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Short communication

MicroRNAs and Tooth Development; Model for Organogenesis?

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Abstract

The developing tooth can be regarded as a typical vertebrate organ starting as an epithelial bud that subsequently develops into a mature organ, a process that entails extensive epithelial-mesenchymal interactions. Functions of various signaling molecules and gene expression during odontogenesis have appealed considerable interest. Although it now is evident that microRNAs (miRNAs) are implicated in tooth development, their regulative role remains essentially unknown. MicroRNAs have been studied in the context of most ectodermal organs but only a few functional studies of their role in tooth development have been published so far. The fine-tuning of this epithelial-mesenchymal network via the miRNAs` regulation is still poorly understood. In view of similarities between development of the tooth and other organs of ectodermal origin, studies of miRNAs function during tooth development will likely have wide biological relevance.

Introduction

Knowledge of which and when specific genes and molecules are expressed during differentiation, development and mineralization of dental tissues is important for understanding tooth development and for being able to devise molecular approaches in diagnosis and treatment of developmental aberrations. Therefore, how dental tissues, teeth, and dentitions are formed is not only an intriguing question in molecular and developmental biology, but also one of clinical significance. Furthermore, research on the molecular biology of tooth formation has importance in fields other than odontology, for instance in biology, zoology, and medicine, serving as a model in organ development.

Ectodermal organs are useful models for the analysis of genetic network regulation because of the complex inter-tissue and intra-tissue molecular dialogues and their accessibility for classic culture studies. Ectodermal organs, such as hair, skin, cornea, mammary glands, salivary glands, and teeth are widely studied due to powerful clinical translation and importance. At embryogenesis, the mammalian tooth develops out of cells originating from stomodeal ectoderm that forms the epithelium and underlying neural-crest ectomesenchyme[1]. Interactions between the epithelial and mesenchymal tissues constitute an essential regulatory mechanism of tooth development, in which growth factors play a major role[2]. The tooth is therefore a convenient experimental model for studying the basic mechanisms of organ development, including differentiation, cellular interaction, morphogenesis, and production and mineralization of extracellular matrices.

Tooth development requires the use of more than 2400 genes, integrated both in terms of time and space[3]. Today, we know that during evolution developmental regulatory genes have been conserved to a great degree. The regulative gene networks implicated in odontogenesis are also involved during development of other vertebrate organs. Most of these genes may be connected to signaling pathways regulating cell-cell interactions, e.g. signaling molecules, receptors and transcription factors. Gene knock-out mice for some of these genes exhibit arrested tooth development, and developmental defects also in other tissues. Mutations in several of these genes in humans have been identified as causes of dental defects, e.g. hypodontia[4].

MicroRNAs are a class of non-protein-coding RNAs (~22 nt in length) that are involved in important biological functions, such as development and cell physiology[5]. Substantial interdependence between miRNAs and activity of signaling pathways is likely, as suggested by evidence of miRNA dependent regulation of cell cycle and of apoptosis[6]. It is evident that miRNAs combinatory regulate the expression of about 30% of protein-coding genes by cleavage or translational regulation of their specific messenger RNAs (mRNAs)[7]. MicroRNAs provide a convenient and efficient pathway for regulation of gene expression at a posttranscriptional level, and they exert their effects by base pairing with the target mRNAs in a much more compact and energy-efficient manner compared to regulatory molecules like enzymes and hormones[8]. MicroRNAs are often organized in tandem and closely clustered on the chromosome. This arrangement can have particular significance in the control

of gene expression. Clustered miRNAs with similar sequences may regulate a set of mRNA targets, and these closely related characteristics may allow miRNAs to function as powerful regulators at the cellular level. In some cases miRNAs have been proposed to act relatively promiscuously and to inhibit multiple targets[9]. Many different miRNAs can target several genes in their 3ÙTRs. In this way it is possible for combinatorial action of multiple miRNAs in order to significantly inhibit translation. For most miRNAs, however, these predictions have not yet been tested. The prediction of target genes for particular miRNAs are a difficult computational challenge, and is impaired by the lack of data describing alterations in levels of individual protein in response to changes in levels of miRNAs.

The miRNAs involvement in the tooth genetic network fine-tuning was already suggested in 2008[10]. The expression of miRNAs in developing tooth germ is highly dynamic. Some miRNAs exhibit high levels of expression during the early stages of odontogenesis, while other miRNAs are abundantly expressed at later developmental stages. Lately, investigation has shown that miRNAs probably regulate tooth morphogenesis and ameloblast differentiation, perhaps largely by fine-tuning conserved signaling networks[11]. Another study suggested discrete sets of miRNAs to be expressed in molars compared with incisors, as well as in epithelium compared with mesenchyme[12]. Conditional knockout of *Dicer1* in the dental epithelium of the *Pitx2-Cre* mouse results in multiple and branched enamel-free incisors and cuspless molars together with changes in incisor patterning and in incisor and molar size and shape[12]. These observations indicated an involvement of miRNA in tooth morphogenesis and patterning, as well as in the terminal cell differentiation and tissue homeostasis. Although available evidence suggests that miRNAs may profoundly influence tooth germ development, the mechanism by which this is achieved remains unknown.

