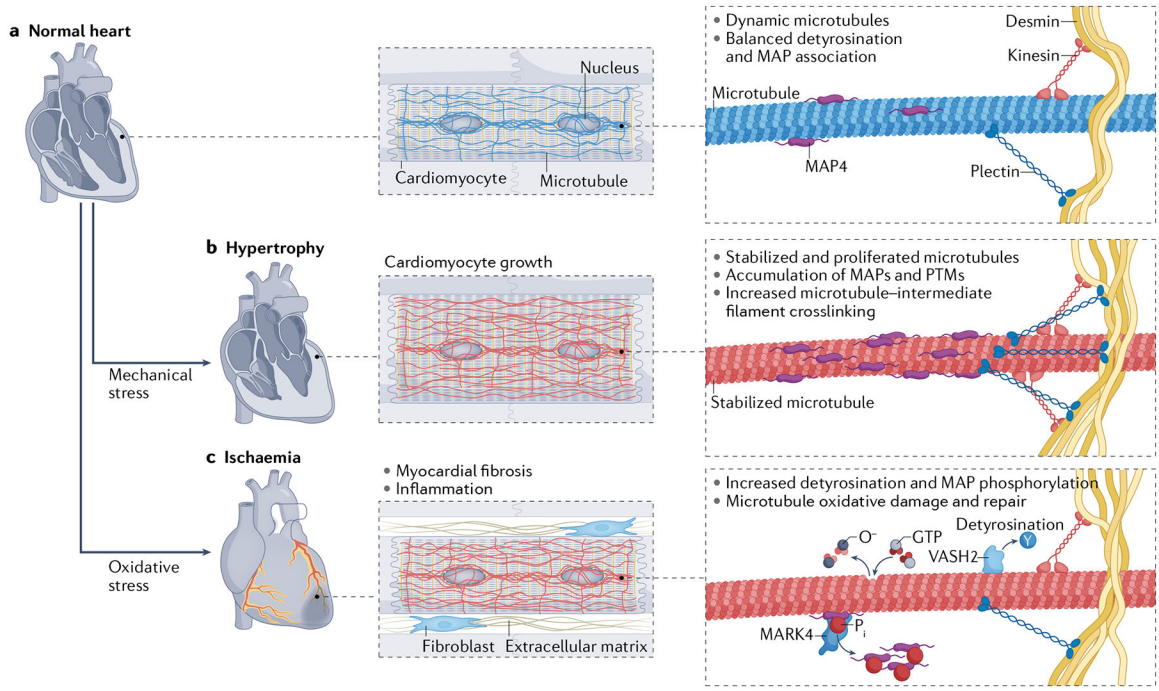

Fig. 4 |. Microtubule remodelling in heart failure.

a | In the healthy heart, the microtubules are dynamic and undergo balanced detyrosination and binding to microtubule-associated proteins (MAPs), such as MAP4. Microtubule network stabilization and detyrosination are hallmarks of heart disease, but arise from divergent processes in response to mechanical or oxidative stress. **b** | Mechanical stress such as haemodynamic overload leads to cardiac hypertrophy driven by cardiomyocyte growth. At the microtubule level, mechanical stress induces hypophosphorylation of MAP4, driving MAP4 accumulation on the microtubule and subsequent microtubule stabilization. Long-lived microtubules accumulate post-translational modifications (PTMs), such as detyrosination and acetylation, which further stabilize the microtubules and increase crosslinking between the microtubule network and desmin intermediate filament networks, which can occur through kinesin, plectin and other MAPs (TABLE 1). **c** | In the ischaemic heart, oxidative stress and inflammation lead to myocardial fibrosis and cardiomyocyte remodelling. At the microtubule level, hyperphosphorylation of MAP4 by MAP–microtubule affinity-regulating kinase 4 (MARK4) dissociates MAP4 from the microtubule, allowing increased vasohibin 2 (VASH2) binding and microtubule detyrosination. Oxidative stress also damages the microtubule and promotes repair by GTP–tubulin, which exerts a stabilizing effect on the microtubule. P_i, inorganic phosphate.

