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Altered myofilament stoichiometry in response to heart failure in a cardioprotective α-myosin heavy chain transgenic rabbit model

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Abstract

Purpose—Decreases in alpha myosin heavy chain (α-MHC) is a common feature of human heart failure (HF), while α-MHC overexpression in transgenic (TG) rabbits is cardioprotective against tachycardia-induced cardiomyopathy (TIC). Hypothesizing that MHC isoform content alterations would impact sarcomere and mitochondrial energetics protein complement, we investigated the impact of α-MHC overexpression on global cardiac protein expression.

Experimental Design—Protein expression was assessed by two-dimensional gel electrophoresis and mass spectrometry on extracts from TG and non-transgenic (NTG) rabbits under TIC or sham-operated conditions.

Results—We observed significant changes in the levels of actin, myosin light chain 2 and desmin between the LV tissue of TG and NTG animals. The proteome was broadly impacted, with significant changes in mitochondrial energetics and chaperone protein families. No changes were observed in total cellular MHC or in myofibril-associated MHC. In myofibrils isolated from TG_{Sham} animals, only actin levels were altered in TG_{sham} compared to NTG_{sham} animals, suggesting careful myofibril assembly regulation.

Conclusions /Clinical Relevance—These data suggest myofibril protein composition may protect against TIC, emphasizing protein interconnectivity and demonstrating the need for broad-based proteomic studies in understanding targeted genetic manipulations. This study identifies targets for future development of cardioprotective agents and elucidates tachycardia-induced HF pathways.

Keywords

two dimensional gel; mass spectrometry; myofilament; α myosin heavy chain

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Introduction

Heart failure (HF) is a pathophysiological state characterized by insufficient cardiac output partially due to decreased contractility.[1] Cardiac contraction results from protein interactions between the thin and thick filaments.[2] Thick myofilaments consist of the myosin complex, comprised of a pair of myosin heavy chains (MHC) and two pairs each of myosin light chain 1 and 2 (MLC-1, MLC-2), and associated proteins such as myosin binding protein C[3]. The thin filaments are comprised of α-actin, α-tropomyosin (Tm) and the troponin complex, comprised of troponin I, troponin T (TnT) and troponin C. The sarcomere also contains associated proteins, including desmin and α-actinin. The MHC isoforms α-MHC or β-MHC are classified as either V1 myosin or V3 myosin, respectively.[4] V1 myosin has a high rate of ATP hydrolysis[5, 6], whereas V3 myosin has a slower rate of ATP hydrolysis, but generates more force per unit of ATP.[7] V1 myosin predominates in the rodent ventricle, whereas V3 myosin is the predominant isoform in human and rabbit ventricles.[8]

Although α-MHC levels are low in the human and rabbit ventricle, decreases in α-MHC is a common hallmark of human [9, 10], rabbit [11], and rodent [12] heart failure. Transgenic (TG) rabbits with increased α-MHC expression displayed cardioprotection against tachycardia-induced cardiomyopathy (TIC) based on echocardiographic and hemodynamic parameters. These changes included increased pressure development, relaxation, and shortening fraction.[11] Whether protective effects are attributable solely to α-MHC or to broader myocyte alterations resultant from transgene expression is not clear. We hypothesized that alterations to a single MHC isoform would result in general sarcomeric protein complement alterations and mitochondrial energetic changes in response to the increased enzymatic activity of α-MHC. To address this hypothesis we performed 1- and 2-dimensional gel electrophoresis (1DE and 2DE, respectively) studies on cardiac tissue, from TG and non-transgenic (NTG) rabbits that were either sham-operated or underwent TIC. Since functional thick and thin filament proteins must be incorporated into myofibrils, we sought to determine whether increased α-MHC in TG animals was fully incorporated into myofibrils, and whether overexpression of α-MHC altered expression of other myofibrillar proteins. We also examined whether expression of a recently described MHC protein chaperone, UNC-45B, was impacted in TG animals.

Materials and Methods

Animal experiments

The experimental cohort consisted of 28 rabbits, 14 TG and 14 NTG (7 TIC and 7 sham, each group). Epicardial pacing lead placement and pacemaker implantation with sequential increases in LV pacing was performed over a 30-day period to induce cardiomyopathy with severe ventricular dilation as previously described.[11] Sham animals were treated identically, but the pacemaker generator was not activated. All animals were treated humanely and in accordance with the Animal Welfare Act regulations of the U.S. Department of Agriculture and with the Guide for the Care and Use of Laboratory Animals.

Sample preparation

From each animal, 100 mg of tissue was homogenized in 10 volumes per mass of tissue in whole tissue extraction buffer (20 mM Tris-HCl (pH 6.8), 7 M urea, 2 M thiourea, 4% ASB-14 (tetradecanoylamidopropyl-dimethylammonio-butanesulfonate), 50 mM NaF, 1μM leupeptin, 1 μM pepstatin, 2.5 mM EDTA, 0.36 μM aprotinin and 0.2 mM Na_3VO_4) using a dounce homogenizer. Extracts were centrifuged and supernatant stored at -80°C. For the analysis of the myofibril subproteome, myofibrils were purified from both failing NTG

