Journal of Dental Sciences 19 (2024) 2150–2156

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Original Article

Small RNAs and tooth development: The role of microRNAs in tooth agenesis and impaction

Agnese Giovannetti [a], Rosanna Guarnieri [b], Francesco Petrizzelli [c], Sara Lazzari [d], Gabriella Padalino [b], Alice Traversa [d,e], Alessandro Napoli [c], Roberto Di Giorgio [f], Antonio Pizzuti [a,d], Chiara Parisi [g], Tommaso Mazza [c], Ersilia Barbato [b], Viviana Caputo [d*]

[a] *Clinical Genomics Laboratory, Fondazione IRCCS Casa Sollievo della Sofferenza, S. Giovanni Rotondo (FG), Italy*
[b] *Department of Oral and Maxillofacial Sciences, School of Dentistry, Sapienza University of Rome, Rome, Italy*
[c] *Bioinformatics Laboratory, Fondazione IRCCS Casa Sollievo della Sofferenza, S. Giovanni Rotondo (FG), Italy*
[d] *Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy*
[e] *Dipartimento di Scienze della Vita, della Salute e delle Professioni Sanitarie, Università degli Studi "Link Campus University", Roma, Italy*
[f] *Department of Sense Organs, Sapienza University of Rome, Rome, Italy*
[g] *Institute of Biochemistry and Cell Biology, CNR-National Research Council, Monterotondo Scalo, Rome, Italy*

Received 3 January 2024; Final revision received 11 March 2024
Available online 22 March 2024

KEYWORDS
microRNAs;
Odontogenesis;
Tooth abnormalities

Abstract *Background/purpose:* Tooth development, or odontogenesis, is a complex process in which several molecular pathways play a key role. Recently, microRNAs, a class of approximately 20-nucleotide small RNA molecules that regulate gene expression, have been implicated in the odontogenesis process. This study aimed to assess the role of miRNAs in odontogenesis anomalies, specifically agenesis and impaction.
Materials and methods: We analyzed a manually curated list of 82 miRNAs associated with human odontogenesis, sourced from literature data. Employing two different approaches to validate findings, we conducted functional enrichment analysis to evaluate the cell pathways, diseases, and phenotypes enriched for those miRNAs.

* Corresponding author. Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Margherita 261, Rome, 00198, Italy.
E-mail address: viviana.caputo@uniroma1.it (V. Caputo).

https://doi.org/10.1016/j.jds.2024.03.013

Results: Our findings indicate that the analyzed miRNAs regulate pathways linked to tooth anomalies, including the TGFβ and Wnt signaling pathways, and those governing the pluripotency of stem cells, known to mediate various cellular processes, and interconnected with odontogenesis-related pathways. Furthermore, the analysis disclosed several pathways associated with tumors, including small cell lung and gastric cancer. These results were confirmed also by diseases and phenotypes enrichment evaluation. Moreover, cell network analysis disclosed that miRNAs are embedded and interconnected in networks associated with dental diseases and cancer development, thus confirming the functional enrichment analyses.
Conclusion: In summary, our results offer a quantitative measure of the potential involvement of miRNAs in regulating pathways crucial for developmental processes, notably odontogenesis, and provide results suggesting potential association with oncogenesis processes as well.

Introduction

Tooth development, or odontogenesis, is a complex process involving key stages: tooth formation, eruption, and embedding, facilitated by interactions between the dental epithelium and mesenchyme. It begins with the oral epithelium thickening, forming the dental lamina. Multiple dental placodes form within it, each initiating tooth germ development. Then, the placodes bud into the mesenchyme, inducing condensation, and the epithelium continues to extend, shaping the tooth. Crown formation follows, detaching the tooth from the oral epithelium, followed by root formation that connects teeth to nerves, blood vessels, and the alveolar bone. This initiates vertical movement, facilitating eruption.

In this complex scenario, several molecular pathways involving a large number of genes play a key role.[1] "Wnt/β-catenin", "BMP", "FGF", and "Sonic Hedgehog (SHH)" are among the most evolutionary conserved pathways, which are tightly regulated and closely intertwined in all the steps of tooth development. In particular, the "Wnt/β-catenin" and "SHH" pathways regulate all the tooth development transition steps, making them crucial for the whole odontogenesis process.

