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Review

Advances in tooth agenesis and tooth regeneration

V. Ravi [a, †], A. Murashima-Suginami [a, b, d, †], H. Kiso [a, b, d], Y. Tokita [c], C.L. Huang [e], K. Bessho [d], J. Takagi [f], M. Sugai [g], Y. Tabata [h], K. Takahashi [a, b, d, *]

[a] Toregem BioPharma Inc., Kyoto, Japan
[b] Department of Oral and Maxillofacial Surgery, Tazuke Kofukai Medical Research Institute, Kitano Hospital, Osaka, Japan
[c] Department of Disease Model, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Aichi, Japan
[d] Department of Oral and Maxillofacial Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[e] Department of ThoracicSurgery, Tazuke Kofukai Medical Research Institute, Kitano Hospital, Osaka, Japan
[f] Laboratory of Protein Synthesis and Expression, Institute for Protein Research, Osaka University, Osaka, Japan
[g] Department of Molecular Genetics, Division of Medicine, Faculty of Medical Sciences, University of Fukui, Fukui, Japan
[h] Laboratory of Biomaterials, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

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ABSTRACT

The lack of treatment options for congenital (0.1%) and partial (10%) tooth anomalies highlights the need to develop innovative strategies. Over two decades of dedicated research have led to breakthroughs in the treatment of congenital and acquired tooth loss. We revealed that by inactivating *USAG-1*, congenital tooth agenesis can be successfully ameliorated during early tooth development and that the inactivation promotes late-stage tooth morphogenesis in double knockout mice. Furthermore, Anti- *USAG-1* antibody treatment in mice is effective in tooth regeneration and can be a breakthrough in treating tooth anomalies in humans. With approximately 0.1% of the population suffering from congenital tooth agenesis and 10% of children worldwide suffering from partial tooth loss, early diagnosis will improve outcomes and the quality of life of patients. Understanding the role of pathogenic *USAG-1* variants, their interacting gene partners, and their protein functions will help develop critical biomarkers. Advances in next-generation sequencing, mass spectrometry, and imaging technologies will assist in developing companion and predictive biomarkers to help identify patients who will benefit from tooth regeneration.

Contents

Abbreviations: USAG-1, Uterine sensitization associated gene-1; BMP, bone morphogenetic protein; CEBPB, CCAAT enhancer binding protein beta; EDA, Ectodysplasin A.

* Corresponding author. Department of Oral and Maxillofacial Surgery, Tazuke Kofukai Medical Research Institute, Kitano Hospital, 2-4-20, Ohgimachi, Kita-ku, Osaka, 530-8480, Japan. Fax: +81-6-6312-8867.
E-mail address: katsu-takahashi@kitano-hp.or.jp (K. Takahashi).

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† These authors contributed equally to this work.

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1. Introduction

Loss of teeth due to congenital or acquired diseases or accidents is a common health condition in almost all age groups, especially in the aging populations. Current approaches to treating tooth loss include prostheses, transplantations, and dental implants. Therefore, to address the unmet needs of oral care, new strategies and therapeutic alternatives, such as tooth regeneration, are required for patients to regain normal food intake and lifestyle. However, this issue remains a challenge for dental researchers and the dental industry. Over the past decade, the integration of health and life science fundamentals with advanced chemistry and engineering has provided alternatives and advanced therapeutics for tooth regeneration [1]. Several regenerative methods, including scaffold-based tissue regeneration, cell and tissue engineering, activation of the third dentition, and gene-edited tooth regeneration in animal models are being developed to improve the chances of regenerating lost teeth. Regeneration of teeth by activating the third dentition has proven to be a scientifically viable approach [2]. In this review, we present the scientific progress toward tooth regeneration that has resulted from almost two decades of research by the Takahashi group and other investigators.

2. Classification of tooth agenesis

Depending on the number of missing congenital teeth, tooth agenesis can be classified as hypodontia, oligodontia, and anodontia [3,4]. Another classification of tooth agenesis includes syndromic and non-syndromic forms, based on the accompanying syndromes of tooth loss. The syndromic form of the disease is associated with various systemic conditions and syndromes [5–8]. Patients with syndromic tooth agenesis may have accompanying anomalies such as delayed tooth formation and eruption, canine transposition, and enamel hypoplasia [3]. Other possible clinical indicators of tooth agenesis include ectodermal dysplasia, cleft lip, cleft palate, Down syndrome, and Van der Woude syndrome [9].

