Alternative RNA splicing of transcripts encoding protein serine/threonine kinases downstream of PDGFR signaling in the facial mesenchyme

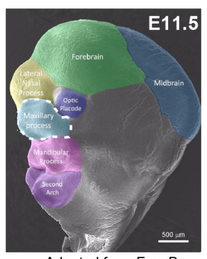
Cassandra B. Minne[1], Brenna J.C. Dennison[1], Eric D. Larson[2], and Katherine A. Fantauzzo[1,3]

[1]Department of Craniofacial Biology; [2]Department of Otolaryngology – Head and Neck Surgery; [3]RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO

ABSTRACT

Craniofacial development is a complex morphogenetic process, disruptions in which result in highly prevalent human birth defects. Signaling through the platelet-derived growth factor receptors (PDGFRs) plays critical roles in this process in humans and mice. However, the gene expression changes that mediate cellular activity downstream of PDGFRα and/or PDGFRβ signaling are incompletely understood. Here, we performed sequencing of maxillary process mesenchyme RNA from E11.5 mouse embryos that lack *Pdgfra*, *Pdgfrb* or both in the neural crest lineage to examine the transcriptional output in each context. DESeq2 analysis identified 23, 20 and 25 genes that were differentially expressed between *Pdgfra*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$, *Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ and *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ samples as compared to wild-type, respectively. In contrast, rMATS analysis detected over 5,000 differential alternative RNA splicing (AS) events per genotype compared to wild-type samples, with the majority of events involving skipped exons. Gene ontology (GO) analysis of the genes encoding the transcripts in the skipped exon category of each genotype revealed an enrichment for protein serine/threonine kinase activity functioning within the MAPK and/or PI3K signaling pathways. For approximately one third of these events unique to a single genotype, the same transcript was subject to AS in one or more of the remaining genotypes at a different exon. This finding indicates that signaling downstream of the various PDGFR dimers targets an overlapping set of transcripts encoding protein serine/threonine kinases for AS. Together, our results demonstrate that AS is the predominant mechanism of gene expression regulation downstream of PDGFR signaling in the facial mesenchyme.

EXPERIMENTAL DESIGN



Adapted from FaceBase

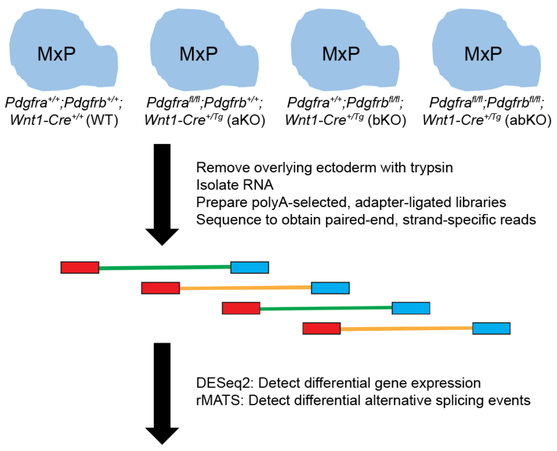


Figure 1. Maxillary process (MxP) mesenchyme RNA from three biological replicates of embryonic day 11.5 (E11.5) *Pdgfra*$^{+/+}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/+}$ (WT), *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) and *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) embryos was harvested and sequenced. The resulting data were analyzed using various bioinformatics pipelines.

RESULTS

Relatively few differentially expressed genes upon loss of *Pdgfra* and/or *Pdgfrb*

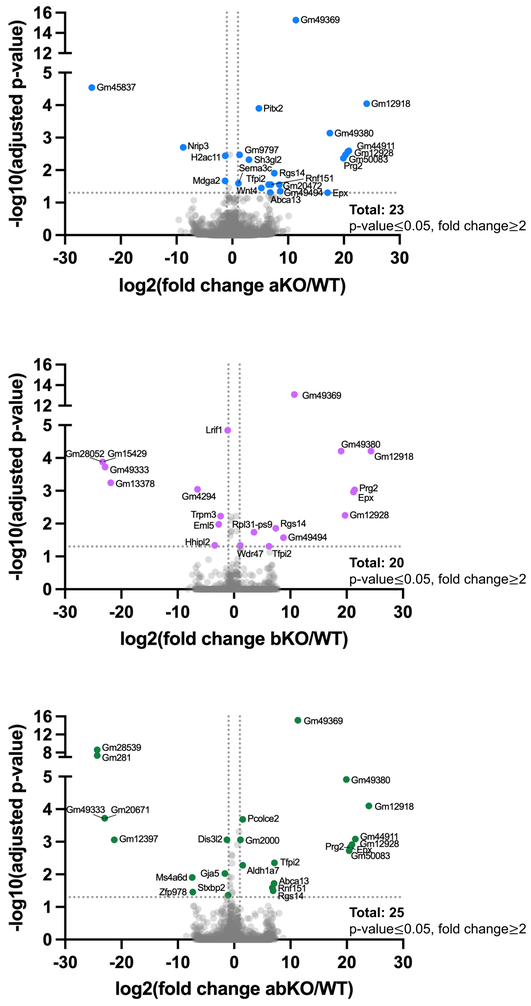


Figure 2. Volcano plots depicting -log10(adjusted p–value) versus log2(fold change) detected by DESeq2 analysis of RNA-seq data from E11.5 *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO) (top), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) (middle) and *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) (bottom) versus *Pdgfra*$^{+/+}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/+}$ (WT) maxillary process mesenchyme. Dots in aqua, lavender and moss have an adjusted p-value ≤ 0.05 and a fold change ≥ 2.

