Epigenetics and dental enamel development: a scoping review

Epigenética e desenvolvimento do esmalte dentário: uma revisão de escopo

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ABSTRACT
Introduction: The aim of this study was to perform a scoping review to systematically map the research done in the role of epigenetic factors on enamel development. Methods: Articles that reported relevant data among the role of epigenetics on enamel development/formation or enamel defects were selected. The data from the included studies were compiled and organized. The search strategy retrieved a total of 263 titles and abstract. Upon exclusion, only 53 articles were eligible for full article assessment. Results: After the selection process, only 12 articles were included in the scoping review. The first studies were published in 2010, while the most recent studies were published in 2021. The studies have different aims and designs and none of them evaluated human population. Conclusion: Future studies in this subject are recommended to unravel the role of epigenetic factors in normal and abnormal enamel development as well as in the development enamel defect.

Keywords: Dental Enamel, Epigenetic, microRNA, Tooth Development

1 INTRODUCTION
The dental enamel formation, also called amelogenesis, involves different phases\textsuperscript{1,2} regulated by ameloblasts that express an important set of genes that encode the production of proteins for enamel formation.\textsuperscript{3} During the secretion phase, ameloblasts synthesize and secrete enamel matrix proteins, such as amelogenin, ameloblastin and enamelin, and enzymes, like the enamelinisin, also called MMP-20.\textsuperscript{1,3} In the maturation phase, the proteins amelotin and apine play an important role in the final development of enamel hardness.\textsuperscript{3} Alterations or deficiencies in one or more of these proteins and / or enzymes can lead to enamel malformation in different severity phenotypes\textsuperscript{1,3,4} These malformations during amelogenesis lead to what is known clinically as developmental
enamel defects and is commonly observed in the clinical practice. Several factors have been implicated in the etiology of DED, including local, systemic, environmental, genetic and epigenetic factors.\textsuperscript{5,6}

The term epigenetics was first cited by Conrad Waddington, in 1942, in an embryonic development study, which inferred that the course of development is determined by the interaction of genes with the environment. Since then, different researchers have discussed perspectives for the term.\textsuperscript{7-9} In 2014, Williams et al.\textsuperscript{10} defined epigenetics as a group of dynamic molecular mechanisms acquired or inherited and potentially transgenerational, which are affected by the environment and act directly on the genome to regulate gene expression. Greally (2018)\textsuperscript{9} inferred the term ‘epigenetics’ is currently ambiguous and over-encompassing. The set of epigenetic changes comprises the so-called epigenome, which is characterized for each cell type. Epigenetic marks provide differentiation mechanisms by regulating the accessibility of genetic information to cells, in addition to silencing repetitive elements, inhibiting their replication and transposition within the genome.\textsuperscript{11} There are three main mechanisms of epigenetic regulation: DNA methylation, post-translational histone modification, and non-coding RNA. Epigenetic factors might play a role in the enamel development as suggested in some previous articles.\textsuperscript{12-18} However, the role of epigenetic factors in amelogenesis are remains unclear (Dos Santos et al., 2016).\textsuperscript{6}

Scoping studies are an emerging approach to reviewing the literature which to date has received little attention in the research literature.\textsuperscript{18,19} Although in the past decades have been performed in different areas to explore the role of epigenetic factors in developmental alterations, in amelogenesis research this subject was poorly explored.\textsuperscript{8,10,11,20} Therefore, the aim of this study is to perform a scoping review to systematically map the research done in the role of epigenetic factors on enamel development, as well as to identify any existing gaps in knowledge. The following question was formulated based on framework Population, Concept, Context (PCC): “What is known from the literature about epigenetic and developmental enamel defects?

2 METHODS

2.1 PROTOCOL AND REGISTRATION

The authors drafted a protocol using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P).\textsuperscript{21} It was registered prospectively with the Open Science Framework on 22\textsuperscript{th} April 2021 (https://www. https://osf.io/ftx9a/.
We followed the steps according to JBI manual for evidence syntheses for scoping review. After finishing the scoping review the paper was described following PRISMA-ScR.