Recently, a peculiar role in the regulation of pathways underlying odontogenesis has been found for a class of non-coding RNAs, microRNAs (miRNAs), small RNA molecules that are ~20 nucleotides long and regulate gene expression binding by complementarity to the 3′UTR of messenger RNAs (mRNAs). miRNAs play crucial regulatory roles in human dental tissues, contributing to the regulation of the complex process of odontogenesis through the targeting of key genes.[2,3]

Odontogenesis perturbation leads to dental anomalies, with dental agenesis being one of the most common, with a prevalence ranging between 0.15% and 16.2%,[4] with cases that can be both familiar and isolated. Further anomalies include ectopic eruption and impaction of teeth, both responsible for malocclusion, with a prevalence of 5.4%[5] and 3.9%,[6] respectively.

Over the past decade, research has identified several molecular pathways associated with these conditions, including "Wnt", "TGF", and "EDA/EDAR/NF-κB".[7–9] These pathways have been implicated through the occurrence of genetic variants or perturbed gene expression.

Conversely, the biological role of miRNAs in tooth anomalies remains less characterised. Recently, deregulation of certain miRNAs has been identified in human dental tissues participating in the odontogenesis process.[2] Additionally, a Single Nucleotide Variant (SNV) affecting the processing of a specific miRNA (miR-605) has been linked to non-syndromic tooth agenesis.[10]

In this study, our attention was directed towards understanding the role of miRNAs in regulating the odontogenesis process, particularly in the context of dental anomalies such as agenesis and impaction. To accomplish this objective, we compiled and analyzed a curated list of miRNAs, assessing their significance in odontogenesis through functional enrichment analyses of diseases and pathways associated with tooth development. Additionally, we explored the recurrence of miRNA-target interactions within cell networks involved in tooth development.

Materials and methods

Tooth anomalies-associated miRNAs and their targets

We gathered miRNAs potentially associated with odontogenesis and with tooth anomalies through a literature search on PubMed (https://pubmed.ncbi.nlm.nih.gov/, NCBI, Bethesda, Maryland, USA), using the terms "miRNA," "dental development," and "odontogenesis." We analyzed the abstracts of the resulting 414 articles, refining the analysis using terms such as "tooth agenesis," "tooth impaction," "tooth eruption," and "tooth movement." Among the resulting 38 articles, we selected the 20 papers reporting functional experimental data, such as miRNA expression analysis in human cells and tissues related to tooth development performed using microarrays, RT-qPCR or in situ hybridization. This analysis resulted in a manually curated list of miRNAs based on experimental data, making them particularly reliable in terms of their involvement in tooth-associated cellular mechanisms. miRNA nomenclature was compliant with miRBase v22.

We identified target genes of miRNAs resulting from the literature search using miRWalk 3.0 (release_2022_01),[11] a web tool that provides predicted and experimentally validated miRNAs' targets. We selected only miRNA-gene interactions showing the highest interaction probability (score = 1), predicted by TargetScan,[12] and experimentally validated according to miRTarBase.[13] In this way, we specifically considered miRNA-targeted genes deemed more reliable, presenting a more plausible depiction of the biological context. This addresses the challenge associated with in silico miRNA target prediction, which usually provides a high number of potential targets, with a substantial number that may lack complete reliability.

Tooth anomalies-associated genes

We updated a list of genes known to be associated with isolated and syndromic phenotypes characterized by tooth anomalies (Supplementary Table 1),[14] searching in the literature (PubMed, NCBI) and in disease databases (OMIM, Online Mendelian Inheritance in Man, https://www.omim.org;[15] HPO, the Human Phenotype Ontology, http://human-phenotype-ontology.github.io).[16] The search terms were: "hypodontia," "primary failure of tooth eruption," "selective tooth agenesis," "oligodontia," "anodontia," and "agenesis of permanent teeth." As previously reported, the list included genes associated with tooth agenesis and impaction anomalies, both isolated and syndromic, and validated through functional studies. To assess whether there was a significant enrichment of genes associated with tooth anomalies among the target genes of each miRNA, we employed a Fisher's exact test, considering a $P < 0.05$ as indicative of significance.

Functional enrichment analysis of miRNAs

We performed a functional enrichment analysis using WebGestaltR[17] to evaluate the cellular pathways, diseases, and phenotypes enriched for genes targeted by miRNAs selected in this study and potentially associated with odontogenesis (Supplementary Table 2), and also for tooth anomalies-associated genes (Supplementary Table 1).[14] Regarding the enriched pathways analysis, we used data from the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/kegg/) database.[18] For the analysis of enriched diseases and phenotypes, reference sets were sourced from the following databases: Gene List Automatically Derived For You (GLAD4U, http://glad4u.zhang-lab.org)[19] and Human Phenotype Ontology (HPO).[16] Among approaches available in WebGestalt, we employed the Over-Representation Analysis (ORA) method.

