Connecting enterovirus infection to dystrophin dysfunction in dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is the most common type of cardiomyopathy characterized by dilation of the left ventricle (1). DCM can be caused by various etiologies including genetic variants, coronary artery disease, hypertension, diabetes, drug and alcohol abuse, and infections. Progressive dilatation of the left ventricle can lead to heart failure and sudden arrhythmogenic cardiac death, both leading causes of cardiovascular mortality in the US and Europe (2).

Up to 30% of DCM patients exhibit evidence of an infection with enteroviruses (EV) such as for example Coxsackievirus B3 (3,4). Moreover, EVs have been detected in 20% to 40% of patients who died suddenly after acute myocardial infarction, suggesting that EV infection increases the susceptibility of the heart to injury and catastrophic dysfunction (5). An infection by EV initially leads to myocarditis, during which the EV itself as well as expression of EV-encoded genes cause direct cytopathic effects (6,7).

This concept has been demonstrated in transgenic mice *in vivo*, since cardiac-restricted expression of Coxsackieviral proteins is sufficient to induce DCM independent of viral replication (6,7). Within cardiomyocytes, Coxsackievirus produces proteases, such as protease 2A, that have an important role in viral replication, but also affect host cell proteins such as dystrophin (6,8). Cleavage of dystrophin may have a role in the release of virus from the myocyte, since viral counts are increased in the absence of dystrophin (8). Badorff *et al*. (6) proposed that enteroviral proteins interact with and adversely affect myocyte proteins to induce myocyte dysfunction and cardiomyopathy. In particular, it was proposed that Coxsackievirus protease 2A can cleave dystrophin. Xiong *et al*. (9) subsequently demonstrated that inducible cardiac-restricted expression of enteroviral protease 2A is sufficient to induce DCM, due to disruption of the sarcolemmal membrane and cleavage of dystrophin. Moreover, experiments by Lim *et al*. (10) showed that expression of a 2Apro cleavage–resistant dystrophin peptide could block cardiomyopathy induced by EV infection/2Apro expression, providing evidence that dystrophin is a key downstream target among other 2Apro targets in the pathogenesis of viral-mediated cardiomyopathy. Finally, it has also been shown that the intrinsic innate immune factor known as 'ubiquitin-like modifier interferon-stimulated gene of 15 kDa' (ISG15) significantly contributes to the suppression of viral replication and infectious virus yield in the heart (11). Mice deficient in ISG15 exhibited a profound exacerbation of myocarditis and a significantly increased mortality due to heart failure.

Dystrophin is a large intracellular protein that links the actin cytoskeleton to the extracellular matrix via the transmembrane dystroglycan complex (DGC). By means of its connection with the DGC, dystrophin provides mechanical stability to the cell and prevents damage from muscle contractions (12). Absence or loss-of-function variants of dystrophin caused Duchenne muscular dystrophy (DMD) and Becker's muscular dystrophy (BMD), genetic disorders characterized by progressive skeletal muscle weakness and cardiac disease (12). Many DMD patients develop DCM, which is generally manifested around 10 years of age, and is prevalent in most patients by

Page 2 of 3

Wang and Wehrens. Linking enterovirus to dilated cardiomyopathy

20 years of age (13). Dystrophin and DGC dysfunction induce membrane fragility and loss of membrane integrity, which leads to abnormal intracellular calcium (Ca^{2+}) homeostasis, abnormal neuronal nitric oxide synthase (nNOS), and reactive oxygen species (ROS) production, among other things (12,14,15).

The recent paper by Barnabei *et al*. (5) addressed an important outstanding question: the mechanism by which dystrophin cleavage by virus-encoded 2A protease causes DCM. Specifically, the authors set out to determine whether DCM resulted from the overall loss of dystrophin or from the emergence of a dominant-negative acting dystrophin cleavage product. To address this question, two new transgenic mouse models were generated, that overexpress either the C-terminal dystrophic fragment (CtermDys) or the N-terminal fragment (NtermDys), respectively (5). Overexpression of the sarcolemma-localized CtermDys fragment but not NtermDys caused enlarged hearts, myocardial fibrosis and inflammation. Furthermore, it was shown that CtermDys overexpression reduced dystrophin expression at the plasma membrane, while this peptide—which contains the DGC binding domain—also nucleated key DGC proteins and increased expression of α-, β-, and γ-dystroglycan. However, this paper did not explore whether those CtermDys-DGC protein aggregates are cytotoxic or contribute in other ways to the pathogenesis of DMD.

Because EV infection has been associated with an increased risk of sudden cardiac death following acute myocardial infarction (16), the authors also assessed whether the CtermDys fragment altered the risk of death after ischemic injury and cardiac stress (5). It was shown that CtermDys transgenic mice were more susceptible to ischemia/reperfusion injury and increased mechanical stress induced by chronic isoproterenol administrations. Therefore, reduced membrane stability in CtermDys mice might underlie the enhanced mortality in CtermDys mice.

In a final set of experiments, Barnabei *et al*. (5) demonstrated that overexpression of human intact dystrophin provided protection in CtermDys mice following cardiac stress. In mice with a higher-fold level of expression of human dystrophin (hDys), there was a significant reduction in CtermDys protein expression with reduced membrane permeability and improved survival. These findings suggest that titrating full-length dystrophin levels could confer myocardial protection in CtermDys mice during stress conditions.

In summary, enterovirus infection can cause severe cardiomyopathy in humans. Barnabe *et al*. (5) demonstrated for the first time that a virus-encoded 2A protease–mediated carboxyl-terminal dystrophin cleavage fragment (CtermDys) is sufficient to cause marked dystrophic cardiomyopathy in mice. A two-hit dominant-negative disease mechanism was proposed, in which first membrane-tethered CtermDys mechanically uncouples the key actin-DGC linkage, and subsequently CtermDys inhibits full-length dystrophin and compensatory utrophin from binding at the membrane. This elegant study provided new therapeutic insights as membrane-bound CtermDys emerged as a novel translational target—in addition to overexpressing full-length dystrophin—for the treatment of virus-mediated cardiomyopathy.

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Footnote

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