rabbits (n=3) and failing TG rabbits (n=3) in a protocol modified from Chandra *et al.*[13]. Tissue (100 mg) was rinsed in 10 volumes per mass of Relax buffer (75 mM KCl, 10 mM imidazole (pH 7.2), 2 mM $MgCl_2$, 2 mM EGTA, 1 mM NaN_3, 4 mM phosphocreatine, 1 mM ATP, 50 mM BDM, 1 mM DTT, 1 mM benzamidine-HCl, 0.1 mM PMSF, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 1% TX-100) and cut into small pieces. The buffer was then altered to include 10 mM EDTA and the tissue was homogenized using a Polytron (Kimatica AG, Littau-Lucerne, Switzerland) homogenizer at 55% of maximum speed for 12 seconds, five times. The homogenized sample was centrifuged at 3,000 × *g* at 4°C, the supernatant was discarded while the pellet was resuspended in 10 volumes / weight of Standard buffer (5 mM KCl, 10 mM imidazole (pH 7.2), 2 mM $MgCl_2$, 2 mM EGTA, 1 mM NaN_3, 1% TX-100). The suspended pellet was homogenized with a Dual glass-on-glass homogenizer and then centrifuged at 3,000 × *g* for 8 minutes at 4°C. The supernatant was discarded and the pellet was suspended again in Standard buffer (10 volumes / weight) and allowed to sit on ice for 5 minutes to solubilize membranes and non-myofilament proteins. The suspension was centrifuged at 3,000 × *g* at 4°C, and the pellet suspended in Standard buffer as before, which was repeated 4 times. The last two times, the pellet was suspended in Standard buffer without TX-100. Following the final wash, the pellet was highly enriched for myofibrils (myofilament subproteome). The myofibrils were suspended in K-60 buffer (60 mM KCl, 20 mM MOPS, 2 mM $MgCl_2$) and stored at 4°C for ATPase assays or solubilized into whole tissue extraction buffer for 1-DE. Protein concentrations were determined using a BCA protein assay (Pierce Biotech, Rockford, IL)

Protein electrophoresis and western blotting

For 1DE analysis, ~1 μg of myofibril extracts from NTG_{TIC} (n=3) and TG_{TIC} rabbits (n=3) were labeled with cyanine dye (Cy3) (as previously described[14]) and applied to 4-12% bis-Tris acrylamide gels (Invitrogen) with MOPS running buffer (Invitrogen). A higher load (10 μg protein) preparative equivalent gradient gel was run and silver stained.[14] For 2DE analysis, 15 μg of tissue from each rabbit whole tissue extract was first labeled with cyanine dye (Cy3 or Cy5). Proteins were isoelectric focused in 7 cm pH 4-7 Drystrips (GE Healthcare) in IEF buffer (7 M urea, 2 M thiourea, 4% CHAPS, 1% DTT, 1.5% HED). The second dimension separation (SDS-PAGE) was performed using 4-12% bis-Tris gels (Invitrogen) and MES running buffer (Invitrogen). IPG strips with a pH gradient of 4-7 were used as we were primarily interested in the changes in the myofilament proteins located in this pH range. A preparative 2D gel for protein identification (250 μg unlabeled protein extract from a NTG sham rabbit) was made by isoelectric focusing in 18 cm pH 4-7 Drystrips (GE Healthcare) and a second dimension separation was performed using 20 cm × 20 cm bis-Tris SDS-PAGE gels with a resolving gel containing 10% acrylamide and a stacking gel containing 4% acrylamide.[15] Proteins were separated in MOPS running buffer. Western blotting was performed using the NuPAGE western blotting system and apparatus as described by the manufacturer (Invitrogen), with chicken polyclonal IgY antibodies to UNC-45 (1:10,000 dilution, Genway), donkey anti-chicken alkaline phosphatase secondary antibodies (1:10,000), incubated with substrate (Pierce, Lumiphos) and exposed to film.

Gel image analysis

For Cy3/5 labeled proteins, gels were scanned using a Typhoon 9400 Imager (GE Healthcare) and analyzed by Ludesi Inc. (Lund, Sweden) for spot matching and quantification. For 1DE, individual protein bands were quantitated using Progenesis Workstation 2005 (Nonlinear Dynamics). For 2DE, the average integrated intensity for each spot was determined for each group of rabbits and four comparisons were performed between the experimental groups. Statistical significance was determined between groups using both a 1-way and 2-way ANOVA to examine differences between groups as well as

interaction between transgene incorporation and tachycardia. In order to visualize protein spots that would be analyzed by MS, 2DE gels were silver stained using a non-glutaraldehyde protocol adapted from the protocol of Shevchenko *et al* as previously described.[16] Proteins observed as multiple protein spots, possibly due to post-translational modification, were further analyzed by MS to confirm their identity as altered forms of the original protein. When multiple isoelectric forms of a protein were identified, the abundance of a particular isolectric form of a protein was compared to the total abundance of that protein.

MS analysis

For MS identification, protein spots (2DE) or bands (1DE) were excised from silver stained gels, destained and washed multiple times with 50% (v/v) ACN in water (supplemental methods), dried via speedvac (Savant) and analyzed by MS. For MALDI-TOF MS analysis, dried peptides were reconstituted in 5 μL of 50% (v/v) ACN, 0.1% (v/v) TFA. Equal volumes (0.5 μL) of peptide solution and matrix (10 mg/ml α-cyano-4-hydroxy-trans-cinnamic acid in 50% (v/v) ACN and 0.1% (v/v) TFA) were dried on a target plate. Dried plates were analyzed using an ABI-4800 TOF-TOF MS (ABI Technologies) and searched against the NCBInr database using Mascot software (Matrix Science). For LC-MS analysis, dried peptides were solubilized in 0.1% formic acid and loaded onto a C18 column connected to an Eksignent nano-2DLC pump as previously described.[17] Eluted peptides were analyzed with a LTQ-LC/MS/MS (ThermoElectron) and queried against the NCBInr database using Mascot software (Matrix Science) searching against mammals.

Ca^{2+} activated myofibrillar ATPase assay

Myofibrillar ATPase activity was determined for myofibrils purified from NTG_{TIC} (n=3) and TG_{TIC} (n=3) rabbits as previously described [18]. ATPase rates under positive Ca^{2+} conditions (7 mM EGTA, 7 mM $CaCl_2$) and negative Ca^{2+} (7 mM EGTA) were examined.