Non-syndromic tooth agenesis is more common than the syndromic form [5]. Patients with this form of tooth agenesis primarily present with congenitally missing teeth, which is the only apparent symptom. In addition, non-syndromic tooth agenesis can be sporadic or familial [7]. In sporadic cases, hypodontia (<6 missing teeth, usually 1–3 missing teeth) can be caused by environmental or genetic factors. This condition could signify tooth agenesis in patients without other associated syndromes [4]. In familial cases of tooth agenesis, hypodontia can serve as the only clinical indicator or as part of an associated syndrome [6] with autosomal dominant inheritance.

In addition to the congenital, acquired, and environmental factors, other factors that cause tooth agenesis include *Rubella* virus infection and tooth agenesis due to orofacial trauma during odontogenesis [3]. Several studies have quantified craniofacial anomalies in patients with syndromic tooth agenesis. As a result of advances in molecular, next-generation sequencing, and imaging technologies, different underlying causes have been reported regarding the role of genetics and genomic variants in non-syndromic tooth agenesis and their influence on tooth agenesis and associated medical conditions [10–16].

The timeline in Fig. 1 depicts the seminal findings over the past two decades, before identifying *USAG-1* as a potential therapeutic target for tooth regeneration. This study established a system for efficient gene delivery to cranial neural crest cells using a recombinant adenovirus [17]. Subsequently, our group successfully demonstrated cartilage regeneration in cranial neural crest cells using a dominant-negative mutant that ectopically suppressed the function of *MSX2* [18]. Further research was conducted to determine the number of teeth that could be regenerated and identify the molecular factors associated with tooth agenesis. Gene therapy and recombinant adenovirus system were used to achieve the desired results. A breakthrough came in 2007, when Suginami et al. first reported mice with *USAG-1* deficiency and supernumerary teeth. This condition results from the active role of mesenchymal cells [19], which would otherwise have been lost due to apoptosis. A year later, the same group identified a significant role of BMP and Wnt signaling pathways in *USAG-1*-deficient mice that resulted in supernumerary teeth [20]. Tooth development is under both control of partner genes and interactive signaling between the oral epithelium and cranial mesenchyme [21]. Over the next five years, this group reported several cutting-edge findings that involve the interactions and role of *USAG-1* and other partner genes, such as *BMP7* and *Runx 2* genes, in tooth development [22,23]. The focus on finding novel causative variants in Japanese patients with congenital tooth agenesis resulted in the discovery of *WNT10A* variants that play a crucial role in the development of lateral incisors, which are more sensitive to WNT and β-catenin signaling from other teeth [24]. Owing to the role of *Sox 2* in stemness and proximity of *CEBPB* to *Sox2*, further functional analysis of *CEBPB* and *Runx2* in knockout (KO) mouse models demonstrated their role in supernumerary tooth formation in adults [25]. With over a decade of evidence and research on understanding the reasons behind supernumerary teeth in *USAG-1*-deficient mice, Kiso et al. provided another breakthrough. They determined whether these results could be applied to human tooth anomalies. Following reports that the third dentition in humans could result in supernumerary teeth formation, Kiso et al. performed a computed tomography scan study to evaluate the role of third dentition in supernumerary teeth

