Enteroviral Cardiomyopathy: Bad News for the Dystrophin-Glycoprotein Complex

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Abstract

Genetic deficiency of the dystrophin-glycoprotein complex causes hereditary dilated cardiomyopathy. Enteroviruses can also cause cardiomyopathy and we have recently described a potential molecular mechanism for enterovirus-induced dilated cardiomyopathy. The coxsackieviral protease 2A proteolytically cleaves and functionally impairs dystrophin. Additionally, during infection with coxsackievirus B3, the dystrophin-glycoprotein complex becomes disrupted and the sarcolemmal integrity is lost.

This review article discusses the importance of the dystrophin cleavage for the development of increased sarcolemmal permeability and potential pathways for mechanisms by which the dystrophin cleavage during coxsackieviral infection may contribute to dilated cardiomyopathy.

Key Words: Heart failure · Cardiomyopathy · Dystrophin · Myocarditis · Coxsackievirus

Enterovirale Kardiomyopathie: Schlechte Nachrichten für den Dystrophin-Glykoprotein-Komplex

Zusammenfassung

Ein Gendefekt des Dystrophin-Glykoprotein-Komplexes ist eine Ursache der hereditären dilatativen Kardiomyopathie. Enteroviren können gleichfalls eine Kardiomyopathie verursachen. Wir haben vor kurzem einen möglichen molekularen Mechanismus für die enterovirusinduzierte dilatative Kardiomyopathie beschrieben. Die Protease 2A der Coxsackie-Viren spaltet proteolytisch Dystrophin, sodass die Funktion von Dystrophin gravierend beeinträchtigt wird. Zusätzlich wird bei einer Infektion mit Coxsackie-Virus B3 der Dystrophin-Glykoprotein-Komplex aufgebrochen, was mit einem Verlust der sarkolemmalen Integrität einhergeht.

In dieser Übersichtsarbeit wird die Bedeutung der Dystrophin-Spaltung für die Permeabilitätssteigerung des Sarkolemms ebenso diskutiert wie potentielle Mechanismen, über die die Dystrophinspaltung zur Entstehung einer dilatativen Kardiomyopathie während einer Coxsackie-Virusinfektion beiträgt.

Schlüsselwörter: Herzinsuffizienz · Kardiomyopathie · Dystrophin · Myokarditis · Coxsackie-Virus

"Viruses are nucleic acid surrounded by bad news."
(P. Medawar, 1960 Nobel Prize Winner in Medicine and Physiology)

For a coordinated contractile function of the heart, the many mechanical forces generated within the sarcomeres of individual cardiomyocytes need to be transmitted to the extracellular matrix. For this purpose, cardiomyocytes possess a highly specialized extrasarcomeric cytoskeleton [10] that includes the dystrophin-glycoprotein complex [41]. Dystrophin and the dystrophin-associated glycoproteins α-, β-, γ- and δ-sarcoglycan, α- and β-dystroglycan as well as the recently described sarcospan [12] compose a multiprotein-complex that collectively connects the internal F-actin based cytoskeleton to laminin-2 of the extracellular matrix [41]. In addition to its structural role, the dystrophin-glycoprotein complex may have signal transduction properties such as the ecto-ATPase activity that was recently described for α-sarcoglycan [7].

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The importance of the dystrophin-glycoprotein complex for normal cardiac function in man and rodents is highlighted by hereditary dystrophin- and sarcoglycan-mutations that cause dilated cardiomyopathy. For example, dystrophin mutations in Duchenne and Becker muscular dystrophy [28] are associated with a high incidence of dilated cardiomyopathy [6]. Additionally, a mutation in dystrophin causes an X-linked dilated cardiomyopathy in patients with minimal skeletal myopathy [29, 43]. Genetic defects in α-, β-, γ-, or δ-sarcoglycan are the cause of human limb-girdle muscular dystrophy type 2D, 2E, 2C and 2F, respectively [26]. Sarcoglycan deficiency can cause dilated cardiomyopathy in these patients [44] as well as in the cardiomyopathic hamster that has a δ-sarcoglycan deletion [30]. These studies and others [1, 30, 33] have led to the paradigm that familial dilated cardiomyopathy may occur secondary to defective transmission of mechanical force from the sarcomere to the extracellular matrix [42].

