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Lysine methyltransferase Smyd2 regulates Hsp90-mediated protection of the sarcomeric titin springs and cardiac function☆

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ABSTRACT

Protein lysine methylation controls gene expression and repair of deoxyribonucleic acid in the nucleus but also occurs in the cytoplasm, where the role of this posttranslational modification is less understood. Members of the Smyd protein family of lysine methyltransferases are particularly abundant in the cytoplasm, with Smyd1 and Smyd2 being most highly expressed in the heart and in skeletal muscles. Smyd1 is a crucial myogenic regulator with histone methyltransferase activity but also associates with myosin, which promotes sarcomere assembly. Smyd2 methylates histones and non-histone proteins, such as the tumor suppressors, p53 and retinoblastoma protein, RB. Smyd2 has an intriguing function in the cytoplasm of skeletal myocytes, where it methylates the chaperone Hsp90, thus promoting the interaction of a Smyd2–methyl-Hsp90 complex with the N2A-domain of titin. This complex protects the sarcomeric I-band region and myocyte organization. We briefly summarize some novel functions of Smyd family members, with a focus on Smyd2, and highlight their role in striated muscles and cytoplasmic actions. We then provide experimental evidence that Smyd2 is also important for cardiac function. In the cytoplasm of cardiomyocytes, Smyd2 was found to associate with the sarcomeric I-band region at the titin N2A-domain. Binding to N2A occurred in vitro and in yeast via N-terminal and extreme C-terminal regions of Smyd2. Smyd2-knockdown in zebrafish using an antisense oligonucleotide morpholino approach strongly impaired cardiac performance. We conclude that Smyd2 and presumably several other Smyd family members are lysine methyltransferases which have, next to their nuclear activity, specific regulatory functions in the cytoplasm of heart and skeletal muscle cells. This article is part of a Special Issue entitled: Cardiomyocyte Biology: Cardiac Pathways of Differentiation, Metabolism and Contraction.

1. Introduction

Cardiac and skeletal muscles contain in their unitary building blocks, the sarcomeres, a highly ordered array of cytoskeletal proteins, which include myosin, actin, and the largest known protein titin. The maintenance of these muscle proteins is a continuous process, which intensifies during states of stress and illness. Deregulation of muscle protein homeostasis occurs with aging and disease and directly contributes to morbidity and mortality. To maintain protein homeostasis of the sarcomeric protein network, specialized quality-control mechanisms must be in place. These include the three major pathways of proteolysis, the ubiquitin–proteasome system, the autophagy-lysosomal pathway, and the calpain system [1]. An additional element of the protein quality-control machinery is the chaperones [2]. As regards the heart, we are already beginning to understand important proteolytic pathways to mechanistic detail, but comparatively little is known about the role of chaperones [3].

1.1. Sarcomere proteins as chaperone substrates

Molecular chaperones form a class of proteins essential for protein homeostasis in living systems. Many chaperones are also termed heat shock proteins (HSPs) due to their responsiveness to elevated temperatures. Most HSPs share fundamental biochemical properties: the ability to prevent aggregation of partially unfolded proteins and to maintain partially unfolded proteins in a state competent for refolding. Some

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sarcomere proteins are known substrates of HSPs. Translocation of chaperones belonging to the class of small HSPs (such as alpha-B-crystallin, Hsp20 and Hsp27) from the cytosol to the sarcomeres has been observed in cardiomyocytes under mechanical or biochemical stress [4]. This translocation might exert a protective effect on cardiac sarcomeric structure, e.g., via protection of actin, desmin and titin [3–5]. The ubiquitous, ATP-dependent, larger HSPs, such as Hsp70 and Hsp90, play a role in striated muscle, as well. From work mainly on skeletal muscle we know that Hsp70 and Hsp90 act in conjunction with co-chaperones to protect myosin [6]. The co-chaperone Unc45b is exclusively expressed in striated muscle and together with Hsp90 regulates myosin stability and turnover and sarcomeric A-band organization [7,8], in a stress-dependent manner [9]. A role for this chaperone system is also likely in cardiomyocytes [10]. However, additional cardiac and skeletal muscle chaperone systems must exist to help maintain the intricate and elaborate protein structures of the sarcomeres.

Our recent work has established a novel mechanism whereby the I-band region of the skeletal muscle sarcomere is protected in an Hsp90-dependent manner [11]. Specifically, we showed that the lysine methyltransferase (KMT) Smyd2 monomethylates cytoplasmic Hsp90, thus promoting the interaction of a Smyd2–Hsp90 complex with the N2A-domain of titin. Upon knockdown of Smyd2 and concomitant reduction of methylated Hsp90 in zebrafish the sarcomeric I-band region (where the N2A-domain is located) became disrupted. Whether this mechanism is in place also in the cardiac muscle cell has not been investigated.

1.2. Linking Hsp90 and Smyd2 methyltransferase activity to titin protection: our rationale

The aim of the present study is twofold: 1) to provide experimental evidence that the protection mechanism via Smyd2–methyl-Hsp90, shown for skeletal muscle [11], is also relevant in cardiac myocytes; and 2) to discuss the role of Smyd2 and other Smyd family members in striated muscle, highlighting their cytoplasmic actions. We begin with a short overview of Smyd family members and cover suggested Smyd2 functions in more detail, before presenting new original data about the Smyd2–methyl-Hsp90–N2A-titin link in cardiomyocytes and relating our findings to the literature. Our experimental approach includes immunofluorescence-based localization tests in cardiac myocytes, protein–protein interaction assays, as well as a zebrafish Smyd2-knockdown model.