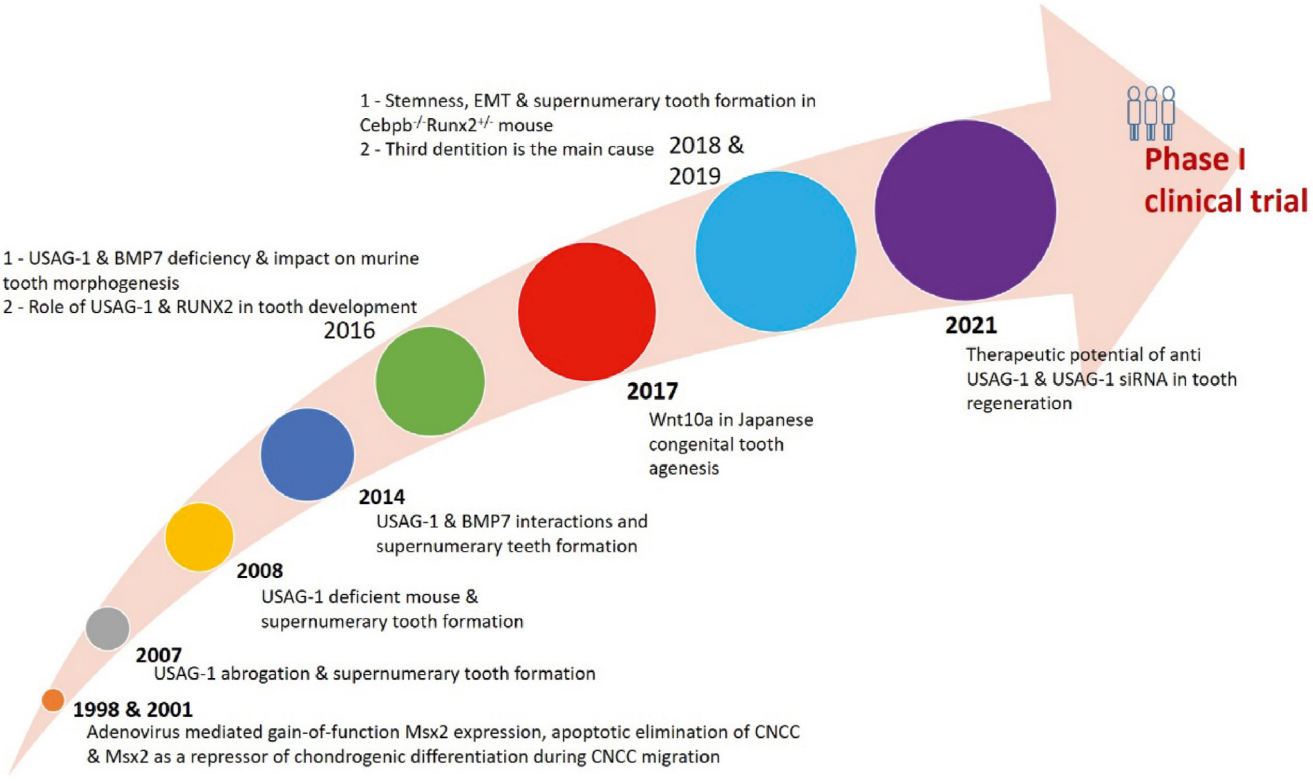

Fig. 1. Timeline of tooth regeneration breakthroughs in Takahashi's lab.

formation. They concluded that third dentition drives supernumerary tooth formation in humans, predominantly in male patients [26]. A year later, the same team reported using an antibody and siRNA against *USAG*-1 for tooth regeneration in mouse models [27]. This team is currently validating the efficacy of *USAG-1* antibody treatment in other mammalian models of tooth agenesis before beginning a phase 1 clinical trial.

3. Genetics of tooth agenesis

Understanding tooth development is critical for examining and identifying the genetic factors that regulate the interactions between epithelial and mesenchymal cells. The number of teeth in all species is usually determined and evolutionarily conserved based on the form and function of teeth in the dentition. However, researchers have made breakthroughs by demonstrating how rudimentary incisor teeth survive and grow as supernumerary teeth due to the knockout of *USAG-1* (Fig. 1). Although this was a mouse study, the prospect of similar results in humans is still promising. Establishing these results in humans would support the suggestion that "third dentition," when activated, can form new teeth and occur in addition to permanent dentition [1].

In contrast to non-congenital reasons, congenitally missing permanent teeth are rare. Regular scientific and clinical reports on syndromic tooth agenesis and other abnormalities are available. Genetic factors play a crucial role in tooth development and are usually the cause of tooth agenesis. Over the last few decades, tooth transplantation and dental implant procedures have become treatment options for tooth agenesis.

Nearly 200 genes involved in different pathways are expressed at different sites and stages during tooth development [28]. Several animal models, primarily mice, serve as model organisms to elucidate the role of specific functional mutants and provide insights into the underlying biological and molecular mechanisms that lead to supernumerary tooth formation. With the current knowledge of supernumerary teeth biology, genetic components are thought to play a role in the partial or total activation of the third dentition in humans. Candidate genes are expected to play a role in stimulating embryonic teeth or in controlling the number and type of regenerated teeth. Thus, when activated or dysregulated, testing the biological role of candidate genes provides an excellent opportunity to improve and develop applications to successfully grow new teeth successfully [29].

