2023 FaceBase Forum

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Whole Genome Sequencing Identifies Pathogenic Variants in Cleft Case-parent Trios

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Background: Orofacial cleft results from defective craniofacial tissue fusion during embryonic development. The etiology of OFC is multifactorial involving genetics and environmental factors.

Purpose: To investigate contributions of *de novo* and inherited (novel and rare) variants in craniofacial genes using whole genome sequencing (WGS) data of European cleft case-parent trios.

Methods: WGS was conducted on 15 case-parent trios. Variants were annotated using GATK.

Annotated variants were filtered based on genotype quality ≥20 and read depth ≥10. Novel

(MAF=0) and very rare (MAF < 0.001) *de novo* and inherited variants in exons were identified.

Variants predicted to be deleterious across polyphen, SIFT and CADD were prioritized. Genes harboring these variants were assessed for contributions to craniofacial development using Mouse Genome Informatics (MGI), Online Mendelian Inheritance in Man (OMIM) and CleftGeneDB. Genes involved in craniofacial development in any of the 3 databases were prioritized and their implication in OFC assessed with DECIPHER and literature search.

Results: We identified a very rare pathogenic *de novo* variant rs781121274 (MAF= 0.0001) in *VCAN* gene that is expressed in relevant craniofacial tissues. We also identified 8 very rare deleterious inherited variants in *CFAP45*, *NISCH*, *CDKN1A*, *NOTCH1*, *FUZ*, *COL6A2*, *IL11RA*, and *AKAP6.* OFC is reported in individuals with deletion of loci that contain *CFAP45, NISCH, NOTCH1, COL6A2* or *AKAP6*.

Conclusions: Our study provides additional evidence for the role of *VCAN*, *CFAP45*, *NISCH, NOTCH1*, *FUZ*, *COL6A2* and *AKAP6* in OFC development. This is the first time *CDKN1A* and *IL11RA* are implicated in OFC. Future functional experiments to validate these observations are needed.

The International Family Study of Nonsyndromic Orofacial Clefts: Design and Methods

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Background: The majority of research to understand the risk factors of nonsyndromic orofacial clefts (NSOFCs) has been conducted in high-income populations. Although patients with NSOFCs in low- and middle-income countries (LMICs) are at the highest risk of not receiving care, global health infrastructure allows innovative partnerships to explore the etiologic mechanisms of cleft and targets for prevention unique to these populations.

Purpose: The International Family Study (IFS) is an ongoing case-control study with supplemental parental trio data designed to examine genetic, environmental, lifestyle, and sociodemographic risk factors for NSOFCs in 8 LMICs (through August 2020).

Methods: Interview and biological samples are collected for each family. The interview includes demographics, family history of cleft, diet and water sources, maternal pregnancy history, and other lifestyle and environmental factors.

Results: Seven of 8 countries are currently summarized (2012-2017) for a total of 2955 case and 2774 control families with 11 946 unique biological samples from Vietnam, Philippines, Honduras, Madagascar, Morocco, Democratic Republic of the Congo, and Nicaragua. The phenotype distribution was 1641 (55.5%) cases with cleft lip and palate, 782 (26.5%) with cleft lip (CL), and 432 (14.6%) with cleft palate (CP).

Conclusion: The International Family Study is the largest case set of NSOFCs with an associated biobank in LMICs currently assembled. The biobank, family, and case-control study now include samples from 8 LMICs where local health care infrastructure cannot address the surgical burden of cleft or investigate causal mechanisms. The International Family Study can be a source of information and may collaborate with local public health institutions regarding education and interventions to potentially prevent NSOFCs.

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Investigation of Mesenchymal Lineage Diversification During Embryonic Palatal Development

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Background: Cleft palate is a common birth defect that occurs due to palatal shelf formation defects during embryonic developmental stages. The development of the embryonic palate is a complex process which requires precise spatial and temporal regulation at the cellular level. The detailed regulatory mechanism of this process remains to be elucidated.

