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| 学位の種類 | 博士（医学） |
| 学位記番号 | 博士第９８６号 |
| 学位授与の要件 | 学位規則第４条第１項 |
| 学位授与年月日 | 令和６年３月２１日 |
| 学位論文題目 | Transmembrane protein 168 mutation reduces cardiomyocyte cell surface expression of Nav1.5 through αB-crystallin intracellular dynamics<br>（変異型 Transmembrane protein 168 はαB-クリスタリンの細胞内動態を障害して心筋細胞膜上における Nav1.5 の発現を抑制する） |
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別紙様式４（課程博士・論文博士共用）

論 文 内 容 要 旨

| ※整理番号 | 986 | (ふりがな)<br>氏　　名 | (グエンリ キム チー)<br>Nguyen Le Kim Chi |
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| 博士論文題目 | Transmembrane protein 168 mutation reduces cardiomyocyte cell surface expression of $Na_v1.5$ through αB-crystallin intracellular dynamics<br>(変異型 Transmembrane protein 168 はαB-クリスタリンの細胞内動態を障害して心筋細胞膜上における $Na_v1.5$ の発現を抑制する) | | |

＜目的＞ **(Purpose)**

Transmembrane protein 168 (TMEM168) was found to be localized on the nuclear membrane. A heterozygous mutation (c.1616G>A, p.R539Q) in TMEM168 was identified with whole exome sequencing in the Brugada syndrome (BrS) patient family. BrS is a non-structural heart disease that often provokes fatal ventricular arrhythmias, such as ventricular tachycardia/fibrillation. To investigate the mechanisms by which the TMEM168 mutation induced the cardiac phenotype, the knock-in (KI) mice with the mutation (1616G>A) were generated by the CRISPR-Cas9 genome editing technology. The KI mice exhibited ventricular tachycardia by pharmacological stimulation. The expression of $Na_v1.5$, an α subunit of the $Na^+$ channel, and inward $Na^+$ current were reduced in the cardiomyocytes isolated from the KI mice. $Na_v1.5$ is encoded by the *SCN5A* gene, and mutations of the gene are often observed in patients with BrS. The $Na_v1.5$ reduction in Tmem168 mutant-KI cardiomyocytes was mediated by the increased association of Nedd4-2 E3 ubiquitin ligase with $Na_v1.5$, and the subsequent protein degradation of $Na_v1.5$. However, the detailed molecular mechanisms by which the Tmem168 mutant increased the association between Nedd4-2 and $Na_v1.5$ remain unclear. To elucidate the mechanisms, we focused on a small heat shock protein, αB-crystallin, which can bind to $Na_v1.5$ and Nedd4-2 and interfere with the association of both proteins. The purpose of this study is to reveal how Tmem168 mutant enhanced the $Na_v1.5$–Nedd4-2 interaction by regulating the molecular association among $Na_v1.5$, Nedd4-2 and αB-crystallin, compared with wild-type (WT) Tmem168.

＜方法＞ **(Method)**

Immunohistochemistry of the frozen heart samples from Tmem168 mutant-KI and control mice was performed to examine the co-localization of αB-crystallin, Nedd4-2 and $Na_v1.5$. Co-immunoprecipitation experiments using the lysates from the mice heart was performed to examine the mode of association of αB-crystallin with Tmem168 WT or mutant. Cultured HL-1 cardiomyocytes were also used to test the association. Subcellular fractionation experiment was performed to investigate the association in more detail. αB-crystallin was knocked down in HL-1 cells to further elucidate the

（備考）１．論文内容要旨は，研究の目的・方法・結果・考察・結論の順に記載し，２千字程度でタイプ等で印字すること。

２．□印の欄には記入しないこと。

αB-crystallin-regulated expression of $Na_v1.5$ on the cell surface of cardiomyocytes. A proteasome inhibitor, MG-132, was administered in HL-1 cells for elucidation of the stability of $Na_v1.5$ expression by ubiquitin-proteasome system. The intermolecular docking analysis between the region (aa 526–543) of either Tmem168 WT or mutant and the HSP domain of αB crystallin was conducted using the Molecular Operating Environment (MOE).

