Developmental Biology 426 (2017) 97–114

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Original research article

Systems biology of facial development: contributions of ectoderm and mesenchyme

Joan E. Hooper[a,b,*], Weiguo Feng[a,c], Hong Li[c], Sonia M. Leach[d], Tzulip Phang[b,e], Charlotte Siska[b], Kenneth L. Jones[f], Richard A. Spritz[g], Lawrence E. Hunter[b,h], Trevor Williams[a,c]

[a] Department of Cell and Developmental Biology, University of Colorado School of Medicine, 12801 E 17th Avenue, Aurora, CO 80045, USA
[b] Computational Bioscience Program, University of Colorado School of Medicine, 12801 E 17th Avenue, Aurora, CO 80045, USA
[c] Department of Craniofacial Biology, University of Colorado School of Dental Medicine, 12801 E 17th Avenue, Aurora, CO 80045, USA
[d] Department of Biomedical Research, National Jewish Health, 1400 Jackson Street, Denver, CO 80206, USA
[e] Department of Medicine, University of Colorado School of Medicine, 12801 E 17th Avenue, Aurora, CO 80045, USA
[f] Department of Pediatrics, University of Colorado School of Medicine, 12801 E 17th Avenue, Aurora, CO 80045, USA
[g] Human Medical Genetics and Genomics Program, University of Colorado School of Medicine, 12800 E 17th Avenue, Aurora, CO 80045, USA
[h] Department of Pharmacology, University of Colorado School of Medicine, 12801 E 17th Avenue, Aurora, CO 80045, USA

ARTICLE INFO

Keywords:
Transcriptome
Functional genomics
Co-expression modules
Epithelial-mesenchymal interaction
Orofacial clefts

ABSTRACT

The rapid increase in gene-centric biological knowledge coupled with analytic approaches for genomewide data integration provides an opportunity to develop systems-level understanding of facial development. Experimental analyses have demonstrated the importance of signaling between the surface ectoderm and the underlying mesenchyme are coordinating facial patterning. However, current transcriptome data from the developing vertebrate face is dominated by the mesenchymal component, and the contributions of the ectoderm are not easily identified. We have generated transcriptome datasets from critical periods of mouse face formation that enable gene expression to be analyzed with respect to time, prominence, and tissue layer. Notably, by separating the ectoderm and mesenchyme we considerably improved the sensitivity compared to data obtained from whole prominences, with more genes detected over a wider dynamic range. From these data we generated a detailed description of ectoderm-specific developmental programs, including pan-ectodermal programs, prominence- specific programs and their temporal dynamics. The genes and pathways represented in these programs provide mechanistic insights into several aspects of ectodermal development. We also used these data to identify co-expression modules specific to facial development. We then used 14 co-expression modules enriched for genes involved in orofacial clefts to make specific mechanistic predictions about genes involved in tongue specification, in nasal process patterning and in jaw development. Our multidimensional gene expression dataset is a unique resource for systems analysis of the developing face; our co-expression modules are a resource for predicting functions of poorly annotated genes, or for predicting roles for genes that have yet to be studied in the context of facial development; and our analytic approaches provide a paradigm for analysis of other complex developmental programs.

1. Introduction

Facial development requires finely choreographed growth and morphogenesis of bilaterally paired nasal, maxillary and mandibular prominences that converge to form the nose and the upper and lower jaws. In the mouse (reviewed in Depew et al., 2002), the discrete prominences arise by embryonic day (E) 10, originating as mesenchymal bulges encased in an overlying layer of ectoderm and surrounding the primitive oral cavity. By E13 these separate structures have fused to form an integrated unit (summarized in Fig. S1). Facial mesenchyme is derived from both the mesoderm and the neural crest cell populations and eventually forms the bone, cartilage, connective tissues and muscles of the face. The cranial ectoderm gives rise to the epidermis as well as the lining of the oral and nasal cavities. Moreover, via

