Blockade of sarcolemmal TRPV2 accumulation inhibits progression of dilated cardiomyopathy

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Aims Dilated cardiomyopathy (DCM) is a severe disorder defined by ventricular dilation and contractile dysfunction. Abnormal Ca²⁺ handling is hypothesized to play a critical pathological role in DCM progression. The transient receptor potential vanilloid 2 (TRPV2) has been previously suggested as a candidate pathway for enhanced Ca²⁺ entry. Here, we examined the sarcolemmal accumulation of TRPV2 in various heart-failure model animals and DCM patients, and assessed whether presently available inhibitory tools against TRPV2 ameliorate DCM symptoms.

Methods and results Immunological and cell physiological analyses revealed that TRPV2 is highly concentrated and activated in the ventricular sarcolemma of DCM patients and three animal models—δ-sarcoglycan-deficient hamsters (J2N-k), transgenic mice over-expressing sialytransferase(4C30), and doxorubicin (DOX)-induced DCM mice. Over-expression of the amino-terminal (NT) domain of TRPV2 could block the plasma membrane accumulation and influx of Ca²⁺ via TRPV2. Transgenic (Tg) or adenoviral expression of the NT domain in DCM animals caused effective removal of sarcolemmal TRPV2 along with reduction in the phosphorylation of calmodulin-dependent protein kinase II (CaMKII) and reactive oxygen species (ROS) production, which were activated in DCM; further, it prevented ventricular dilation and fibrosis, amelioratedcontractile dysfunction in DCM, and improved survival of the affected animals. The TRPV2 inhibitor tranilast markedly suppressed DCM progression.

Conclusion Sarcolemmal TRPV2 accumulation appears to have considerable pathological impact on DCM progression, and blockade of this channel may be a promising therapeutic strategy for treating advanced heart failure.

Keywords Dilated cardiomyopathy • Heart failure • Therapeutic tool • Ca²⁺-permeable channel • DOX-induced cardiomyopathy

1. Introduction

Dilated cardiomyopathy (DCM) is a severe disorder defined by ventricular dilation and cardiac dysfunction.¹⁻³ Although a considerable proportion of DCM cases develop because of inflammatory, metabolic, or toxic effects from medications, 30–48% of DCM cases are caused by genetic mutations.⁴ Some affected genes encode sarcomeric or cytoskeletal proteins, including the components of the dystrophin–glycoprotein complex (DGC).⁵⁻⁶ For example, δ-sarcoglycan-deficient hamsters (J2N-k) provide an animal model of human limb-girdle muscular dystrophy-associated DCM. However, little information is available regarding the pathways by which heterogeneous genetic defects and/or various causes lead to DCM symptoms.

The calcium ion (Ca²⁺) plays a pivotal role in the pathogenesis of cardiac disease.⁷⁻⁸ Ca²⁺-handling abnormalities have been found in various forms of heart failure, including DCM.⁹⁻¹¹ Further Ca²⁺-permeable transient receptor potential (TRP) channels have recently been recognized as key molecules in pathological cardiac hypertrophy and heart failure.¹²⁻¹⁴ We have previously reported that cardiac-specific over-expression of TRP vanilloid 2 (TRPV2) results in DCM with outstanding ventricular dilation,¹⁵ suggesting that chronic elevation in cytosolic Ca²⁺ concentrations ([Ca²⁺]) is critical in DCM pathogenesis. However, it is unclear whether TRPV2 activity is a risk factor for DCM in humans as well as animals and whether TRPV2 inhibition can be beneficial against DCM progression, because of the limited number of methods for specific TRPV2 inhibition.

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Therefore, we examined the role of TRPV2 in DCM. We also assessed the effect of TRPV2 blockades on cardiac dysfunction and DCM progression in several animal models.

2. Methods

Detailed methods are available in the Supplementary material online.

