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The Regulatory Role of ADAM28 in Tooth Development

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Abstract

Objectives: *ADAM28 gene was screened from the patients with congenital hypoplasia of tooth root (CHTR). According to clinical feature, CHTR was separated into dentin developmental anomaly of tooth root, cementum developmental defect of root, root paramorphia, and root adhesive organ dysplasia. Nowadays, no therapy of CHTR has been detected in the world, and its potential mechanisms are unknown. ADAM28 has been regarded as one of the possible virulence genes for CHTR. The objective of this study was to outline the influence of ADAM28 on proliferation, differentiation, and apoptosis of human odontogenic mesenchymal cells and the regulatory role of ADAM28 in tooth development and action mechanism.*

Methods: *Immunological, cell biological and molecular biological techniques were used for detecting the effects of ADAM28 on odontogenic mesenchymal cells.*

Results: *ADAM28 may actively participate in tooth root development process as a major regulator of proliferation, differentiation, and apoptosis of human odontogenic mesenchymal cells by interacting with Notch signal pathway. The action mechanism might be that ADAM28 release IGF-1 and lead to activation of IGF signaling pathway, and further accelerate cell proliferation. ADAM28 AS-ODN and shRNA interference vector might inhibit catalytic cracking of metalloproteinase and disintegrin domain, and suppress the promoting cell proliferation of EGF–like domain, block Notch signal transmission and participate in negative regulation of apoptosis.*

Conclusions: *ADAM28 metalloproteinase inhibitor could be an effective therapeutic target against CHTR. ADAM28 inhibition can reduce inflammation due to diminished IGFBP-3 cleavage and TNF-α shedding, and may be served as a means of treating CHTR.*

Keywords: *A disintegrin and metalloprotease 28 (ADAM28); congenital hypoplasia of tooth root (CHTR); Proliferation ; Differentiation ; Apoptosis.*

Abbreviations: *ADAM, a disintegrin and metalloprotease; CHTR, congenital hypoplasia of tooth root; HDFCs, human dental follicle cells; HDPCs, human dental papilla cells; HDPSCs, human dental pulp stem cells; HPDLCs, human periodontal ligament cells; HDCLECs, human dental cervical loop epithelial cells; RT, reverse transcriptase; PCR, polymerase chain reaction.*

1. Introduction

A Disintegrin and metalloprotease (ADAM) families are large membrane-anchored glycoproteins composed of diverse domains exerting multiple functions including proteolysis, cytokine regulation, cell adhesion, cell fusion, intracellular signaling, fertilisation, muscle development, neurogenesis and protein ectodomain shedding[1,2].

ADAMs are associated with pathological conditions such as cancer and meanwhile can manipulate various processes including differentiation, proliferation, apoptosis, migration and invasion of tumor cells as well as angiogenesis [3,4,5].

It is known that ADAM28 is a member of the ADAM family in humans and murine with autocatalytic activity. [6,7,8] Previous studies have shown that ADAM28 could facilitate cell proliferation via IGF-1 (Insulin-like Growth Factor-1) signaling axis, and that the IGF system has an essential role in protecting cells from apoptosis [7]. Researches indicated that Zhao et al. screened ADAM28 gene from the patients with congenital hypoplasia of tooth root (CHTR), and further studied the relationship between ADAM28 and tooth development from different levels [9]. According to the clinical

situation, CHTR is separated into dentin developmental anomaly of tooth root, cementum developmental defect of tooth root, root paramorphia (absence, short cone shape), and root adhesive organ dysplasia [10]. Nowadays, no effective therapy of CHTR has been found in the world, and its potential mechanisms are still not clear. Therefore, ADAM28 has been regarded as one of the possible virulence genes for CHTR. CHTR has been proposed to relate with the proliferation and differentiation disorder of odontogenic mesenchymal stem cells [11, 12, 13, 14, 15].

Teeth develop as a result of sequential and reciprocal interactions between the oral ectoderm and the neural-crest-derived mesenchyme. The regulatory gene of tooth development controls advancing morphogenesis and cell differentiation by sequential inductive interactions between the epithelium and mesenchyme [16].

