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Review article

Cardiac phenotype of Duchenne Muscular Dystrophy: Insights from cellular studies

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ABSTRACT

Dilated cardiomyopathy is a serious and almost inevitable complication of Duchenne Muscular Dystrophy, a devastating and fatal disease of skeletal muscle resulting from the lack of functional dystrophin, a protein linking the cytoskeleton to the extracellular matrix. Ultimately, it leads to congestive heart failure and arrhythmias resulting from both cardiac muscle fibrosis and impaired function of the remaining cardiomyocytes. Here we summarize findings obtained in several laboratories, focusing on cellular mechanisms that result in degradation of cardiac functions in dystrophy. This article is part of a Special Issue entitled "Calcium Signaling in Heart".

Contents

Abbreviations: ACE, Angiotensin converting enzyme; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CaV3, Caveolin 3; CICR, Ca^{2+}-induced Ca^{2+} release; DMD, Duchenne muscular dystrophy; ECC, Excitation–Contraction Coupling; ISO, Isoproterenol; NCX, Na^{+}–Ca^{2+} exchanger; NOX, Nicotinamide adenine dinucleotide phosphate-oxidase; NOS, Nitric oxide synthase; PDE, Phosphodiesterase; PKA, Protein kinase A; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; RyR, Ryanodine receptor; SAC, Stretch-activated channel; SOC, Store-operated Ca^{2+} entry pathways; SERCA, Sacrcoplasmic reticulum Ca^{2+} ATP-ase; SR, Sarcoplasmic reticulum; TRPC, Transient receptor potential channel.

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1. Introduction

Duchenne Muscular Dystrophy (DMD) is an inherited lethal muscular disease that affects primarily adolescent males. DMD was named after the French physiologist Guillaume-Benjamin Duchenne who presented several cases of infants with dystrophy in the mid 19th century [1]. DMD is usually diagnosed in early childhood. For a long time it was considered to be predominantly a skeletal muscle illness clinically associated with progressive debilitating muscle weakness, skeletal deformities and breathing disorders. Cardiac complications of this disease became prominent only recently as the life of DMD patients could be prolonged with improved therapies, such as assisted ventilation and corticosteroid treatment. About 95% of the patients with DMD develop cardiomyopathy by 20 years of age, and for approximately 20% of these patients it is limiting for survival. Abnormalities in the electrocardiogram and sinus tachycardia are found in a majority of DMD patients at early age. Later, echocardiography reveals motion abnormalities of the left ventricular walls in areas of fibrosis. Progressive spreading of fibrosis throughout the ventricular wall mediates a gradual enlargement of the ventricle, thinning of the wall and consequently a loss of contractility and heart failure. In addition to the dilated cardiac myopathy many DMD patients also develop arrhythmias that may lead to a sudden death [2–4]. Further prolongation of survival and amelioration in the quality of life for DMD patients depends not only on improving skeletal muscle performance but also on the development of therapies that slow down the progression of the cardiac disease and enhance cardiac function. This requires a mechanistic understanding of the nature of the cardiac defects, which can be obtained from studies of the cellular phenotype of the disease. This review focuses on our current view of cellular and molecular pathomechanisms of the dystrophic cardiomyopathy.

