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Review

A novel scheme of dystrophin disruption for the progression of advanced heart failure

Tomie Kawada[a], Fujiko Masui[b], Asaki Tezuka[b], Takashi Ebisawa[b], Hiroyuki Kumagai[c], Mikio Nakazawa[d], Teruhiko Toyo-oka[b,c,*]

[a]*Division of Pharmacy, Niigata University of Medical and Dental Hospital, Niigata, 951-8520, Japan*
[b]*Department of Pathophysiology and Internal Medicine, University of Tokyo, Tokyo 113-0033, Japan*
[c]*Department of Molecular Cardiology, Tohoku University, Biomedical Engineering Research Organization (TUBERO), Sendai 980-8587, Japan*
[d]*Department of Medical Technology School of Health Science, Faculty of Medicine, Niigata University, Niigata 951-8510, Japan*

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Abstract

The precise mechanism of the progression of advanced heart failure is unknown. We assessed a new scheme in two heart failure models: (I) congenital dilated cardiomyopathy (DCM) in TO-2 strain hamsters lacking δ-sarcoglycan (SG) gene and (II) administration of a high-dose of isoproterenol, as an acute heart failure in normal rats. In TO-2 hamsters, we followed the time course of the histological, physiological and metabolic the progressions of heart failure to the end stage. Dystrophin localization detected by immunostaining age-dependently to the myoplasm and the in situ sarcolemma fragility evaluated by Evans blue entry was increased in the same cardiomyocytes. Western blotting revealed a limited cleavage of the dystrophin protein at the rod domain, strongly suggesting a contribution of endogenous protease(s). We found a remarkable up-regulation of the amount of calpain-1 and -2, and no change of their counterpart, calpastatin. After supplementing TO-2 hearts with the normal δ-SG gene in vivo, these pathological alterations and the animals' survival improved. Furthermore, dystrophin but not δ-SG was disrupted by a high dose of isoproterenol, translocated from the sarcolemma to the myoplasm and fragmented. These results of heart failure, irrespective of the hereditary or acquired origin, indicate a vicious cycle formed by the increased sarcolemma permeability, preferential activation of calpain over calpastatin, and translocation and cleavage of dystrophin would commonly lead to advanced heart failure.

Keywords: Dystrophin; δ-Sarcoglycan (SG); Gene therapy; Heart failure; Proteolysis; Calpain

1. Background

Advanced heart failure is the most prevalent cause of death or hospitalization in developed countries. Although several pharmacological agents have improved its mortality or morbidity of the patients [1–4], no treatment is available to completely prevent its progression, with the exception of cardiac transplantation. However, this therapy encompasses a variety of socioeconomic and medical limitations. Specifically, in infantile and juvenile cases, cardiac transplantation is accompanied with some problems such as mismatch in organ size. End-stage DCM is the most frequent cause of heart transplantation in Japan.

The precise mechanism remains unknown of the progression of advanced heart failure. Its clarification is urgently required for prevention or treatment. We conducted a comprehensive study of the progression of cardiac dysfunction in advanced heart failure, and propose our paradigm that the disruption of dystrophin or dystrophin-associated proteins (DAP) may aggravate heart failure. DAP forms a complex connecting dystrophin at the subsarco-

Abbreviations: CM, cardiomyopathy; DCM, dilated cardiomyopathy; δ-SG, δ-sarcoglycan; DAP, dystrophin-associated proteins; HCM, hypertrophic cardiomyopathy; LVP, left ventricular pressure; rAAV, recombinant adeno-associated virus

* Corresponding author. Tel.: +81 3 5841 2570; fax: +81 3 3813 2009.
E-mail address: toyooka_3im@hotmal.com (T. Toyo-oka).

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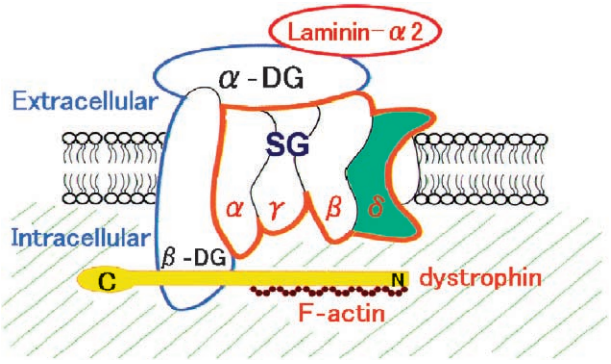

Fig. 1. A scheme of dystrophin-associated proteins (DAP). Mutations in DAP, which cause DCM in human cases, are shown in red characters. SG: sarcoglycan; DG: dystroglycan.

lemma with laminin α-2 at the extracellular matrix (Fig. 1). Cardiac muscle repeats contraction and relaxation throughout its life and the sarcolemma should be more resistant to the expansion–shrinkage cycling in cardiac than in skeletal muscle. The hereditary origin of DCM is estimated to account for about 20% to 30% of all patients [5,6]. A gene defect in DAP and subsequent dysfunction of the corresponding protein commonly induce DCM, as is the case in Duchenne or Becker type muscular dystrophy [7].