2.1 Molecular biology

All plasmid construction involving TRPV2 was carried out via a PCR-based strategy using the full-length mouse TRPV2 cDNA cloned into the pIRES expression vector (Invitrogen). Restriction enzyme-digested PCR products corresponding to the amino-terminal (NT) (amino acids (aa) 1–387) and carboxyl-terminal (CT) (aa 633–756) domains of TRPV2 were cloned into the p3Xflag-CMV-14 expression vector (Sigma-Aldrich). For adenoviral gene transfer, we inserted the haemagglutinin (HA)-tagged NT domain of TRPV2 (amino acids 1–387) cDNA into the pAd/CMV/V5-DEST viral vector (Invitrogen).

2.2 Animals and drug administration

DCM mice (4C30) were produced as described previously.16 Heart-specific NT-transgenic (Tg) mice were generated from C57BL/6J mice according to the standard procedures.15 J2N-k hamsters, and age-matched normal controls (J2N-n) were purchased from Japan SLC. J2N-k hamsters were orally administered tranilast for 14 days at a dose of 30 or 300 mg/kg per day. In the DOX experiment, wild-type (WT) and NT-Tg mice were chronically treated with either phosphate-buffered saline (PBS; control) or doxorubicin (DOX) (Pfizer) by four intraperitoneal (i.p) injections (d 0, 2, 4, and 6) at a dose of 4 mg/kg (cumulative dose totalling 16 mg/kg). All animal experiments were performed in accordance with the Guidelines for Animal Experimentation of the National Cerebral and Cardiovascular Center (NCVC), and procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH; NIH Publication, 8th Edition, 2011).

2.3 Histology, immunoblotting, and immunohistochemistry

Animals were anaesthetized with 5% isoflurane in an anaesthesia chamber until unresponsive to nose pinch, and the heart was harvested for

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Figure 1 TRPV2 accumulation and activation in the sarcolemma of cardiomyopathic hearts. (A) Immunohistochemical analysis of frozen ventricular sections from 8-week-old J2N-n and J2N-k hamsters with TRPV2 and β-catenin antibodies. β-catenin-positive intercalated discs or sarcomemal regions are shown by arrowheads or arrows, respectively. Scale bar: 50 μm. (B) Immunoblots (40 μg/lane) of TRPV2-immunostained cardiac muscles. Age-dependent increases in the expression level of TRPV2 are observed. The data represent mean ± SD values (n = 4–6 hamsters/group); *P < 0.05 vs. 4 weeks, †P < 0.05 vs. J2N-n. (C) Phase-contrast micrographs of freshly isolated ventricular cardiomyocytes. Scale bar: 30 μm. (D) Confocal micrographs of TRPV2-immunostained isolated cardiomyocytes. TRPV2 is extensively localized to the sarcolemma in J2N-k cells but to the intracellular compartment and intercalated disc (arrow) in J2N-n cells. Scale bar: 30 μm. (E) Intracellular Ca²⁺ increase in response to extracellular Ca²⁺ (5 mM) and 2-APB (500 μM) in cardiomyocytes loaded with fura-2. (F) Intracellular Ca²⁺ concentration calculated from three independent experiments. The data represent mean ± SD values (n = 7–10 cardiomyocytes/group); *P < 0.05.
biochemical assays. These experiments were conducted as described previously.15,17,18

2.4 Echocardiography
Cardiac function was evaluated by echocardiography using a Hewlett Packard Sonos 5500 ultrasound system with a 12-MHz transducer and M-mode imaging. Animals were sedated with tribromoethanol [i.p., 350 mg/kg of body weight (BW)] during the procedure.

2.5 Cardiomyocyte isolation and [Ca\textsuperscript{2+}]\textsubscript{i} measurement
Single-ventricular cardiomyocytes were freshly isolated from adult mouse and hamster hearts using standard enzymatic techniques.19 The [Ca\textsuperscript{2+}]\textsubscript{i} was measured at room temperature via a ratiometric fluorescence method using fura-2 or indo-1 acetoxymethyl ester.17,19

2.6 Human tissues
Cardiac tissue samples were obtained from patients with DCM (Supplementary material online, Table). Written informed consent was obtained from all living patients, and the experiments on human tissues were approved by the Institutional Review Board of the NCVC. The investigation conforms to the principles of the Declaration of Helsinki.