To control for false-positive results, we restricted the number of enriched genes for each pathway, disease, and phenotype to a range of 5–200.[20] Moreover, we applied the Benjamini-Hochberg procedure to correct all P in case of multiple comparisons and set a significant threshold of 0.05 (indicated as False Discovery Rate, FDR).

We also analyzed the lists of genes targeted by miRNAs and genes related to tooth anomalies, using Ingenuity Pathway Analysis (IPA; www.qiagen.com/ingenuity, QIAGEN, Redwood City, CA, USA), in order to evaluate pathways enrichment and compare results with that of the WebGestalt analysis. Even in this case, we applied the Benjamini-Hochberg procedure to correct all P and set a significant threshold of 0.05 (indicated as False Discovery Rate, FDR). We also evaluated the networks interconnecting miRNAs and tooth anomalies-associated genes through IPA. The IPA's knowledgebase contains predicted transcriptional regulators, functions, and pathways, together with z-scores to infer their activating or inhibiting states. It also contains functional networks, which are associated with specific biological functions or diseases. Their degree of association is quantified by a score. The higher the score of a network, the lower the probability of finding the observed number of molecules in a given network by chance.[21] The IPA's knowledgebase is manually curated and mostly fed with experimental evidence obtained from the literature. For this reason, it can be considered orthogonal to the other ontologies queried in this work.

Results

Genes and miRNAs associated with tooth anomalies

We compiled a list of 82 miRNAs functionally linked to the development and eruption of teeth, drawing from literature data (Supplementary Table 2). These 82 miRNAs exhibited altered expression in tissues engaged in odontogenesis and were potentially implicated in the regulation of tooth development and tooth movement.

Employing miRWalk and following the criteria outlined in the Methods section, we found that the 82 miRNAs overall targeted 1846 transcripts, corresponding to 842 genes, through 2611 interactions.

We additionally used a list of genes associated with isolated and syndromic tooth anomalies as a reference set of known associated genes, relying on an updated version comprising 102 genes (Supplementary Table 1).[14]

To determine whether there was a significant occurrence of tooth anomalies-associated genes among each miRNA's target genes, we conducted a statistical analysis. Our findings revealed two tooth anomalies-associated genes, *KREMEN1* ($P = 0.00659$) and *PIK3R1* ($P = 0.02319$), which are targeted by two miRNAs, hsa-let-7a-5p and hsa-miR-103a-3. These results suggest that these two miRNAs may play a biological role in odontogenesis by targeting genes with established functional significance in this developmental process.

Functional enrichment analysis of miRNAs

We employed different approaches to unveil enriched pathways among the selected miRNAs linked to tooth development, using the WebGestalt approach and the IPA orthogonal ontological knowledgebase to validate findings. As a proof of concept, we also used both approaches to analyze tooth anomalies-associated genes disclosing some pathways known to play a role in odontogenesis, as the "Hedgehog signaling pathway" (Supplementary Tables 3 and 4).

The functional enrichment analysis of pathways (KEGG) of the 842 miRNA's target genes through WebGestalt and

confirmed using IPA, disclosed numerous enriched pathways (Supplementary Tables 5 and 6), several with a well-established role in odontogenesis, such as the "TGFβ signaling pathway" and the "Wnt signaling pathway"[1] (Fig. 1, Supplementary Tables 5 and 6). We also identified, using both approaches, pathways for which a role in odontogenesis has been suggested, such as the "Hippo signaling pathway," "mTOR signaling pathway," and "Adherens junction"[8] (Fig. 2, Supplementary Tables 5 and 6).

Among pathways shared with tooth anomalies-associated genes, we found "pathways regulating pluripotency of stem cells", which are interconnected with pathways known to be involved in odontogenesis (Fig. 2, Supplementary Tables 3, 4, 5 and 6). Interestingly, miRNAs found in these pathways target different genes (Fig. 3).

These results demonstrate, for the first time to our knowledge, that miRNAs play a significant role in odontogenesis by targeting several genes involved in cell networks associated with developmental processes and whose role in tooth development has been established.