Purpose: The aim of this project is to perform gene profiling at single-cell resolution for the developing palatal mesenchymal cells to better understand the molecular regulatory mechanism required for proper palatal mesenchymal fate determination during embryonic stages.

Methods: Palatal shelves of embryonic day (E) 12.5, E14.5, E15.5, and E18.5 mouse embryos were collected for analysis. Single-cell RNA sequencing analysis was performed to compare cell-type specific gene expression profiles in E12.5 to E18.5 cells from the palatal shelves.

Results: We performed integration analysis of scRNA-seq data from E12.5-E18.5 palatal cells. Using molecular markers associated with individual cellular types, we mapped the in vivo identities of different cell clusters in the developing palatal shelves. We focused on the palatal mesenchymal cells and identified hard palate-associated osteogenic and odontogenic cell clusters as well as soft palate-associated perimysial clusters. We have also identified a previously unreported mesenchymal cell population that decreases as development proceeds, thus likely associated with the putative palatal mesenchyme progenitors. We are currently performing lineage trajectory analysis to identify lineage commitment for palatal mesenchymal cells as well as regulon analysis for putative lineage-specific and location-specific makers and regulators.

Conclusion: scRNA-seq analysis is a valuable tool to enable the understanding of how morphologically identical palatal mesenchymal cells undergo different fate decisions during embryonic development.

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Trp53 supports mesenchymal stem cell homeostasis through regulating vascular architecture

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Background: Mesenchymal stem cells (MSCs) reside in niches within stromal tissues, where they serve as a reservoir for generating mesenchymal derivatives during tissue homeostasis, repair, and regeneration. Microenvironmental cues provided by stem cell niches are important for regulating the fate of MSCs, and the detailed mechanisms of the crosstalk between them are of significant interest. Blood and lymphatic vasculature have well-known roles in transporting oxygen and nutrients, as well as removing waste and CO2. However, the vasculature's role as a niche component in regulating MSCs remains largely unclear.

Purpose: To investigate the role of vasculature in regulating stem cell homeostasis in adult tissue.

Methods: The transgenic mouse model used in this study is Gli1-CreERT2;Trp53fl/fl, in which Trp53 was deleted in Gli1+ cells and their progenies. Cellular and molecular experiments used in this study included immunohistochemistry, in situ hybridization (RNAscope), CoIP, RNA-seq, scRNA-seq and ChIP-qPCR.

Results: In this study, we use adult mouse incisors as a model to reveal the functional significance of Trp53 in regulating the vascular architecture to maintain tissue homeostasis. Our study shows that the loss of Trp53 in GLI1+ progeny reduces THBS2, which leads to alterations in the vascular architecture including an increase of arteries and a decrease of other vessel types. These changes further result in an increased deposition of artery-derived PDGFA and PDGFB at the proximal MSC region, where they interact with PDGFRA and PDGFRB. More importantly, PDGFRA+ and PDGFRB+ cells are subpopulations of MSCs.

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Joubert syndrome protein Arl13b impacts palatogenesis and craniofacial myogenesis

Eva Janečková, Jifan Feng, Tingwei Guo, Aileen Ghobadi, Angelita Araujo-Villalba, Heliya Ziaei, Thach-Vu Ho, and Yang Chai

Background: The craniofacial region determines our identity and is essential for our daily activities, communication, and intellect. Craniofacial malformations represent 75% of congenital disorders, with the most common being cleft palate. Furthermore, craniofacial phenotypes are associated with 30% of ciliopathies. However, detailed mechanisms underlying the relationship between cilia and craniofacial development is not known.

Purpose: To analyze the detailed mechanism by which cilia serve as key mediators of tissue-tissue interactions among cranial neural crest (CNC)-derived mesenchymal and mesoderm-derived muscle cells during palate and muscle development.

Methods: Histology, RNAScope, immunofluorescence and MicroCT analyses of Wnt1-Cre;Arl13bfl/fl, and Arl13bfl/fl mice.