2.2 ELIGIBILITY CRITERIA

We selected articles that reported relevant data among the role of epigenetics on enamel development/formation or enamel defects considering the framework PCC: Population – humans, animals, in vitro; Concept: developmental enamel defects Context – epigenetic disorders.

Papers about revision case reports paper or if they did not fit into the conceptual framework of the study were excluded.

2.3 INFORMATION SOURCES

A broad literature search was performed until June 30th, 2021, in the following databases: MEDLINE (PubMed), Web of Science, Scopus and Lilacs.

In addition, to ensure a comprehensive literature search the gray literature was assessed. Hand searching was also conducted to identify studies that could be missed by the primary electronic search.

2.4 SEARCH

MeSH (Medical Subject Headings) terms (https://www.ncbi.nlm.nih.gov/pubmed), Health Sciences Descriptors terms (http://decs.bvs.br), related terms and free terms were included. The Boolean operators “AND” and “OR” were applied to combine the keywords “epigenetics” OR “DNA methylation” OR “histone modification” OR “non-coding RNA” OR “ncRNA” OR “long non-coding RNA” OR “lncRNA” AND “enamel” OR “amelogenesis”. The final search results were exported into EndNote, and duplicates were removed.

2.5 SELECTION PROCESS

Two independent reviewers (CPT and LAP) performed the selection of the articles. To increase consistency among reviewers, two reviewers screened 20 publications, applying the eligibility criteria. Concordance between authors were obtained.
(kappa statistic of 0.8). The articles that generated disagreement among the reviewers were discussed and reviewed by a third author (EKC), reaching an agreement.

2.6 SOURCES OF EVIDENCE, DATA CHARTING PROCESS, DATA ITEMS

Before beginning screening for this review a data-charting form was jointly developed by two reviewers (CPT and EKC) to determine which variables to extract. The three reviewers (CPT, JGS and LAP) independently charted the data, discussed the results, and continuously updated the data-charting form in an iterative process. Disagreements on study selection and data extraction by consensus and discussion with other reviewers (EKC and ESR) if needed.

2.7 DATA ITEMS AND SYNTHESES OF THE RESULTS

The data from the included studies were compiled and organized according to the studies objectives, method design, explored outcomes and important results reported by the authors.

3 RESULTS

Our initial search strategy retrieved a total of 263 titles and abstract. Upon exclusion, only 53 articles were eligible for full article assessment. After the selection process, only 12 articles were qualified for final analysis. PRISMA flow diagram (Fig. 1) summarizes the study selection process.
The characteristics of the included articles are presented in the table 1. The first studies were published in 2010\textsuperscript{24,25} and the most recent studies were published in 2021\textsuperscript{26}. The studies have different aims and designs.
Table 1. Characteristics of the included articles