Dephosphorylation 2DE

Myocardial tissue extract (50 ug) from NTG rabbits was diluted into 50 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM Mg Cl_2, 1 mM DTT and incubated overnight at 37°C with or without the addition of 20 units of calf intestinal alkaline phosphatase (AP, New England Biolabs). AP treated samples were labeled with Cy3 whereas untreated sample was labeled with Cy5. Samples were combined and run on a single 2D gel and scanned as previously described.

Results

Total protein extracts from LV tissue obtained from sham-operated or TIC NTG and TG animals were subjected to 2D-DIGE (n = 7/condition) (Figure S1). A comparison was carried out using both pooled samples (n=3/gel) and also individual animals. Figure 1A shows the quantitative data for the myofilament proteins Tm, TnT, MLC-1 and MLC-2, α-actin and desmin. Significant changes were observed for 55 spots representing 17 proteins, and direct comparisons between all experimental groups are presented in Table 1. The thick filament protein MHC, due to its high molecular mass, is not amenable to quantitative analysis by 2DE. There were no significant changes observed in abundance or ratio of the thin filament associated proteins Tm or TnT following transgene incorporation or TIC. A common effect of TIC in both TG and NTG animals was an increase in MLC-1 (P=0.04) and MLC-2 (P<0.01, Figure 1A). Although the absolute ratios of MLC-1 and MLC-2 differed between the TG and NTG animals, TG rabbits possess a higher MLC-2:MLC-1 ratio compared to NTG rabbits, regardless of TIC status. MHC isoform composition largely modulates catalytic activity of cardiac myosin; the data therefore suggest changes in force

generation.[19] Although there were no significant differences in protein abundance of MLC-2 between NTG_{TIC} and TG_{TIC} animals, there was a change in the most basic form of MLC-2 (Spot 4, pI=4.9). As confirmed by treatment with calf alkaline phosphatase, this was due to differential phosphorylation (spot 4; pI = 4.9, unphosphorylated form of MLC-2) (Figure 2). Actin underwent a differential response depending upon transgene expression. Under sham conditions, NTG rabbits possessed 1.2-fold more actin than TG rabbits (P=0.03). TG_{TIC} rabbits showed increased actin, whereas NTG_{TIC} rabbits showed a reduction (Figure 1A, Table 1).

The intermediate filament protein desmin, which is associated with the sarcomere through its interaction at the Z-disk, [20] showed increased abundance in both NTG_{TIC} and TG_{TIC} rabbits (Figure 1), although desmin increases in TG_{TIC} animals did not reach the same level as NTG_{TIC} animals (P=0.01). Desmin levels were ~35% lower in TG_{TIC} rabbits compared to the NTG_{TIC} rabbits (Figure 1A, P=0.03). We also observed a reduction in the abundance of the most basic form of desmin in TG_{sham} and TG_{TIC} animals (Table 1, Figure S2).

Using 2DE-DIGE it was not possible to determine whether the changes in the myofilament proteins reflected changes in intact myofilaments or in the soluble, non-incorporated protein pool. We hypothesized that the effects of altered α-MHC would be most pronounced in myofilaments, so myofibrils were isolated and compared to total cellular extracts of the myofilament proteins from the LV-free wall tissue of NTG_{TIC} and TG_{TIC} rabbits (Figure 3A and B). Absolute and relative levels of MHC, MLC-1, and MLC-2 were unchanged in intact myofibrils between NTG_{TIC} and TG_{TIC} rabbits. Thus, the increase in MLC-2 observed in the 2DE experiments for the TG rabbits is most likely due to increases in the soluble protein pool. As α-MHC levels are maintained in TG_{TIC} animals (but undetectable in NTG_{TIC} animals),[11] increases in the soluble pool of α-MHC due to TG expression suggests that proteins involved in chaperoning myosin assembly might be elevated during TIC in TG animals. We therefore focused on a newly identified co-chaperone for α-MHC, UNC-45. [21] UNC-45 is essential for formation of thick filament in *C elegans*.[22-24]. Among vertebrates, there are two UNC-45 homologues: UNC-45A and UNC-45B. UNC-45A is ubiquitous, while UNC-45B is found only in cardiac and skeletal muscle, and has a role in sarcomere formation.[25] Western blot analysis indicates that UNC-45B was upregulated >5-fold in TG_{TIC} animals compared with TG_{Sham} animals (Figure S3). Similar to the findings from total cell extracts, there was also higher actin levels in TG_{TIC} rabbits compared to NTG_{TIC} rabbits (20%; P=0.01) from isolated myofibrils. TnT levels were elevated in myofibrils (24%, P=0.04) but not whole cell extracts.

These data suggest that there is a selective increase in at least some of the thin filament proteins relative to the thick filament (e.g. MHC) in the myofibrils of TG rabbits following pacing. Since several key myofilament proteins are differentially regulated, we investigated whether this affected myofibrillar ATPase acvitivty. Ca^{2+}-activated myosin ATPase was increased in TG_{TIC} myofilaments compared to NTG_{TIC} myofilaments (P<0.005; Figure 3C), consistent with increased incorporation of α-MHC, as described by James *et al.*[11]

Energetic and stress response proteins

We observed changes in proteins involved in cellular energetics and stress responses among TG_{TIC} animals (Figures 4 and S2). The majority of these proteins were from electron transport chain Complex I or Complex III. In Complex I, there was 37% less of the 75 kDa subunit, but 21% more of the 30 kDa subunit in TG_{Sham} rabbits compared to NTG_{Sham} rabbits. Following TIC, the level of the 75 kDa subunit decreased in NTG_{TIC} rabbits but not in TG_{TIC} rabbits, whereas the 30 kDa subunit increased in NTG_{TIC} rabbits, but decreased in TG_{TIC} rabbits. The 24 kDa subunit of Complex I was approximately 25% lower in NTG_{Sham} animals, but significantly reduced in TG_{TIC} animals, whereas levels were unchanged in