1.3. Smyd protein family of lysine methyltransferases, focus on Smyd2

In the Smyd (SET and MYND domain containing) protein family, KMT capacity is provided by a (split) SET domain, originally identified in *Drosophila* suppressor of variegation [Su(var)3-9], enhancer of zeste [E(z)] and trithorax. A zinc finger-containing MYND domain (after myeloid translocation protein-8, Nervy, and DEAF-1) mediates interactions with proteins involved in transcriptional regulation and signal transduction. The Smyd protein family has five members, Smyd1 to 5. Smyd1, 2 and 3 are highly homologous, whereas Smyd4 has an additional NH_2-terminal extension of ~240 amino acids [12]. Smyd1 to 4 have in common a tetratricopeptide (TPR) like domain at their respective COOH-termini, which is lacking in Smyd5 [12]. TPR domains are often involved in protein–protein interactions and also in Smyd1 to 4 they mediate ligand binding, e.g., to Hsp90 [12]. Crystal structures are available for Smyd1, Smyd2, and Smyd3 [13–20].

Smyd1 is a histone methyltransferase regulating downstream gene transcription specifically in skeletal and cardiac muscles, where this KMT is most highly expressed [21]. The protein assumes a critical role in early striated muscle development as a myogenic regulator directly targeted by serum response factor and myogenin [22]. Constitutive knockout of Smyd1 in mice disrupts cardiac differentiation and morphogenesis, resulting in embryonic lethality [21]. Knockdown of Smyd1 isoforms a and b in zebrafish using morpholino antisense oligonucleotides causes severe myofibrillar disorganization and malfunction of cardiac and skeletal muscles [23,24]. Importantly, Smyd1 also binds myosin and is localized at the M-band region in the center of the sarcomere [24,25]. A nonsense mutation in the *Smyd1* gene is responsible for A-band disruption in the zebrafish mutant, flatline (fla), causing disturbed sarcomere assembly in the heart and fast-twitch skeletal muscle [24]. Taken together, Smyd1 is established as a striated muscle-specific regulator of myogenic differentiation and sarcomeric A-band (myosin) assembly.

Smyd3 is best known for its role in cancer development. Knockdown of Smyd3 in cancer cells using siRNA suppresses cell growth suggesting that Smyd3, via its histone methyltransferase activity, promotes the proliferation of cancer cells [26]. These findings have since been confirmed and extended [27,28]. Interestingly, Smyd3 also methylates a non-histone protein in the cytoplasm, specifically, lysine 831 within the cytoplasmic portion of vascular endothelial growth factor receptor-1 [29]. Smyd3 is expressed in various tissue types and is also found in the heart and in skeletal muscles [26,30]. Recently, Smyd3 has been demonstrated to be required for cardiac and skeletal muscle development in zebrafish [31], suggesting some of its functions may be similar to those of Smyd1.

Relatively little is known yet about Smyd4 and Smyd5. Suppression of Smyd4 in human mammary epithelial cells by siRNA promotes cell growth, such that Smyd4 may act as a potential tumor suppressor [32]. However, Smyd4 has also been suggested as a muscle-specific transcriptional modulator involved in development [33]. Smyd5 is the least studied member of this protein family. Unlike Smyd1 to 3, and probably Smyd4, Smyd5 does not bind Hsp90 [12]. In contrast to Smyd 1 to 4, which show more cytosolic than nuclear expression, Smyd5 is mainly a nuclear protein [11]. In the nucleus, Smyd5 establishes histone-4 lysine-40 trimethylation as a repression checkpoint at toll-like receptor-4-responsive promoters [34].

Smyd2 initially gained attention as a histone-3 lysine-36 (H3K36) methyltransferase highly expressed in the heart, brain, and skeletal muscle [30]. Whereas H3K36 is usually associated with active transcription, Smyd2 participates in a Sin3A histone deacetylase repressor complex linked to H3K36 methylation, potentially explaining why dimethylation of H3K36 by Smyd2 can repress transcriptional activity [30]. Smyd2 also monomethylates histone-3 at lysine-4 [35]. Smyd2 methyltransferase activity and specificity for H3K4 are enhanced by interaction with Hsp90α—a property Smyd2 shares with Smyd1 and Smyd3 [23,26,35]. HSP90α-dependent H3K4 methylation by Smyd2 promotes genes involved in cell cycle, chromatin and transcription regulation [35]. In the absence of HSP90α the activity of Smyd2 for H3K36 dimethylation is weak, hinting at H3K4 as a predominant site of methylation in vivo [35]. Unexpectedly, then, a cardiac-specific knockout of Smyd2 in mice shows unaltered H3K4 and H3K36 methylation patterns [36]. This Smyd2 knockout does not impede cardiac development and reveals no basal heart phenotype.

Smyd2 also methylates non-histone proteins, among them the tumor suppressor p53, at lysine K370, which represses p53-dependent transcriptional regulation [37,38]. Monomethylation via Smyd2 causes dissociation of p53 from promoter regions of p53-target genes, including *p21* (a cyclin-dependent protein kinase important for cell-cycle control) and *mdm2* (an E3 ubiquitin ligase), increasing their expression [37]. Evidence thus suggests that Smyd2 is involved in apoptosis control, transcriptional inhibition, and p53-dependent cell-cycle arrest. However, Smyd2-deficient mouse hearts show unaltered p53 protein stability and functional dysregulation of p53 by Smyd2 in vivo may be unlikely for the heart [36]. Other non-histone targets of Smyd2 are the retinoblastoma tumor suppressor RB, which is methylated at lysine K860 [39], and cytoplasmic Hsp90, which is methylated at K616/K615 in mice/men [11,12]. Importantly,