<table>
<thead>
<tr>
<th>Author(s) and Year</th>
<th>Title</th>
<th>Objectives</th>
<th>Study design and Outcome measurements</th>
<th>Important results</th>
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<tbody>
<tr>
<td>Li et al. (2021).26</td>
<td>Bisphenol A (BPA) exposure disrupted Enamel Formation via EZH2-Mediated H3K27me3</td>
<td>To identify the effects of BPA exposure on the enamel formation, the histone modification and its regulators in dental epithelial stem cells.</td>
<td>The morphological structure of the mouse incisor was analyzed searching for morphological and molecular malformations. Dental stem cells were isolated and cultured, and from that culture some molecular interactions were tested. Such as immunofluorescence staining, RNA-seq and PCR, Western blotting, ChIP-seq and ChIP-qPCR.</td>
<td>- BPA exposure leads to enamel defects in mouse incisors. - BPA exposure promotes dental epithelial progenitor proliferation in vitro and in vivo. - BPA exposure drives upregulation of repressive mark H3K27me3 in dental epithelial progenitors. - EZH2 is required for the effect of BPA in dental epithelial progenitors. - EZH2 inhibitor GSK126 partially rescues BPA - Induced enamel defect.</td>
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<td>Raduljovic et al. (2020).27</td>
<td>Epigenetic drug 5-azacytidine impairs the potential for odontogenesis but improves tooth morphogenesis in the transplanted embryonic mandible</td>
<td>To investigate if 5-azacytidine affects odontogenesis in embryonic mandible ectopically transplanted in vivo.</td>
<td>Mandibles at 15 and 14 days old from rat embryos were transplanted under the kidney capsule of adult males. The horts were treated with the epigenetic drug 5-azacytidine for three days after the procedure and after two weeks the differentiation was analyzed by histology and immunohistochemistry.</td>
<td>- Embryonic mandibles still developed teeth at the ectopic site, but odontogenesis was more advanced in day-old mandibles. - In these mandibles the epigenetic drug 5-azacytidine impaired potential for odontogenesis, but the teeth that developed reached a higher stage of organogenesis.</td>
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<td>Neupane et al. (2020).27</td>
<td>Signaling Modulations of miR-206-3p in Tooth Morphogenesis</td>
<td>To examine the expression pattern of miRNA206-3p in tooth morphogenesis using ex-vivo culture methods.</td>
<td>In order to identify the miRNA, in situ hybridization was used. The embryologic nodular tissue were dissected from 13 day old mice embryos and then cultured and transplanted to the renal cavity of male adult mice, which were submitted to immunohistochemical tests. The expression pattern were measured by quantitative real time PCR. A TUNEL assay identified apoptosis regions.</td>
<td>- Changing the expression of miRNA206-3p altered the expression patterns of dental related signaling molecules. - There were changes in cellular events like apoptosis and proliferation that resulted in altered crowns and pulp morphogenesis in renal-capsule-calcified teeth. - Authors suggested that miRNA206-3p regulates cellular physiology in tooth morphogenesis through the Wnt, Bmp, Fgf, and Shh signaling pathways.</td>
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<tr>
<td>Kamiunten, et al.</td>
<td>Essential roles of G9a in cell proliferation and development in mice</td>
<td>To observe tooth development in mice in which the G9a enzyme was knocked down</td>
<td>Knocking out the gene that translates G9a mimics a scenario where the methylation would not occur, therefore it is possible to</td>
<td>- Smaller tooth germs and cervical loops were observed in knock out G9a mice. - The cell proliferation was significantly</td>
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<th>Source</th>
<th>Description</th>
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<tr>
<td>Le et al. (2016)</td>
<td>Annelogenin exon4 forms a novel miRNA that directs ameloblast and odontoblast differentiation. To confirm if in the occurrence of alternative splicing of Annelogenin Exon4 a new miRNA would be formed. The experiment consisted in the analyzes of mice teeth. RNA and miRNA extractions were performed. Also a different lineage of cells was cultured at a minimus and then had the RNA and miRNA extracted as well. The expression was characterized by quantitative PCR. A potential role of miRNA-exon4 in differentiation of ameloblasts and odontoblasts as well as other cells regulated by RUNX2 was observed. mRNA-exon4 was produced from the annelogenin gene. 80% of Exon4 was spliced and available for mRNA processing. mRNA-exon4 was present in bone developing enamel organs. RUNX2 expression was upregulated by mRNA-exon4 mimic in L88 cells. mRNA-exon4 was significantly downregulated in Ansl KO mice. Reduction of mRNA-exon4 resulted in downregulation of RUNX2 in amel knock out mice.</td>
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<tr>
<td>Yoshihara et al. (2015)</td>
<td>The dynamics of DNA methylation and hydroxymethylation during amelogenesis. To associate the spatial distribution of 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) to epigenetic events in mice teeth. Mice mandibular incisors were used. The tissue was submitted to immunohistochemical tests and then observed in fluorescence microscope. The genome wide levels of 5-mC and 5-hmC in early and late stage development was confirmed by Dot blotting. The mRNA levels were obtained by quantitative PCR. The incisors of positive 5mC mice presented a different spatial distribution. The genome wide did not have significant differences, but when paired with the data from the immunohistochemical tests, they suggest that the hydroxilation of 5mC in the epigenome changes throughout the amelogenesis. The levels of DNMT1 were higher in the early stages of development.</td>
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<tr>
<td>Yin et al. (2014)</td>
<td>Genome-wide analysis of miRNA and mRNA transcriptomes during amelogenesis. Identify miRNA and its corresponding target genes that can potentially change in the transition process from secretory to maturation stages of the ameloblasts during amelogenesis. mRNAs and mRNAs of rat enamel organs from secretory and maturation stages of the ameloblasts during amelogenesis were submitted to a genome-wide transcriptional profiling. 39 mRNAs were differentially expressed in the maturation stage in comparison with the secretory stage. 1729 mRNA transcript were expressed differentially in the maturation stage. 5.8% (629 total) of those genes were considered as potential targets for 39 miRNAs. Those same 629 genes were enriched.</td>
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The data indicates that miRNAs present a dynamic pattern during the transition from secretory to maturation states of ameloblasts.