2.7 Statistical analysis
We used an unpaired Student’s t-test and one-way ANOVA followed by Dunnett’s test for statistical analysis. *P < 0.05 indicates statistical significance.

3. Results
3.1 TRPV2 is concentrated and activated in cardiomyopathic hearts
To study the role of TRPV2 in DCM, we first examined the expression and subcellular distribution of TRPV2 in the ventricles of δ-sarcoglycan-deficient (J2N-k) hamsters. In their normal control counterparts (J2N-n, 8-week-old hamsters), most of the TRPV2 expression was observed in the cell interior and in regions co-stained with the intercalated disc marker β-catenin (Figure 1A). In contrast, age-matched J2N-k ventricles showed increased TRPV2 expression in the peripheral sarcolemma as well as in parts of the intercalated discs (Figure 1A). With disease progression, total TRPV2 expression levels gradually increased only in J2N-k ventricles (from 9-week-old hamsters; Figure 1B). Although apparently similar rod shapes and sizes were noted in isolated cardiomyocytes from both types of hamsters (Figure 1C), stronger TRPV2 surface expression was observed in J2N-k cardiomyocytes than in controls (Figure 1D).

In J2N-k cardiomyocytes expressing TRPV2 in the sarcolemma, a rapid and large increase in [Ca\textsuperscript{2+}]\textsubscript{i} was elicited by exposure to the TRPV2 channel activator 2-aminoethoxydiphenyl borate (2-APB) as well as a high-Ca\textsuperscript{2+} solution, whereas increases in [Ca\textsuperscript{2+}]\textsubscript{i} were marginal in control J2N-n cardiomyocytes (Figure 1E and F); this increase was inhibited by the TRPV channel antagonist ruthenium red (data not shown). Although TRPV1–3 are known to be activated by 2-APB,20 cardiac tissues did not show detectable TRPV1 or TRPV3 expression (data not shown). Furthermore, although 2-APB is known to inhibit the intracellular Ca\textsuperscript{2+}-release channel inositol 1, 4, 5-trisphosphate receptor (IP\textsubscript{3}R) and other TRP channel family proteins,21 we saw an
increase in [Ca\(^{2+}\)], which is why TRPV2 was considered the principal candidate involved in the 2-APB-induced [Ca\(^{2+}\)]\(_i\) increase in J2N-k cardiomyocytes. In addition, we observed that 2-APB often induced abnormal Ca\(^{2+}\) elevation accompanied by loss of regular Ca\(^{2+}\)-transients under electrically stimulated conditions in J2N-k cardiomyocytes (but not in J2N-n control cardiomyocytes; unpublished observations). These data suggest that sarcolemmal TRPV2 accumulation contributes to the increased [Ca\(^{2+}\)]\(_i\) levels in J2N-k cardiomyocytes.

In addition to the J2N-k hamster, other animal models with DCM display sarcolemmal accumulation of TRPV2. One such model is the 4C30 mouse, which over-expresses β-galactoside α-2,3-sialyltransferase II (ST3Gal-II) and was recently developed as a model for human DCM; the other is DOX-induced DCM, a widely familiar model, although its precise mechanism of cardiotoxicity remains debatable. In hearts from these two animal models, total expression (see Figures 4A and 5E) and sarcolemmal accumulation of TRPV2 (see Figures 4B and 5D and Supplementary material online, Figures S1 and S2) were found to be largely increased when compared with WT mice. In 4C30 mice, expression of TRP canonical (TRPC6), but not that of TRPC1 and TRPC3, was slightly increased (Supplementary material online, Figure S3); however, expression of α-dystroglycan and α-sarcoglycan were greatly reduced (Supplementary material online, Figure S4).