In this review, we elaborated the regulatory role of ADAM28 in tooth development process from prokaryotic expression, eukaryotic expression, antisense oligonucleotide and shRNA interference system and explored the action mechanism.

2. ADAM28 STRUCTURE FEATURES

The nucleotide sequence data about human ADAM28 in this study appear in the GenBank Nucleotide Sequence Databases under accession number NM-014265. The human ADAM28 gene is located on chromosome 8p24.2 whereas the mouse ADAM28 gene is discovered on chromosome [17]. The ADAM28 protein exists as two different isoforms: a classical transmembrane form (ADAM28m) and a shorter (secreted) form, lacking the transmembrane domain (ADAM28s) [6, 7]. Both isoforms, ADAM28m and ADAM28s, are initially produced as proforms of 87 and 65 kDa, respectively, which can be further processed to generate active forms of 55/57 and 42 kDa, respectively. Membrane-bound ADAM28 displays the common structure of an ADAM protease and is composed from N- to C-terminus of the following domains: a signal peptide, a pro-domain, a metalloproteinase domain, a disintegrin, a cysteine-rich, an EGF-like, a transmembrane and a cytoplasmic domain [6, 7, 8]. Structure of secreted ADAM28 is similar to transmembrane ADAM28 except that the cysteine-rich domain is shorter while EGF-like, transmembrane and cytoplasmic domains are missing [6, 7, 8]. Proteolytic activity of ADAM28 has been firstly demonstrated in vitro through the cleavage of recombinant MBP (myelin basic protein) [18]. Other substrates have been further identified upon several studies such as CTGF (Connective Tissue Growth Factor) [19], IGFBP-3 (Insulin-like Growth Factor-Binding Protein-3) [7, 20], vWF (von Willebrand Factor) [17] and Notch2 [21]. IGFBP-3 is the main carrying protein that transports IGFs (Insulin-like Growth Factors) in the blood circulation and regulate their bioavailability for tissues.

ADAM28 releases IGF-1 through the cleavage of IGFBP-3 and leads to activation of the IGF signaling pathway and stimulate cell proliferation [7]. Besides, ADAM28 could also influence hemostasis and angiogenesis in physiological conditions via vWF processing. Actually, vWF exhibits pro-apoptotic properties by inducing tumor cell death and therefore, reducing metastasis formation suggesting an involvement of ADAM28 in cell survival [22].

Moreover, ADAM28 could be involved in Notch2 processing and could play a role in various development mechanisms since Notch2 controls cell fate decisions [21]. Protein sequence analysis indicates that ADAM28 and SVMPs (Snake Venom MetalloProteinases) structures are closely related indicating that they could cleave common substrates such as components of the extracellular matrix (type IV collagen, laminin, fibronectin and proteoglycans) suggesting a distinct role of ADAM28 in tissue reconstruction [6, 23].

Catalytic activity of ADAM28 proteases is regulated by Tissue Inhibitors of Metalloproteinases (TIMPs). Moreover, ADAM28 proteases mediate cell-cell and cell-matrix interactions through the binding of their disintegrin domain to various molecules including integrins. Particularly, ADAM28 binds to α4β1, α4β7 and α9β1 integrins and promotes adhesion of lymphocytes to leukocytes and endothelial cells as well as their migration [24, 25, 26, 27]. Whereas ADAM28 proteolytic activity is found completely inhibited by TIMP-3 and TIMP-4 [7, 18].

3. ADAM28 PROKARYOTIC EXPRESSION SYSTEM

ADAM28 mRNA expression has been primarily identified in human lymphocytes extracted from lymph nodes but its expression is also detected in lymphocytes from blood and other secondary lymphoid tissues such as the spleen [28].