4. Third dentition

Humans are typically diphyodonts that develop two successive sets of teeth, namely deciduous and permanent dentition. In addition to the permanent dentition in humans, a "third dentition" with one or more teeth can occur. In some cases, this third dentition is thought to develop as a partial dentition following permanent dentition [19,30,31]. Diphyodont dentition occurs in both mammals and humans [32]. In humans, deciduous or milk teeth are the first set of teeth. Except for molars, permanent teeth belong to the second generation. In the dental community, the term "third dentition" refers to an extra set of teeth that occur in addition to the primary and permanent teeth. Early reports of rudimentary third dentition in a few mammals were published in the 19th century [33]. In humans, a rudimentary epithelial form of the third dentition has been identified [34,35]. After almost a century, Ooë et al. observed that the epithelium that helps form the third dentition develops lingual to the permanent tooth germs

[31]. In addition, when the epithelial anlagen was found, the permanent tooth germ was reportedly bell-shaped [31]. Detection of the third dentition during early childhood facilitates the visualization and characterization of hyperdontia in the mouth of infant and some fetuses. Thus, identifying the third dentition is a valuable tool for exploring its potential for successful tooth regeneration.

5. MSX1 anomalies and tooth agenesis

MSX1 encodes a DNA-binding protein that is located on chromosome 4 [36]. MSX1 interacts with TATA box-binding protein [37] and other transcription factors to regulate transcription rate [38–40]. The MSX1 protein is known to regulate gene expression, which is essential for initiating tooth development during the early phase of growth. The DNA-binding domains in MSX-1 regulate other interacting gene partners associated with pathways leading to tooth formation [41]. Alterations in *MSX1* and *PAX9* expression are associated with autosomal dominant inheritance of tooth agenesis and oligodontia and a decrease in tooth size, respectively [42]. Defects in *MSX1* and *PAX9* disrupt the early phase of tooth formation, leading to the loss of different teeth [43–49]. The nature, presence, and location of mutations in homeobox genes result in altered tooth agenesis phenotypes. For example, missense mutations in *MSX1* result in familial tooth agenesis, nonsense mutations lead to aggravated tooth agenesis and nail abnormalities, and the absence of the C-terminal sequence in MSX1 results in orofacial clefts [50]. *MSX1* and *PAX9* variants are observed in <1% of patients, whereas *WNT10A* variations are frequently detected in 25–50% of patients with congenital tooth agenesis.

6. *PAX9* anomalies and tooth agenesis

PAX9 encodes a transcription factor essential for the natural arrangement and structure of teeth [51–53]. The DNA-binding domain was found in exon 2 of *PAX9*. Mutations in the paired domain of *PAX9* cause tooth agenesis [54,55]. The absence or low expression of or mutations in the start codon of *PAX9* are known to cause critical defects in the premolars [56,57].

7. Other genes anomalies associated with tooth agenesis

A few other genes are known to play a role in oligodontia or other types of tooth agenesis, such as *EDA, WNT10A, AXIN2, LTBP3,* and *TP63* [58].

8. Tooth regeneration in murine models

Following the successful identification and reporting of mice with *USAG-1* deficiency and supernumerary teeth in 2007, Takahashi et al. used mouse models and molecular techniques to demonstrate successful tooth regeneration. Mating mice with congenital tooth agenesis and supernumerary teeth revealed phenotypic changes in a double-t KO mouse. Development of both the maxillae and mandibles was arrested in the early stages in *USAG-1*$^{+/+}$/*Msx1*$^{-/-}$ mice. However, histological observations revealed that all mice lacking both *USAG-1* and *Msx1* had regular third maxillary molars. Following these findings, researchers considered the genomic and functional significance of *EDA1* in tooth agenesis and analyzed *EDA1*$^{-/-}$/*USAG-1*$^{-/-}$ mice for tooth regeneration. *EDA1*$^{-/-}$/*USAG-1*$^{-/-}$ mice had normal teeth, hyperdontia, or combined mandibular molars. Molar hypodontia in the mandible was detected in 75% of the female *USAG-1*$^{+/+}$/*EDA1*$^{-/-}$ and male *USAG-1*$^{+/+}$/*EDA1*$^{+/-}$ mice. Phenotypes such as hair loss and tail kinks, typically associated with tabby mice, were also detected in all *USAG-1*/*EDA1* double KO mice. This study further revealed that by inactivating *USAG-1*, congenital tooth agenesis can be successfully ameliorated during early tooth development and that this inactivation promotes late-stage tooth morphogenesis [59].