Analyzing miRNAs target genes through both approaches, we also identified cancer related pathways such as "Thyroid cancer" and "Melanoma" (Supplementary Tables 5 and 6), some of them shared with tooth anomalies-associated genes, as "Small cell lung cancer," and "Gastric cancer" (Fig. 2, Supplementary Tables 3, 4, 5 and 6).

Additionally, we conducted a functional enrichment analysis to identify over-represented diseases (GLAD4U) and phenotypes (HPO) among miRNAs and known genes. Among miRNAs, several terms were associated with tumors, including "Neoplasms, hormone-dependent," "Uterine neoplasms", "Neoplasm of the large intestine", and "Rectal neoplasms" (Supplementary Tables 7 and 8). Among known tooth anomalies-associated genes, we disclosed, as expected, that genes were enriched for diseases and phenotypes involving "Tooth malformation," "Anodontia," "Partial congenital absence of teeth," "Reduced number of teeth," "Hypodontia," and "Abnormality of dental morphology" but also cancer related terms as "Giant cell tumors," "Teratoma" and "Squamous cell carcinoma", some of which were also shared with miRNAs such as "Neoplasms, Basal Cell," "Carcinoma, Large Cell," "Neoplasm of the skin" and "Fibrous tissue neoplasm" (Supplementary Tables 7, 8, 9 and 10).

Based on the above mentioned results, to evaluate potential miRNAs and tooth anomalies-associated genes cell networks, we performed a network analysis using IPA, which disclosed that genes and miRNAs were part of the same cell networks associated with dental disease (Supplementary Figure 1 and Supplementary Table 11), and cancer development (Supplementary Figure 2 and Supplementary Table 11).

Discussion

This study aims to explore the involvement of miRNAs, a class of non-coding molecules, in odontogenesis through in silico analyses. The biological role of miRNAs in odontogenesis is currently emerging as crucial regulators of the intricate process of dental development.[2,3] However, to date, substantial evidence demonstrating the involvement of this class of small non-coding RNAs has not been conclusively established. Leveraging a manually curated literature search, we identified 82 miRNAs potentially associated with dental development and tooth anomalies, based on miRNA expression analysis in human cells and tissues relevant to odontogenesis.

An initial, preliminary assessment of highly reliable genes targeted by these miRNAs provided valuable insights into the role of miRNAs in regulating odontogenesis through the modulation of genes known to be involved in this process. Specifically, two miRNAs, hsa-let-7a-5p and hsa-miR-103a-3, previously found to be deregulated in gingival tissue of patients with periodontitis,[2] target *KREMEN1* and *PIK3R1*. Notably, these two genes have been identified as mutated in cases of syndromic oligodontia and hypodontia, respectively.[22,23]

The primary analysis focused on the functional enrichment of pathways that encompass miRNAs' target genes. The involvement of miRNAs in odontogenesis through the targeting of genes pivotal for these developmental processes was confirmed using a different approach, thus substantiating the results through a quantitative assessment that provided statistically significant results.

This analysis revealed that several genes were significantly enriched in pathways with established functional roles in dental development, such as the "TGFβ" and "Wnt signaling" pathways. These networks regulate several cellular processes, including cell growth, differentiation, apoptosis, and tissue homeostasis, playing a pivotal role in both embryonic development and adult tissue maintenance. Upon activation, a cascade of intracellular events occurs, leading to the activation of downstream proteins and the regulation of gene expression. Dysregulation of these pathways is associated with different diseases, including cancer, fibrosis, developmental disorders, and degenerative diseases. Their specific role in dental development has been established through the identification of genetic variants in different genes within these pathways, leading to potential disruptions in tooth formation.[1] For instance, within the "Wnt signaling pathway", missense variants in *LRP6* have been discovered in familial cases of oligodontia,[24] while mutations in *WNT10A* have been linked to both familial and sporadic cases of syndromic and non-syndromic hypodontia.[7]

Moreover, among other enriched pathways that were significantly enriched for miRNAs' target genes there are "Hippo", "mTOR", and "Adherens junction" pathways. The "Hippo" and "mTOR signaling" pathways, both evolutionarily conserved, govern organ size (Hippo) and control lipid metabolism, autophagy, and the actin cytoskeleton (mTOR).[8,25] Adherens junction pathway serves as crucial regulator of tissue architecture, cell polarity and proliferation and has recently been suggested to play a role in tooth development and oral pathologies.[26] For instance, missense variants in genes involved in these pathways, such as *BMP4* and *FGFR1*, have been associated with isolated and syndromic tooth eruption anomalies.[27,28] Additionally, other genes like *AXIN2* have been linked to oligodontia-colorectal cancer syndrome.[29] Notably, upon comparing the enriched pathways of miRNAs' target genes with those