A polyclonal antibody (pAb) against ADAM28 was preparared successfully by zhao et al. previously [9], and the expression and localisation of ADAM28 were firstly examined in murine tooth germ and human dental mesenchymal cells [9]. Immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR) showed that ADAM28 was expressed at each stage of tooth germ development at different levels [9]. The results disclosed that positive staining was found in oral epithelia and mesenchymal cells , while the enamel organ was negatively stained at the bud stage (E13d), This may be clarified by the fact that ADAM28 contains an EGF-like domain, which may have certain functions of EGF such as the motivation or maintenance of undifferentiated cell proliferation during embryonic development [29]. The patterns of EGF-like domain binding in different tissues suggest that EGF may play a key role in the odontogenesis by stimulating epithelial proliferation during initial epithelial bud generation and branching morphogenesis [29].

Up to the cap stage (E16d), positive staining was found in the enamel knot, basement membrane, dental papilla and dental follicle, and negative staining was found in the outer and inner enamel epithelium [9]. These data suggest that the main tendency to tooth development has directed to mesenchymal differentiation, and ADAM28 may be involved in formation of the enamel knot and basement membrane. This is supported by the fact that the primary enamel knot is a signalling center that forms at the tip of the epithelial tooth bud. The signals of the enamel knot also have important roles, together with mesenchymal signals, in regulating the patterning of the cusps and hence the shape of the tooth crown. In molar teeth, secondary enamel knots appear in the enamel epithelium at the sites of the future cusps [30]. This evidence suggests that ADAM28 may be an important signal molecule in the regulation of tooth crown shape. Moreover, the formation of dental papilla starts at the onset of transition from the bud to the cap stage of tooth morphogenesis, and this is regulated by epithelial signals from the primary enamel knot. Dental papilla cells and dental follicle cells have the ability to differentiate into odontoblasts and cementoblasts, respectively, and the advancing differentiation within the odontoblast cell lineage is regulated by sequential epithelial signals [30]. Therefore, it is proposed that ADAM28 may link cell differentiation to morphogenesis.

Recent data suggest that Notch signalling pathway is also important for proper odontogenesis [31] and controls cell fate during the development of a wide range of tissues and organs. [32] Furthermore, the Notch gene encodes a transmembrane receptor with an extracellular domain carrying multiple EGF-like repeats and a cytoplasmic domain required for signal transduction [33].

As mentioned above, ADAM28 protein contains a transmembrane domain, a cytoplasmic domain and an EGF-like domain, which include components of the extracellular matrix and may play an important role in signalling transduction, intracellular protein maturation, or localisation to sites of activity [6].

In addition, the basement membrane, which is part of the extracellular matrix that separates the inner enamel epithelium from the dental papilla in the early stages of tooth development, is believed to participate in epithelial—mesenchymal interactions during organogenesis [34]. Previous work showed that basement membranes in both monkey and shark teeth at early stages of development are specialised for functions such as anchoring and firm binding, which are essential for the successful growth and differentiation of odontoblasts, and for better understanding of the mechanism of development and maintenance of the tooth, and specialisations of tooth basement membranes in relation to their roles [35]. This evidence reveals that ADAM28 may participate in tooth morphogenesis, mesenchymal cell differentiation and signal transduction at the cap stage by interacting with multiple signal molecules such as Notch signalling.

However, at the early bell stage (E18d), weakly positive was only found in stellate reticulum cells of enamel organ, which suggested that ADAM28 could mainly keep the maturation of the enamel organ [9].

At the late bell stage (P2d), positive staining was found in ameloblasts, enamel matrix, HERS and dental papilla cells [9]. It is well known that the epithelial root sheath is the germinal center of tooth root development. Therefore, ADAM28 may be involved in root development. Differentiation of dental papilla cells and dental follicle cells was directly associated with development of HERS [36]. Amelogenin has been shown to have signalling activity and may

play a crucial role in the terminal differentiation of both ameloblasts and odontoblasts [37]. Previous study indicated that ameloblastin is likely to participate in the attachment of ameloblasts to the enamel surface and mineralisation of enamel [38]. These data suggested that ADAM28 might distinctly enhance proliferation and differentiation of ameloblasts and dental papilla cells, and further influence the secretion of enamel matrix.

