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Research Article

Ascribing novel functions to the sarcomeric protein, myosin binding protein H (MyBPH) in cardiac sarcomere contraction

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ABSTRACT

Myosin binding protein H (MyBPH) is a protein of unknown function, which shares sequence and structural similarities with myosin binding protein C (cMyBPC), a protein frequently implicated in hypertrophic cardiomyopathy (HCM). Given the similarity between cMyBPC and MyBPH, we proposed that MyBPH, like cMyBPC, could be involved in HCM pathogenesis and we therefore sought to determine its function. We identified MyBPH-interacting proteins by using yeast two-hybrid (Y2H) analysis. The role of MyBPH and cMyBPC in cardiac cell contractility was analysed by measuring the planar cell surface area of differentiated H9c2 rat cardiomyocytes in response to β-adrenergic stress after siRNA knockdown of MyBPH and cMyBPC. Individual knockdown of either protein had no effect on cardiac contractility, while concurrent knockdowns reduced cardiac contractility. These proteins therefore functionally compensate for one another and are critical for cardiac contractility. We further show that both proteins co-localise with the autophagosomal membrane protein LC3, suggesting that both proteins are involved in autophagosomal membrane maturation processes. The results of this study ascribe *novel* functions to MyBPH, which may contribute to our understanding of its role in the sarcomere. This study provides evidence for a potential role of MyBPH in HCM, which warrants further investigation.

Abbreviations: MyBPH, myosin binding protein H; cMyBPC, cardiac myosin binding protein C; LVH, left ventricular hypertrophy; HCM, hypertrophic cardiomyopathy; ALP, autophagy-lysosome pathway; AbA, Aureobasidin A; *HIS3*, imidazoleglycerolphosphate dehydratase; *ADE2*, phosphoribosylaminoimidazole carboxylase; *MEL1*, alpha-galactosidase; MYH7, cardiac β-myosin heavy chain; FLNC, filamin c; MTRNR2L2, MT-RNR2-like 2 protein; ACTC1, cardiac actin; FAF1, fas (TNFRSF6) associated factor 1; FBN1, fibrillin 1; UPS, ubiquitin-proteasome system

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Introduction

Left ventricular hypertrophy (LVH) is a major risk factor for cardiovascular morbidity and mortality [1,2], and is a feature of common diseases, such as hypertension and diabetes. It is therefore vital to understand the underlying mechanisms influencing its development. However, investigating the mechanisms underlying LVH in such complex disorders can be challenging. For this reason, many researchers have focused their attention on the autosomal dominant cardiac muscle disorder, hypertrophic cardiomyopathy (HCM), since it is considered a model disease in which to study the causal molecular factors underlying isolated cardiac hypertrophy.

Mutations in cardiac myosin-binding protein C (cMyBPC) are frequent causes of hypertrophic cardiomyopathy (HCM). Identification of proteins that interact with cMyBPC has led to improved insights into the function of this protein and its role in cardiac hypertrophy [3]. However, very little is known about another member of the myosin binding protein family, myosin binding protein H (MyBPH).

Cardiac MyBPC is one of three isoforms of MyBPC, namely fast skeletal, slow skeletal and cardiac, each of which are encoded by separate genes [4–6]. This multi-domain sarcomeric protein (approximately 130 kDa) resides in the thick filaments of the striated muscle. It is arranged within the C-zone along the length of the A-band in nine to 11 transverse stripes that are 43 nm apart, with approximately two to four molecules of cMyBPC associated with each myosin cross-bridge [7,8].

Myosin binding protein H is approximately 55 kDa in size and is encoded by a single gene (*MyBPH*). *MyBPH* encodes a single isoform that is expressed in both fast skeletal and cardiac muscle cells, including those that differentiate into Purkinje fibres [4,5,9]. MyBPH was found to localise to stripe 3 in the C-zone (counting from the M-line) in the majority of psoas fibres, fast-twitch (Type II) muscle fibers [7].