- The overexpression led to a repression of Bmp4-1a and Bmp4-1b, both targets of miRNA135a.
- The correlation between the microRNA and BMP was confirmed.

When microRNA135a was ectopically expressed there was no tooth formation.

Transgenic mice miRNA200c knock out were analyzed by microCT and histology searching for enamel defects.

- miRNA200c represses nogggin in dental epithelial-like cells.
- Endogenous Pitx2 binds to the miRNA200c 5'-flanking chromatin and activates miRNA200c expression.
- miRNA200c and BMP signaling form a regulatory positive-feedback loop.

Pitx2 is highly expressed in the incisor cervical loop stem cell.

- miRNA200c expression increases cell adhesion in L-S-S dental epithelial-like cells.
- miR-200c/141 knockout mice have defects in enamel and in cell adhesion.

The authors identified by microarray technology, over fifty miRNAs differentially expressed in cervical loops of mouse incisor, and more differentially expressed transcripts.

- The miRNAs were differentially expressed in cervical loops.
- miRNAs have a role in the renewal and differentiation in adult stem cells during incisor growth.

There was a differential expression of miRNAs in the different regions. The late stage showed more expression of...
The dental enamel formation occurs within a series of precisely regulated and timed molecular, biochemical and cellular events. Epigenetic modifications of key developmental genes in enamel formation may be closely connected to a network of molecular events. However, the role of epigenetic regulation in amelogenesis remains poorly. Our scoping review demonstrated that only few studies have been focusing to explore epigenetic events in enamel development and developmental enamel defects.

Developmental enamel defects are common developmental alterations in humans and are associated with an increased risk of dental caries, dental sensitivity, malocclusion and esthetic problems that could lead to a social and psychological problems affecting child’s quality of life. Therefore, research focusing in unraveling the etiological aspects of developmental enamel defects are extremely important.

The specificity of the epigenetic mechanisms makes them a source to point some biomarkers that could contribute to improve clinical diagnosis and prognosis of human diseases. Despite the substantial number of studies that have identified epigenetic disorders in different inflammatory diseases, few have explored the possible relationship between epigenetic changes and developmental enamel defects.

**Scoping review**

Scoping reviews are a type of knowledge synthesis that systematically pull evidence on a specific subject matter, identifying key concepts, theories, sources of evidence, and research. Scoping reviews are also useful way of mapping fields of study.

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**4 DISCUSSION**

<table>
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<th>Study</th>
<th>Gene expression and dental enamel structure in developing mouse incisor</th>
<th>RNA from tooth germs was extracted from two different periods of development (Embryonic and postnatal). Microarray analysis was used to measure miRNAs and mRNAs. The expression was validated by real time PCR. Bioinformatics analysis and western blotting were used to verify the findings.</th>
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</table>
| Selic, et al. (2010) | The layers of enamel produced in mice incisors present different thickness along the tooth. The aim was to correlate gene expression pattern and mRNA in these regions and the enamel secretion. | - Knock out of epithelial mature miRNAs resulted in incisors lacking enamel.  
- miRNAs control molar cusp formation.  
- miRNAs Regulate Dental Epithelial Cell Differentiation  
- Aβ, Aβ, Aβ, Ena, Klk4, Mmp20, and Calb1 are expressed in the two segments of incisor tooth germ.  
- miRNAs are expressed in the two segments of incisor tooth germ. |

Note: BPA means Bisphenol A; PCR means polymerase chain reaction; mRNA means microRNA; microCT means microtomography.
where it is difficult to visualize the range of material that might be available, such as epigenetic and enamel formation.