2. Merits of TO-2 strain hamsters for the study of DCM

Animal models are very significant for both the assessment of pathological processes and the development of new treatments [8]. The cardiomyopathy (CM) hamster is a valuable model of human hereditary CM [9] and shows hypertrophic CM in the BIO 14.6 strain [9–11] and DCM in the TO-2 strain of hamsters [11]. Many pathological and physiological features have been reported, including oncosis, apoptosis and necrosis of myocardial cells, interstitial fibrosis and calcification (Fig. 2). We showed that both HCM and DCM hamsters share a common defect in δ-SG gene, a component of DAP (Fig. 1, Refs. [11,12]), and determined the breakpoint of the δ-SG gene in both strains [11]. δ-SG makes a complex with other α-, β- and γ-SGs and connects dystrophin with the extracellular matrix via α- and β-dystroglycan [13–15]. In the BIO 14.6 myocardium at the early stages of heart failure, both the β- and δ-SG proteins were missing, but α- and γ-SG were weakly and heterogeneously expressed in cardiomyocytes. In contrast, the TO-2 strain shows loss of other SGs from the onset [16]. We conclude that TO-2 was suitable for developing gene therapy of the hereditary DCM.

Similar mutations of the δ-SG gene have been reported in patients of four families with DCM, one of who underwent cardiac transplantation [17]. Accordingly, the same δ-SG gene mutation causes DCM in both humans and hamsters. Most gene mutations in animals have been demonstrated in humans. This is the evidence that the genes of all animals have mutated to differentiate or adapt to changing environments. At present, mutations of cardiac F-actin, α-, β-, γ-, and δ-SG or lamin A/C have been reported to cause DCM in humans [7,17–21].

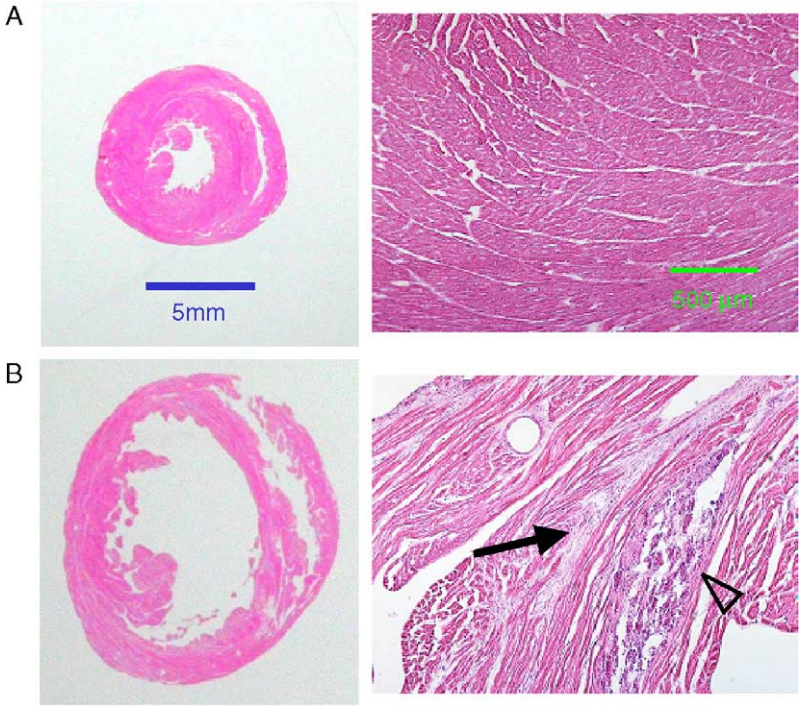

Fig. 2. Pathological features of normal (A, F1B) and DCM (B, TO-2) hamster hearts. TO-2 showed the enlarged cardiac chamber. The arrow and arrowhead denote interstitial fibrosis and calcified lesion, respectively.