学位論文の内容の要旨

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<td>Oculofaciocardiodental syndrome: novel BCOR mutations and expression in dental cells (眼・顔面・心臓・歯症候群:新規 BCOR 遗伝子変異の同定とヒト歯由来細胞における発現)</td>
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Abstract

Oculofaciocardiodental (OFCD) syndrome is a rare X-linked dominant condition. Mutations in *BCL-6-intercting corepressor (BCOR)* have been described as causal in OFCD syndrome. Almost all *BCOR* mutations result in premature termination codons (PTCs); therefore, nonsense-mediated mRNA decay (NMD) might have an important role in pathogenesis. The purpose of this study was to identify *BCOR* mutations in two OFCD patients, if it present, and to clarify the pathogenesis of radiculomegaly using one OFCD patient’s pulp and periodontal ligament (PDL) cells. In our genetic analysis, two novel *BCOR* mutations were found. We also examined the transcript levels and the effects of NMD using cultured pulp and PDL cells from one affected patient. *BCOR* expression was normal in pulp but reduced in PDL cells, which is consistent with the higher rates of NMD in PDL cells. The mutant PDL cells had unstable mutant transcripts and proliferated faster than did wild-type cells, but mutant pulp cells appeared normal by these measures. In summary, the nonsense and frameshift mutations, which introduce PTCs, were found to contribute to OFCD syndrome in our two patients. Furthermore, in PDL cells, the mutation resulting in a PTC corresponded to greater NMD, unstable mutant transcripts and increased cell proliferation, which may contribute to hyperactive root formation.

Introduction

Oculofaciocardiodental (OFCD, MIM 300166) syndrome is a rare, X-linked dominant hereditary trait with skewed X inactivation in heterozygous females. Males with this syndrome cannot survive because of embryonic lethality. OFCD syndrome is characterized by microphthalmia, congenital cataracts, facial dysmorphic features, congenital heart defects and dental anomalies. Most affected OFCD patients have remarkable dental anomalies, including radiculomegaly with prolonged dental roots and widely open apices, most often in the canine roots. Recently, mutations in the *BCOR* gene, which encodes the BCL6 corepressor, have been found to cause OFCD syndrome.

*BCOR* has multiple roles in the complex process of human development. *BCOR* expression has
been detected in both the dental epithelium and mesenchyme during tooth development in early embryogenesis, and BCOR expressed in the mesenchyme has an important role in proper tooth formation. Moreover, mutant BCOR increased the osteo-dentinogenic potential of mesenchymal stem cells (MSCs) by inducing AP-2a, and it increased the cell proliferation rate. However, the mechanism of hyperactive root formation in OFCD syndrome is still unclear.

Almost all of the mutations identified in individuals with OFCD syndrome result in predicted premature termination codons (PTCs) and have similar phenotypic consequences. The PTC-carrying mRNA will be degraded by a protective mechanism in the cell called nonsense-mediated mRNA decay (NMD) to shield cells from dominant-negative activities of mutant proteins. The efficiency of NMD is an inherent characteristic and varies among different cells. Here, for the first time, we report alterations in the transcript levels of BCOR due to NMD in cultured pulp and periodontal ligament (PDL) cells from one affected patient.

**Materials and Methods**

1. Subjects and mutation analysis

Two female OFCD patients (aged 27 years and 18 years) and three healthy subjects were patients of the Department of Orthodontics, Dental Hospital, Tokyo Medical and Dental University, Japan. The healthy patient 1’s parents and the healthy patient 2’s mother were also included in the genetic analysis. Genomic DNA was extracted from buccal swabs. Coding exons and flanking introns of the BCOR gene were amplified by conventional PCR and directly sequenced. The mutations were evaluated for their disease-causing potential based on sequence alterations by the ‘Mutation Taster’ and confirmed with the Japanese Single Nucleotide Polymorphisms Database.

2. Analysis of BCOR expression and NMD

The PDL surrounding the apical 1/3 of the root and apical pulp tissues were harvested from the extracted first premolars of patient 2 and three healthy subjects. Transcript levels of vimentin (VIM) and keratin 14 (KRT14) were measured. BCOR mRNA expression was analyzed by real-time PCR. The levels of mutant transcripts following treatment with cycloheximide (CHX), a general inhibitor of translation elongation that also indirectly inhibited NMD, were evaluated.

3. Analysis of RNA stability and cell proliferation rate

Total RNA was isolated from the cells at 0, 3 and 6 h after adding actinomycin D, and BCOR expression was evaluated using real-time PCR. To monitor cell proliferation, MTT assays were performed.

**Results**

1. Clinical findings

The two patients were diagnosed with OFCD syndrome based on a distinct pattern of eye,
craniofacial, heart and dental anomalies. According to their family pedigrees, their parents and siblings are healthy, so both patients had sporadic mutations. The record of an oral examination of patient 1 (first visit: 16Y6M) showed nine missing and six impacted teeth, persistent primary teeth, delayed secondary dentition, radiculomegaly, malformed teeth and cleft palate. Patient 2 (first visit: 10Y9M) had similar but less severe oral phenotypes. She had only two missing teeth, one impacted tooth and submucous cleft palate.