Other important aspect of the scoping reviews is that they are useful tools to identify research gaps in the existing literature. In our scoping review we were able to note the lack of studies evaluating human populations and consequently evaluating human enamel defect as an outcome. This fact should be considered by the researchers’ team that have been investigation group of patients with a variety of enamel defects.

4.1 CHARACTERISTICS OF THE INCLUDED STUDIES

The elicitation of enamel defects by environmental factors has been the focus of research and debate. The included study from Li et al. (2021) the authors explored the impact of a common environmental factor in the enamel formation using an in vivo and in vitro design. They explored the connection between Bisphenol A (BPA), which is a well-known endocrine-disrupting chemical, with enamel defect. Their study suggested that a developmental effect of BPA on enamel formation is partially mediated by upregulation of repressive mark H3K27me3. The increase of EZH2 expression and function demonstrated after exposure to BPA was considered a potential mechanism for the increased risk of enamel defect.

Rodents were widely used in the investigation of the included studies. Le et al. (2016) used wild-type and Amel knockout mice to investigate and determine that a novel miRNA (miR-exon4) was produced from amelogenin exon4 when it was spliced out by alternative splicing of amelogenin gene and was present in ameloblasts. Amelogenin is the predominant extracellular enamel matrix protein and plays a pivotal role in enamel formation.

MicroRNAs are a family of small, endogenous, non-coding, and single-stranded RNAs that regulate gene expression post-transcriptionally. (Taft, 2010; Bartel, 2004). MicroRNAs are known to regulate innumerable gene functions in many tissues and organs. Many studies included here focused in microRNAs investigation, and, although it is not a canonical epigenetic mechanism as discussed by Taft et al. (2010) and others, we included in our review. Cao et al (2010), Sehic, et al. (2010) and Jheon et al. (2011) highlighted the important function of microRNAs during odontogenesis (including enamel formation) a decade ago, showing that tooth development is tightly controlled by microRNAs and that specific microRNAs regulate tooth epithelial stem cell differentiation. Cao et al (2013) concluded that Pitx2 induces a transcriptional
program involving microRNA200c. More recently, the study from Yin. et al (2014)\(^3\) showed that microRNAs exhibit a dynamic expression pattern during the transition from secretory-stage to maturation-stage of the enamel formation. Kim et al. (2014)\(^3\) suggested that BMP signaling (a key pathway in enamel development) regulates tooth formation via microRNA135a. Neupane et al (2020)\(^2\) observed that changing the expression of microRNA206-3p altered the expression patterns of well-known dental-related signaling molecules such as Axin2, Bmp2, Fgf4, Lef1 and Shh and suggested that microRNA206-3p regulates cellular physiology in tooth morphogenesis through these signaling pathways.

It is important to highlight that although microRNAs represent only one of numerous mechanisms influencing gene activities, microRNAs specific to the maturation stage could be involved in regulating several key processes of enamel maturation by influencing mRNA stability and translation leading to an enamel defect in humans.

5 CONCLUSION
5.1 FUTURE PERSPECTIVES

Dental research, especially enamel development research, can significantly benefit from new studies in epigenetic area. Future studies could lead to a range of opportunities for diagnosis, prognosis, treatment, and prevention of this condition as well as the understanding of the connection between enamel defects and systemic conditions. Briefly, the growing field of epigenetic regulations in enamel development provides excellent opportunities to identify novel enamel-related disease biomarkers and to explore the potential therapeutic methods. Additional epigenetics studies have much to offer to the knowledge regarding enamel development defects.
REFERENCES