Dilated cardiomyopathy is a multifactorial disease that encompasses acquired etiologies in addition to hereditary forms. Dilated cardiomyopathy is characterized by an enlargement of ventricular chamber(s) and often heart failure, a leading cause of cardiovascular mortality in the US and Europe [13, 38]. A subset of human acquired dilated cardiomyopathy, as much as 30%, is associated with an enteroviral infection of the heart, in particular coxsackie B viruses [2]. In addition, transgenic expression of coxsackie-B3-viral genomes and protein in the heart of mice is sufficient to induce dilated cardiomyopathy [46]. Among the coxsackieviral proteins are 2 proteases, protease 2A and protease 3C, both of which are essential for the viral life cycle [36]. The overall folding of the viral protease 2A resembles chymotrypsin-like serine proteases but it possesses a cysteine as the nucleophile at the active site [34]. Substrate recognition by the enteroviral protease 2A depends on a degenerate amino acid pattern upstream of the cleavage site. In addition, a glycine residue at the C-terminal side of the scissile bond of the cleavage site occurs in all known natural substrates of the enteroviral protease 2A. Since the viral proteases can affect host cell proteins this amino acid pattern was used by Blom et al. [8] to establish a neural network algorithm for the computer prediction of potential novel cellular protease 2A-substrates.

Despite the extensive epidemiological studies that demonstrate a role for enteroviruses in human dilated cardiomyopathy [2], the molecular pathogenic mechanism(s) through which enteroviruses can induce dilated cardiomyopathy are poorly understood. In analogy to many other virus-mediated illnesses [31], both direct viral effects as well as the host's immune response play an important role in the pathogenesis of viral heart disease. On one hand, coxsackievirus B3 clearly has direct myocytopathic effect(s) and expression of coxsackieviral genome and proteins is sufficient to induce dilated cardiomyopathy [46]. On the other hand, the immune response – although necessary to eliminate virally infected cells – may contribute to the tissue damage by inappropriately attacking uninfected cardiomyocytes [21].

The data from hereditary cardiomyopathies demonstrate that abnormalities in the dystrophin-glycoprotein complex can cause cardiomyopathy. In addition, we recently demonstrated that enteroviral protease 2A can cleave dystrophin leading to disruption of the dystrophin-glycoprotein complex suggesting a potential mechanism by which viral infection may contribute to the induction of cardiomyopathy [4].

Disruption of the Dystrophin-Glycoprotein Complex in Coxsackieviral Cardiomyopathy

Given the importance of the dystrophin-glycoprotein complex in hereditary dilated cardiomyopathy, and the known role for enteroviruses in acquired cardiomyopathy, it is conceivable that coxsackievirus B3 induces dilated cardiomyopathy through alterations of dystrophin and/or sarcoglycans.

Based on a bioinformatic approach, we utilized the protease 2A cleavage site prediction server established by Blom et al. [8] *(http://www.cbs.dtu.dk/services/NetPicoRNA/)*. Based on a computer search of the human Swiss protein databank, they identified dystrophin as one of the human proteins most likely to be cleaved by enteroviral protease 2A. We applied the full dystrophin sequence to their prediction algorithm and identified a second site for potential cleavage of dystrophin by protease 2A [4]. The computer-predicted protease 2A-cleavage site that we identified lies in the dystrophin hinge 3, a region that has been previously demonstrated to be accessible to proteolytic cleavage [22]. Since the hinge 3 region lies between the actin-binding sites and the β-dystroglycan anchoring motif of dystrophin [4], cleavage at this site would disconnect the (actin-bind-

ing) N-terminal fragment from the sarcolemma (where the C-terminal fragment binds β-dystroglycan). Additionally, the viral protease 2A is N-myristoylated [36] and thus membrane-associated, as is dystrophin [41]. Thus, both protease and substrate are in close proximity to each other, which should facilitate their interaction. It is important to note that the enteroviral protease 2A is a highly specific protease with very few substrates. Cis-cleavage of the viral polyprotein is required for viral replication [36]. The only cellular proteins known to be cleaved *in trans* by the protease 2A are the eukaryotic translation initiation factors 4G I [24] and II [16] and the poly-A binding protein [19], all proteins that are important for translation of host proteins.

In order to determine whether enteroviral protease 2A could cleave dystrophin at the computer predicted cleavage sites, we added purified protease 2A to neonatal rat and human myocyte extracts. As we previously reported, dystrophin is proteolytically cleaved by purified protease 2A and functionally impaired following enteroviral infection as demonstrated by dissociation of the N-terminal rod domain fragment from the plasma membrane in cultured cardiomyocytes infected with coxsackievirus B3 [4]. Antibody epitope mapping of the protease 2A cleavage site in human and mouse dystrophin demonstrated that the coxsackieviral protease 2A cleaves dystrophin in the hinge 3 region [3]. Cleavage at the other computer predicted site in the third spectrin repeat appears to occur more slowly and may depend on initial cleavage in the hinge 3 region. The slower cleavage in spectrin repeat 3 might be explained by its triple-helical secondary structure [22] that may make that region less accessible to cleavage by protease 2A.