We next studied TRPV2 expression in ventricular samples from DCM patients (Supplementary material online, Table S1). TRPV2 and TRPC1 expression was significantly higher in DCM patients than in controls (Figure 2A and B), but expression of TRPC3 and TRPC6 was not significantly different. All DCM samples also exhibited reduced dystrophin and syntrophin expression levels (Figure 2A and B), suggesting that DCM is somehow linked to abnormal cytoskeletal organization. Similar to the findings in J2N-k ventricles, strong TRPV2 immunostaining was detected only in the peripheral sarcolemma of DCM cardiomyocytes (Figure 2C). Part of the TRPV2 expression was co-localized with the sub-sarcolemmal cytoskeletal protein dystrophin in human DCM ventricles, although dystrophin was detected only in a limited number of myocytes (Figure 2D).

### 3.2 Over-expression of the NT domain effectively blocks plasma membrane accumulation of TRPV2

We hypothesized that sarcolemmal TRPV2 accumulation is a common factor leading to Ca\(^{2+}\)-induced muscle degeneration in various heart diseases. Therefore, translocation of TRPV2 from the sarcolemma to the cell interior could be a promising therapeutic method. To study such ‘back-translocation’ of TRPV2, we used HEK293 cells, because they always recruit TRPV2 to the plasma membrane, independent of growth conditions, upon its heterologous expression (Figure 3A). We examined over-expression of several functional domains of TRPV2 together with full-length TRPV2 to identify the part of the TRPV2 molecule required for plasma membrane accumulation. We found that when the NT domain of TRPV2 was over-expressed, the majority of TRPV2 molecules moved from the sarcolemma to the cell interior (Figure 3A); such translocation was not observed with CT domain over-expression (Figure 3A). Consistent with this, over-expression of the NT but not the CT domain dramatically inhibited 2-APB-induced [Ca\(^{2+}\)]\(_i\) increase (Figure 3B and C). Thus, the NT domain may be a useful tool for abrogating the sarcolemmal TRPV2 accumulation, thereby inhibiting the sustained increase in [Ca\(^{2+}\)]\(_i\) in agonist-stimulated cells. To examine the effect of NT domain over-expression on DCM symptoms, we used 4C30 mice and DOX-induced DCM mice.

### 3.3 Tg expression of the NT domain ameliorates cardiomyopathy in 4C30 mice

We generated Tg mice expressing the HA-tagged NT domain (Figure 4A) under the control of the α-myosin heavy chain (α-MHC) promoter. NT-Tg mice were apparently healthy as evidenced by normal heart morphology (Figure 4C), cardiac function (Figure 4G and H) and life span (Figure 4I). The NT domain was introduced into the hearts of 4C30 mice by crossing them with NT-Tg mice. Interestingly, elevated expression level of endogenous TRPV2 in 4C30 mice was decreased to control levels in 4C30/NT-Tg mice (Figure 4A). The exogenous NT domain was mostly localized to intercalated discs in both NT-Tg and 4C30/NT-Tg mice (Figure 4B). As expected, the sarcolemmal localization of TRPV2 in 4C30 mice was dramatically reduced following NT domain over-expression (4C30/NT-Tg) (Figure 4B), potentially leading to a reduction in sustained [Ca\(^{2+}\)]\(_i\) increase. Consistent with this idea, CaMKII phosphorylation was markedly reduced in the 4C30/NT-Tg mice (Figure 4A). In addition, the expression level of modulatory calcineurin inhibitory protein-1 (MCIP) was increased in 4C30 mice but reduced in 4C30/NT-Tg mice (Figure 4A), further suggesting that blockade of TRPV2 results in a reduction in sustained [Ca\(^{2+}\)]\(_i\) increase.