Understanding of miRNA functions likely requires loss-of-function studies *in-vivo*. It has been shown that intravenous administration of a novel class of chemically engineered oligonucleotides termed "antagomirs" or "anti-miRs" resulted in a marked decrease on levels of corresponding miRNA levels in tissues[9]. Injection of an anti-miR to miR-122 resulted in up-regulated expression of hundreds of genes predicted to be repressed by miR-122 based on the presence in these genes of a miR-122 recognition motif in their 3´UTR region. Paradoxically, anti-miR treatment also revealed a significant number of down-regulated genes that may be activated by miR-122. The mechanisms through which miRNAs activate gene expression (transcription) may be 1) direct (e.g. via chromatin remodeling) and 2) indirect (e.g. the suppression of a transcriptional repressor)[9].

In tooth germs miR-214 exhibit a higher level of expression at post-natal stages of odontogenesis[10]. In order to study the function of miR-214 during murine tooth development Sehic et al. selected this miRNA as a target for silencing by *in-vivo* transfection with anti-miR-214. The effects on gene expression and tooth phenotype were consequently studied[13]. In developing tooth germ, transfection of 50 pmol anti-miR-214 resulted in significantly lower levels of expression of miR-214 compared to the levels of scrambled control[13]. About 1200 mRNAs and 6 miRNAs were found differentially expressed following in vivo transfection with 50 pmol of anti-miR-214. Altered levels of mRNAs were also reflected in corresponding changes in levels of the corresponding encoded proteins. Furthermore, the enamel of the resulting mature tooth exhibited evidence of hypomineralization[13]. Bioinformatic analysis suggested that inactivation of miR-214 markedly diminished enamel biosynthesis, while biosynthesis of contractile proteins (e.g. myosins, actins) was stimulated. Levels of associated transcription factors, such as Ezh2 and Twist1, were found to have increased both the mRNA and protein level. The levels of miR-199a, miR-199b were significantly increased in transfected tooth germs[13]. Both of these miRNAs, together with miR-214, are encoded by *Dnm30os*, a "non-coding" RNA transcript derived from the 14th intron of *Dnm3*. These three miRNAs are therefore encoded as a polycistronic gene, transcribed as a single RNA transcript, which is subsequently processed into the three mature miRNAs. This is a phenomenon shared by several other miRNAs, including those of the miR-17-92 or miR-106a-363 clusters[14]. Recent studies on miR-17-92 cluster demonstrated that this cluster is down-regulated during odontogenesis, and it was suggested that the miR-363, one of the members from the miR-106a-363 cluster, may be implicated in this process[15,16]. Further investigation on the interplay between the clusters and their mRNA targets in genetic network regulation of tooth development is therefore of outmost importance.

Conclusion

MicroRNAs are the key regulators during embryonic development and are also implicated in disease. Understanding the biological function of miRNAs requires mapping of their expression time and space as well as identification of mRNA targets. Ultimately, an improved understanding of miRNA functions may lead to novel therapeutic tools both in dentistry and medicine. Therefore, the involvement and mechanisms of miRNAs in tooth development should be further studied.

References

1. Thesleff, I., Sharpe, P. Signalling networks regulating dental development. (1997) Mech Dev 67(2): 111-123.
2. Thesleff, I., Vaahtokari, A. The role of growth factors in determination and differentiation of the odontoblastic cell lineage. (1992) Proc Finn Dent Soc 88 (Suppl 1): 357-368.
3. Landin, M. A., Shabestari, M., Babaei, E., et al. Gene Expression Profiling during Murine Tooth Development. (2012) Front Genet 3: 139.
4. Simmer, J. P., Hu, J. P. Dental enamel formation and its impact on clinical dentistry. (2001) J Dent Educ 65(9): 896-905.
5. Bushati, N., Cohen, S. M. microRNA functions. (2007) Annu Rev Cell Dev Biol 23: 175-205.
6. Jovanovic, M., Hengartner, M. O. miRNAs and apoptosis: RNAs to die for. (2006) Oncogene 25(46): 6176-6187.
7. Filipowicz, W., Bhattacharyya, S. N., Sonenberg, N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? (2008) Nat Rev Genet 9(2): 102-114.
8. Mattick, J. S., Makunin, IV. Small regulatory RNAs in mammals. (2005) Hum Mol Genet 14(Spec No 1): R121-132.
9. Krutzfeldt, J., Rajewsky, N., Braich, R., et al. Silencing of microRNAs in vivo with 'antagomirs'. (2005) Nature 438(7068): 685-689.
10. Jevnaker, A. M, Osmundsen, H. MicroRNA expression profiling of the developing murine molar tooth germ and the developing murine submandibular salivary gland. (2008) Arch Oral Biol 53(7): 629-645.
11. Michon, F., Tummers, M., Kyyronen, M., et al. Tooth morphogene-

sis and ameloblast differentiation are regulated by micro-RNAs. (2010) Dev Biol 340(2): 355-368.
12. Cao, H., Wang, J., Li, X., et al. MicroRNAs play a critical role in tooth development. (2010) J Dent Res 89(8): 779-784.
13. Sehic, A., Risnes, S., Khuu, C., et al. Effects of in vivo transfection with anti-miR-214 on gene expression in murine molar tooth germ. (2011) Physiol Genomics. 43(9): 488-498.
14. Mendell, J. T. miRiad roles for the miR-17-92 cluster in development and disease. (2008) Cell 133(2): 217-222.
15. Jevnaker, A. M., Khuu, C., Kjole, E., et al. Expression of members of the miRNA17-92 cluster during development and in carcinogenesis. (2011) J Cell Physiol 226(9): 2257-2266.
16. Khuu, C., Jevnaker, A. M., Bryne, M., et al. An investigation into anti-proliferative effects of microRNAs encoded by the miR-106a-363 cluster on human carcinoma cells and keratinocytes using microarray profiling of miRNA transcriptomes. (2014) Front Genet 5: 246.

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