In the intact mouse heart, dystrophin is proteolytically cleaved and its sarcolemmal localization is disrupted in CVB3 infected cardiac myocytes. Additionally, the sarcolemmal integrity in these cells is impaired as determined by tracer dye uptake in a manner similar to that observed in muscular dystrophy [4]. Since genetic dystrophin deficiency leads to a loss of dystrophin-associated glycoproteins [14], we investigated whether dystrophin cleavage during coxsackie B virus infection would similarly affect the sarcoglycans. The sarcoglycan complex becomes physically, morphologically and functionally disrupted during coxsackievirus B3 (CVB3) infection of cultured cardiomyocytes and mice as determined by staining for α-sarcoglycan and β-dystroglycan [4].

In analogy to findings from patients with dilated cardiomyopathy due to Duchenne muscular dystrophy, we have previously proposed that the cleavage of dystrophin with its subsequent biochemical, morphological and functional disruption of the dystrophin-glycoprotein complex during coxsackievirus B3 infection initiates a cascade of events that contributes to the pathogenesis of dilated cardiomyopathy [4]. Since the coxsackieviral protease 2A cleaves the human dystrophin hinge 3 region [3], the dystrophin cleavage is potentially relevant for the pathogenesis of human viral heart disease (Figure 1).

How Could Dystrophin Cleavage Lead to Enterovirus-Induced Dilated Cardiomyopathy?

Although the dystrophin cDNA was cloned more than a decade ago [23], the exact physiological role of the dystrophin protein still remains unknown. The current model assumes a structural role for dystrophin [41] because its N-terminus as well as an epitope in the rod domain bind F-actin [37] whereas a more distally located motif in the cysteine-rich region near the carboxy-terminus binds to β-dystroglycan [35]. Dystrophin, therefore, connects the internal cytoskeleton to the sarcolemma via its transmembrane glycoprotein complex (see Figure 1). Genetic dystrophin deficiency leads to a loss of the dystrophin-glycoprotein complex with increased myocyte permeability [14, 41]. However, the "downstream" effectors that ultimately lead to myocyte death are unknown even in Duchenne muscular dystrophy. Several mediators, including reactive oxygen species [9] and lymphocyte-mediated myocyte damage [40], have been proposed.

Our findings in enteroviral myocarditis/cardiomyopathy suggest a pathogenic link between virally induced dystrophin abnormalities and genetic dystrophin deficiency. The protease 2A cleaves dystrophin during coxsackievirus B3 infection, adversely affecting myocytes by impairing transmission of mechanical force and increasing cell permeability [4]. At the organ level, myocyte "drop-out" and/or dysfunction and loss of contractile force may ultimately contribute to the induction of dilated cardiomyopathy.

However, important distinctions exist between virus-induced cardiomyopathy and the hereditary absence of dystrophin. In particular, in contrast to a genetic defect the infection is focal in viral-mediated

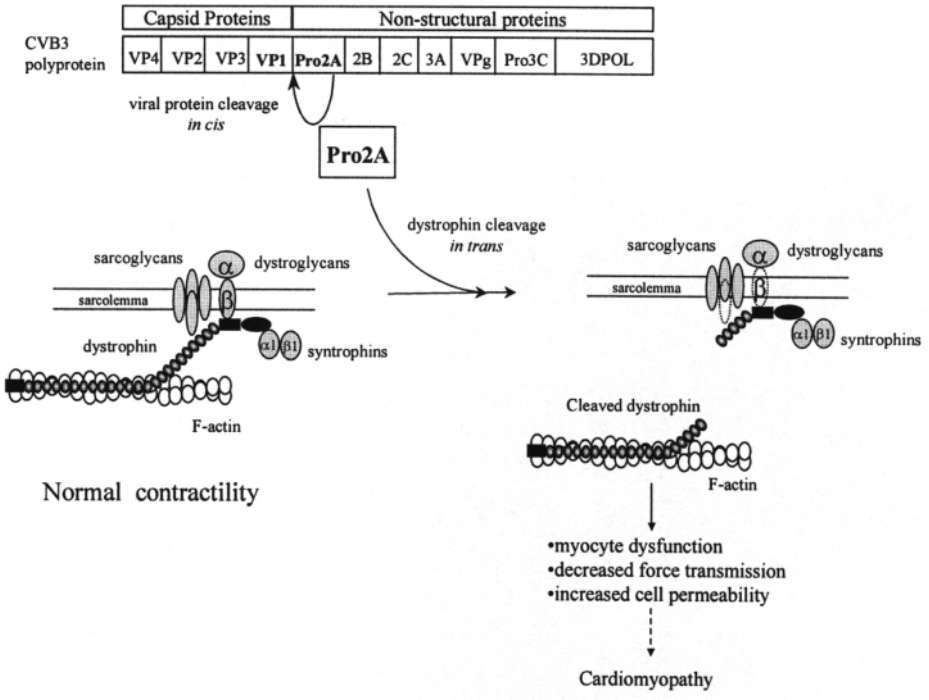

Figure 1
Schematic diagram of effect of dystrophin cleavage by protease 2A.