NTG_{TIC} animals. There was 39% more core protein I in NTG_{Sham} rabbits compared to TG_{Sham} rabbits ($P<0.005$), but following TIC there was 45% more in TG_{TIC} rabbits ($P=0.04$). We also observed changes in oxidoreductase protein 3-hydroxyisobutyrate dehydrogenase (3-HIBD) and the TCA cycle protein ATP-specific succinyl-CoA ligase (SCL) following TIC. There was an increase of 110% for 3-HIBD in TG_{Sham} rabbits compared to NTG_{Sham}, but there was no significant difference between TIC groups. While there was not a significant difference in ATP-specific SCL between TG_{Sham} and NTG_{Sham} rabbits, there was a 24% increase in TG_{TIC} rabbits. The only significant change for GTP-specific SCL was in NTG_{TIC} rabbits, with a 22% reduction after TIC.

The ER stress response proteins glucose regulated proteins 78 and 94 (GRP78/GRP94) also showed changes. There were higher levels of GRP 78 (20%; $P<0.005$) in TG_{sham} rabbits (Figure 4) than NTG_{Sham} rabbits. There were higher levels of GRP 78 (49%; $P=0.002$), GRP 94 (58%; $P=0.02$) and HSP 71 (21%; $P=0.02$) in NTG_{TIC} versus TG_{TIC} rabbits. HSP60 levels were only significantly different in NTG_{TIC} animals by 1-way ANOVA and underwent a 26% reduction ($P<0.001$). Although not different between NTG and TG rabbits, a common increase in transglutaminase 2 was also observed following TIC. The cytosolic antioxidant protein peroxiredoxin 6 (Prx6) was higher (24%; $P<0.005$) in the TG_{Sham} rabbits compared to NTG_{Sham} rabbits, with no detectable difference after TIC.

Discussion

This study investigated the effects of MHC isoform changes in a transgenic rabbit model with cardiac isoform content reflective of the human heart. These rabbits possess increased α-MHC content from 5% (NTG levels) to 40% of the total MHC pool, but have similar hemodynamics to NTG rabbits under normal physiological conditions.[11] TG rabbits showed significant cardioprotection against TIC, as they have less ventricular dilation and better systolic performance than NTG animals. We hypothesized that this was due to differential protein changes resultant from altered protein expression. Our observations demonstrate that TG rabbits possess a distinct protein expression pattern under basal conditions and furthermore exhibit a differential response to TIC.

Technical Limitations

The study design utilized 7cm 4-7 IPG strips enabling use of the large numbers of samples required to support our statistical interpretation of the data. This pI range is very robust for isoelectrical focusing and aided in data analysis, many proteins, including a majority of mitochondrial proteins are underrepresented[26].

Thick Filaments

Following TIC, MLC-1, MLC-2, and desmin levels increased in both NTG and TG animals compared to sham-operated animals within each group. TG animals had higher MLC-2 in comparison to NTG animals under either sham or TIC conditions, whereas no significant differences in MLC-1 were found when comparing TG to NTG animals in either sham or TIC conditions. Furthermore, differences in MLC-2 phosphorylation were observed between TG and NTG groups. Compared to NTG_{SHAM} MLC-2 was less phosphorylated in TG_{Sham} rabbits; following TIC, TG_{TIC} animals exhibited increased phosphorylation compared to NTG_{TIC} animals. Phosphorylation of MLC-2 can increase myofilament force production [27] and this could be a mechanism by which TG rabbits are able to maintain myocardial contractile force. In contrast, dephosphorylated MLC-2 has been previously associated with human heart failure, and was shown to have a higher calcium sensitivity in terms of force production (pCA(50)).[28] We expect that the effects of transgene expression on MLC-2 are divorced from MLC-1, as the latter was neither increased in abundance nor was its

phosphorylation status altered with either TIC or transgene incorporation. Since the functional myosin complex is known to contain 2:2:2 molar ratios of MHC and the two light chains, these data imply that either there was a reduced quantity of MLC-1, which limited the amount of myosin complex formed, or that the non myofilament associated pool of MLC-2 is increased. We believe the latter, as purified myofibrils contained an equal stoichiometry of both MLC-1 and MLC-2 with respect to MHC (Figure 2). This suggests that in TIC, MLC2 has either increased steady state mRNA levels or decreased protein turnover rates. Careful regulation of UNC-45 abundance is likely important as over- or under-expression of UNC-45 results in a decrease in myosin levels.[21, 29] Landsverk and colleagues hypothesize that increased or decreased levels of UNC-45 prevent proper folding of myosin, resulting in increased myosin degradation via ubiquitinylation and degradation via the proteosome.[21] We observed increases in UNC-45B levels in TG_{TIC} animals compared with TG_{Sham} controls, suggesting an association with MHC expression. Given the protective effect of α-MHC among TG_{TIC} animals, and the reported need for optimal UNC-45 expression for the proper function of myosin, it is not surprising that increased UNC-45 protein expression was observed in these animals.

Thin filaments

Actin abundance changes were prominent in NTG_{TIC} or TG_{TIC} animals, with increased actin in TG_{TIC} animals versus a decrease in NTG_{TIC} animals. In 2DE we cannot differentiate between myofilament, soluble or cytoskeletal actin; however, these results were also observed for myofilament-associated actin. Regardless, there were no derangements in sarcomeric regularity (electron micrograph data not shown), so the impact of this change is difficult to interpret.

