The role of the cytoskeleton in heart failure

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Abstract

The cytoskeleton of cardiac myocytes consists of actin, the intermediate filament desmin and of \(\alpha\)- and \(\beta\)-tubulin that form the microtubules by polymerization. Vinculin, talin, dystrophin and spectrin represent a separate group of membrane-associated proteins. In numerous experimental studies, the role of cytoskeletal alterations especially of microtubules and desmin, in cardiac hypertrophy and failure (CHF) has been described. Microtubules were found to be accumulated thereby posing an increased load on myocytes which impedes sarcomere motion and promotes cardiac dysfunction. Other groups were unable to confirm microtubular densification. The possibility exists that these changes are species, load and chamber dependent. Recently, damage of the dystrophin molecule and MLP (muscle LIM protein) were identified as possible causes of CHF. Our own studies in human hearts with chronic CHF due to dilated cardiomyopathy (DCM) showed that a morphological basis of reduced contractile function exists: the cytoskeletal and membrane-associated proteins are disorganized and increased in amount confirming experimental reports. In contrast, the contractile myofilaments and the proteins of the sarcomeric skeleton including titin, \(\alpha\)-actinin, and myomesin are significantly decreased. These changes can be assumed to occur in stages and are here presented as a testable hypothesis: (1) The early and reversible stage as present in animal experiments is characterized by accumulation of cytoskeletal proteins to counteract an increased strain without loss of contractile material. (2) Further accumulation of microtubules and desmin to compensate for the increasing loss of myofilaments and titin represents the late clinical and irreversible state. We suggest, based on a structural basis for heart failure, an integrative view which closes the gap between changes within cardiac myocytes and the involvement of the extracellular matrix, including the development of fibrosis. These factors contribute significantly to structural ventricular remodeling and dilatation finally resulting in reduced cardiac function. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Heart failure was defined by Braunwald as the pathophysiological state of impaired cardiac function rendering the heart unable to maintain an output sufficient for the metabolic requirements of the body’s tissues and organs. Heart failure occurs either because of a decreased myocardial capacity to contract or because an excessive pressure–stroke–volume load is imposed on the myocardium [1]. Major causes of heart failure include ischemic heart disease, longstanding valvular defects, hypertension and the cardiomyopathies [2]. The pathophysiological situations of reduced myocardial perfusion and of volume or pressure overload can be studied in animal experiments but the situation of prolonged chronically reduced function as in the cardiomyopathies, is more difficult to reproduce experimentally and is therefore mostly studied in human hearts obtained at the time of transplantation.

There is established evidence that heart failure has a morphological basis, i.e. myocyte degeneration resulting in cellular atrophy and interstitial fibrosis, which represent structural correlates of reduced ventricular function [3–5]. One particular aspect of degeneration, apart from the

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reduction of myofilaments, are cytoskeletal alterations occurring as primary or secondary events which will be discussed in this minireview.

2. The cytoskeleton

The proteins which contribute to cell shape, mechanical resistance, and morphological integrity of cardiomyocytes can be subdivided on the basis of their structural and functional properties into four different groups [6]:

- **Sarcomeric skeleton:** titin, C-protein, α-actinin, myomesin, and M-protein.
- **True ‘cytoskeletal’ proteins:** tubulin, desmin and actin.
- **Membrane-associated proteins:** dystrophin, spectrin, talin, vinculin, ankyrin.
- **Proteins of the intercalated disc:** desmosomes consisting of desmoplakin, desmocollin, desmoglein and desmin; adherens junctions with N-cadherin, the catenins and vinculin and gap junctions with connexin.

The cytoskeleton is a complex network of filaments and tubules which transmit mechanical and chemical stimuli within and between cells [7,8]. It contributes substantially to cell stability by anchoring subcellular structures, such as mitochondria, Golgi apparatus, nuclei, and myofibrils. The action of the cytoskeleton as a stabilizing force and as mechanotransducer is supported by membrane-associated proteins, especially dystrophin that binds to both, intracellular actin and extracellular laminin [9]. A close integrin-cytoskeleton linkage system exists and allows cells to respond to physical and biochemical influences exerted by the extracellular matrix: When the matrix resists movement, the linkage to the cytoskeleton is strengthened via an increased number of integrins [7]. At the intercalated disc, the cytoskeleton is anchored to sites of cadherin-mediated adhesion between adjacent plasma membranes via catenins and desmoplakins [10].