2. Mutation analysis

The nonsense mutation, c.*4794G4A (p.W1598*), in patient 1 and the frameshift mutation, c.3668delC (p.S1223Wfs*15), in patient 2 were found to contribute to OFCD syndrome. Disease-causing potential analyzed by Mutation Taster showed that the nonsense and frameshift mutations are predicted to cause disease, and no single nucleotide polymorphisms in the altered region were found.

3. BCOR expression and analysis of NMD

The BCOR mutations did not affect the expression of VIM and KRT14 in our cultured cells. The cultured PDL cells also showed higher BCOR expression than pulp cells, whether wild-type or mutant. Compared with wild-type, BCOR expression was normal in pulp but reduced in PDL cells, which was consistent with our finding of higher rates of NMD in PDL.

4. Analysis of RNA stability and cell proliferation rate

In PDL but not pulp cell cultures, the mutant PDL had unstable mutant transcripts and proliferated faster than wild-type cells.

Discussion

To date, a total of 34 mutations in BCOR have been found, most of which result in premature termination of the protein with deletion of the carboxy-terminal domain; therefore, NMD might have an important role in pathogenesis. Interestingly, the efficiency and sensitivity of the NMD mechanism differ by cell type. The effect of NMD on a disease phenotype depends on both the affected gene and the location of the disease-causing PTC. NMD can also contribute to a disease phenotype when it inhibits the expression of partially functional proteins. In the case of BCOR mutations, all of the affected patients have similar phenotypes, irrespective of the location of the mutation, although the phenotypes differ in severity. This observation suggests that the pathogenesis might be haploinsufficiency rather than a dominant negative effect of mutant proteins. It was reported that the truncated OFCD protein in affected patients showed repressor effects equivalent to those of wild-type BCOR. Therefore, we hypothesized that the mutant BCOR proteins would have partially functional effects and that the abnormal phenotypes observed in the affected tissues were the result of NMD of mutant RNAs, which would result in haploinsufficiency.
Different tissues have different NMD efficiencies. This situation could result in a selective effect in which only NMD-sensitive tissues become abnormal, which is consistent with the typical characteristics of OFCD syndrome. We focused our study on the tooth root, which becomes hyperactive and much longer than normal in OFCD-affected patients. During tooth root development, all functional hard tissues are formed by three types of cells: Hertwig’s epithelial root sheath, dental papilla mesenchymal and dental follicle cells, which form developing apical complexes. Thus, we used the PDL cells surrounding the apical 1/3 and apical pulp cells, which are assumed to comprise all developing apical complexes, for studying hyperactive root formation in OFCD patients. We found that BCOR mutations did not affect the expression of the mesenchymal and epithelial cell markers VIM and KRT14, respectively, in our cultured cells. Both markers were more highly expressed in cultured PDL cells than in pulp culture. Moreover, cultured PDL cells also showed higher BCOR expression than pulp cells, whether wild-type or mutant. It was previously reported that BCOR expressed in the mesenchyme has an important role in proper tooth formation. Therefore, our results suggested that BCOR is highly expressed in the mesenchyme and that our cultured PDL expressed BCOR at higher levels than pulp cells because of the predominance of mesenchymal cells in the PDL cultures. Compared with wild-type, BCOR expression was normal in pulp but reduced in PDL cells, which was consistent with our finding of higher rates of NMD in PDL. Moreover, in PDL but not pulp cell cultures, the mutant PDL had unstable mutant transcripts and proliferated faster than wild-type cells. This evidence suggests that the mutant PDL cells have insufficient BCOR function to repress target genes, resulting in the promotion of cell proliferation that contributes to hyperactive root formation.

Conclusion

The nonsense mutation in patient 1 and the frameshift mutation in patient 2 were found to contribute to OFCD syndrome. A variation in phenotype severity was observed, and thus, further studies of factors such as X-chromosome inactivation or epigenetic modifications are needed to clarify the genotype–phenotype relationship. In patient 2, the mutation leading to the PTC-induced NMD mechanism in PDL cells caused unstable mutant transcripts and increased cell proliferation, which may be involved in hyperactive root formation.
和文による要約