9. Use of *USAG-1* antibodies for tooth regeneration

Single systemic and dose-dependent administration of USAG-1-targeting antibodies in *EDA1*-deficient and wild-type mice [59] ameliorated tooth agenesis and promoted normal tooth formation. These findings established a significant role for USAG-1 and USAG-1-targeted antibodies in promoting tooth regeneration. The antibodies generated by neutralizing *USAG-1* action on BMP signaling and reducing low *Lrp5/6* dosage recovered the *USAG-1*-null phenotype, including hyperdontia [59,60]. However, several mice died in this *Lrp5/6* study, thereby obscuring any information on Wnt signaling regulation. Thus, Takahashi's group aimed to overcome these shortcomings by performing further analyses, including detailed protein analysis of additional USAG-1–targeting antibodies. Observations from such experiments have revealed associations between causal genes, including *Msx1* and *USAG-1*, and successful tooth regeneration in congenital tooth agenesis mice.

A single systemic administration of *USAG-1*-targeting antibodies did not cause any side effects in this mouse lineage. Notably, *USAG-1* abrogation prevented the development of cleft palates by regulating Wnt signaling in *Pax 9*-deficient mice [61]. In addition, small-molecule Wnt agonists reportedly correct cleft palate in *Pax9*-deficient mice [62]. *EDA* controls BMP activity [63] and *EDAR* targets *Wnt* genes [64,65]. However, USAG-1-targeting antibodies did not result in tooth recovery in any of these cases. Nonetheless, genes and mutations associated with congenital tooth agenesis may be potential biomarkers for patient selection.

A single systemic dose of EDA antibody rescued congenital tooth agenesis in *EDA*-deficient canines [66]. Likewise, administering a *USAG-1*–neutralizing antibody, which targets BMP signaling, but not Wnt signaling, can rescue congenital tooth agenesis. Thus, *USAG-1*-targeting antibodies can be tailored to focus on specific signaling pathways. Next-generation sequencing and imaging technologies can identify molecular vulnerabilities and thereby focus on *USAG-1*-antibody selection and use for treating congenital tooth agenesis. *USAG-1*-neutralizing antibody did not prevent tooth loss in any of the cases. Nonetheless, genes and mutations associated with congenital tooth agenesis may be potential biomarkers for patient selection.

Takahashi et al. successfully generated new teeth using *USAG-1*-targeted antibodies [59]. After receiving these antibodies, no abnormal symptoms apart from the usual phenotypic changes were observed in wild-type mice compared to *USAG-1*-KO mice. This suggests that, in *EDA1*-deficient mice, the third dentition can be activated using USAG-1-targeting antibodies, which regenerate regular teeth. After analyzing 78 patients with supernumerary teeth, researchers concluded that third dentition was the cause of these additional teeth [26]. This finding suggests strategies to monitor outcomes in patients receiving targeted molecular therapy to stimulate the third dentition. The researchers also demonstrated that systemic application of *USAG-1*-targeting antibodies in ferrets could regenerate a tooth similar to the third dentition. This result is encouraging given that ferrets share dental patterns similar to those of humans. However, the clinical application of *USAG-1*-

targeting antibodies to regenerate lost teeth requires further safety and efficacy validation in nonrodent models.

The inhibition of BMP signalling in early mandible by exogenous Noggin protein resulted in ectopic Barx-1 expression in the distal presumptive incisor mesenchyme and transformation of tooth identity from incisor to molar [67]. However, any specific factors for all the 28 types of human teeth are not identified. It is possible to control the eruption of regenerated tooth with accurate morphology, adequate calcification, correct eruption timing and region by administration of anti- *USAG-1* antibody [29,59]. Because *USAG-1* protein has only the potential to rescue the developmental arrested tooth germ, that had been programmed the certain tooth type [1,2,19,20,22,23,29,59]. Furthermore, our strategy of tooth regeneration is acellular system [59]. It is enough to administrate only anti-USAG-1 antibody [59]. Our observation demonstrates that the morphology of supernumerary teeth is depended on the position. If it erupts in the incisor or molar region, its shape is incisor or molar [59].