3. Tubulin

The tubulin molecule is a heterodimer of an α- and β-isofrom with a molecular weight of 55 kDa per monomer. Tubulin occurs in cells as organized microtubules with a diameter of 25 μm. A constant turnover of microtubules by polymerization and depolymerization takes place. In cardiomyocytes, only 30% of total tubulin is present in the polymerized form as microtubules whereas 70% occurs as non-polymerized cytosolic protein [11]. Microtubules can be detected with the electron microscope in the perinuclear area of the cardiomyocyte where contractile filaments are absent [12]. Microtubular associated proteins (MAPs) bind to α- and β-tubulin and play a significant role in stabilizing microtubules and enabling an interaction with other cellular organelles.

The importance of microtubules in the development of hypertrophy and heart failure has been investigated and reviewed by Rappaport [13] who reported tubulin accumulation which may contribute to myofibrillogenesis and possibly play a role in protein synthesis as a transient phenomenon in the development of pressure overload hypertrophy in rats. The role of microtubules in cardiac hypertrophy and failure was further investigated intensively by Cooper and coworkers in numerous experimental studies. In a model of feline right ventricular hypertrophy resulting from pulmonary artery banding, isolated myocytes showed contractile dysfunction [14,15] and loss of compliance [16]. These changes were accompanied by increased cytoskeletal stiffness characterized by an augmentation of the amount of total tubulin and an elevated degree of polymerization. Reduction of the microtubule hyperpolymerization by colchicine treatment reversed myocyte stiffness and normalized contraction dynamics (a finding questioned by others [17,18]). Taxol treatment of normal myocytes produced microtubule overloading and functional disturbances similar to chronic pressure overload in vivo. In another study, failing feline hearts were shown to exhibit reversible abnormalities of the cytoskeleton that were even more pronounced than in hearts with compensated hypertrophy [19]. Functional abnormalities in cardiac hypertrophy were therefore interpreted to be a consequence of increased intracellular loading due to increased microtubular density causing impediment of sarcomere motion. The conclusion drawn from these elegant studies was that cytoskeletal abnormalities rather than changes of myofilaments are responsible for cellular contractile dysfunction observed in compensated hypertrophied and failing myocardium.

In continuation of this work, the same group recently published data on the role of microtubules in the transition from compensated to decompensated hypertrophy in a canine model of left ventricular pressure overload due to aortic banding [11]. In myocytes isolated from failing hearts, contractile function was depressed and microtubules were increased, both of which could be reversed by reducing the rate of tubulin polymerization with colchicine. The 2:1 ratio of non-polymerized to polymerized tubulin typical of controls and hypertrophic hearts was reversed in heart failure. An augmentation of tubulin at the protein level in combination with an elevated degree of microtubule stabilization was discussed as a possible mechanism for the increased densification [20].

The view that increased microtubular stabilization may be an important factor in causing cytoskeletal stiffness and contractile dysfunction is shared by Wang et al. examining the transition from hypertrophy to failure in guinea pig hearts after aortic banding [21]. These authors found an increased microtubule density by quantitative confocal microscopy, but no increase in total tubulin in Western
blots [21] and believe that because of increased microtubu-
lar stabilization the cytoskeleton (including desmin and
titin) may be involved in causing ventricular dysfunction.

On the other hand, Collins et al. using the same guinea-
pig model of hypertrophy and failure [22] came to the
conclusion that neither the level of tubulin nor the degree
of polymerization are involved in functional changes
occurring in cardiac hypertrophy. The role of tubulin
accumulation and polymerization was also questioned by
Bailey [17] who did not observe an effect of colchicine on
contraction dynamics in isolated feline myocytes from
hypertrophied hearts and by de Tombe studying the effects
of colchicine and taxol on rat trabeculae contractility [18].

In pigs with heart failure induced by supraventricular
tachycardia, absence of changes in mRNA levels of
myosin or actin, but abundance of α-tubulin mRNA and
microtubules (while β-tubulin was unchanged), were ob-
served and may have contributed to remodeling of the
cytoskeletal architecture [23].

In failing human myocardium a distinct increase of
cytoskeletal proteins has consistently been observed by our
group [5]. Our more recent findings (unpublished) in
human explants using confocal microscopy indicate that
tubulin is clearly increased as compared to control tissue
from normal human hearts while contractile filaments are
reduced. It should be noted, however, that these changes
occur in a heterogeneous manner across the left ventricular
wall varying in intensity from cell to cell. Furthermore,
using Western blot and Northern hybridization, our group
observed increased protein and mRNA levels for tubulin
(unpublished results).