* Correspondence to: 12801 E 17th Avenue, rm 12103. University of Colorado School of Medicine, Aurora, CO 80045, USA.
E-mail addresses: Joan.hooper@ucdenver.edu (J.E. Hooper), wgfeng2009@gmail.com (W. Feng), Hong.Li@ucdenver.edu (H. Li), Leachs@njhealth.org (S.M. Leach), tzu.phang@ucdenver.edu (T. Phang), charlotte.siska@ucdenver.edu (C. Siska), kenneth.l.jones@ucdenver.edu (K.L. Jones), Richard.Spritz@ucdenver.edu (R.A. Spritz), Larry.Hunter@ucdenver.edu (L.E. Hunter), trevor.williams@ucdenver.edu (T. Williams).

http://dx.doi.org/10.1016/j.ydbio.2017.03.025
Received 31 October 2016; Received in revised form 23 March 2017; Accepted 23 March 2017
Available online 29 March 2017
0012-1606/

placodal intermediates (reviewed in Singh and Groves, 2016), the ectoderm provides critical components of sensory organs, exocrine glands, and teeth.

Although mesenchymal tissues make up the bulk of the embryonic facial prominences, crucial patterning information is relayed to these cells by surrounding tissues including the ectoderm, endoderm and neural tube (Adameyko and Fried, 2016; Chai and Maxson, 2006; Singh and Groves, 2016). The facial ectoderm provides both permissive and instructive signals that are required for normal development of the underlying mesenchyme. Thus, manipulation of the embryonic facial ectoderm by either genetic or surgical means results in major developmental defects, including orofacial clefts (OFCs). The ectoderm also receives critical signaling input from mesenchyme to regulate its growth, to maintain its competence to drive face formation and to diversify its derivatives. Human clinical analyses and animal model studies have shown that normal face formation requires the integration of multiple signals between the ectoderm and mesenchyme (reviewed in Van Otterloo et al., 2016; Yuan et al., 2016). Some of these – including Fibroblast Growth Factors (Fgfs), Bone Morphogenetic proteins (BMPs), Wnts, Hedgehogs (Hhs), Platelet Derived Growth Factors (PDGFs), Retinoic Acid (RA), and endothelin – are well known, but others remain to be identified. This intricate signaling crosstalk coordinates the convergent growth and morphogenesis of the facial prominences that is essential for aligning these structures prior to fusion (e.g. Geetha-Loganathan et al., 2014; Green et al., 2015; Hu et al., 2015; Linde-Medina et al., 2016; Suzuki et al., 2016). Subsequent interactions between the apposed epithelia then consummate lip and palate fusion (reviewed in Kousa and Schutte, 2016). The complex interplay of signaling, growth, morphogenesis and fusion that occur during face formation, presumably accounts for the susceptibility of this process to genetic and environmental insults, reflected by the prevalence of human craniofacial defects (reviewed in Dixon et al., 2011; Twigg and Wilkie, 2015).

Despite the importance of the ectoderm in facial morphogenesis, surprisingly little is known about the genetic programs that define the facial ectoderm and its derivatives, how those programs are influenced by mesenchymal or environmental cues or how the signals from the ectoderm influence the genetic programs of the adjacent mesenchyme. To understand the ectodermal genetic programs and how they coordinate and integrate with the mesenchymal programs to drive facial development, it is important to obtain a comprehensive understanding of the cellular and molecular changes that occur in each of these tissues during the early stages of facial development. Previous transcriptome studies of human, mouse or chick facial development have provided considerable information concerning gene expression in discrete tissues or regions at specific developmental stages or have generated gene expression profiles of whole prominences over a defined time course (Bhattacherjee et al., 2007; Brinkley et al., 2016; Brugmann et al., 2010; Brunskill et al., 2014; Buchtová et al., 2010; Cai et al., 2005; Ding et al., 2016; Feng et al., 2009; Garaffo et al., 2013; Han et al., 2014; Iwata et al., 2012; Mima et al., 2013; Musselmann et al., 2011; O'Connell et al., 2012; Potter and Potter, 2015; Warner et al., 2014). However none of these datasets have sufficient temporal breadth and spatial resolution to identify the contributions of the ectoderm to the genetic and signaling programs that are crucial for facial morphogenesis. To address this gap, we have generated a transcriptome resource from separated ectoderm and mesenchyme of the developing mouse face at 24 h intervals between E10.5 and E12.5 – a period that is critical for establishing overall facial shape as well as for the fusion of the lip and primary palate (Fig S1). This has allowed us to identify an ectodermal program that is radically different from the mesenchymal program that has dominated previous studies. In addition we have leveraged the spatial, temporal and tissue-specific dimensions of the data, using co-expression to generate functional modules specific to facial patterning. These modules can be used to predict functions of individual genes, to generate hypotheses about the developmental processes represented by the modules and to investigate how module member genes may act together to effect those processes.