TG rabbits have differential intermediate filament protein accumulation

The intermediate filament protein desmin was increased in the total protein extracts from both NTG_{TIC} and TG_{TIC} rabbits, although the extent differed. Both human dilated cardiomyopathy (DCM) patients and a number of HF animal models have demonstrated increased desmin compared to appropriate controls.[30-32] Desmin increases are most likely a protective rather than a pathogenic response, as TG mice that overexpress desmin specifically in the heart are unaffected and have preserved cardiac function.[33] Post-translational modifications of desmin may also be involved in this response, as TG rabbits show greater amounts of dephosphorylated desmin in total protein extracts (Figure S2). In contrast, equal amounts of desmin were present in isolated myofibrils from both NTG_{TIC} and TG_{TIC} rabbits (Figure 3A and B), suggesting an increased pool of non-sarcomere associated protein with maintenance of the myofilament-associated desmin. In TG mice that over-express desmin, the protein shows normal incorporation and is localized to the z-disc, whereas in DCM patients, desmin is disorganized and distributed amorphously in the cytoplasm.[31, 34] We also observed a reduction in the abundance of the dephosphorylated form of desmin in TG_{Sham} and TG_{TIC} animals. This form of desimin has recently been reported by Agnetti and colleagues to be protective in a canine HF model and in human HF. [35]

ATP hydrolysis rates and mitochondrial proteins

As expected, rates of ATP hydrolysis were dramatically increased in myofibrils isolated from TG animals versus NTG animals; therefore, it was not surprising that there was differential expression of proteins involved in energy generation between these groups. Complex I 75 kDa subunit and CP1 levels decreased in NTG_{TIC} animals, whereas the 30 kDa and the 24 kDa Complex 1 subunits decreased in the TG_{TIC} rabbits. The oxidoreductase protein 3-HIBD was increased 2.1 fold higher in TG_{Sham} animals as compared to NTG_{Sham} animals. Thus, while proteins involved in maintaining the ATP pool may be reduced at a

time when higher levels of ATP should be necessary, the base increase in 3-HIBD may offset the decreases under pacing conditions. This could result from the communication/integral response between the myofilament requirement for ATP and the mitochondria. Since 3-HIBD is involved in the degradation of aliphatic amino acids, it is also possible that protein catabolism may be an additional source of energy for the TCA cycle by acting as a source of succinyl CoA in TG rabbits. It is also interesting to note that the differential abundances of Complex I and Complex III that occurred in the TG_{Sham} rabbits and the change in ATP-specific SCL under TIC have also been observed in rabbit cardiomyocytes preconditioned with adenosine.[36]

Stress-associated protein changes

The stress response proteins GRP 78, GRP 94 and HSP 71 responded differentially between NTG and TG rabbits. All increased during TIC in NTG animals but decreased in TIC in TG animals. GRP 78 and 94, molecular chaperones induced by glucose starvation, are involved in the unfolded protein response and may be involved in apoptosis regulation. Notably, increases in GRP78 are associated with HF.[37]

Conclusion

Collectively these results suggest that modifications in the relative α-MHC levels results in a broad cellular impact on other members of the cellular proteome. These changes, which range from myofilament proteins to proteins in energetics and redox handling, demonstrate that despite extreme specificity in genetic manipulations, the final outcome on phenotype can be impacted by changes in several protein networks and systems. Therefore, it is very difficult to determine the singular effect of increased α-MHC on the function of the rabbit myocardium either at rest or following TIC outside the scope of other protein changes.

In vitro motility analysis of myosin isolated from end-stage HF patients demonstrated that incorporation of physiological levels of V1 myosin versus V3 myosin had a broad impact on other proteins.[38] This is consistent with our results, in that many of the changes of increased V1 myosin in the cell may not be attributed to myosin expression alone, but rather reflects compensatory and/or auxiliary protein changes occurring in different areas of the cell. Our results also highlight the importance of determining the target sub-proteome of interest. For example, by 2DE there was discordance in the changes of the contractile proteins MHC, MLC-1, and MLC-2 in tissue lysates versus these same proteins in isolated myofibrils, suggesting that protein abundance changes for these proteins in the soluble pool did not carry over into assembled sarcomeres. Taken together, these results suggest that α-MHC interacts with multiple networks, including energetic proteins, contractile proteins and protein chaperones, which collectively confer a protective phenotype against tachycardia-induced heart failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MHC	myosin heavy chains
α-MHC	alpha myosin heavy chain
TG	transgenic
NTG	non-transgenic rabbits
HF	heart failure
TIC	tachycardia-induced cardiomyopathy
TnT	troponin T
Tm	tropomyosin
MLC-1	myosin light chain 1
MLC-2	myosin light chain 2
1DE	1-dimensional gel electrophoresis
2DE	2-dimensional gel electrophoresis

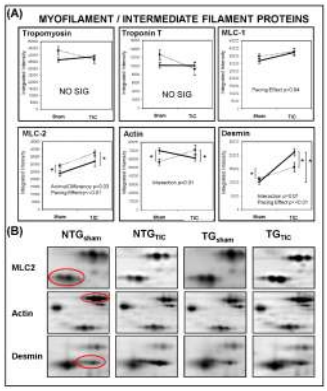

Figure 1.

(A) Quantification of the myofilament proteins identified from 2-DE of rabbit whole tissue extracts from sham operated (Sham) and TIC rabbits. Solid line illustrates changes in NTG rabbits. Dashed line illustrates changes in TG rabbits. *P<0.01 by 1-way ANOVA; 2-way ANOVA P-value is shown. (B) Representative sections of 2-DE changes in actin, MLC-2 and desmin between the four groups.