4. Desmin

Desmin (MW 53 kDa) belongs to the family of inter-
mediate filaments with a diameter of 12–15 nm ranging
between microtubules (25 nm) and actin filaments (8–10
nm). Using electron microscopy, the occurrence of inter-
mediate filaments (called 100 A filaments at that time) in
the myocardium was meticulously described by Ferrans
and Roberts [4]. Currently, desmin filaments are most
effectively visualized using monoclonal antibodies and
immunohistochemistry.

Muscle specific desmin counteracts external stresses, as
postulated by Lazarides [24] and later shown by Fuchs and
Cleveland [25]. Gross perturbations of the intermediate
filament system are incompatible with cell survival [25]. In
fibroblasts, the mesenchymal cell specific intermediate
filament vimentin is crosslinked to microtubules by plec-
tins that also bind to actin [26]. This linkage most probably
also exists in muscle cells and would explain why both
components of the cytoskeleton are altered concomitantly
in hypertrophied and failing myocardium. Cytoskeletal
control of myogenesis has been demonstrated in skeletal
and smooth muscle cells using desmin null mutations in
embryoid bodies, but there were no obvious effects on
cardiac cell differentiation [27]. However, using desmin
knockout mice, desmin was found to be essential for
myofibrillar functional integrity and the maintenance of
general cellular integrity, e.g. for the position of the
nucleus [28]. In mice with desmin null mutations, degene-
ration of cardiac muscle was observed indicating the
essential role of desmin for cell survival and sar-
comerogenesis [29,30].

Few studies have investigated the role of desmin in
hypertrophy and failure. In an early study, it was shown
that desmin was increased during the development of
hypertrophy, possibly to keep sarcomeres in register [13].
On the other hand, in hypertrophy and failure in feline or
canine hearts, the organization and amount of desmin
filaments were found to be normal [11,15]. In contrast,
others reported increased mRNA levels for desmin and
Western blot analysis showed an increased expression of
desmin [22]. A progressive increase of desmin protein and
filaments was observed during the transition from hy-
pertrophy to failure in guinea pig hearts after aortic
banding [21].

In explanted failing human myocardium we observed an
increase and disorganization of desmin filaments using
immunocytochemistry. The irregular distribution of desmin
and its mRNA by in situ hybridization in individual
myocytes coincided with the occurrence of Z-line stream-
ing and with a lack of contractile filaments as observed by
electron microscopy [3,4,31]. We concluded that the abnor-
mal wall stress on myocytes in heart failure is counteracted
by an overexpression of myocyte scaffold proteins.

5. Membrane-associated proteins

These proteins have also been considered as belonging
to the cytoskeleton [32]. To define them as a separate
family of proteins seems, however, more appropriate
because they are all localized at or close to the cellular
membrane and are functionally different from the ex-
tramyo filament cytoskeleton [6]. These proteins are in-
volved in fixation of sarcomeres to the lateral sarcolemma
and stabilization of the T-tubular system [33]. They
represent the non-integrin component connecting the in-
tracellular milieu with the extracellular matrix [6,9,34].
Recently, it was shown that a dystrophin missense
mutation may cause X-linked dilated cardiomyopathy [35].
This view was enforced by a recent report demonstrating that
cleavage of dystrophin by an enteroxiral protease 2A
results in cytoskeletal disruption and post-myocarditis
cardiomyopathy [36]. It was postulated that whereas
hypertrophic cardiomyopathies may be a sarcomeric dis-
ease, the possibility exists that mutations or other distur-
bances of cytoskeletal proteins are the cause of dilated
cardiomyopathy [37,38]. This would implicate mutations
of the membrane-associated protein dystrophin and probably disturbances of other cytoskeletal proteins as a cause of heart failure. This is in contradistinction to our own results showing that an accumulation of the cytoskeletal components tubulin and desmin in the presence of a reduction of the contractile material are the prevailing alterations of structural proteins in hearts failing because of DCM. Further studies are needed to clarify this problem.

Vinculin (MW 116 kDa) links the cell membrane and actin filaments via talin, paxillin and α-actinin. According to our confocal microscopy studies, vinculin is not localized at the Z-disc as claimed earlier [39] but is part of the T-tubular membrane thereby giving the impression of being localized in the Z-disc [33].