Oculofaciocardiodental (OFCD) syndrome は X 連鎖性優性遺伝形式の女性のみに発症するまれな疾患で、特異的顔貌、心臓および眼の異常、ならびに長い歯根といった特徴を認める。本研究では、東京医科歯科大学歯学部付属病院矯正歯科外来を受診した OFCD 患者 2 例において、原因遺伝子である BCOR (BCL-6-interacting corepressor) の変異の同定ならびに、うち 1 例から得られた歯髄・歯根膜細胞を用い、歯根長に異常を生じる原因を明らかにすることを目的とした。結果として、症例 1 および症例 2 に BCOR の新規変異; ナンセンス変異 (c.4794G > A, p.W1598*)、フレームシフト変異 (c.3668delC, p.S1223Wfs*14) をそれぞれ同定した。また、症例 2 より歯科矯正治療のため便宜抜歯された下顎第一小臼歯から得られた培養歯髄・歯根膜細胞において、BCOR の mRNA の発現量を正常コントロールと比較したところ、歯根膜細胞において減少を認めた。さらにシクロヘキシンミドを添加し、ナンセンス変異依存 mRNA 分解機構 (NMD) の関与の可能性について検討したところ、患者由来の培養歯根膜細胞において、BCOR の発現量が増加した。また、患者由来の培養歯根膜細胞では、mRNA の不安定性ならびに細胞増殖能の増加を認めた。以上より、BCOR の変異により培養歯根膜細胞において認められた NMD ならびに BCOR mRNA の不安定性、細胞増殖能の増加が歯根長に異常を生じた原因となっている可能性が示唆された。
歯科矯正治療や歯の再植・移植といった治療や炎症により、歯根吸収が惹起され、動揺や脱落といった転帰をたどるケースは少なくない。歯根形成のメカニズムを解明することは一端吸収が進んだ歯根の再生や歯根吸収の抑制といった治療法の開発に向けて期待されるテーマである。

Oculofaciocardiodental (OFCD) 症候群は、特異的顔貌、心臓および眼の異常、ならびに長い歯根といった特徴を呈する X 連鎖性優性遺伝形式の女性のみに発症するまれな遺伝性疾患である。近年、原因遺伝子として BCL-6-interacting corepressor (BCOR) が同定されたが、歯根形成における BCOR の機能は明らかにされていない。

現在まで報告されている OFCD 症候群に認められる BCOR の変異は、すべてナンセンス変異ならびにフレームシフト変異である。それらの変異により、本来の終止コドンよりも 5' 上流に終止コドンが形成される場合、異常タンパクの発現を抑制するために、ナンセンス変異依存 mRNA 分解機構 (NMD) が働くことが知られている。また、NMD が遺伝子変異に由来する形質発現に大きく影響することも知られており、OFCD 症候群における歯根形態異常に関しても NMD が関与している可能性が考えられる。

そこで、本研究では東京医科歯科大学歯学部付属病院矯正歯科外来を受診した OFCD 症候群患者 2 例において、詳細な臨床症状を報告するとともに、BCOR の変異解析ならびに、うち 1 例から得られた歯髄・歯根膜細胞を用い、歯根長に異常を生じる原因を明らかにするこ
とを目的とした。

方法に関しては、まず OFCD 症候群患者 2 例からゲノム DNA を抽出し、BCOR 遺伝子の全翻訳領域とエクソン/イントロン境界の塩基配列を決定した。また、症例 2 より歯科矯正治療のため便宜抜歯された下顎第一小臼歯から得られた培養歯髄・歯根膜細胞における BCOR の mRNA の発現量を、3 名の歯根長に異常を来さない患者の小臼歯から得られた培養歯髄・歯根膜細胞（正常コントロール）と比較した。またシクロヘキシンを添加し、NMD の関与の可能性について検討した。さらに、患者由来の培養歯髄・歯根膜細胞の mRNA の安定性ならびに細胞増殖能を正常コントロールと比較検討した。

研究結果として、以下の知見を得ている。

1. 症例 1、2 とともに特異的顔貌、眼の異常といった典型的な臨床症状を呈していたが、心臓の異常は症例 1 のみに認められた。また口腔内所見として、2 症例ともに主症状である長い歯根の他、先天性欠如歯、埋伏歯、口蓋裂を認めたが、症例 1 の方がより重篤な
表現型を示す傾向が認められた。


3. 正常コントロールならびに患者由来の培養細胞において、ともに培養歯根膜細胞の方が培養歯髄細胞と比較して、BCOR の mRNA の発現を強く認めた。

4. 患者由来の培養歯髄・歯根膜細胞において、BCOR の mRNA の発現量を正常コントロールと比較したところ、培養歯根膜細胞においては減少を認めた。

5. シクロヘキシミドを添加し、NMD の関与の可能性について検討したところ、患者由来の培養歯根膜細胞において、BCOR mRNA の発現量が増加した。また、シクロヘキシミド添加前後でヘテロ接合性に認められる BCOR の変異に変化は認められなかった。

6. 患者由来の培養歯髄細胞において正常コントロールと比較して、mRNA の安定性ならびに細胞増殖能に有意な差を認めなかったが、患者由来の培養歯根膜細胞においては mRNA の不安定性ならびに細胞増殖能の増加を認めた。

本研究により、東京医科歯科大学歯学部付属病院矯正歯科外来を受診した OFCD 患者 2 例において、BCOR の新規変異を同定し、さらに BCOR の変異により培養歯根膜細胞において認められた NMD ならびに BCOR mRNA の不安定性、細胞増殖能の増加が歯根長に異常を生じた原因となっている可能性が示唆された。

以上より、本研究の着眼点とその成果は高く評価され、基礎および臨床歯学の発展に大いに寄与することが期待される。したがって、本論文は博士（歯学）の学位を申請するに十分値するものと認められた。