Abbildung 1
Schematisches Diagramm der Konsequenzen einer Dystrophin-Spaltung durch die Coxsackie-virale Protease 2A.

cardiomyopathy. The percentage of virally infected myocytes is very different in the various stages of the disease. In the acute stage, 7 days after infection with coxsackievirus B3, we regularly find more than 10% of infected myocytes in our experiments with C3H mice (data not shown). This percentage may be high enough that acute cleavage of dystrophin could significantly affect overall cardiac function. A similar mechanism could occur in acute, fulminate myocarditis as is observed in children and young adults. In the chronic stage of murine infection, the percentage of cells with viral persistence appears to be much lower [20]. A similarly low percentage has been reported in human heart samples from patients with dilated cardiomyopathy [18].

So, how can enteroviruses induce dilated cardiomyopathy if the percentage of cells that express viral proteins such as the protease 2A is very low? First, the number of infected cells in any sample is only indicative of the number of cells infected at the point in time when the tissue was harvested for analysis. A replicating virus, even if only few cells are infected at any given time, can infect many cells over a prolonged period of time and thus cause substantial myocyte loss. The same concept has been applied to myocyte apoptosis where the percentage of apoptotic nuclei is several-fold increased in human heart failure but the absolute numbers of apoptotic cells are very low [32]. Second, increased sarcolemmal permeability that occurs with cleavage of dystrophin can expose myocyte contents to immunoregulatory cells. For instance, low-level expression of coxsackieviral genomes in cultured cardiomyocytes results in LDH release from the cells [45]. "Leaking" myocyte proteins from even very few infected cells could act as autoantigens and may initiate an autoreactive response. T and B lymphocytes activated in such a way may recognize diverse myocardial antigens such as myosin, the adenine-nucleotide translocator or the sarcolemma, all of which have been reported as autoantigens in human dilated cardiomyopathy [25, 27, 39]. This inappropriate immune response may attack and destroy many uninfected myocytes and thus cause substantial myocyte loss or dysfunction [21]. In this regard, it is noteworthy that increased lymphocytic infiltrates and enhanced expression of cytolytic mediators such as perforin and TIA-1 have been reported in endomyocardial biopsies from patients with dilated cardiomyopathy [5].

Another difference between hereditary dystrophin deficiency and cleavage of dystrophin by protease 2A is the chronic versus acute loss of dystrophin. In hereditary dystrophin deficiency, dystrophin is absent in embryonic muscle and throughout the animal's life.

This allows for compensatory mechanisms such as up-regulation of the related utrophin as has been observed in dystrophin deficient mice [17]. However, in virally infected cells there is acute cleavage of dystrophin that is likely to occur in a cell that cannot promptly compensate for the loss of dystrophin since host cell translation mechanisms are impaired in the virally infected cells. How this would affect the cell is not known, but it may contribute to the more dramatic loss of sarcolemmal integrity that is observed in virally infected cells [4].

Recently, an alternative mechanism by which disruption of the dystrophin-glycoprotein complex can cause cardiomyopathy has been proposed. In mice with genetic disruption of α-sarcoglycan there is disruption of the typical sarcolemmal organization in the cardiac myocyte, but not in the vascular smooth muscle cells; however, genetic knockout of δ-sarcoglycan leads to disruption of the sarcoglycans in both cardiac and vascular smooth muscle cells. In these 2 models, significant cardiomyopathy occurs only in the mice with genetic disruption of δ-sarcoglycan and disruption of the sarcoglycan complex in vascular smooth muscle cells. This is associated with arteriolar constriction leading to areas of apparent myocardial ischemia and cell death that is increased with exercise [11]. Since coxsackievirus can infect smooth muscle cells [15], it is conceivable that enteroviral infection of smooth muscle cells can lead to disruption of the dystrophin-glycoprotein complex and focal areas of arteriolar vasospasm and cell loss through oxygen supply-demand mismatch. Such a paradigm would not require that a large number of myocytes be simultaneously infected to affect a significant amount of myocardium.

Taken together, the exact pathway of how disruption of the dystrophin-glycoprotein complex during coxsackieviral infection induces cardiomyopathy is still unknown. Nevertheless, cleavage of dystrophin by protease 2A is the first potential molecular mechanism that relates the pathogenesis of familial cardiomyopathy to events occurring in acquired cardiomyopathy. Ultimately, a complete understanding of the mechanisms by which cleavage of dystrophin by protease 2A can contribute to cardiomyopathy will depend on an improved understanding of how genetic loss of the dystrophin-glycoprotein complex causes muscular dystrophy and dilated cardiomyopathy.

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