Metavinculin, an isoform of vinculin, has the same localization as vinculin: the intercalated disc, the costameric part of the sarcolemma, and the T-system. In one patient with heart failure due to DCM, Maeda et al. found a metavinculin deficiency though its mRNA was present and they concluded that changes of the cytoskeleton may significantly contribute to ventricular remodeling observed in this disease [40]. Recently, it has been shown that the ‘actin-based cytoskeleton protein (MLP)’ which binds to vinculin and myomesin is essential for the maintenance of myocyte architecture [41]. MLP−/− mice developed the clinical picture of DCM followed by heart failure. It was concluded from this study that MLP may be a crucial component for the lateral alignment of myofilaments and their anchorage to the vinculin containing costameres.

In failing human hearts vinculin was increased [5] as were dystrophin, talin, and spectrin as observed in our more recent studies using confocal microscopy [5,6].

6. Titin

Titin (MW 3000 kDa) is a giant protein that spans in a spring-like fashion from the Z-disc (binding sites to α-actinin [42]) to the M-band (binding sites to myomesin [43]) as one single molecule, and it is also connected to myosin via the C-protein. It is the largest molecule known and it has been completely sequenced [44]. Ig-like and fibronectin-like domains have been identified as that part of the molecule that is responsible for its elastic behavior. Titin filaments ensure the elasticity and extensibility of the sarcomere and also its capability to restore original sarcomere length after application of passive stretch [45–47]. Besides its mechanical properties, deposition of titin filaments has been found to be a prerequisite for sarcomerogenesis and lack of titin may therefore contribute to the reduction of the contractile apparatus in failing hearts [48].

Only a few experimental studies exist on the role of titin in cardiac hypertrophy and failure. Collins reported an increase in titin mRNA with hypertrophy and loss of titin in heart failure [22] which was confirmed by Wang [21]. In the failing human heart loss of titin was evident [49,50]. This adds to increased ventricular stiffness observed in failing hearts because of a loss of sarcomere elasticity.

7. General comments and conclusion

Changes within the cytoskeleton of cardiac myocytes are important in cardiac hypertrophy and failure. However, whether these changes are specific for the experimental hypertrophy model or whether they can be extrapolated to heart failure in general is still under discussion. In a recent editorial it was stated that: “the results (from Cooper’s group) seem to be load, species, and chamber specific” [51] (also critically discussed by ter Keurs [52]). The stage of failure, whether compensatory or decompensatory may also be important.

Our findings in human cardiomyopathic heart tissue confirm the association between cytoskeletal changes and contractile dysfunction. We believe the accumulation of tubulin, desmin and membrane-associated proteins to be a compensatory mechanism typical of heart failure independent of the underlying cardiac disease.

Based on our current knowledge we would propose the following scenario: The transition from hypertrophy to failure can be differentiated into two stages: (1) An early reversible stage, as observed in animal experiments, where an accumulation of cytoskeletal proteins counteracting the increased strain on the myocardium occurs. (2) A later irreversible stage characterized by loss of the contractile filaments in the presence of microtubule densification and desmin disorganization. This may be beneficial because it compensates for the loss of myofilaments but on the other hand, it has deleterious effects because it increases the internal load on the already damaged myocytes. Loss of proteins of the sarcomeric skeleton (titin, α-actinin, myomesin) further contributes to ventricular dysfunction. In addition, accumulation of membrane-associated proteins will contribute to cardiomyocyte dysfunction. Alterations of the intercalated disc proteins may also play an important role but have not been studied yet.

The cytoskeleton is not only involved in cellular stability and integrity but it plays a significant role in transmitting signals from the cellular membrane to the nucleus. Integrins can act as mechanoreceptors, and transfer of force from integrins to the cytoskeleton is thought to represent a proximal step in an intracellular mechanical signalling that leads to global cytoskeletal rearrangements [53]. The extracellular matrix controls cytoskeletal mechanics and structure, particularly by binding of fibronectin to integrins [7,8]. An increased number of integrin binding sites for fibronectin leads to increased cytoskeletal stiffness and apparent viscosity [8] which would establish a causative link between the increased amount of extracellular matrix proteins and the changes of the cytoskeleton in cardiac hypertrophy and failure. Though this review has
only discussed intracellular changes of the myocytes and not the problem of fibrosis, it needs to be emphasized that this extracellular phenomenon plays an equally important role as structural component contributing to heart failure [54].

These various processes within the myocytes and in the interstitial space result in ventricular structural and geometric remodelling. Therapeutic approaches should take into account alterations of the cytoskeleton in the development of end-stage heart failure.

References