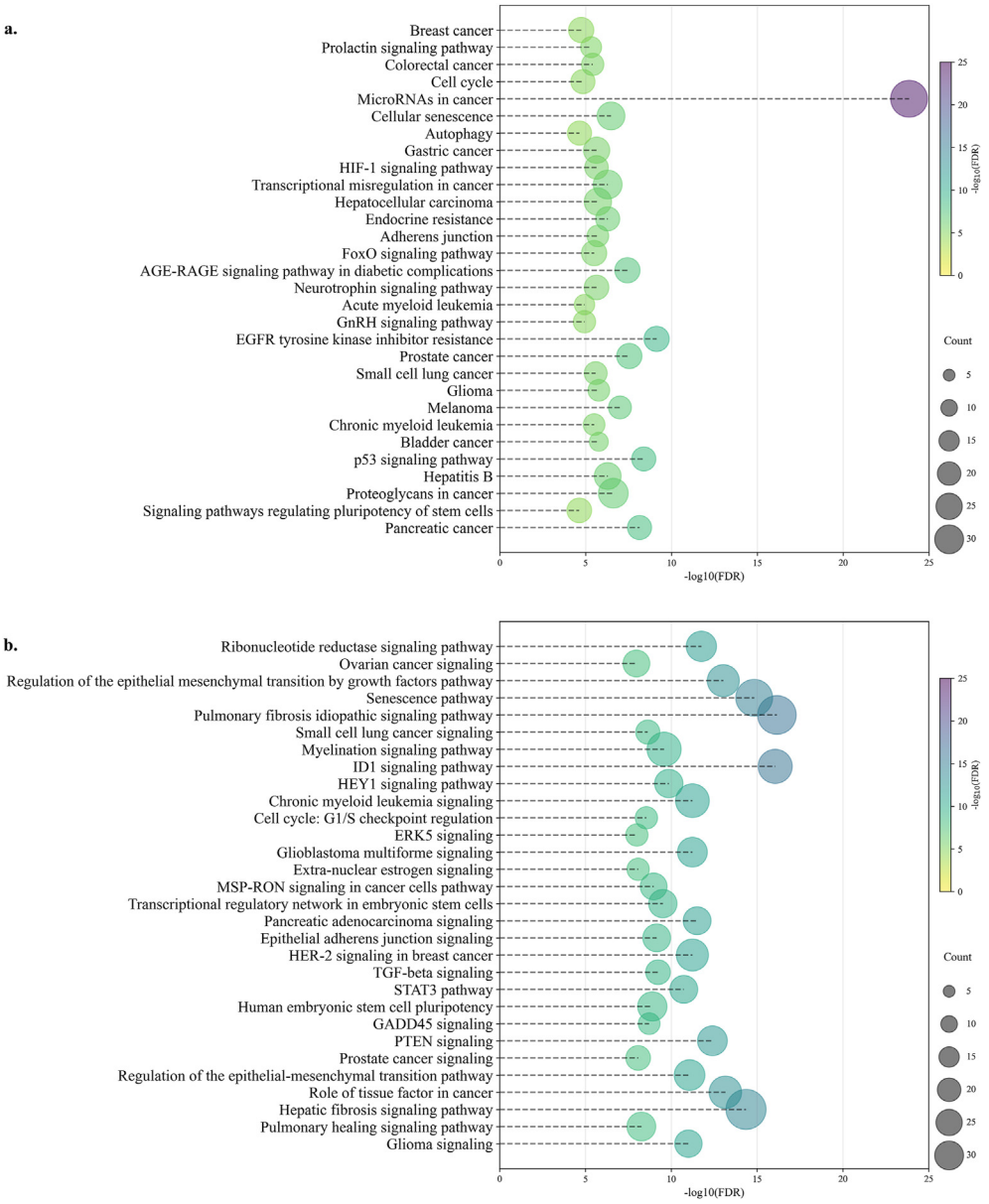

Figure 1 Functional enrichment analysis of miRNAs' target genes for pathways according to WebGestalt (**A**) and IPA database (**B**). For both analyses, the top 30 most significantly enriched pathways are reported. FDR (False Discovery Rate) indicates *P* adjusted by applying Benjamini-Hochberg procedure.

