Mouse NCCs migrate into pharyngeal arches and interact with endoderm and ectoderm.
Neural Crest Cells (NCCs) Differentiate into Cartilage and Bone of the Face
Discrete embryonic prominences form coordinately during vertebrate craniofacial development

Dixon et al (2011)
Nature Review Genetics

ethmoid (FNP)

trabecula (Palate)
Do miRNA sub-populations control region-specific craniofacial morphogenesis?

Aim 1: Deep sequencing to characterize mature miRNA expression in different tissues during craniofacial development
   – Physical dissection in mouse followed by miRNA-seq:
     • FNPs, Maxillae, Palatal shelves
   – “Genetic dissection” in zebrafish

Aim 2: Expression analysis in mouse and zebrafish

Aim 3: Test functional significance of tissue-specific differences
   – Inject zebrafish embryos to test results of gain- and loss-of-function
Uploaded data to Hub so far

1. All raw data and bioinformatic processing from mouse miRNA-seq:
   - E10.5 FNP, MAX
   - E11.5 FNP, MAX
   - E12.5 FNP, MAX, PAL
   - E13.5 FNP, MAX, PAL
   - E14.5 FNP, MAX

2. Biological replicates and new bioinformatic processing-Uploaded in one month

3. Expression of 20 miRNAs examined in mouse frontal sections- 10 sections per probe throughout the head

4. Expression of ~20 miRNAs examine in zebrafish whole mount in situ hybridization

5. ~16 functional analysis in zebrafish by overexpression and knockdown
Overview of miRNA biogenesis

Isolation of mouse midfacial and palatal shelves by microdissection at E10.5-14.5

Iwata, Bringas and Chai, FaceBase
Pipeline

Flowchart

Illumina FASTQ

FASTX-Toolkit

Quality Filter

Remove Adapter/Barcode

Output FASTA

Remove reads < 14bp or > 30bp

Count reads

BLASTn to reference genome

BLAST results

Sequence Binning

Excel/R Output
## Mirbase Annotations

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<tr>
<th>Species</th>
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<td>Human</td>
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<td>Mouse</td>
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<td>Zebrafish</td>
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Mouse Ref. chr. 9

Binning (Full Length Perfect Matches)

AACCCGTAGATCCGAACTTG

100% identity, full-length match, Most abundant

BLAST results
100% identity, full-length match, overhang (or underhang)
Binning (Not Full-Length)

Mouse Ref. chr. 9

BLAST results

41339520

41339542

AACCCGTAGATCCGAACTTG

AACCCGTAGATCCGAACTTT

AACCCGTAGATCCGAACTTGTG

100% identity, not full-length
Binning (One mismatch)

**Mouse Ref. chr. 9**

BLAST results

One mismatch, possibly full-length
# Pileup and Associated Read Depth

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Expression profiles of miRNAs in mice

<table>
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<tr>
<th>miRNA</th>
<th>Embryonic Age</th>
<th>Expression Profile</th>
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<tbody>
<tr>
<td>miR-10b</td>
<td>E13.5</td>
<td>spinal cord, DRG, intestine</td>
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<tr>
<td>miR-20a</td>
<td>E13.5</td>
<td>eye, lung, brain</td>
</tr>
<tr>
<td>miR-23b</td>
<td>E12.5, E13.5</td>
<td>naris, vibrissae, TG, DRG, palatal shelf, maxilla, tongue, nasal and tongue epithelium, tongue muscle, incisor, otic capsule, malleus, diencephalon, limb muscle, mesothelium, intervertebral space, foregut, gastrointestinal neurons</td>
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<tr>
<td>miR-24-1</td>
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<td>vibrissae, TG, DRG, nasal epithelium, maxilla, tongue, incisor, limb muscle, gastrointestinal neurons, mesothelium, intervertebral space, pinna</td>
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<td>miR-34b</td>
<td>E13.5</td>
<td>brain</td>
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<td>miR-122a</td>
<td>E13.5</td>
<td>liver</td>
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<td>miR-124-3</td>
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<td>brain, FNP, TG, pancreas, liver, craniofacial mesenchyme, intestinal neurons</td>
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<td>miR-128-1</td>
<td>E13.5</td>
<td>no expression</td>
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<td>miR-128-2</td>
<td>E12.5, E13.5</td>
<td>maxilla, TG, DRG, tongue, diencephalon, telencephalon, ocular muscles, limb, mesothelium, ribs</td>
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<tr>
<td>miR-133b</td>
<td>E12.5, E13.5</td>
<td>vibrissae, maxilla, TG, tongue, intervertebral space, hyoid, ocular muscles, diencephalon, muscle, mesothelium, ribs</td>
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<td>miR-195</td>
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<td>miR-666</td>
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<td>miR-206</td>
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<td>miR-335</td>
<td>E12.5</td>
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miR-23b is expressed in mouse facial structures at E12.5

