様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Elucidation of the pathology in ARVC caused by Japanese-specific DSG2 mutations using knock-in mice models: searching for the therapeutic targets

研究課題名（英文）Elucidation of the pathology in ARVC caused by Japanese-specific DSG2 mutations using knock-in mice models: searching for the therapeutic targets

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：日本人不整脈原性右室心筋症の二つの創始者効果のある変異、DSG2 p.R292CとD494Aに相当するノックインマウスモデル、dsg2 R297CとD499Aを構築した。HomoのR297Cマウスの生存は野生型やhomoのD499Aやheteroマウスと比較しても悪かった。心エコーやMRIでは両心室の拡大や収縮低下を認めた。運動負荷試験では心室拡大や機能低下を亢進した。病理組織では、特に右心室壁において、著明な線維化を認めた。

研究成果の学術的意義や社会的意義
Phenotype of ARVC caused by variants in DSG2 are different from those in PKP2. Our knock-in mice showed similar phenotype with human ARVC, therefore, we can use our mice to develop effective treatment for Japanese ARVC.

研究成果の概要（英文）：There are two DSG2 founder variants in Japanese ARVC, p.R292C and p.D494A. In human, most patients carry the variants in homozygous or compound heterozygous manner. We constructed knock-in (KI) mice model with these variants, dsg2 R297C and D499A. The survival of homozygous R297C mice was worse than others. In the echo cardiography and cardiac MRI, we could confirm the enlargement of both ventricles and decreased contraction function. Exercise stress training except for homozygous R297C exacerbated cardiac function and enlarged the cavities in mice models. In the histological analysis, we found severe fibrosis, especially in the right ventricular wall.

研究分野：Cardiovascular disease

キーワード：ARVC　dsg2　knock-in mouse

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a devastating disease due to possibility of sudden cardiac death and gradual deterioration of cardiac performance to terminal heart failure. ARVC is gene variants-associated and so far no treatment is available. We designed current study with the purpose to generate two mice models carrying ARVC causative genetic variants in desmoglein 2 gene (Dsg2). These transgenic mice carry equivalent to human the two most frequently encountered variants in Japanese ARVC cohort DSG2 p.R292C and p.D494A. The mouse corresonding locations are Dsg2 p.R297C (RC) and p.D499A (DA). Using in vivo and in vitro tecniques we compared the simirarity between mice and ARVC patients phenotype.

２．研究の目的

The purpose of this project is to establish animal model of ARVC suitable to be used in the testing of chemical compounds during the process of discovery a curative therapy of ARVC. We also attempt to identify therapeutic targets.

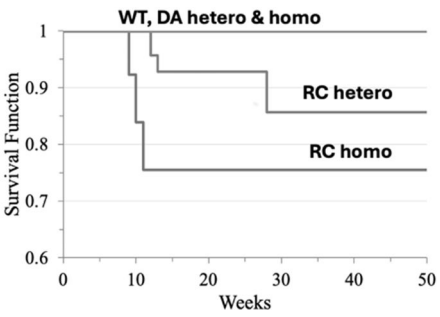
３．研究の方法

The first year of the KAKENHI we generated knock-in (KI) mice harboring Dsg2 RC an DA variants using CRISPR/Cas 9 genome editing. Genotyping by DNA isolation (mice tale piece was cut) and Sanger sequencing confirmed correct position of Dsg2 variants. We produced heterozygous and homozygous knock-in animals for both variants.

４．研究成果

Natural history and morphological change

KI mice were born and grow as wild type (WT) ones. Homozygous RC mice after 45 weeks of age had significantly increased heart weight compared to WT. RC variant produced more severe phenotype than those with DA: homozygous RC mice died suddenly from 9 weeks and older age (Right figure). Suddenly died mice had enlarged hearts with expanded cavities, and in the most cases myocardium was infiltrated with abnormal-looking areas.　Similar morphological lesions were found in sacrificed RC mice. 50 weeks observation and build Kaplan-Meier curve demonstrated lethality in RC but not in WT or DA mice.



Histological analysis

Paraffin sections of sacrificed WT and KI mice were stained with hematoxylin-eosin (HE), Masson's and Gomori trichrome, to demonstrate structure and collagen presence in the heart muscle. The heart sections from 30 weeks wild type mouse had morphological

properties as expected for healthy hearts and were negative for collagen staining. The sections from mice with heterozygous RC or DA stained with HE had morphology as those of WT, but small amount of collagen developed around the vessels and subepicardial tissue revealed by Masson's trichrome. By contrast, in the HE-stained sections of 11 weeks RC homozygous mice, violet-blue aggregations were clearly seen under microscope; these aggregations represent the typical staining of calcium deposits by hematoxylin. Masson's trichrome staining from the same section showed massive fibrosis in weakly HE-stained and calcium-containing areas. In a section from sacrificed 19 weeks mouse with homozygous RC, the segment of left ventricular wall was completely replaced by fibrotic tissue. Gomori trichrome staining of a section from 26 weeks RC homozygous mouse, showed massive fibrosis and calcium deposits. Enlargement of right ventricle and broad fibrotic areas are visible. The percentage of fibrotic areas in homozygous hearts sections were significantly increased compared to WT: 6.7 ± 2.1 and 7.3 ± 3.0% for the homozygous RC and DA, respectively. In heterozygous mice, there was small but significant increase in RC hearts (0.86±0.49%) though DA (0.16±0.16%) showed similar to WT collagen amount. In homozygous mice, the transcription was increased in both cavities. In heterozygous RC hearts, however, significant collagen I mRNA accumulation was noticed only in the right ventricle.