When entering the hard tissue development stages (including crown and root), positive staining for ADAM28 was found in ameloblasts, odontoblasts, predentins, HERS, dental papilla cells and dental follicle cells [9]. Positive staining in dental follicle cells suggested that ADAM28 may play a role in maintaining the structure of the dental follicle and the inducement to cervical loop. The enamel matrix and predentin were further subjected to mineralisation, while HERS and dental follicle cells were proceeding differentiation into cementoblasts, and later mineralisation of cementum. Therefore, it is conceivable that ADAM28, as an active signal molecule expressed in the enamel knot, basement membrane and mesenchymal cells, may induce odontoblast differentiation.

Furthermore, the expression of ADAM28 in human odontogenic mesenchymal cells revealed that strongly positive staining was found in the cytoplasm and cytomembrane of HDPCs, positive staining was found in HDFCs, HPDLCs and HDPSCs [9]. RT-PCR results showed that a specific band of 255 bp was seen in human dental papilla cells （HDPCs), human dental follicle cells (HDFCs), human periodontal ligament cells (HPDLCs), human dental pulp stem cells (HDPSCs) and human dental cervical loop epithelial cells (HDCLECs) at different transcription levels [9].

In a word, it is reasonable to suggest that ADAM28 may participate in tooth development and the regulation of odontogenic mesenchymal cells through progressive inductive interactions between the epithelium and the mesenchyme.

4. Influence of ADAM28 Eukaryotic Expression Vector and AS-ODN on Biologic Features of Odontogenic Mesenchymal Cells

Eukaryotic expression plasmid containing ADAM28 (NM-014265) coding region and ADAM28 antisense oligodeoxynucleotides (AS-ODN) were constructed and synthesized [11]. Then we respectively transfected them into human dental papilla mesenchymal cells (HDPMCs), HDFCs, human periodontal ligament stem cells (HPDLSCs), and HDPSCs and detected their effects on proliferation, differentiation and apoptosis of cells by MTT assay, cell cycle detection, ALP activity and Annexin V-FITC/PI analysis [11,12,13].

4.1. HDPMCs Expressions

The results indicated that overexpression of ADAM28 promoted the proliferation and specific differentiation of HDPMCs, while blockage of ADAM28 exerted the opposite effects and induced apoptosis of HDPMCs. ADAM28 S-ODN transfected HDPMCs stained positively for cytoplasmic bone sialoprotein (BSP), osteopontin (OPN) and dentin sialophosphoprotein (DSPP). Whereas ADAM28 AS-ODN could significantly inhibit the expressions of BSP, OPN and DSPP [11].

The extracellular matrix (ECM) is a biologically active tissue composed of a complex mixture of macromolecules, that in addition to serving a structural function, also widely affect the cellular physiology of an organism.[39] In addition, the ECM relays multiple signals from the cell microenvironment to direct proliferation and differentiation during tissue development,[40] and plays significant roles in tissue physiology through interaction with cells and interstitial fluid transport. These roles include regulating cell morphology, growth and intercellular signaling.[41,42] Cell adhesion, migration, proliferation and differentiation are examples of biological processes influenced by the composition and structural organisation of surrounding extracellular matrices.[43] It is well known that BSP, OPN, DSPP and type III **collagen** are thought to be closely related to cytodifferentiation, matrix secretion, mineralisation and bone formation, BSP and OPN have also been regarded as critical markers for **osteoblast** differentiation [44], formation and remodelling of the mineralised tissue matrix [45].

Since DSPP represents specific phenotypic markers for odontoblast secretory stage, and under certain circumstances dental papilla mesenchymal cells have the capability of osteogenic differentiation [46], this result would be indicative that ADAM28 is indispensable for the lineage-specific differentiation of **HDPMCs** [11].

These evidence identified an unrecognized hypothesis that ADAM28 may function as

positive regulator of growth and differentiation of **HDPMCs** and act as an important molecule mediating reciprocal epithelial– mesenchymal signaling transmission during tooth root development [11].