Table 1 |

Microtubule crosslinkers

Microtubule-associated protein	Cytoskeletal filaments or organelles to which the microtubule is linked	Refs
Microtubule–actin crosslinking factor 1 (MACF1)	Actin	73
Microtubule-associated protein 4 (MAP4)	Actin	71
Profilin	Actin	74
Microtubule-associated protein tau	Actin	71
Plectin	Actin and intermediate filaments	66
Kinesin 1	Intermediate filaments	68
A-kinase anchor protein (AKAP)	Nucleus	77
CAP-Gly domain-containing linker protein 1 (CLIP170)	Transverse tubule	25
Microtubule-associated protein RP/EB family member 1 (EB1)	Transverse tubule and intercalated disc	25,33
Cytoskeleton-associated protein 4 (CLIMP63)	Sarcoplasmic reticulum	28

Table 2 |

Targeting stable microtubules in heart disease

Species	Disease model	Therapy	Outcomes	Ref.
LV pressure overload				
Dog	Aortic stenosis	Colchicine (hours, high dose)	Restored cardiac output	113
Rat	Transverse aortic constriction	Colchicine (days, high dose)	Prevented LV hypertrophy and preserved LV function	41
Mouse	Transverse aortic constriction	Transgenic: hypostabilized microtubules	Prevented LV hypertrophy and preserved LV function	100
Mouse	Transverse aortic constriction	Transgenic: hyperstabilized microtubules	Promoted LV hypertrophy and reduced LV function	100
Mouse	Transverse aortic constriction	Colchicine (days, high dose)	Prevented LV hypertrophy and transverse tubule remodelling, preserved LV function and increased survival	40
Rat	Angiotensin II-induced	Parthenolide	Prevented LV hypertrophy	105
Mouse	Phenylephrine-induced	Colchicine (days, high dose)	Prevented LV hypertrophy	44
Hypertension				
Rat	Spontaneously hypertensive rat	Colchicine (days, high dose)	Blocked progression of LV hypertrophy	42
Right ventricular pressure overload				
Rat	Pulmonary hypertension (monocrotaline)	Colchicine (days, high dose)	Restored right ventricular function, reduced right ventricular hypertrophy and increased exercise capacity	114
Myocardial infarction				
Mouse	Myocardial infarction	Colchicine (hours–days, high dose)	Improved survival and preserved LV function	129
Rat	Ischaemia–reperfusion	Parthenolide	Reduced infarct size, inflammation and markers of oxidative stress	130
Atrial fibrillation				
Rabbit	Coronary artery ligation	Colchicine (days, high dose)	Restored LV function and reduced atrial fibrillation	116
Muscular dystrophy				
mdx mouse	Dystrophic cardiomyopathy	Colchicine (days, high dose)	Corrected gap junction remodelling	138
mdx mouse	Dystrophic cardiomyopathy	LC-1	Prevented workload-induced arrhythmia	80
mdx mouse	Dystrophic cardiomyopathy	Colchicine (days, high dose)	Corrected transverse tubule remodelling	136
Laminopathy				
Mouse	Laminopathy (LMNA-H222P)	Paclitaxel	Corrected gap junction remodelling	139
All-cause cardiovascular disease				
Human	Cardiovascular disease	Colchicine	30% reduction in adverse cardiovascular outcomes	117

Species	Disease model	Therapy	Outcomes	Ref.
Inflammatory cardiomyopathy				
Human	Pericarditis	Colchicine	50% reduction in the risk of recurrence	[117]

This table summarizes studies to date that have tested the in vivo therapeutic efficacy of targeting microtubule stability in various forms of heart disease. Modifications to microtubule stability (using colchicine or paclitaxel) and detyrosination (using parthenolide or LC-1) have been explored in diverse animal models of heart failure. In humans, low-dose colchicine is cardioprotective, but probably via anti-inflammatory effects. The use of high-dose colchicine in animal models to destabilize cardiac microtubules can both protect against pathological remodelling and improve the performance of the diseased heart. New compounds that can more specifically and potently manipulate cardiac microtubules are needed. LV, left ventricular.