10. Advances from teeth atlas

Regenerative therapies require an extensive understanding of the human organs. Whole organ and tissue functional reconstitution and regeneration depend primarily on stem cell composition. In human dental pulp and periodontium, the expression of different genes, including *FRZB*, *THY1*, and *MYH11*, aid in the characterization and classification of mesenchymal stem cells (MSCs). *FRZB* as a reliable marker based on FRZB's ability to markedly identify periodontal region to dental mesenchyme from early stages of odontogenesis, by allowing Wnt molecules and thus regulating the Wnt-dependent transcription [68]. CD90 (also known as Thy-1) as a positive marker to identify dental pulp stem cells and as a cell surface marker proposed to identify mesenchymal stem cells [69–71]. Similar differentiation patterns and results in bone tissues further help identify differences between MSCs from the dental pulp and periodontium [72–75]. Despite these differences, periodontal and pulp MSCs exhibit the same migratory behavior when cultured independently.

Notably, when periodontal and dental pulp MSCs are co-cultured, periodontal MSCs divide rapidly and migrate towards dental pulp MSCs. Thus, both cell types exhibit different proliferation and migration abilities [76]. Complex intercellular interactions rather than transcriptional differences in periodontal and dental pulp MSCs determine the extent of proliferation and migration [74,76]. However, MSCs express different proteins and factors that determine their roles in tooth formation.

Homology analysis revealed that periodontal fibroblasts and MSCs highly express genes encoding collagens, matrix metalloproteinases, and osteonectin [77]. Periodontal fibroblasts express matrix GlA protein and have high affinity for calcium ions [78]. Epithelial-like cells form most periodontal cell types. These epithelial-like cells express signaling proteins such as follicular dendritic cell secreted protein (FDCSP) and WNT10A, which play significant roles in controlling the proliferation and differentiation of periodontal MSCs [79–81].

Additionally, periodontal dental epithelial stem cells have the potential to initiate and develop tooth-associated hard tissues, including the alveolar bone, dentin, and enamel [82,83]. Notably, the signals sent by epithelial stem cells influence the interactions and roles of periodontal MSCs.

Accordingly, cell states, gene, or protein expression, and other unique signatures can explain the dynamic remodeling of the periodontium. This dynamic remodeling is closely associated with tooth masticatory capability. Along with these unique signatures, standard collagen levels, extracellular matrix remodeling, and mineralization prevention are required for the active role of the periodontium [84]. The dental pulp and periodontium are heterogeneous microenvironments that exhibit unique characteristics in each tissue. These special features, including different kind of cells including mucosal immune cells, cellular interactions, oral microbiome, salivary pH and the role of food intake can all become indicators of the tissue microenvironment. The unique microenvironment of the periodontium and dental pulp can drive MSC differentiation to achieve fibroblast-like and osteogenic fates, respectively.

Aside from regeneration efforts, recent studies have also shown that understanding the tooth status can inform various other health conditions, like stress [85], cognitive impairment, and dementia [86]. Single-cell analysis of pulp and periodontal tissues to better understand these conditions may lead to breakthroughs that could advance cell-based regenerative treatments and help identify predictive biomarkers.

However, stem cell therapy should be pursued with caution. Technical challenges involved in these therapies and the associated costs must be considered before using stem cells for tooth regeneration. These considerations can limit stem cell use in tooth regeneration, providing an excellent opportunity for antibody-based drug discovery and single-dose vaccines to treat tooth anomalies.

11. Beyond conventional approaches: Antibody-based tooth regeneration therapeutics

Over the past three decades, extensive research has been conducted using tissue engineering techniques [87,88] to identify standard treatment methods. Owing to cost, safety, and technical limitations, current therapies are ineffective in promoting tooth regeneration. Takahashi's group noted the presence and benefits of activating the third dentition, which provided new research impetus and hope for potential therapeutics to regrow lost teeth in humans, as well as in animal care. They demonstrated the role of USAG-1 in the development of tooth primordia and subsequent tooth regeneration. Furthermore, their research revealed that a lack of tooth development results from congenital tooth agenesis, which is associated with different genetic abnormalities.

For this reason and its associated limitations, the traditional approach involving tissue engineering in regenerative medicine is less commonly used in tooth regeneration. As described in earlier sections, Takahashi's research outcomes suggest that targeting USAG-1 activates the third dentition and effectively treats the different clinical presentations of congenital tooth agenesis. Molecular biomarker discovery could reduce the gulf, improve patient selection for targeted therapies, and achieve precision in tooth regeneration. The latest advances in precision medicine and technologies will promote further discoveries that facilitate tooth regeneration and fulfill patient demands. Further genomic sequencing-driven studies across different ethnic groups are needed to determine and understand the heterogeneous nature of individuals with tooth anomalies. This in-depth understanding would help improve standard care and genetic counseling practices, especially in cases of familial or congenital tooth agenesis.