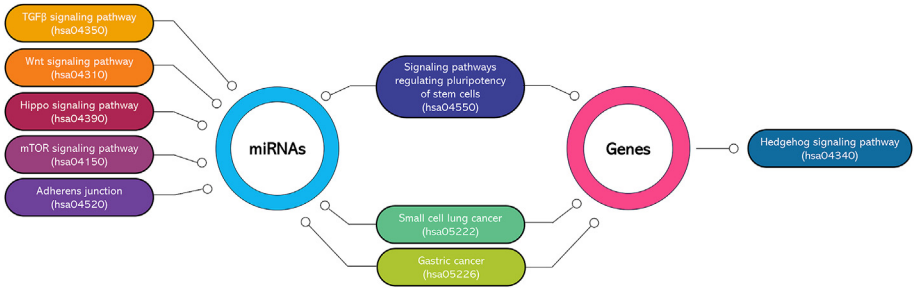

Figure 2 Most important enriched pathways related to odontogenesis and cancer for both miRNAs and tooth anomalies-associated genes identified through WebGestalt and IPA. The pathways reported in the center are shared between miRNAs' target genes and tooth anomalies-associated genes. The KEGG identifier for each pathway is reported in brackets.

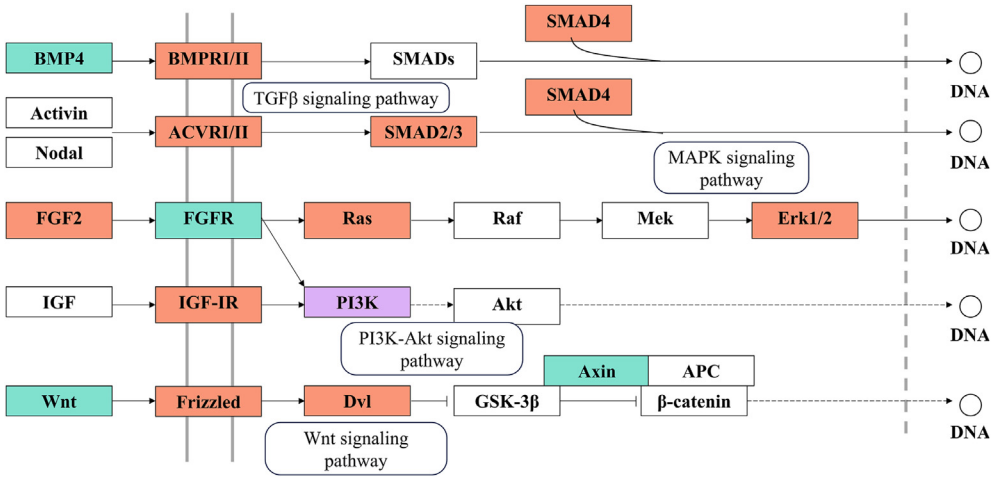

Figure 3 A section of "Signalling pathways regulating pluripotency of stem cells" as represented by KEGG. As it can be observed, this pathway is interconnected with "Wnt" and "TGFβ signaling" pathways, known to be related to odontogenesis. In light blue are reported tooth anomalies-associated genes, in orange miRNA's target genes and in purple the genes belonging to both sets of genes.

of odontogenesis-related genes, we observed shared pathways, as "pathways regulating pluripotency of stem cells", which can mediate multiple cellular processes and exhibit interconnections with the "Wnt" and "TGFβ signaling pathways".[1] We noted that the enrichment was due to different genes. This observation suggests that miRNAs may contribute biologically to developmental pathways governing tooth development and eruption, primarily by targeting the expression of different pivotal genes involved in these mechanisms. This can occur through a multi-layered system of regulation of the same cellular pathways through an integrated mechanism due to genes and miRNAs fine-tuning the regulation of these processes.

Further studies are necessary, through gene expression analyses, sequencing, or genotyping screening, along with functional in vitro and in vivo approaches, to evaluate the potential role of miRNAs in tooth development and to assess how their perturbation, in terms of expression, biogenesis, and targeting, could contribute to tooth anomalies such as agenesis and impaction.

Another notable finding is the identification of several cancer-related pathways using both approaches among the miRNAs' enriched pathways, some of them shared with known genes, such as "Small cell lung cancer," and "Gastric cancer".