4.2. HDFCs Expressions

Whereas overexpression of ADAM28 accelerated the HDFCs proliferation and inhibited differentiation of HDFCs, moreover ADAM28 could significantly inhibit the secretion of OPN and type III collagen of HDFCs [12]. Research indicated that OPN is a highly acidic secreted phosphoprotein predominantly accumulating at tissue interfaces in bone and teeth [47] and implicated as being an interfacial adhesion molecule [48]. Moreover, OPN has multiple functions in mineralization via the contributions of osteoblasts and osteoclasts [49]. OPN promotes the attachment of osteoblasts, allowing them to perform the functions necessary for osteogenesis [49]. Type I and Type III collagen are regarded as important factors mediating chemotaxis, cell attachment and migration. They probably serve as developmental signals, which may be critical to the regulation of interactions between periodontal fibroblasts and the root surfaces, and thus influence the regeneration and repair of the periodontium [45]. Moreover, type III collagen is present in reticular fibres which provide elasticity to tissues and the characteristics that type III collagen imparts to tissues in vivo make it a worthwhile molecule to study for tissue engineering applications including odontogenic tissue and cell regeneration [50].

This is probably due to the process that ADAM28 eukaryotic plasmid might enhance its catalytic activity of metalloproteinase, cleave matrix protein and rebuild tissue structure to regulate cell proliferation liveness [18]. Moreover, ADAM28 comprises an EGF-like domain, which may possess certain functions of EGF such as the stimulation or maintenance of undifferentiated cell proliferation during embryonic development [51].

4.3. The Expressions of HPDLSCs and HDPSCs

On one hand, ADAM28 eukaryotic plasmid group showed the highest expression level in HPDLSCs, whereas the AS-ODN group displayed the lowest. Furthermore, the overexpression of ADAM28 enhanced the proliferation of HPDLSCs and inhibited the specific differentiation of HPDLSCs, whereas the inhibition of ADAM28 induced apoptosis. ADAM28 AS-ODNs were able to significantly inhibit CAP expression, and ADAM28 had a positive correlation with CAP [13]. Cementum attachment protein (CAP) is the only cementum-specific protein, which plays a critical role in cementogenesis during regeneration of periodontal tissues and wound healing [52, 53].

The application and results from a number of antisense inhibition and shRNA interference strategies serve to provide efficient and specific methods for suppressing gene expression during mandibular morphogenesis, early tooth development and root formation [54].

On the other hand, ADAM28 had a negative regulatory effect on the proliferation of HDPSCs. ADAM28 eukaryotic plasmid could significantly inhibit the HDPSC proliferation, promote specific differentiation of HDPSCs, induce apoptosis, and enhance the DSPP expression, whereas ADAM28 AS-ODN produced the opposite effects [14]. The results coincidentally showed that ADAM28 could inhibit HDPSCs proliferation during the process of differentiation enhancement, which was in accordance with the previous concept that proliferation and differentiation are oppositely correlated with each other because of the existence of dual-function regulators involved in controlling both the processes [55]. Therefore, it is reasonably proposed that some components of ADAM28 could act not only as inhibitors for the HDPSCs proliferation but also as promoters for cytodifferentiation [14].

DSPP was first cloned from developing teeth and thought to be tooth specific [56]. Moreover, DSPP may be implicated in the initial mineralization process of the dentin matrix collagen [57] based on the defective dentin formation in DSPP-null mice [58]. The expression levels of DSPP were directly correlated with a dentin developmental defect of tooth root. ADAM28 played a positive regulatory role in the expression level of DSPP in HDPSCs, which suggested that the ADAM28 gene could significantly promote the functional differentiation of HDPSCs into odontoblasts and further promote dentin development.

As demonstrated above, the biological function of the HDPSCs was almost contrary to that of the HPDLSCs [13], which suggested that ADAM28 might have a positive regulatory effect on the proliferation of HDPSCs and

display negative correlations with differentiation and apoptosis of HPDLSCs.