In 4C30 mice aged more than 120 days, we observed thinner ventricular walls and greater ventricular dilation accompanied by fibrosis (Figure 4C), with increased serum cardiac troponin I (cTnl; a heart
injury marker) levels (Figure 4D). Furthermore, reactive oxygen species (ROS) production measured by dihydroethidium (DHE) staining (Figure 4E) and the extent of lipid peroxidation estimated by measurement of 4-hydroxynonenal (4-HNE) adducts (Figure 4F) were significantly higher in the 4C30 mice, suggesting high oxidative stress in these DCM hearts. The 4C30/NT-Tg mice showed marked suppression of these symptoms (Figure 4C–F). Echocardiographically, the 4C30 mice showed increased left-ventricular diastolic and systolic dimensions (LVDd and LVDs, respectively), with decreased fractional shortening (FS) and ejection fractions (EF) (Figure 4G and H). However, the 4C30/NT-Tg mice had significantly improved cardiac functions. Moreover, the 4C30 mice progressively died at 200–300 days after birth, but 4C30/NT-Tg mice had a much longer life span, particularly the female mice (Figure 4I). We suspect that the amelioration in DCM symptoms may have resulted from the removal of endogenous TRPV2 from the sarcolemma.

3.4 Beneficial effects of NT domain over-expression in DOX-induced DCM

The effects of NT domain over-expression were next examined in DOX-induced DCM. In WT mice, DOX treatment resulted in ventricular dilation, reduced FS, and higher mortality (Figure 5A–C). In contrast, DOX-treated NT-Tg mice demonstrated better cardiac morphology and function and better survival (Figure 5A–C). Indeed, upon DOX treatment, the NT-Tg mice showed lower TRPV2 expression and sarcolemmal accumulation of TRPV2 (Figure 5D and E), demonstrating reduced CaMKII phosphorylation (Figure 5E) and oxidative stress (Figure 5F and G). These results suggest that TRPV2 also plays an important pathologic role in non-genetic heart failure, such as DOX-induced DCM.

3.5 Adenoviral expression of the NT domain ameliorates cardiac dysfunction in J2N-k hamsters

We next addressed whether the effects of the NT domain could be seen in J2N-k cardiomyopathy following over-expression via adenoviral transfer. We injected an adenovirus carrying either β-galactosidase (β-gal as a control) or the NT domain into the hearts of 9-week-old J2N-k hamsters, at which age sarcolemmal TRPV2 translocation and cardiac dysfunction had already been observed. At 14 days post-infection, we detected NT domain expression in the ventricles by immunoblotting with the HA antibody (Figure 6A). Detection of green fluorescent protein (GFP) in cardiac homogenates (Figure 6A) and sections (Figure 6B) confirmed that the adenoviral vector had reached the

Figure 4 Over-expression of the NT domain blocks TRPV2 surface expression and ameliorates morphological and biochemical symptoms of cardiomyopathy in 4C30 mice. (A) Representative immunoblot of heart homogenates with the indicated antibodies (upper panel). Data represent values from four independent experiments (n = 3–4/group) *p < 0.05 vs. WT mice, †p < 0.05 vs. 4C30 mice. (B) Representative immunohistochemical data from longitudinal cardiac sections from each group of mice. Scale bar: 100 μm. (C) Cardiac sections from 150-day-old mice stained with Masson’s trichrome. Scale bar: 5 mm. (D) Level of cTnI in serum (n = 10 mice/group). (E) Superoxides produced in the hearts were analysed by staining the ventricular tissues with DHE. (F) Immunostaining data of the hearts with 4-HNE antibody were analysed (n = 3 mice/group) *p < 0.05 vs. WT mice, †p < 0.05 vs. 4C30 mice. (G) Representative echocardiograms of each group of mice. (H) Echocardiographic analysis of cardiac function. (I) Kaplan–Meier survival analysis of each group of mice (n = 25–30 mice/group).
cardiac muscles and that the surface membrane expression of TRPV2 was decreased by NT domain over-expression. Echocardiography revealed that NT domain over-expression resulted in good amelioration of cardiac dysfunction, with improved FS and EF and reduced fibrosis (Figure 6C–E). These results demonstrated that NT domain-induced prevention of the sarcolemmal localization of TRPV2 can greatly ameliorate gene-defective DCM.