12. Use of predictive biomarkers

Biomarkers can predict treatment responses, identify potential individuals who may benefit from a clinical trial, and monitor treatment responses. Genomic and functional biomarker discovery has led to the growth of precision medicine, allowing researchers and clinicians to tailor treatment alternatives for patients. Understanding the role of pathogenic mutations in signaling and

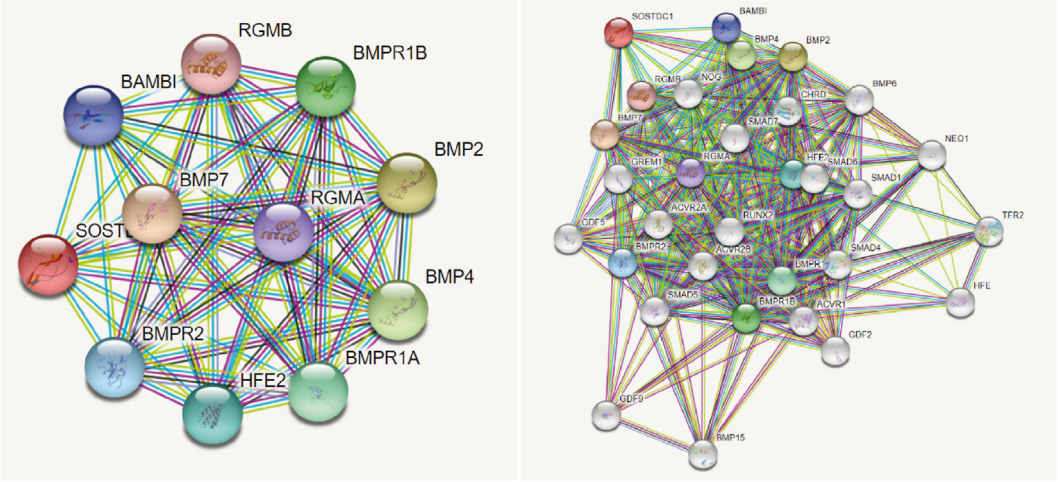

Fig. 2. Picture on the left shows interacting partners of *USAG-1* gene with the default settings in STRING ver 11.5. Picture on the right shows *USAG-1* interacting partners including *SMAD* family genes, *RUNX2* and others.

activation pathways is vital for understanding the response of patients to specific treatments. Next-generation sequencing, mass spectrometry, and imaging technologies can create companion and/or predictive diagnostic markers to identify patients who are most likely to benefit from a specific treatment. There is a clear unmet medical need for a companion and/or predictive diagnostic test that helps identify and stratify patients (based on age, sex, and other clinical features) who are most likely to respond to USAG-1 antibody treatment. Developing a functional biomarker assay could also assist in precision treatment by examining receptor-protein binding interactions under different conditions of tooth agenesis. Analysis of *USAG-1* and its interacting partners will help identify several mutation-linked post-translational modifications and membrane attachments, demonstrating the potential for understanding the functional consequences of genetic mutations and the need to examine the protein-level effects of mutations. The dbSNP search for *USAG-1* resulted in approximately 2,368 variants with or without molecular consequences (missense, frameshift, and/or synonymous), based on their genomic location (such as in introns and upstream).

Consequently, predicting interacting partners at the residue level is crucial for understanding the role of mutations in USAG-1 activity. A brief analysis of the interacting partners of *USAG-1* using STRING [89] helped identify (Fig. 2) partner candidates, such as *BMP* family genes. Furthermore, adding more nodes to the network revealed other significant interacting partners such as the *SMAD* family of genes and *RUNX2*. Understanding the functional relevance of mutations at the protein level has rapidly improved cancer treatment [90]. Thus, translating these innovations into tooth regeneration research could improve our understanding of the role of mutations in the respective proteins. This will reveal the mechanisms by which the differentially expressed isoforms and wild-type proteins play distinct roles in identifying protein components in specific subcellular compartments or interaction partners. Additionally, comparing $^{mut/wt}$ and $^{wt/wt}$ genotypes and protein levels for any post-transcriptional and translational modifications, analyzing gain or loss of function that might affect subcellular localization, and altering downstream signaling might help distinguish diseases associated with the wild type or isoform. A MalaCards search for “tooth agenesis” resulted in ClinVar data with 418 genetic disease variations with a list of genes, such as *LRP6, EDAR, MSX1, PAX9*, and *WNT* family genes [91].