From the study mentioned above [13], we analyzed that the mechanism might be that the HPDLSCs and HDPSCs originally come from the dental follicle cells and dental papilla cells, respectively, whose biological features have significant differences. Meanwhile, the ADAM28 expression distribution in diverse odontogenic cells was also different, which could lead to the developmental defect of tooth root.

5. Influence of ADAM28 AS-ODN on Proliferation, Differentiation and Apoptosis of Human Gingival Fibroblasts (HGFs)

The detection of ADAM28 AS-ODN on the biological features of HGFs indicated that AS-ODN group displayed the lowest expression level in HGFs, meanwhile the ADAM28 S-ODN group showed the highest. Furthermore, blocking of ADAM28 could inhibit the proliferation of HGFs, enhance HGFs differentiation and induce apoptosis of HGFs.

Whereas, overexpression of ADAM28 generated the opposite effects and inhibited apoptosis. ADAM28 AS-ODN was able to notably suppress the expressions of core binding factor α1 (cbfα1) and CEMP1, and ADAM28 had positive correlations with cbfα1 and CEMP1 [15]. Researches indicated that cbfα1 is an essential transcription factor for osteoblast differentiation and bone formation [59], and Cbfα1 isoform is closely related to the development and differentiation of tooth germ [59]. Furthermore, Cbfα1 has been detected in odontoblasts, periodontal ligament cells and cementoblasts [60]. In vitro experiments showed that the cementum Protein 1 (CEMP1) promotes cell attachment and differentiation [61,62].

Moreover, overexpression of CEMP1 induces expression of bone and cementum-matrix proteins in non-osteogenic cells such as human gingival fibroblasts [63]. Previous report demonstrated that CEMP1 can promote osteoblastic and/or cementoblastic cell differentiation of HGF in vitro, and participate in the mineralization process of human putative cementoblasts in vitro [61]. These evidence suggested that CEMP1 might have a potential function in cementum and bone formation [63]. These provided conspicuous evidence that ADAM28 may play a crucial role in root development as a potential regulator of growth, differentiation, and apoptosis of HGFs.

6. Effects of ADAM28 shRNA Interference Vector on Biologic Feature of HPDLSCs

PGPU6/GFP/Neo-ADAM28-shRNA1-4 interference vector were successfully constructed and efficiently transfected into HPDLSCs, and ADAM28-shRNA1 displayed the highest inhibition efficiency. Furthermore, ADAM28-shRNA1 could significantly suppress proliferation and differentiation, and induce apoptosis of HPDLSCs. ADAM28-shRNA1 was able to significantly inhibit the expression levels of CAP and Pax9, and enhance expression levels of Bcl-2 and Bax proteins [64]. Similarly, ADAM28 AS-ODN could significantly induce apoptosis of HPDLSCs and inhibit CAP expression, and ADAM28 had a positive correlation with CAP.

It is well known that CAP promotes cell attachment of fibroblasts and other periodontal cells, and its activity is mediated by α5ß1 integrin receptors on cell surfaces [65]. PAX9 gene is strongly expressed in the oral mesenchyme and required for the mesenchymal expression of BMP4, MSX1, and LEF1 during tooth development [66]. Moreover, PAX9 is essential for the development of a variety of organs and skeletal elements. Mutations in PAX9 are the most common genetic cause of tooth agenesis [67].

Functionally, the Bcl-2 family of proteins play an important role in the control of apoptosis and Bcl-2 gene codes for a 25-26 kDa protein. [68, 69, 70]. Previous experiments have established that Bcl-2 is capable of blocking a wide range of apoptotic stimuli in a variety of different cell types [71]. Bax was isolated as a Bcl-2 binding protein in immunoprecipitation experiments and Bax is a 21 kDa protein that is known to play an essential role in apoptosis [72, 73]. In overexpression experiments, Bax committed the cell to apoptosis and could antagonise the protective effect of Bcl-2.

These evidence suggested that ADAM28 shRNA interference vector and AS-ODN could both participate in negative feedback mechanism of apoptosis, and inhibit the catalytic cracking of metalloproteinase and disintegrin-like domain, thus suppress the promoting cell proliferation of epidermal growth factor (EGF)–like domain.