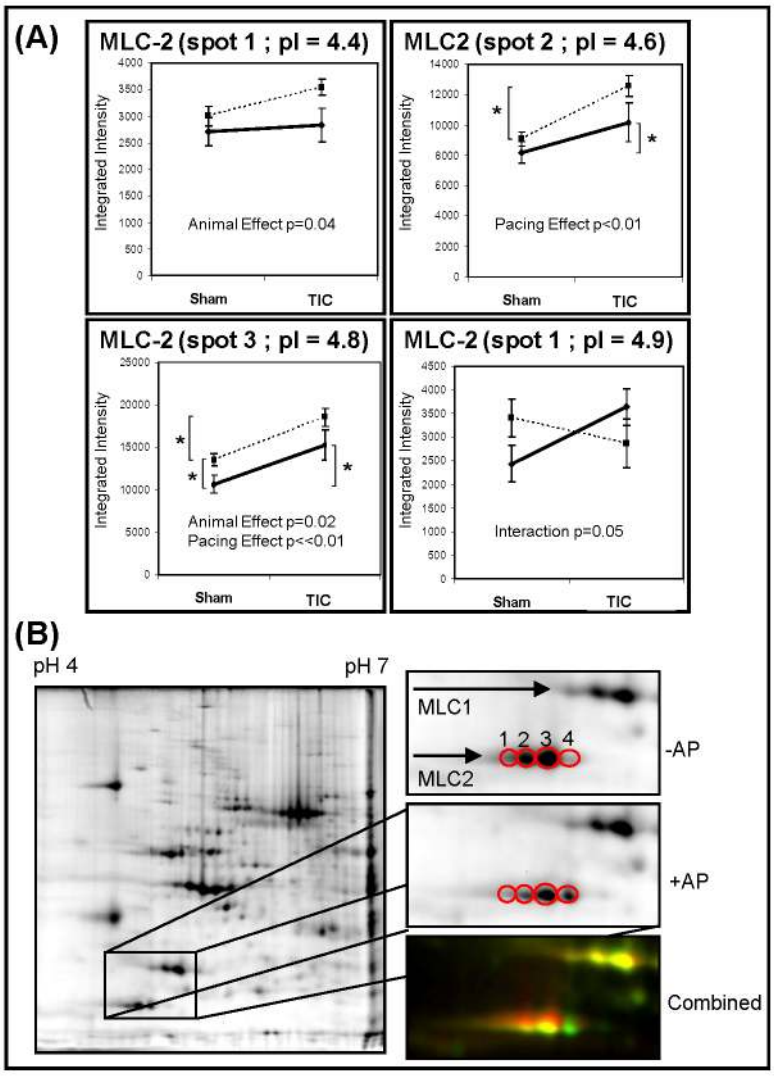

Figure 2.

(A) Graphical illustration of change in abundance of 2-DE spots corresponding to MLC-2 isoforms with different pI. Only spot 4 had significant ($p \leq 0.05$) interaction between TIC and transgene by 2-way ANOVA. *$p < 0.05$ between individual groups. (B) 2-DE of whole tissue from NTG_{TIC} rabbit with and without alkaline phosphatase (AP) treatment.

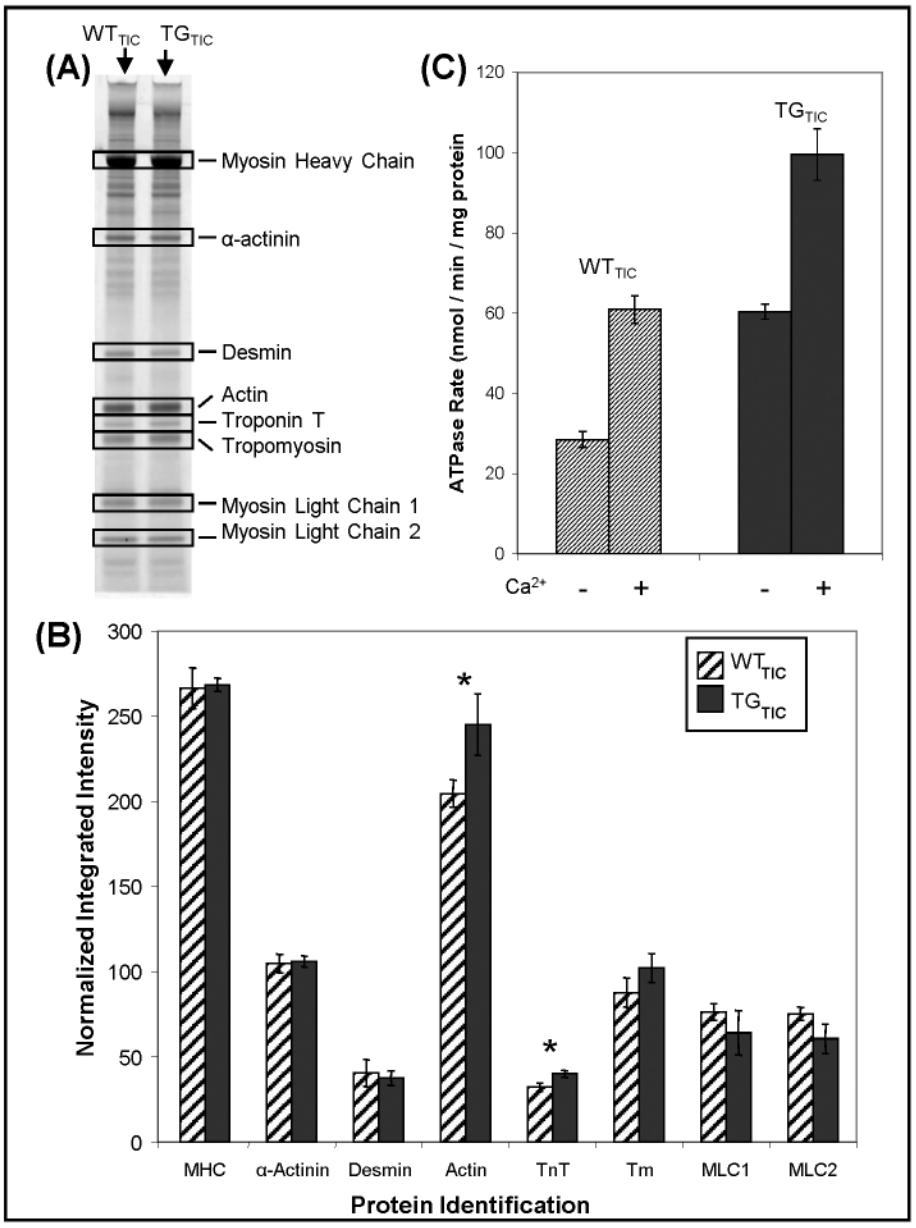

Figure 3.