3.6 Tranilast prevents cardiomyopathy in J2N-k hamsters

We previously found that tranilast, which is known to be a non-selective cation channel blocker, effectively inhibits TRPV2. Tranilast inhibited 2-APB-induced [Ca\(^{2+}\)]\(_i\) increases with half-maximal inhibition at about 30 μM, in HEK293 cells expressing TRPV2 (Supplementary material online, Figure S5), but it has almost no effect on HEK293 cells expressing TRPV1, TRPV3, or TRPC1 (Supplementary material online, Figure S5). A recent study reported that tranilast inhibits TRPV2 ion channel activity. Thus, tranilast is one of the better inhibitors presently available against TRPV2. We observed that tranilast reduced the amount of surface TRPV2 and abnormal Ca\(^{2+}\) mobilization by 2-APB in DCM cardiomyocytes (J2N-k, 4C30; unpublished observation). Oral administration of tranilast to J2N-k hamsters resulted in the effective removal of TRPV2 from the sarcolemma of J2N-k hearts (Figure 6F), similar to the effect of TRPV2 NT domain over-expression. Tranilast markedly reduced ventricular dilation and muscle fibrosis in J2N-k hearts (Figure 6G). Furthermore, it improved cardiac contraction, as evidenced by a decrease in echocardiographic parameters (LVDD and LVDS) to control levels (Figure 6H) and improved FS (Figure 6H).

4. Discussion

The present results suggest, for the first time, the pathological significance of TRPV2 in DCM development. First, TRPV2 was observed to be extensively localized to the ventricular sarcolemma in DCM patients as well as in animal models of heart failure (J2N-k, 4C30, and DOX-induced cardiomyopathic mice), whereas it localized to the intracellular compartments and intercalated discs in normal ventricles. Second, Tg or adenoviral NT domain over-expression significantly reduced the sarcolemmal accumulation of TRPV2 and simultaneously ameliorated cardiac dysfunction, preventing DCM progression and improving survival in the animal models. Third, the TRPV2 inhibitor tranilast effectively prevented DCM progression in J2N-k hamsters. Based on these findings, we
hypothesize that the amelioration of DCM resulted from the inhibition of the Ca\textsuperscript{2+} influx through TRPV2; therefore, TRPV2 may be a potential upstream target against abnormal Ca\textsuperscript{2+} handling.

The DCM phenotype results from a broad variety of primary and secondary aetiologies. Despite the various underlying causes, there are many similarities in the final structural, functional, biochemical, and molecular phenotypes related to the long-lasting mechanical stress and neurohormonal activation observed in DCM.\textsuperscript{24} CaMKII is an ideal nodal molecule for transducing Ca\textsuperscript{2+} signals into downstream events such as apoptosis and necrosis, leading to clinical phenotypes of congestive heart failure and sudden death.\textsuperscript{25} In addition to CaMKII activation, ROS production is frequently observed in DCM hearts, with detrimental effects on cardiomyocytes.\textsuperscript{26} We found that increased CaMKII phosphorylation and ROS production observed in DCM hearts were attenuated by over-expression of the NT domain of TRPV2 (Figures 4 and 5), suggesting that TRPV2 may be an upstream regulator of Ca\textsuperscript{2+} influx and ROS production as well as an important mediator of various stress signals, including those arising from genetic defects, mechanical stress, and cardiotoxic drugs, leading to Ca\textsuperscript{2+}-induced cell death. In addition, calcineurin is known to be an important Ca\textsuperscript{2+}-dependent signalling molecule leading to cardiac hypertrophy.\textsuperscript{27} Certainly, cardiomyocyte-specific over-expression of calcineurin causes hypertrophy\textsuperscript{28} and cardiomyopathy, but conflicting results are reported on the effects of calcineurin inhibition.\textsuperscript{29} In our DCM models, calcineurin was slightly activated, as determined by the increase in the expression level of MCIP protein (Figure 4) as well as CaMKII activation and inhibition of TRPV2 suppressed both Ca\textsuperscript{2+}-signalling pathways (Figure 4). These findings suggest TRPV2 as a putative therapeutic target for the treatment of heart failure.