7. THE RELATIONSHIP BETWEEN PROLIFERATION, DIFFERENTIATION AND APOPTOSIS DURING ADAM28 EXPRESSIONS IN ODONTOGENIC MESENCHYMAL CELLS

As described above, ADAM28 dramatically regulates the proliferation, differentiation and apoptosis of human odontogenic mesenchymal cells from different levels.

Cell proliferative kinetics is an essential feature during tooth morphogenesis since high cell proliferative activity is desirable for rapid increase in volume of the tooth germ and tooth cusp formation [74]. During dentinogenesis, the extracellular matrix synthesised by the odontoblasts coincides with their expression level of ALP.33 ALP activity is generally used for an important reference index in detecting odontogenic mesenchymal cytodifferentiation and matrix mineralization [75]. ADAM28 could promote HDFCs proliferation during the process of differentiation inhibition, which was in accordance with the previous concept that proliferation and differentiation are inversely correlated with each other due to the existence of dual-function regulators participating in controlling both the processes.35 Therefore, it is reasonably proposed that some components of ADAM28 could act not only as promoters for cell proliferation but also as inhibitors for cytodifferentiation [12].

Apoptosis is a key process in the embryological development of the tooth, periodontal ligament and supporting oral tissue in the progression of oral disease, bone resorption, immunological response and inflammation, and in wound healing and certain pharmacological effects [76].

Previous studies indicated that epithelial–mesenchymal tissue interactions prevent apoptosis, cell-apoptosis and proliferation are interconnected and interact with each other in root development, and they both participate in shaping of teeth. Understanding of apoptosis regulation in the vestigial tooth primordia can help to elucidate the mechanism of their suppression during evolution [77]. Moreover, Apoptosis represents an important process in teeth morphogenesis and remodelling during tooth development. Hence, apoptosis may be a general mechanism for the silencing of embryonic signalling centres including odontogenesis signal transmission [78].

In addition, Notch signaling pathway has been proved to be of paramount importance for proper odontogenesis [79, 80] and control cell fate during development of lots of organs [81,82,83]. The structural similarity between Notch and ADAM28 is in that both contain transmembrane domain which carries extracellular epidermal growth factor (EGF)-like repeats renders the latter possible role in parallel to the Notch signaling pathway. Differently, expression of Notch signaling molecules is restricted within dental epithelial cells that give rise to the ameloblasts, while ADAM28 has a propensity to be ubiquitously expressed in almost all tissues and cells of developing tooth germ by our studies [11-15].

8. SUPPOSED ACTION MECHANISM

Based on above findings, we propose the action mechanism might be that ADAM28 release IGF-1 and lead to activation of the IGF signaling pathway, and further accelerate cell proliferation. ADAM28 inhibition may reduce inflammation due to diminished IGFBP-3 cleavage and TNF-α shedding, and may be considered to be a means of treating CHTR. Moreover ADAM28 AS-ODN and shRNA interference vector might inhibit the catalytic cracking of metalloproteinase and disintegrin-like domain, and suppress the promoting cell proliferation of EGF–like domain, block Notch signal transmission and participate in the negative regulation of apoptosis.

9. CONCLUSION

In a word, this study overviews convincingly that ADAM28 may be instrumental in tooth root development as a major regulator of proliferation, differentiation, and apoptosis of human odontogenic mesenchymal cells by interacting with Notch signal pathway, and ADAM28 metalloproteinase inhibitor could be an effective therapeutic target against CHTR.

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11. AUTHOR CONTRIBUTIONS

Zheng Zhao: Conceptualization, Methodology, Formal analysis, Investigation, Supervision,

Funding acquisition, Writing – Original draft preparation, Writing – Reviewing and editing.

Di Yin: Data curation, Formal analysis, Investigation, Software.

Fei Zhao: Project administration, Resources, Validation.

Jie Li: Methodology, Software, Supervision.

Fei Qin: Data collection，Visualization, Proof reading.

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