2. Genetic and molecular underpinning of DMD

To date, 25,267 variants of the dystrophin gene are known [5]. The gene is on the Xp21 chromosome locus and with 2.2 megabases is one of the longest human genes known. Skeletal and cardiac muscles of DMD patients either completely lack or express a truncated form of dystrophin, which in muscle is a ~427 kDa protein. Alternatively spliced isoforms expressed in a variety of other tissues can be substantially smaller, many are ~70 kDa. Dystrophin links the sarcomeric cytoskeleton to a complex of transmembrane proteins (called dystrophin-associated protein complex), which interacts with extracellular matrix [6,7]. In muscle, the dystrophin network covers almost the entire cytoplasmic surface of the plasma membrane. Dystrophin is also present in T-tubular membranes in cardiac myocytes [8,9]. Thus, it is strategically placed to serve in a variety of mechanical roles, such as maintenance of membrane stability and the transduction of mechanical force to the extracellular matrix. Indeed, it is widely accepted that the predominant functional consequence of the lack of dystrophin is an increased cellular vulnerability to mechanical stress associated with muscle contraction. One of the early diagnostic tests of DMD is to determine serum creatine kinase (CK) activity. It is increased in DMD patients as creatine kinase leaks through the plasmalemmal membrane of mechanically damaged dystrophin deficient muscle cells. Optical methods also detected accumulation of large molecular weight indicators inside dystrophic muscle cells getting inside through the unstable cellular membrane [10–12]. However, it should be noted that the direct measurement of tensile strength of normal and dystrophic sarcolemma did not reveal a significant difference [13] and the increase in intracellular Ca^{2+} levels, rather the absence of dystrophin was proposed to underly the reduced resilience of the plasmalemma [14]. The precise molecular underpinnings of the membrane vulnerability resulting from the lack of dystrophin are, however, poorly understood at present [7]. In principle, the mechanism could be direct and mechanical, or indirect via Ca^{2+} overload or oxidative stress.

3. Animal models of DMD

3.1. The mdx mouse

The *mdx* mouse is a strain of mice which arose from a spontaneous mutation (*mdx*) in inbred C57BL mice. In more recently identified dystrophin mutants, such as the mdx^{2cv} mdx^{4cv} mdx^{5cv} mice, the spectrum and tissue distribution of the affected isoforms is different, usually more wide spread, and there are fewer mutation reversions [15]. Like DMD patients, *mdx* mice have a total loss of functional dystrophin from the muscle tissue. In contrast to humans, they have a less severe phenotype and a more slowly developing cardiac disease with a near normal life span. Because of their mild phenotype, there were initially some difficulties to validate *mdx* mice as an appropriate model of cardiac dystrophy. However, recent studies clearly demonstrated that *mdx* mice develop dystrophic cardiomyopathy later in life. Hearts of 2 month old *mdx* mice seemed to have normal ventricular function and normal echocardiograms [16]. Nevertheless, even these young *mdx* hearts are more susceptible to damage when subjected to mechanical overload [17]. By 8 months of age, hearts from *mdx* mice are dilated, hypertrophied, somewhat fibrotic and poorly contracting [16,18]. Additionally, electrocardiographic (ECG) deviations gradually increase in *mdx* mice and by the age of 6 months significant abnormalities are revealed in the cardiac conduction system [19,20]. Altogether, *mdx* mice have some but not all symptoms of DMD cardiomyopathy. The manifestation is not as pronounced as in DMD and depends on mechanical challenges. However, because of their slowly developing phenotype *mdx* mice can be a useful model for studies of the progression of DMD and can help to identify the cellular sequence of events leading from the genetic defect (lack of functional dystrophin) to the onset of cardiac disease.

3.2. The utrophin deficient mdx mouse

Utrophin is a cytoskeletal protein which was originally described as dystrophin-like in 1989 because of its partial homology [21]. It is widely expressed in differentiating skeletal muscle with the same cellular distribution as dystrophin. In adult tissue utrophin disappears from the sarcolemma, being replaced by dystrophin and it remains primarily localized to the neuromuscular junction. Utrophin expression dramatically increases in muscle from DMD patients and in *mdx* mice. It is believed that utrophin can partially compensate for the loss of dystrophin and to some extent explain the mild phenotype of *mdx* muscle (especially in skeletal muscle). $Mdx/utrn^{-/-}$ double knockout mice (DKO) exhibit most of the clinical signs of DMD patients, such as short stature, kyphosis, limb weakness and breathing problems by 6 weeks of age [22]. All DKO mice succumb to premature death by 20 weeks of age. Compared to senescent *mdx* mice, their cardiac dystrophic phenotype is similar, but it's onset is much earlier [23]. By 10 weeks of age DKO mice not only exhibit multiple histological defects, such as myocyte degeneration, inflammation and some fibrosis, but also abnormal ECGs and contractile dysfunction [24]. Therefore, the $mdx/utrn^{-/-}$ DKO mouse is another useful model of DMD. However, DKO mice are more difficult to breed. The homozygous animals do not survive birth and the