＜結果＞ **(Results)**

After confirming the reduction of $Na_v1.5$ and the enhancement of $Na_v1.5$–Nedd4-2 interaction on the cell surface of cardiomyocytes in the Tmem168 mutant-KI heart, it was found that the cell surface localization of αB-crystallin was remarkably inhibited in the Tmem168 mutant-KI heart. Instead, αB-crystallin was highly accumulated in the perinuclear region where it co-localized with Tmem168 mutant. In HL-1 cells transfected with human TMEM168 mutant or WT, αB-crystallin was preferentially co-immunoprecipitated with TMEM168 mutant in the nuclear subfraction. This was confirmed in the mouse heart samples in which the immunoprecipitation of αB-crystallin with Tmem168 mutant was significantly higher than that with Tmem168 WT. The molecular docking simulation using MOE software suggest that the additional generation of α-helix by TMEM168 mutation can increase the hydrogen bonds between αB-crystallin and TMEM168 mutant. Next, αB-crystallin was knocked down in HL-1 cells, and it was confirmed that the amount of $Na_v1.5$ was decreased on the cell surface. MG-132 treatment rescued the reduction of $Na_v1.5$ expression induced by knockdown of αB-crystallin, suggesting that the Nedd4-2-mediated protein degradation is the main cause of $Na_v1.5$ expression regulation through αB-crystallin.

＜考察＞ **(Discussion)**

In this study, the applicant identified that altered intracellular localization of αB-crystallin due to TMEM168 mutation affected the balance of molecular interactions among $Na_v1.5$, Nedd4-2, and αB-crystallin. This alteration resulted in the enhancement of the association between $Na_v1.5$ and Nedd4-2, which led to increased ubiquitination and degradation of $Na_v1.5$. For clinical applications, the applicant would propose the repair of the abnormal molecular interactions by several procedures and/or recovery of $Na_v1.5$ reduction by the inhibition of Nedd4-2 function in the heart. The applicant assumes that this study may give rise to clew for potential therapy for BrS patients with the TMEM168 mutation.

＜結論＞ **(Conclusion)**

TMEM168 mutation reduces the cell surface expression of $Na_v1.5$ by increasing the interaction of TMEM168 mutant and αB-crystallin, leading to the enhancement of $Na_v1.5$–Nedd4-2 interaction. This may play a role in the pathogenesis of BrS.

別紙様式９（課程博士・論文博士共用）

博士論文審査の結果の要旨

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（博士論文審査の結果の要旨）

同研究グループは以前の研究で、*TMEM168*遺伝子の1616G>A変異によって心筋細胞における$Na_v1.5$発現が低下していることを見出しており(Shimizu et al., 2020)。本研究はその分子機序を以下の実験によって明らかにした。

1）TMEM168ノックイン(KI)心筋細胞では正常な心筋細胞に比べて、Nedd4-2と$Na_v1.5$の共局在の割合が増加している。

2）αBクリスタリンとTMEM168の共局在の割合が正常心筋細胞に比べKI心筋細胞で増加して、同時にTMEM168の変異によってαBクリスタリンの核への局在が増加する。

3）HEK293T細胞を用いた過剰発現実験によってαBクリスタリンのTMEM168への結合が変異によって増加する。

4）HL-1細胞でαBクリスタリンノックダウン(KD)により$Na_v1.5$とNedd4-2の結合と$Na_v1.5$のユビキチン化が亢進する。

5）αBクリスタリンKDによる$Na_v1.5$発現はプロテオゾーム阻害によってレスキューされる。

本論文は、ブルガタ症候群患者で発見された核に局在するTMEM168タンパク質の変異が細胞膜におけるナトリムチャネルの発現量に影響を与える分子機構を明らかにしたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士(医学)の学位論文に値するものと認められた。

（５８５文字）

（ 2023年　8月　23日）