様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　２ 年　 ６ 月 ３０ 日現在

機関番号：１６１０１
研究種目：若手研究
研究期間：2018～2019
課題番号：１８Ｋ１７２６１

研究課題名（和文）The role of ROGDI during enamel formation

研究課題名（英文）The role of ROGDI during enamel formation

研究代表者
ＭＩＴＳＵＩ　Ｓ．ＮＡＯＭＩ（MITSUI, S. Naomi）

徳島大学・大学院医歯薬学研究部（歯学域）・徳島大学専門研究員

研究者番号：７０７８６１５２

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）：Kohlschutter-Tonz 症候群は、てんかん、精神運動発達退行 、エナメル質形成不全を特徴とする常染色体劣性遺伝性疾患である。本研究では、Kohlschutter-Tonz 症候群の原因遺伝子であるRogdiをCRISPR/Casシステムを用いてマウスでRogdiを欠失させたところ、人間と同様にエナメル形成不全が認められた。Rogdiはマウスのエナメル芽細胞および長骨の成長板での発現が認められたことからエナメル質形成および骨形成に重要な役割を果たすことが示唆された。

研究成果の学術的意義や社会的意義
Due to the absence of prior publication regarding Rogdi knockout mice, the obtained results may not only help to elucidate the molecular mechanism involved in amelogenesis imperfect observed in KTS, but also serve as a basis to understand the mechanism of other symptoms present in the disease.

研究成果の概要（英文）：Kohlschutter-Tonz syndrome (KTS) is an autosomal-recessive disorder characterized by epilepsy, psychomotor regression and amelogenesis imperfecta, reported to be caused by mutation of the Rogdi gene. However the role of Rogdi during enamel development is poorly understood. To investigate the role of ROGDI in enamel formation, an animal disease model was created using CRISPR/Cas system. Rogdi deficient mice showed hypocalcified type of amelogenesis imperfecta as reported in patients with KTS. The results suggest that the phenotype is caused by defects in the maturation stage, where the polarization and ameloblast modulation were disturbed. Although bone mineralization defects were not reported in human Rogdi mutations previously, our results suggest that Rogdi plays an important role not only during amelogenesis, but also during bone formation in mice.

研究分野：歯科矯正学

キーワード：Amelogenesis imperfecta　Tooth development　Genome editing

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Enamel is the hardest and a unique mineralized ectodermal tissue that covers the teeth of vertebrates, scales and dermal bones of many fossil lobefins. Ameloblasts secrete enamel protein during the secretory stage, such as Amelogenin, Ameloblastin and Enamelin into the enamel matrix, which undergoes enzymatic modification by Enamelysin and Kallikrein 4 in the following transition and maturation stages. As the result, mature enamel is formed, composing mainly of hydroxyapatite crystallites and a minor amount of residual proteins.

Amelogenesis is highly regulated at the molecular level, involving thousand of genes and their products interacting in developmental and regulatory pathways. Perturbations in some of these sensitive processes may lead to enamel defects. Amelogenesis imperfecta is a genetic disorder characterized by abnormal enamel formation, varying in phenotype and inheritance pattern, observed either as isolated trait or in association with other symptoms.

Kohlschütter-Tönz syndrome (KTS) was first described as a genetic disorder in 1974. It is an autosomal-recessive disorder characterized by global developmental delay, epilepsy, amelogenesis imperfecta and psychomotor regression. The neurological symptoms associated with KTS usually occur within the first two years of life. Epileptic seizures are often resistant to pharmacological treatment. Amelogenesis imperfect is the most striking feature of KTS. It is used as a clinical marker, which is frequently observed as yellowed teeth and abnormal enamel. Although the molecular mechanism leading to KTS has not yet been elucidated, recent studies have suggested that is caused by loss-of-function mutations in the ROGDI gene.

ROGDI gene is known to play an important role in tumorigenesis and the cell cycle. Recently, it has been demonstrated that *Drosophila* homolog of *Rogdi* acts as a novel sleep-promoting factor by supporting a specific subset of gamma-aminobutyric acid transmission. In addition, Rogdi has been reported as a presynaptic protein, in which its dysfunction may arise the neurological symptoms associated with KTS.