Cardiac function and morphological changes in *Dsg2* knock-in mice. Characteristics of cardiac function and morphology were assessed by echocardiography and cardiac MRI. Echocardiography was performed in adult mice of various ages starting from 9-week-old. Declines of left and right ventricular function were observed in all KI mice with significant variability and worsening with age. A WT mouse kept normal heart size and functions in both ventricles Left ventricular ejection fraction (LVEF) and tricuspid annular plane systolic excursion (TAPSE) in an RC heterozygous mouse at 45 weeks showed mild decrease. In addition, we could observe a highly echoic region in the left ventricle. Homozygous DA mouse showed enlarged right ventricle and decreased cardiac functions. These results were confirmed by cardiac MRI measurements of chamber volumes and functions in 16 to 19-week-old RC mice. Compared to WT, heterozygous and homozygous mice showed significantly enlarged left ventricular volumes though those of right ventricles in heterozygous ones were not significantly different. We found that these two *Dsg2* variants produced cardiac dysfunction and heart enlargement in our mouse model.

Arrhythmogenic substrate recorded by telemetry.

As shown in the disease name, ARVC is characterized by ventricular arrhythmias originated from right ventricles. We evaluated the occurrence of arrhythmias by continuous ECG recording using telemetry system. WT, hetero- and homozygous RC, four per group, were implanted with subcutaneous telemetry transmitter. Evaluation of recordings found intermittent electrical abnormalities in two homozygous RC. In a homozygous 15 weeks one, second degree atrio-ventricular (AV) block were recorded. Premature ventricular contractions and ventricular tachycardia were observed in 15 weeks and 18 weeks

homozygous ones. In other mice including two 17 weeks homozygous ones, no abnormal electrical findings were recorded.

Effect of physical exercise on the phenotype of KI mice

Physical exercise is a well-known detrimental factor in ARVC patients, therefore we assessed the effect of exercise by treadmill system for mice. Because some of the homozygous RC mice died suddenly after 9 weeks, we performed exercise stress test for heterozygous RC and hetero-and homozygous DA, most of which were healthy until 20 weeks. Eleven-week-old mice of both heterozygous and DA homozygous were trained for 8 weeks and cardiac morphology and function was followed by echography before and after the experiment. In WT, there was no change in the size of left ventricle and functions in both ventricles (Figure 6A left). In contrast, all the KI mice we trained showed enlarged left ventricles and decreased functions in left and right ventricles. These data suggested the worsening of cardiac function by exercise observed in ARVC patients.

Aspects of cellular characteristics of myocardium in KI mice.

Protein expression of desmoglein 2 is compared among WT and KI mice using immunohistochemistry and Western blot techniques. Compared to WT, the green fluorescence intensity of desmoglein 2 between cardiomyocytes have decreased in both homozygous KI mice, but not in heterozygous ones. Relative fluorescence intensity in homozygous hearts was decreased to 0.3±0.12 and 0.57±0.06 in RC and DA, respectively. The protein level in whole cardiomyocyte were evaluated by Western blotting in mice between 22 and 43 weeks of age, and we found the reduced band densities in samples from both homozygous adult KI mice but not from heterozygous ones: to 0.29±0.03 in RC and 0.49±0.012 in DA. To know whether the decrease of desmoglein 2 is present from the new-born period, we evaluated the samples from new-born DA homozygous mice, but there was clear band comparable with WT. To evaluate the apoptosis of cardiomyocytes affected by *Dsg2* variants, we performed nuclear staining by terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL). In the positive control by WT, we could observe clear red staining showing the apoptosis, but not in negative control. In samples from both hetero- and homozygous KI mice there was red staining suggesting apoptotic process related to *Dsg2* variants.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Soh Joanne Ern Chi、Shimizu Akio、Molla Md Rasel、Zankov Dimitar P.、Nguyen Le Kim Chi、Khan Mahbubur Rahman、Tesega Wondwossen Wale、Chen Si、Tojo Misa、Ito Yoshito、Sato Akira、Hitosugi Masahito、Miyagawa Shigeru、Ogita Hisakazu	４．巻 299
２．論文標題 RhoA rescues cardiac senescence by regulating Parkin-mediated mitophagy	５．発行年 2023年
３．雑誌名 Journal of Biological Chemistry	６．最初と最後の頁 102993～102993
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jbc.2023.102993	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Byambajav Tserenlkham, Koichiro Takayama, Dimitar Zankov, Minoru Horie, Seiko Ohno
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３．学会等名 Heart Rhythm Soceity Meeting 2023（国際学会）
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１．発表者名 Zankov Dimitar
２．発表標題 Human-specific desmoglein 2 mutations in mice models of arrhythmogenic right ventricular cardiomyopathy reproduce patients' phenotype
３．学会等名 Europeean Heart Rhythm Association（国際学会）
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関