This result aligns with a recent hypothesis suggesting a close connection between odontogenesis and cancer.[30,31] Indeed, according to literature data, there is evidence, although sometimes discordant,[32,33] supporting an association between tooth agenesis and cancer.[34,35] For instance, genes involved in odontogenesis are expressed in tumor cells,[36,37] some variants in genes related to tooth development are associated with cancer,[29] and altered methylation of those genes is observed in some tumors.[38] Additionally, both tooth development and cancer are characterized by rapid cell growth. These observations have led to the proposal that tooth anomalies could serve as early indicators of cancer risk, and variants identified in genes associated with tooth anomalies may be suggested as predictive markers of cancer.

In conclusion, our study provides initial insights into the role of miRNAs in tooth development through an in silico approach. The assessment of enriched pathways supports preliminary and fragmentary evidence that this class of non-coding molecules is intricately involved in the same cellular networks known to underlie tooth development. Moreover, it provides which genes can play a role in this developmental process, as targets of specific miRNAs, whose perturbation can potentially lead to tooth anomalies, such as agenesis and impaction. These findings shed light on the molecular determinants of tooth development, with potential implications for the clinical management of patients with tooth anomalies.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by Sapienza University of Rome (Ricerca di Ateneo 2021) to V. C.; Italian Ministry of Health (Ricerca Corrente 2022–2024) to A. P.; Italian Ministry of Health (Ricerca Corrente 2022–2024) and "5 x 1000" voluntary contribution to T. M.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2024.03.013.

References

1. Hermans F, Hemeryck L, Lambrichts I, Bronckaers A, Vankelecom H. Intertwined signaling pathways governing tooth development: a give-and-take between canonical Wnt and Shh. *Front Cell Dev Biol* 2021;9:758203.