Results: Conditional deletion of Arl13b, a Joubert syndrome-associated gene, specifically in CNC-derived mesenchymal cells using Wnt1-Cre caused severe defects leading to death at birth. Decreased numbers of ciliated cells were observed in Wnt1-Cre;Arl13bfl/fl mice. Focusing on the craniofacial region, complete or soft palate cleft was observed, coupled with changes in maxillary, palatine, and mandibular bones. Furthermore, we observed defects in the tongue and soft palatal muscles. Surface area and volume of the tensor veli palatini, palatoglossus, and levator veli palatini were reduced in Wnt1-Cre;Arl13bfl/fl mice. These results suggest that cilia in CNC-derived mesenchymal cells mediate signaling to the mesoderm-derived myogenic cells through tissue-tissue interactions.

Conclusion: This study presents a mouse model of Joubert syndrome and an opportunity to expand knowledge about regulatory inputs of primary cilia, their interactions and complexity. This will contribute to understanding the precise etiology of craniofacial malformations which present with very high incidence in humans.

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Piezo1 is indispensable for dental mesenchymal progenitor cell fate

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Background: Piezo1 is a large mechanosensitive ion channel protein that is activated by mechanical stimuli, triggering intracellular chemical signals. The conversion of mechanical stimuli to biochemical signals and the resultant activation of downstream signaling pathways have been shown to play a crucial role in the regulation of cellular functions and behaviors. Although mechanosensitive signals play an essential role in sensing and responding to environmental cues during embryological development, the regulation of these signals remains poorly understood.

Purpose: The aim is to analyze the role of Piezo1 in dental mesenchymal progenitor fate determination during tooth root development.

Methods: This study explores these mechanisms of Piezo1 using mouse tooth roots as a model.

Results: During tooth root development, Piezo1 is expressed in the mesenchyme and mediates mechanical responses that are required for dental mesenchymal progenitor fate. The results of this study show that loss of Piezo1 in Glil+ root progenitor cells can lead to shortened roots, defective dentin, and defective periodontal tissue development.

Conclusion: These results illustrate the importance of these mechanosensitive ion channel proteins for dental mesenchymal progenitor fate, making Piezo1 indispensable in tooth root development.

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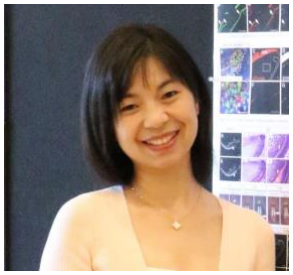

Sensory nerve regulates progenitor cells via FGF-ETV5/PTCH1-Hh axis in morphogenesis

Fei Pei, Li Ma, Tingwei Guo, Mingyi Zhang, Quan Wen, Jifan Feng and Yang Chai

Background: Nerves play important roles in organ development and morphogenesis. Stem and progenitor cells are self-renewing and can differentiate into different cell lineages responsible for building the craniofacial organs. The mechanism by which nerves regulate stem/progenitor cell behavior in organ morphogenesis has not yet been deeply explored.

Purpose: Here, we use tooth root development as a model to investigate the molecular mechanism by which sensory nerves regulate fate decisions of cranial neural crest-derived progenitor cells to modulate organ morphogenesis.

Results: We find that nerves are enriched in the apical papilla and reach the coronal papilla in the molar at the initiation of tooth root development. Through scRNAseq analysis of the trigeminal ganglion and molar, we reveal several signaling pathways that connect the sensory nerve with the molar, of which FGF signaling appears to be the most important. We show that Fgfr2 is expressed in the mesenchymal progenitor cells during tooth root development. The loss of FGF signaling leads to shortened roots with compromised proliferation and differentiation of progenitor cells. Furthermore, Hh signaling is impaired in Fgfr2 mutant mice. Mechanistically, ETV5 downstream of FGF signaling binds to the promoter region of Ptch1 to regulate Hh signaling and proliferation. Modulation of Hh signaling can rescue the tooth root defects in Gli1-CreER;Fgfr2fl/fl mice.