Maxilla enriched:
miR-23b is expressed in zebrafish facial structures as well as notochord and trunk muscle
miR-23b overexpression results in a cleft or size reduction in the ethmoid plate in zebrafish.
miR-23b knockdown results in a size reduction in Meckel’s cartilage and the ethmoid plate in zebrafish.
miR214/199 are expressed in developing cartilage
miR214/199 are expressed in an opposite pattern to *sox9a*, but is co-expressed with *sox9b*
miR214/199 overexpression results in Trabeculae and Ceratobranchial defects

UIC

miR-214 OE
30-50uM

Trabeculae junction and midline defects

miR-199 OE
10-30uM

Trabeculae junction and ep shape

miR214/199 co-OE
3/1uM

Increased phenotype

Mostly loss of 1cb

Mostly loss of 1cb

Profound delay in formation
Summary

• RNA-seq data illustrates the complexity in miRNA expression in midfacial region.
• miRNA expression in mouse and zebrafish shows specific expression.
• Functional analysis of selected miRNAs in zebrafish suggests a role for miR133b in palatal development, miR23b in cartilage formation and miR214/199 in Trabeculae and Ceratobranchial development.
• Co-expression studies with genes that are potential targets suggests gene regulatory networks in midface development.
Potter, Chai
mRNA-seq

Predicted targets

miR-1

Spoke project interactions
Potter, Chai
mRNA-seq

Predicted targets

miR-1

PAL  FNP

1  2  3

... 1000

PAL  FNP

PAL  FNP

PAL  FNP

perfect match
mRNA degraded

Spoke project interactions
Potter, Chai
mRNA-seq

Predicted targets

miR-1

PAL FNP

1
2
3
...
1000

PAL FNP

PAL FNP

PAL FNP

PAL FNP

target

some mismatch

translation inhibited

downstream target

of miR target

Spoke project interactions
Acknowledgements

University of Colorado Anschutz Medical Campus
• David Clouthier
• Kristin Artinger
• Hai-Lei Ding

University of Oregon
• John Postlethwait
• Peter Batzel
• Thomas Desvignes
• Brian Eames
• John Letaw
Neural Crest Cells form Multiple Derivatives during Development

1 Specification

2 Specified cranial derivatives

3 Specified trunk derivatives

Migration

Specification

Rohon-Beard/MesV sensory neurons

Neurons and glia of cranial ganglia

Cartilage and bone

Connective tissue

Sympatho-adrenal cells

Pigment cells

Sensory neurons and glia

Modified from Knecht and Bronner-Fraser, 2002

Differentiation
Mouse NCCs migrate into pharyngeal arches and form the craniofacial skeleton

Clouthier et al, 2010 AJMG 152A, 2967
Ternary plot of miRNA abundance in different midfacial tissues at E13.5
Palatal Development in Mammals

Craniofacial Defects and Cleft Lip and Palate Thomason and Dixon, 2009
Palatal Development in Mammals
**miR-133b is expressed in mouse facial structures at E12.5**

Palate enriched:

- G: mv
- H: mx
- I: t, ps
- J: t, ps
- K: t
- L: t, oc
miR-133b is expressed in zebrafish trunk and facial muscle

miR-23b