Disease-linked mutations, including somatic and germline variants, are more likely to affect protein–protein interactions. Although no clinically relevant mutations and dysfunctional residues in USAG-1/SOSTDC1 have been reported in ClinVar and

Table 1
Analysis of few gene variants highly associated with tooth agenesis.

Gene	UniProtKB accession id	Variant id	ClinVar id	ClinVar significance	Residue change	Effect on Protein
EDA1	Q92838	rs132630319	11044	Pathogenic	R65G	The mutant residue is more hydrophobic and may disturb the rigidity of the protein at this position
EDA1	Q92838	rs132630320	11045	Pathogenic	Q358E	Residue change might disturb the interaction between the binding domains and could affect protein function.
MSX1	P28360	rs121913129	14879	Pathogenic	R202P	The residue is located in a DNA binding region and will affect the function of the protein
MSX1	P28360	rs104893850	14881	Pathogenic	Q193*X*	Truncated and unstable protein
MSX1	P28360	rs121913130	14886	Pathogenic	M67K	This mutation introduces a charge, which can cause the repulsion of ligands or other residues with the same charge.
MSX1	P28360	rs1553877821	14887	Pathogenic	Gly28fs	NA
MSX1	P28360	rs515726227	127273	Pathogenic	NA	NA
WNT10A	Q9GZT5	NA	36972	Germline & Pathogenic	G95K	Deleterious [92]
WNT10B	O00744	rs766021478	253058	Pathogenic	W262*X*	Truncated and unstable protein
WNT10B	O00744	rs779326570	253057	Pathogenic	R211Q	Change from positive to neutral residue might disturb the binding function
SOSTDC1	Q6X4U4	rs34016012	NA	NA	Q189H	occasionally deleterious

UniProt, respectively, further understanding of its interaction with significantly associated tooth agenesis genes, such as *EDA*, *MSX1*, and *Wnt* family genes, will be valuable. Genes associated with tooth agenesis and pathological variants reported in ClinVar are listed in Table 1. The functions of the proteins encoded by these genes were analyzed using PROVEAN [93] and HOPE [94]. Although ClinVar has assigned pathogenic status to all the variants in Table 1, PROVEAN analysis showed a deleterious status only for the rs121913129 and rs121913130 variants of *MSX1*. Protein structural analysis using the HOPE tool [94] has revealed the significant role of individual mutations in protein structure and function. Future studies could analyze how USAG-1 and its partnering protein variants differ between responders and non-responders to anti-USAG-1 antibody treatment. Furthermore, these bioinformatics tools can help us understand the contribution of these variants to tooth agenesis, thereby validating the accuracy of *in vitro* experiments, patient samples, and previous analyses.

Technological advances in imaging will play a prominent role in the early identification of patients who may benefit from treatment with anti-USAG-1 antibodies. Toregem BioPharma has undertaken a study to develop imaging-based biomarkers for this treatment, and the results will be published for a more comprehensive scientific and public interest. Understanding the incidence rate of a pathological variant, its charge change, mutation-bearing domains, and the resulting disturbances in its interactions with binding partners will play a role in identifying genomic and functional biomarkers. Treating tooth agenesis as a complex disease while understanding hereditary patterns can help elucidate the role of multiple genes in tooth microenvironment and regeneration and the interactions of their transcribed proteins. A comprehensive approach for defining the genomic and functional basis of tooth agenesis provides a more precise and potentially personalized approach for treating tooth anomalies. Thus, examining the active role of pathogenic variants in interacting genes could contribute to the development of specific biomarker assays and identify patients who would benefit from targeted therapies for tooth regeneration.

13. Conclusions

Further research is required to develop more effective treatment strategies for tooth agenesis. Compared to dental implants and dentures, antibody-based treatment is more cost-effective and uses a naturally existing third dentition in humans at certain ages. Anti-USAG-1 antibody treatment in mice is effective for tooth regeneration and can be a breakthrough in treating tooth anomalies in humans. Companion and predictive biomarker discovery will assist in selecting patients who can benefit from precision treatment with anti-USAG-1 antibodies.

Authors' contribution

RV and AM-S prepared the manuscript. HK, YT, C-LH, KB, JT, MS, YT, and KT reviewed the manuscript. All coauthors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable Consent for publication.

Consent for publication

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Conflict of interest

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