(A) 1-DE of representative myofibril preparation and identity of the most abundant bands. (B) Relative quantitation of band abundance for NTG_{TIC} and TG_{TIC} rabbits. (C) Average *in vitro* ATPase rates for myofibrils from NTG_{TIC} and TG_{TIC} rabbits. Values were calculated with Ca2+ (7 mMCaCl2) and without Ca2+ (7 mMCaCl2+ 7 mM EDTA). *p<0.05.

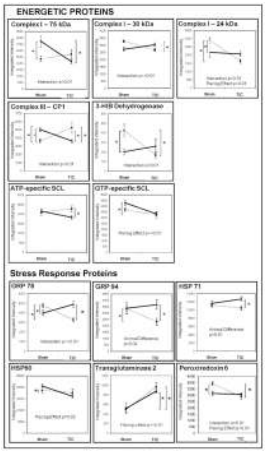

Figure 4.

Quantification of (A) energetic proteins and (B) stress response proteins identified from 2-DE of rabbit whole tissue extracts from sham-operated and TIC rabbits. Solid line illustrates changes in NTG rabbits. Dashed line illustrates changes in TG rabbits. 2-way ANOVA P-value based is shown. *P<0.05 by 1-way ANOVA.

Table 1

MS identification of 2-DE spots which had significant abundance differences between groups of rabbits. Changes are highlighted if they were increased (green) or decreased (yellow).

										Relative Change in Spot Integrated Intensity			
Protein ID	**Accession Number**	**Theoretical**		**Observed**		**Average Integrated Intensity**				**Ratio of TGs : WTs**	**Ratio of WTp : WTs**	**Ratio of TGp : TGs**	**Ratio of TGp : WTp**
		pI	**Mw**	**pI**	**Mw**	**WTs**	**WTp**	**TGs**	**TGp**				
Actin (sum of spots 1-4)										0.81 (p=0.03)	0.87 (p=0.12)	1.26 (p=0.05)	1.16 (p=0.15)
Actin (1)	Q3ZC07	5.2	42.0	4.8	36	9607	8185	8462	10624	0.88 (p=0.33)	0.85 (p=0.20)	1.26 (p=0.09)	1.30 (p=0.04)
Actin (2)	Q3ZC07	5.2	42.0	4.9	36	28681	25754	24439	31058	0.85 (p=0.12)	0.90 (p=0.23)	1.27 (p=0.05)	1.21 (p=0.09)
Actin (3)	Q3ZC07	5.2	42.0	5.0	36	23069	19969	17210	21671	0.75 (p=0.01)	0.87 (p=0.10)	1.26 (p=0.06)	1.09 (p=0.42)
Actin (4)	Q3ZC07	5.2	42.0	5.1	36	8497	7146	6331	7455	0.75 (p=0.01)	0.84 (p=0.09)	1.18 (p=0.27)	1.04 (p=0.77)
Myosin Light Chain 2 (MLC-2) (sum of spots 1-4)										1.21 (p=0.06)	1.33 (p<0.01)	1.29 (p=0.06)	1.18 (p=0.18)
MLC-2 (1)	P10916	4.9	18.8	4.4	18	2705	2836	3006	3543	1.11 (p=0.37)	1.05 (p=0.59)	1.18 (p=0.21)	1.25 (p=0.07)
MLC-2 (2)	P10916	4.9	18.8	4.6	18	8204	10189	9077	12579	1.11 (p=0.34)	1.24 (p=0.04)	1.39 (p=0.04)	1.24 (p=0.13)
MLC-2 (3)	P10916	4.9	18.8	4.8	18	10659	15271	13521	18537	1.27 (p=0.05)	1.43 (p<0.01)	1.37 (p=0.03)	1.21 (p=0.14)
MLC-2 (4)	P10916	4.9	18.8	4.9	18	2433	3641	3408	2863	1.40 (p=0.11)	1.50 (p=0.09)	0.84 (p=0.34)	0.79 (p=0.26)
Desmin (sum of spots 1-3)										1.11 (p=0.34)	2.07 (p<0.001)	1.38 (p=0.05)	0.74 (p=0.03)
Desmin (1)	O62654	5.2	54.0	5.2	54	3211	6515	3419	4695	1.07 (p=0.60)	2.03 (p<0.001)	1.37 (p=0.09)	0.72 (p=0.05)
Desmin (2)	O62654	5.2	54.0	5.3	54	5199	10501	6016	8505	1.16 (p=0.20)	2.02 (p<0.001)	1.41 (p=0.02)	0.81 (p=0.07)
Desmin (3)	O62654	5.2	54.0	5.4	54	1802	4141	1933	2446	1.07 (p=0.64)	2.30 (p<0.01)	1.27 (p=0.19)	0.59 (p=0.02)
Annexin A5	P81287	4.9	36.1	4.7	28	578	708	522	973	0.90 (p=0.72)	1.23 (p=0.40)	1.86 (p<0.01)	1.37 (p=0.05)
Complex I - 75 kDa subunit (sum of spots 1-4)										0.63 (p=0.02)	0.57 (p=0.01)	1.16 (p=0.36)	1.27 (p=0.15)
Complex I - 75 kDa (1)	Q5R911	5.9	79.5	5.4	72	870	287	370	623	0.425 (p<0.01)	0.33 (p<0.01)	1.69 (p=0.02)	2.17 (p=0.01)
Complex I - 75 kDa (2)	Q5R911	5.9	79.5	5.5	72	1546	927	1064	1469	0.69 (p=0.04)	0.60 (p=0.01)	1.38 (p=0.15)	1.58 (p=0.05)
Complex I - 75 kDa (3)	Q5R911	5.9	79.5	5.6	72	3568	2396	2589	2636	0.73 (p=0.06)	0.67 (p=0.03)	1.02 (p=0.90)	1.100 (p=0.52)
Complex I - 75 kDa (4)	Q5R911	5.9	79.5	5.7	72	1493	675	667	714	0.45 (p=0.04)	0.45 (p=0.04)	1.07 (p=0.76)	1.058 (p=0.78)
Complex I - 30 kDa subunit	P23709	6.6	30.3	5.8	23	2722	3040	3285	2671	1.07 (p=0.28)	0.99 (p=0.85)	0.84 (p=0.01)	0.91 (p=0.22)
Complex I - 24 kDa subunit	P19404	8.2	27.4	6.2	21	2411		3223	2500	1.34 (p=0.03)	1.17 (p=0.17)	0.78 (p<0.01)	0.88 (p=0.22)
Complex III - core protein 1 (sum of spots 1-2)										0.72 (p<0.01)	0.72 (p<0.01)	1.45 (p=0.04)	1.45 (p=0.04)
Complex III - CP1 (1)	P31930	5.9	52.6	5.7	48	1320	1003	922	1815	0.70 (p=0.04)	0.76 (p=0.08)	1.97 (p=0.01)	1.81 (p=0.02)