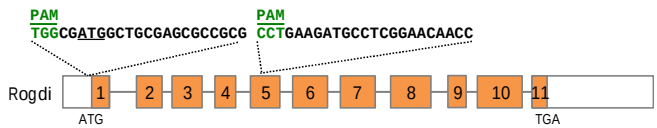
２．研究の目的

ROGDI is highly conserved and has orthologs in many species, suggesting an important functional role. Although it has been reported that ROGDI is highly expressed in adult brain, spinal chord, peripheral blood, and bone marrow, little is known about its role during tooth development. The purpose of the present study is to clarify the role of ROGDI during enamel formation by using Rodgi knockout mice.

３．研究の方法

(1) Rogdi gene targeting using CRISPR/Cas system

Start codon and exon five of Rogdi were targeted using CRISPR/Cas system in mice.



(2) Phenotypic analysis of Rogdi deficient mice

① Micro-CT and Scanning electron microcopy images of adult wild-type and Rogdi deficient mice were analysed to quantify mineral density and to verify the organization of enamel prism.

② Calcein staining of incisors and alcian blue - alizarin red staining of calvarial bones were performed.

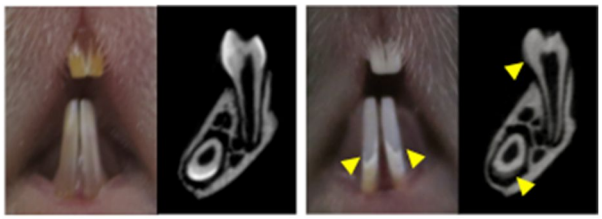
(3) Expression pattern of Rogdi during development

ROGDI expression was analysed by immunohistochemistry.

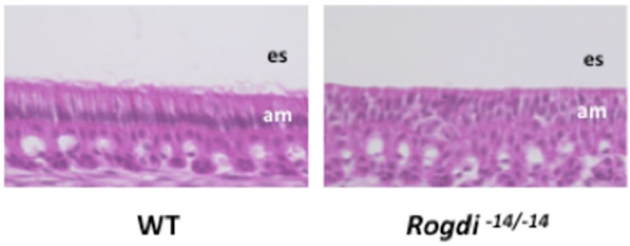
4．研究成果

Start codon and exon five of Rogdi was successfully targeted using CRISPR/Cas system, obtaining alleles with frameshift mutations.

Rogdi deficient mice showed enamel defects similar to patients with KTS carrying Rogdi autosomal-recessive mutations. Mice in the homozygous condition carrying mutant alleles were smaller in size and showed chalky white enamel with partial loss of enamel in the occlusal surface. Seizures were observed sporadically in some Rogdi deficient mice



Although enamel thickness was not altered, enamel mineral density was significantly decreased in Rogdi deficient mice compared with wild-type mice. Moreover, ameloblast polarization at maturation stage and smooth-ended and ruffle-ended ameloblast modulation were disturbed in Rogdi deficient mice. Although bone defects were not previously observed in humans, Rogdi deficient mice showed mineralization defects in the calvaria and long bones.



Ameloblast expression of ROGDI was increased gradually from secretory to maturation stage. ROGDI expression was also found in growth plate of distal femur and proximal tibia.

All these results taken together, indicate that Rogdi deficiency cause hypocalcified type of amelogenesis imperfecta as a consequence of disruption in ameloblasts modulation during enamel maturation. Although several human Rogdi mutations have been reported previously, none of them describe specific bone defect. Our results suggest that Rogdi plays an important role during bone formation in mice.