Conclusion: Collectively, our findings elucidate the nerve-progenitor crosstalk during tooth root morphogenesis, reveal the molecular mechanism of FGF-ETV5/PTCH1-Hh signaling cascade.

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ARID1B Maintains MSC homeostasis through BCL11B-mediated non-canonical Activin signaling

Mingyi Zhang, Jifan Feng, Tingwei Guo, Fei Pei, Junjun Jing, Jiahui Du, Yang Chai

Background: Epigenetic regulation plays a crucial role in controlling stage- and tissue-specific gene expression during development and tissue homeostasis. As a core subunit of the BAF complex, ARID1B acts as a DNA-binding protein that regulates chromatin accessibility. However, the role of ARID1B as an epigenetic regulator in maintaining stem cell homeostasis remains largely unexplored.

Purpose: To investigate the role of Arid1b in regulating cell fate commitment of MSCs and their homeostasis using the adult mouse incisor model.

Methods: Gli1-CreERT2;Arid1bfl/fl mice were generated to study the roles of ARID1B in mouse incisor MSCs.

Results: In this study, we found that loss of ARID1B within the GLI1+ MSC lineage impairs mouse incisor growth and tissue homeostasis. Through comprehensive analysis of single-cell (sc)RNA-seq, scATAC-seq, and bulk RNA-seq data, we identified Bcl11b expression is directly repressed by ARID1B. Moreover, loss of ARID1B leads to upregulation of non-canonical TGF-β/Activin signaling, specifically the p-ERK pathway. BCL11B acts as a mediator, directly regulating the gene expression of Inhba which encodes the subunit for ligand Activin A, thereby modulating non-canonical Activin signaling. Notably, a reduction in Activin signaling completely rescues the phenotype of ARID1B loss.

Conclusion: Our study highlights the critical roles of ARID1B in maintaining MSC homeostasis and its intricate interaction with non-canonical TGF-β/Activin signaling, providing valuable insights into the epigenetic regulatory network and its potential implications for stem cell regulation in the future.

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The Essential Role of Arid1a and Arid1b in Palatogenesis

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Background: Cleft palate is one of the most common craniofacial congenital abnormalities, affecting major physiological functions such as swallowing, breathing, and speech. Palatogenesis requires the timely regulation of specific genes and flawless control of cell-fate identity. The roles of particular transcription factors and protein complexes in the regulation of cell fate identity in hard and soft palate development are of paramount importance. ARID1A and ARID1B are two mutually exclusive subunits of SWI/SNF, a chromatin remodeling complex, that plays an important role in repressing tumorigenesis. Mutation of these two genes is also implicated in various developmental disorders, suggesting they may play important roles in cell fate determination.

Purpose: To analyze the role of Arid1a and Arid1b in palatal mesenchymal cells during hard and soft palate development.

Methods: Histological analyses, RNAScope, immunofluorescence staining, and MicroCT analyses of control and Osr2-Cre;Arid1a fl/fl;Arid1bfl/fl mice.

Results: Deletion of Arid1a and Arid1b in palatal mesenchymal cells utilizing Osr2Cre, which affects palatal mesenchyme, results in severe defects in the palatal region. Osr2-Cre;Arid1a fl/fl;Arid1bfl/fl mice show complete cleft palate with severe defects in the development of soft palatal muscles such as tensor veli palatini, palatoglossus, levator veli palatini, and palatopharyngeus. These results suggest an essential role for Arid1a and Arid1b in palatogenesis through tissue-tissue interactions.

Conclusion: This study emphasizes the regulatory potential of Arid1a and Arid1b in mesenchymal cells during palatogenesis. Our results will contribute to understanding the underlying etiology of craniofacial malformations, specifically cleft palate, and suggest the potential role of Arid1a and Arid1b in other developmental processes.

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