Here, we used 4C30 mice as a model for human idiopathic DCM. Unlike J2N-k hamsters, which gradually develop DCM, 4C30 mice are apparently asymptomatic up to 100 days but thereafter rapidly exhibit DCM symptoms and die within 200 days. Similar to that in 4C30 mice,\textsuperscript{30} sialytransferase expression levels are altered in human DCM.\textsuperscript{30} 4C30 mice also show DGC abnormalities (Supplementary material online, Figure S4), similar to those in DCM patients (Figure 2A and B). These characteristics indicate a pointed resemblance between 4C30 mice and human idiopathic DCM. Dystrophin degradation by the Ca\textsuperscript{2+}-dependent protease calpain was proposed as a pathway in

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**Figure 6** Two inhibitory tools against TRPV2 show comparative amelioration of cardiomyopathy in J2N-k. (A) Representative immunoblot and (B) immunohistochemical data of heart homogenates or sections from 11-week-old J2N-k infected with adenovirus carrying β-galactosidase (Ad-β-gal, control) or the NT domain (Ad-NT). Adenoviral infection was confirmed by staining with GFP. Scale bar: 100 μm. Similar results were obtained from three independent experiments. (C) Echocardiographic analysis of adenovirus-infected J2N-k. Representative echocardiograms (upper panels) and statistical evaluation (lower panels; n = 6 hearts/group); \*P < 0.05 vs. control. (D) Masson’s trichrome staining of cardiac sections from adenovirus-infected J2N-k. (E) Fibrotic areas were analysed (n = 6 hearts/group). (F) J2N-k hamsters were administered none (−) or (+) tranilast (300 mg/kg per day) for 2 weeks, and the obtained tissues were immunostained with TRPV2 antibody. Tranilast prevented the sarcolemmal accumulation of TRPV2. Scale bar: 100 μm. (G) Heart sections from J2N-k hamsters were stained with Masson’s trichrome. Tranilast ameliorated ventricular dilation and reduced fibrosis in these hamsters. Scale bar: 5 mm. (H) Effect of tranilast (TN) (0, 30, 300 mg/kg) on echocardiographic parameters in J2N-k (n = 5–8 hamsters/group); \#P < 0.05 vs. no treatment in J2N-k.
advanced heart failure with DCM symptoms. Therefore, TRPV2 remodelling appears to be linked to sarcolemmal instability caused by DGC defects in various models of advanced heart failure. Thus, the 4C30 mouse appears to be a good animal model to study the connection between Ca\textsuperscript{2+}-abnormality and DCM symptoms.

Plasma membrane TRPV2 translocation is known to be stimulated by receptor agonists or mechanical stress. Stimulation by growth factors or sympathetic transmitters could act as a signal inducing sarcolemmal TRPV2 translocation in DCM, because these stimulants are known to be released into the blood vessels of diseased hearts in response to mechanical load. Considering that the TRPV2 inhibitory tools abrogated the surface expression of TRPV2 (Figure 6), Ca\textsuperscript{2+} influx via TRPV2 may also be important for sarcolemmal TRPV2 accumulation. Although further confirmatory studies are required, we suspect that NT domain over-expression inhibited membrane retention of TRPV2 by disrupting the interaction between TRPV2 and its putative binding partner, which regulates subcellular localization. Recently, a peptide mimetic of the CT domain of connexin 43 was used to disrupt the interaction between connexin 43 and the PDZ2 domain of zonula occludens-1 and reduce gap junction remodelling in injured hearts.

Tranilast prevented ventricular dilation and fibrosis and ameliorated decreased FS by ~50% in J2N-k hamsters (Figure 6). These beneficial effects were comparable to those obtained from the adenosine transfer of the NT domain (Figure 6). These treatments were performed in 9-week-old J2N-k hamsters that had already started displaying DCM symptoms. Therefore, our approach may be useful as a therapeutic intervention against the initial symptoms of DCM. Similar results using 9-week-old J2N-k hamsters that had already started displaying DCM of the NT domain (Figure 6) showed a decrease of FS by 50% in J2N-k hamsters (Figure 6). These beneficial effects were comparable to those obtained from the adenosine transfer of the NT domain (Figure 6).

Conflict of interest: none declared.

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Supplementary material
Supplementary material is available at Cardiovascular Research online.

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