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 Ichihara A, Yasue A, Mitsui SN, Arai D, Minegishi, Oyadomari, Imoto I, Tanaka E	４．巻 -
２．論文標題 The C-terminal region including the MH6 domain of Msx1 regulates skeletal development	５．発行年 2020年
３．雑誌名 Biochemical and Biophysical Research Communications	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bbrc.2020.03.068	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13件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Kunida T, Matsuki Y, Yamamoto t, Takekawa K, Saitoh S, Mitsui N, Iwasa A, Tanaka E
２．発表標題 矯正歯科における歯科衛生士による口腔筋機能療法に関する臨床統計学的検討
３．学会等名 徳島県歯科医学大会
４．発表年 2020年

１．発表者名 Mitsui SN, Yasue A, Horiuchi S, Oyadomari S, Tanaka E
２．発表標題 Rogue plays an important role during bone and enamel development
３．学会等名 第78回日本矯正歯科学会学術大会
４．発表年 2019年

１．発表者名 Ichihara A, Yasue A, Arai D, Sawada M, Mitsui N, Oyadomari S, Tanaka E
２．発表標題 多数歯欠損症の原因遺伝子Msx1のC末端領域は骨形成において重要である
３．学会等名 第78回日本矯正歯科学会学術大会
４．発表年 2019年

１．発表者名
Arai D, Yasue A, Mitsui N, Oyadomari S, Tanaka E

２．発表標題
歯の形態形成におけるMsx1遺伝子MH6ドメインの機能検証

３．学会等名
第78回日本矯正歯科学会学術大会

４．発表年
2019年

１．発表者名
Ichihara A, Yasue A, Mitsui SN, Arai D, Sawada M, Oyadomari S, Tanaka E

２．発表標題
骨形成に関与するMsx1遺伝子C末端領域の機能解析

３．学会等名
第42回日本分子生物学会年会

４．発表年
2019年

１．発表者名
Arai D, Yasue A, Mitsui SN, Ichihara A, Sawada M, Oyadomari S, Tanaka E

２．発表標題
Functional verification of C-terminal domain of Msx1 gene in mice for craniofacial development

３．学会等名
第42回日本分子生物学会年会

４．発表年
2019年

１．発表者名
Arai D, Yasue A, Mitsui N, Oyadomari S, Tanaka E

２．発表標題
In vivo functional analysis of C-terminal domain of Msx1 genein mice for Craniofacial Development

３．学会等名
European Developmental Biology Congress 2019（国際学会）

４．発表年
2019年

１．発表者名
Mitsui SN, Yasue A, Horiuchi S, Tanaka E

２．発表標題
The role of Rogdi during bone and enamel development

３．学会等名
第61回歯科基礎医学会学術大会

４．発表年
2019年

１．発表者名
Mitsui SN, Yasue A, Horiuchi S, Oyadomari S, Tanaka E

２．発表標題
Rogdi plays an important role during enamel mineralization

３．学会等名
第59回日本先天異常学会学術集会-13th World Congress of The International Cleft Lip and Palate Foundation CLEFT2019【合同開催】（国際学会）

４．発表年
2019年

１．発表者名
Sawada M, Iwasa A, Yoshimura M, Ichihara A, Mitsui N, Shioyasono R, Hiasa M, Yasue A,Tanaka E

２．発表標題
シルエットを用いた側貌に対する審美的評価

３．学会等名
第62回中・四国矯正歯科学会大会

４．発表年
2019年

１．発表者名
Ichihara A, Yasue A, Mitsui N, Watanabe Y, Tanaka E

２．発表標題
Mutation detection of PAX9 gene in a patient with oligodontia

３．学会等名
第77回日本矯正歯科学会学術大会

４．発表年
2018年

１．発表者名
Takekawa K, Iwasa A, Mitsui N, Kunida T, Matsuki Y, Yamamoto T, Saito S, Kawai N, Tanaka E

２．発表標題
A survey of oral myofunctional therapy to orthodontic patients by dental hygienists

３．学会等名
第61回中・四国矯正歯科学会学術大会

４．発表年
2018年

１．発表者名
Watanabe M, Kawai N, Shirai M, Hichijo N, Mitsui N, Yasue A, Tanaka E

２．発表標題
外科的矯正治療と矯正単独治療を行った骨格性下顎前突症患者の顎口腔機能評価，

３．学会等名
第61回中・四国矯正歯科学会学術大会

４．発表年
2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考