2. Materials and methods

2.1. Sample preparation, data capture and pre-processing

All animal experiments used inbred C57BL/6 J mice (Jackson Labs) and were performed in accordance with protocols approved by the University of Colorado Denver (UCD) Animal Care and Usage Committee. Animal husbandry, embryo staging by a combination of embryonic day and morphological criteria (Theiler staging), as well as sample preparation, was as described in Feng et al. (2009). Details of the dissections, morphological landmarks and tissue separation are further described in Li and Williams (2013) and its accompanying video presentation. Specifically, ectoderm and mesenchyme from dissected facial prominences were separated by 'peeling' following Dispase II digestion for 15 min at 37 °C (Roche, cat no. 04 942 078 001). Fig. S1 illustrates the tissues taken at each timepoint with the boundary of the maxillary and nasal prominences at the naso-lacrimal groove. The nasal prominences were processed intact, rather than being separated into lateral and medial nasal components to assist with direct comparison to data in Feng et al. (2009). Due to the intimate interdigitation of the olfactory epithelium and the mesenchyme, the nasal prominence mesenchyme samples included the olfactory epithelium. Finally, at E12.5, protruding tongue tissue was trimmed from the mandible samples to avoid excessive muscle gene expression signatures.

Dissected tissues from multiple embryos were pooled to generate at least 5 μg total RNA from each sample; this required an average of 50 embryos at E10.5, 18 embryos at E11.5 and 8 embryos at E12.5. Total RNA was purified using Trizol (Invitrogen, Carlsbad, CA) and the RNAeasy MiniKit (Qiagen, Germantown, MD). 2–5 μg of total RNA was random primed to generate cDNA, from which biotin-labeled cRNA was derived and used to probe Affymetrix MoGene-1.0-st-v1 microarrays (Affymetrix, Santa Clara, CA). We chose this platform because it has been previously used for studies of the earliest stages of mouse face development (Brunskill et al., 2014, see Fig S1). Probe preparation and microarray analyses were carried out by the UCD Gene Expression Core Facility using standard procedures recommended by the manufacturer (Affymetrix). Three biologically independent replicates for each condition allowed for statistical analysis. Raw data were processed with Affymetrix Power Tools (apt-probeset-summarize, v 1.91, Robust Multi-array Average; RMA) to normalize and summarize probeset expression levels, and for DABG (detected above background at $p > 0.05$) analysis. MGI Gene symbols were mapped onto the probesets using MoGene-1_0-st-v1 Transcript Cluster Annotations, Release 34 (4/7/14). Statistics of microarray data are summarized in Table S1 and Fig S2. Gene expression data from this study, both as.cel files and as a probeset-by-sample expression spreadsheet, are available via GEO (GSE62214) and FaceBase (FB00000803) respectively. Plots of expression profiles for each gene across age, prominence and tissue, as well as for the earlier whole prominence data (Feng et al., 2009), are available in an accompanying Data in Brief (Leach et al., Submitted to Journal – Data In brief).

2.2. Statistical analyses

All data and statistical analyses were performed using custom scripts in R, available upon request. After filtering out probesets that were not detected above background in at least one sample, and whose variance was less than the median of all probesets (median filtering), 9457 differentially expressed probesets were identified among the remaining 12072 probesets by 3-way analysis of variance (ANOVA), two-sample *t*-test for significance at $p=0.01$ and correction for multiple testing (p.adj < 0.01; Benjamini and Hochberg, 1995). Hierarchical