2. Sehic A, Tulek A, Khuu C, Nirvani M, Sand LP, Utheim TP. Regulatory roles of microRNAs in human dental tissues. *Gene* 2017;596:9—18.
3. Jin Y, Wang C, Cheng S, Zhao Z, Li J. MicroRNA control of tooth formation and eruption. *Arch Oral Biol* 2017;73:302—10.
4. Rakhshan V. Congenitally missing teeth (hypodontia): a review of the literature concerning the etiology, prevalence, risk factors, patterns and treatment. *Dent Res J* 2015;12:1—13.
5. De Ridder L, Aleksieva A, Willems G, Declerck D, Cadenas de Llano-Pérula M. Prevalence of orthodontic malocclusions in healthy children and adolescents: a systematic review. *Int J Environ Res Publ Health* 2022;19:7446.
6. Laganà G, Venza N, Borzabadi-Farahani A, Fabi F, Danesi C, Cozza P. Dental anomalies: prevalence and associations between them in a large sample of non-orthodontic subjects, a cross-sectional study. *BMC Oral Health* 2017;17:62.
7. Yin W, Bian Z. The gene network underlying hypodontia. *J Dent Res* 2015;94:878—85.
8. Yu T, Klein OD. Molecular and cellular mechanisms of tooth development, homeostasis and repair. *Development* 2020;147:dev184754.
9. Lan R, Wu Y, Dai Q, Wang F. Gene mutations and chromosomal abnormalities in syndromes with tooth agenesis. *Oral Dis* 2023;29:2401—8.
10. Gu M, Yu X, Fan L, et al. Genetic variants in miRNAs are associated with risk of non-syndromic tooth agenesis. *Front Physiol* 2020;11:1052.
11. Sticht C, De La Torre C, Parveen A, Gretz N. miRWalk: an online resource for prediction of microRNA binding sites. *PLoS One* 2018;13:e0206239.
12. McGeary SE, Lin KS, Shi CY, et al. The biochemical basis of microRNA targeting efficacy. *Science* 2019;366:eaav1741.
13. Huang HY, Lin YC, Cui S, et al. miRTarBase update 2022: an informative resource for experimentally validated miRNA-target interactions. *Nucleic Acids Res* 2022;50:D222—30.
14. Barbato E, Traversa A, Guarnieri R, et al. Whole exome sequencing in an Italian family with isolated maxillary canine agenesis and canine eruption anomalies. *Arch Oral Biol* 2018;91:96—102.
15. Amberger JS, Bocchini CA, Scott AF, Hamosh A. OMIM.org: leveraging knowledge across phenotype-gene relationships. *Nucleic Acids Res* 2019;47:D1038—43.
16. Köhler S, Gargano M, Matentzoglu N, et al. The human phenotype Ontology in 2021. *Nucleic Acids Res* 2021;49:D1207—17.
17. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res* 2019;47:W199—205.
18. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res* 2023;51:D587—92.
19. Jourquin J, Duncan D, Shi Z, Zhang B. GLAD4U: deriving and prioritizing gene lists from PubMed literature. *BMC Genom* 2012;13(Suppl 8):S20.
20. Yoshioka H, Wang YY, Suzuki A, et al. Overexpression of miR-1306-5p, miR-3195, and miR-3914 inhibits ameloblast differentiation through suppression of genes associated with human amelogenesis imperfecta. *Int J Mol Sci* 2021;22:2202.
21. Palmieri O, Mazza T, Merla A, et al. Gene expression of muscular and neuronal pathways is cooperatively dysregulated in patients with idiopathic achalasia. *Sci Rep* 2016;6:31549.
22. Issa YA, Kamal L, Rayyan AA, et al. Mutation of KREMEN1, a modulator of Wnt signaling, is responsible for ectodermal dysplasia including oligodontia in Palestinian families. *Eur J Hum Genet* 2016;24:1430—5.
23. Thauvin-Robinet C, Auclair M, Duplomb L, et al. PIK3R1 mutations cause syndromic insulin resistance with lipoatrophy. *Am J Hum Genet* 2013;93:141—9.
24. Massink MP, Créton MA, Spanevello F, et al. Loss-of-Function mutations in the WNT co-receptor LRP6 cause autosomal-dominant oligodontia. *Am J Hum Genet* 2015;97:621—6.
25. Wang J, Martin JF. Hippo pathway: an emerging regulator of craniofacial and dental development. *J Dent Res* 2017;96:1229—37.
26. Kingsley C, Kourtidis A. Critical roles of adherens junctions in diseases of the oral mucosa. *Tissue Barriers* 2023;11:2084320.
27. Mu Y, Xu Z, Contreras CI, McDaniel JS, Donly KJ, Chen S. Phenotype characterization and sequence analysis of BMP2 and BMP4 variants in two Mexican families with oligodontia. *Genet Mol Res* 2012;11:4110—20.
28. Dodé C, Levilliers J, Dupont JM, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet* 2003;33:463—5.
29. Lammi L, Arte S, Somer M, et al. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am J Hum Genet* 2004;74:1043—50.
30. Bonczek O, Krejci P, Izakovicova-Holla L, Cernochova P, Kiss I, Vojtesek B. Tooth agenesis: what do we know and is there a connection to cancer? *Clin Genet* 2021;99:493—502.
31. Medina MCG, Bastos RTDRM, Mecenas P, Pinheiro JJV, Normando D. Association between tooth agenesis and cancer: a systematic review. *J Appl Oral Sci* 2021;29:e20200955.
32. Bonds J, Pollan-White S, Xiang L, Mues G, D'Souza R. Is there a link between ovarian cancer and tooth agenesis? *Eur J Med Genet* 2014;57:235—9.
33. Paranjyothi MV, Kumaraswamy KL, Begum LF, Manjunath K, Litha Basheer S. Tooth agenesis: a susceptible indicator for colorectal cancer? *J Cancer Res Therapeut* 2018;14:527—31.
34. Chalothorn LA, Beeman CS, Ebersole JL, et al. Hypodontia as a risk marker for epithelial ovarian cancer: a case-controlled study. *J Am Dent Assoc* 2008;139:163—9.
35. Fekonja A, Čretnik A, Takač I. Hypodontia prevalence and pattern in women with epithelial ovarian cancer. *Angle Orthod* 2014;84:810—4.
36. Hsu DS, Acharya CR, Balakumaran BS, et al. Characterizing the developmental pathways TTF-1, NKX2-8, and PAX9 in lung cancer. *Proc Natl Acad Sci U S A* 2009;106:5312—7.
37. Menezes ME, Mitra A, Shevde LA, Samant RS. DNAJB6 governs a novel regulatory loop determining Wnt/β-catenin signalling activity. *Biochem J* 2012;444:573—80.
38. Rauch TA, Wang Z, Wu X, Kernstine KH, Riggs AD, Pfeifer GP. DNA methylation biomarkers for lung cancer. *Tumour Biol* 2012;33:287—96.