Protein ID	Accession Number	Theoretical		Observed		Average Integrated Intensity				Relative Change in Spot Integrated Intensity			
		pI	Mw	pI	Mw	WTs	WTp	TGs	TGp	Ratio of TGs : WTs	Ratio of WTp : WTs	Ratio of TGp : TGs	Ratio of TGp : WTp
Complex III - CP1 (2)	P31930	5.9	52.6	5.8	48	3705	2630	2705	3449	0.73 (p<0.01)	0.71 (p<0.01)	1.28 (p=0.10)	1.31 (p=0.08)
GTP Specific Succinyl-CoA Ligase	Q96I99	6.2	46.5	5.9	42	4284	3334	3701	3238	0.86 (p=0.06)	0.78 (p=0.01)	0.88 (p=0.14)	0.97 (p=0.74)
ATP Specific Succinyl-CoA Ligase	Q9P2R7	7.1	50.3	6.1	42	2151	1825	2087	2267	0.97 (p=0.76)	0.85 (p=0.06)	1.09 (p=0.46)	1.24 (p=0.05)
3-Hydroxyisobutrate Dehydrogenase	P31937	8.4	34.3	5.7	29	202	262	424	167	2.10 (p=0.02)	1.30 (p=0.47)	0.39 (p=0.01)	0.64 (p=0.27)
Peroxiredoxin - 6	O77834	6.0	25.1	6.3	23	3134	3068	3885	2869	1.24 (p=0.01)	0.98 (p=0.75)	0.74 (p<0.01)	0.94 (p=0.46)
HSP 60 (sum 1-4)										1.04 (p=0.66)	0.94 (p=0.52)	0.738 (p<0.01)	0.82 (p=0.03)
HSP 60 (1)	P10809	5.7	61.1	5.2	60	1384	1229	1307	1148	0.95 (p=0.72)	0.89 (p=0.27)	0.88 (p=0.46)	0.93 (p=0.57)
HSP 60 (2)	P10809	5.7	61.1	5.3	60	3079	2994	3384	2620	1.10 (p=0.35)	0.97 (p=0.78)	0.77 (p<0.01)	0.88 (p=0.12)
HSP 60 (3)	P10809	5.7	61.1	5.4	60	5481	5177	5713	4151	1.04 (p=0.64)	0.94 (p=0.59)	0.73 (p<0.01)	0.80 (p=0.02)
HSP 60 (4)	P10809	5.7	61.1	5.5	60	1553	1347	1537	892	0.99 (p=0.95)	0.87 (p=0.36)	0.58 (p<0.01)	0.66 (p=0.01)
HSP 71 (sum 1-3)										0.96 (p=0.47)	1.09 (p=0.18)	0.94 (p=0.36)	0.83 (p=0.02)
HSP 71 (1)	P11142	5.4	70.9	5.3	66	1687	2004	1714	1725	1.02 (p=0.75)	1.19 (p=0.01)	1.01 (p=0.92)	0.86 (p=0.05)
HSP 71 (2)	P11142	5.4	70.9	5.4	66	3553	4032	3533	3400	0.99 (p=0.92)	1.14 (p=0.08)	0.96 (p=0.56)	0.84 (p=0.03)
HSP 71 (3)	P11142	5.4	70.9	5.5	66	6427	6683	5922	5373	0.92 (p=0.33)	1.04 (p=0.61)	0.91 (p=0.27)	0.80 (p=0.02)
GRP 78	P11021	5.1	72.3	5.0	68	3986	4869	4766	3278	1.20 (p=0.04)	1.2 (p<0.01)	0.69 (p<0.01)	0.67 (p<0.01)
GRP 94	O18750	4.9	82.6	4.7	94	3384	3665	3341	2321	0.99 (p=0.93)	1.08 (p=0.65)	0.70 (p=0.01)	0.63 (p=0.02)
Transglutaminase 2	P21980	5.1	77.3	5.1	72	1184	1680	553	2370	0.47 (p=0.02)	1.42 (p=0.24)	4.29 (p<0.01)	1.41 (p=0.13)