A Time-course Network Approach to Investigate TF-microRNA Co-regulation During Craniofacial Development

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Abstract

Cleft lip with or without cleft palate (CL/P) is the most common facial malformation. However, elucidating the etiology of CL/P has been hindered by the lack of appropriate biological data during craniofacial development. The objective was to investigate TF and miRNA co-regulation in the facial primordia at embryonic day 10.5 to 14.5. Our study provided a deeper understanding of molecular regulation mechanisms, which can aid in managing malformations for CL/P.

Introduction

Cleft lip with or without cleft palate (CL/P) is the most common congenital malformation during craniofacial development. It is critical to understand the abnormal events resulting in CL/P, thus elucidating the etiology of CL/P. However, this effort has been hindered by the lack of appropriate biological data during craniofacial development. MicroRNAs (miRNAs) are small non-coding RNAs that silence gene expression at the post-transcriptional level. Transcription factors (TFs) are proteins that activate or repress the expression of genes or miRNAs at the transcriptional level. Previous studies indicate that both transcriptional (e.g. TF) and post-transcriptional (e.g. miRNA) regulation plays critical roles in craniofacial development, and its disruption results in craniofacial anomalies, such as CL/P. However, a systematical investigation to identify developmental stage-specific regulation and network, especially in their co-regulation, has not been done yet. In this study, we developed a novel time-course network based analytical approach to clarify the co-regulation of TFs and miRNAs in a temporal-specific manner during craniofacial development.

Methods

The mouse gene/mRNA expression data of 30 samples isolated from the maxillary processes at embryonic days 10.5, 11.5, 12.5, 13.5 and 14.5 (E10.5-E14.5), as well as miRNA expression data of 10 samples, were obtained from the FaceBase Portal. The differentially expressed genes, TFs and miRNAs were identified through the comparison between two adjacent time points. We further developed an integrative method to investigate the co-regulatory mechanisms between genes, TFs and miRNAs, by assembling Feed-Forward Loops (FFLs) in which at least two nodes are differentially expressed.

Results

We identified four co-regulatory networks along the five developmental stages in the maxillary processes of mouse embryos. In the resultant network from E10.5 to E11.5, several genes (e.g. Col1a1, Fzd3), TF (e.g. Foxm1, Hif1a) and miRNAs (e.g. miR-340-5p, miR-129-5p) were identified (Figure 1). Specifically, it was found to be involved in the canonical Wnt signaling pathway, one of the most important pathways that modulate the formation of the upper lip in many studies. The subsequent co-regulatory network (E12.5 vs E11.5) implied the importance of hormones. For example, estrogen can facilitate the growth and migration of cranial neural crest cells, which derive the mesenchyme of lip and palatal shelves. The network in later stages highlighted the importance of bone and tissue development and remodeling, which is concordant with developmental biology with the growth and re-orientation of...
palate shelves during E12.5-14.5. Of note, the miR-129-5p, which was previously reported to regulate epithelial-to-mesenchymal transformation⁴, has been consistently identified as a key regulator in each time-course regulatory network, suggesting its critical role in the facial morphogenesis. Additionally, Egr1 is a key regulator in our co-regulatory networks from E11.5 to 13.5, which has been shown to involve in the development of cranial cartilage in mice and zebrafish⁶.

Figure 1. The TF-miRNA co-regulation network from E10.5 to E11.5 visualized by Cytoscape. A triangle (green) represents a miRNA; a diamond (brown) represents a TF; and a circle (pink) represents a non-TF protein-coding gene. The area of the node is proportional to the degree in the network.

Conclusion
In conclusion, the regulatory networks function differently at the different developmental stages to collectively contribute to normal craniofacial development. Our study provided a deeper understanding of dynamic molecular regulatory mechanisms during facial morphogenesis, which can aid in managing malformations for CL/P. The novel key regulators identified in our analysis provide a new insight regarding to the morphological process of CL/P.

References