Structurally, cMyBPC and MyBPH are very similar, as both are multi-domain proteins consisting of IgI and FnIII domains. The C-terminal of MyBPH shares 50% identity and 17% conserved amino acids with cMyBPC, and consist of four protein modules, FnIII–IgI–FnIII–IgI, which correspond to domains C7–C10 of cMyBPC [4]. Given the similarity in sequence and structure between cMyBPC and MyBPH, and since both proteins are known to bind myosin [4,9] we propose that MyBPH, may play a critical role in the structure and functionality of the cardiac sarcomere. However, the function of this protein remains largely unknown.

Furthermore, the observation of impaired autophagy in cMyBPC knockout mice suggests that cMyBPC may be critical for the functioning of the autophagy-lysosome pathway (ALP) [10]. Protein turnover by autophagy is thought to involve fusion of the outer membrane of the autophagosome with a vacuole or lysosome and in doing so exposes the inner autophagosome membrane and its luminal content to the degradation machinery of the vacuole or lysosome [11]. Impaired autophagy in both the cMyBPC knock-in and knockout mice was suggested to be a result of a blockade of the fusion between autophagosomes and lysosomes [10]. Despite the structural similarities between cMyBPC and MyBPH, it remains largely unclear whether MyBPH is involved in this fusion process.

Here we aimed to identify MyBPH-interacting proteins by using yeast two-hybrid (Y2H) analysis and to verify these interactions using three-dimensional (3D) co-localisation and co-immunoprecipitation (Co-IP) analyses. The role of MyBPH and cMyBPC in cardiac cell contractility were analysed by measuring the planar cell surface area of differentiated H9c2 rat cardiomyocytes in response to β-adrenergic stress after individual and concurrent siRNA-mediated knockdown of MyBPH and cMyBPC. Furthermore, we hypothesised that both MyBPH and cMyBPC may be involved in autophagy. To test this hypothesis, both MyBPH and cMyBPC were analysed for co-localisation with a marker for autophagy, LC3.

Materials and methods

Y2H constructs

The cDNA of MyBPH was PCR amplified from a cardiac cDNA library (Clontech Laboratories, Inc., Palo Alto, California, USA). This fragment was cloned into the *Nde*I and *Eco*RI restriction sites in-frame with the GAL4 DNA binding domain in the Y2H bait yeast expression vector pGBKT7 (Clontech) for use in the Y2H library screen. Integrity of insert sequences, reading frame and cloning sites were verified by means of bi-directional sequencing, after which the pGBKT7-MyBPH construct was transformed into *Saccharomyces cerevisiae* (*S. cerevisiae*) strain GOLD (Clontech).

Primers for Y2H insert screening

Primers flanking the multiple cloning sites of pGBKT7 (Clontech) and pGADT7-Rec (Clontech) Y2H vectors were designed to amplify inserts cloned into the the Y2H cloning vectors. The sequences of these primers are presented in Table 1.

Y2H library screening

The *S. cerevisiae* strain GOLD (Clontech) transformed with pGBKT7-MyBPH was mated with *S. cerevisiae* Y187 pre-transformed with a MATCHMAKER™ cardiac cDNA library (Clontech) and the library screen was performed according to the manufacturer's recommendations. Prey conditions from colonies that were resistant to

Table 1 – Oligonucleotide primers used for the amplification of inserts from cloning vectors.

Primer name	Primer sequence (5′–3′)	T_a (°C)	T_m (°C)
pGBKT7_Forward	TCATCGGAAGAGAGTAG	45	50
pGBKT7_Reverse	TCACTTTAAAATTTGTATACA	45	50
pGADT7_Forward	CGATGATGAAGATACCCCAAA	56	50
pGADT7_Reverse	CACGATGCACAGTTGAAGTGAACT	56	50

Abbreviations: A, adenine; **C,** cytosine; **°C,** degrees Celsius; **G,** guanine; **MyBPH,** myosin binding protein H; **T,** thymine; T_a, annealing temperature; T_m, melting temperature.