Widespread alternative RNA splicing upon loss of *Pdgfra* and/or *Pdgfrb*

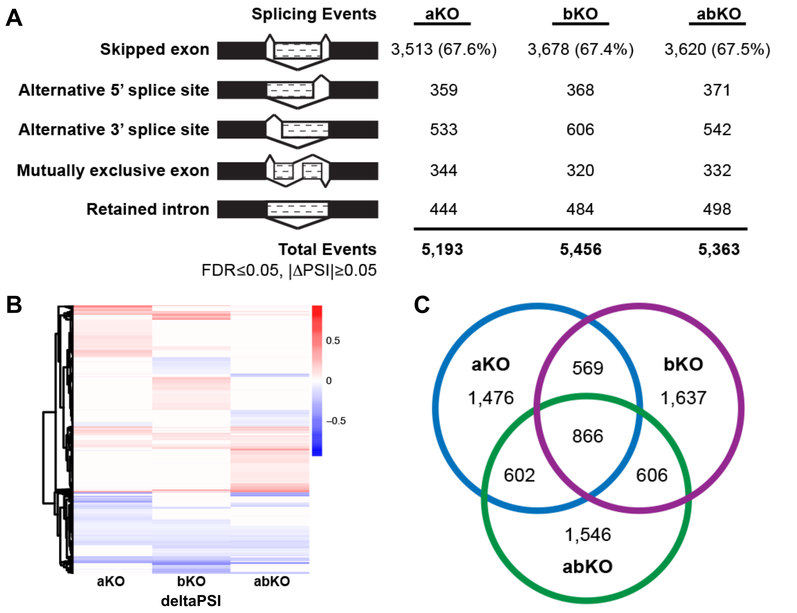


Figure 3. (**A**) Summary of differential alternative splicing events detected by rMATS analysis of RNA-seq data from E11.5 *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) and *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) versus *Pdgfra*$^{+/+}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/+}$ (WT) maxillary process mesenchyme. FDR, false discovery rate; ΔPSI, difference in percent spliced in. (**B**) Hierarchical cluster analysis of skipped exon events across genotypes. Colors correspond to ΔPSI compared to WT. (**C**) Venn diagram representing unique and overlapping skipped exon events between genotypes.

Enrichment for transcripts encoding protein serine/threonine kinases in skipped exon categories

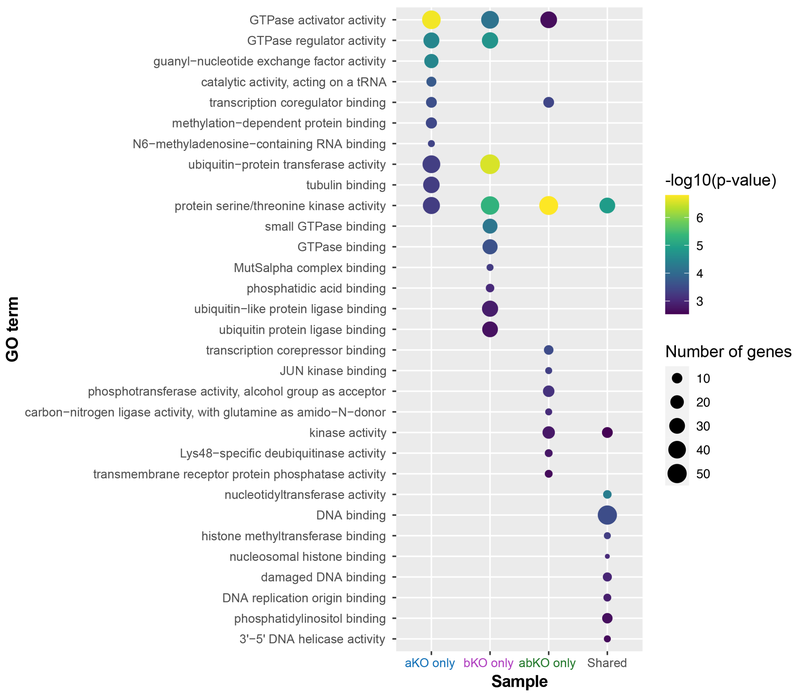


Figure 4. Bubble plot depicting top ten molecular function gene ontology (GO) terms for the genes encoding the transcripts in the skipped exon category of *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) or *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) samples, or common to all three genotypes. Colors correspond to –log10(p-value); sizes correspond to number of genes.

PDGFR signaling targets an overlapping set of transcripts encoding protein serine/threonine kinases for alternative splicing

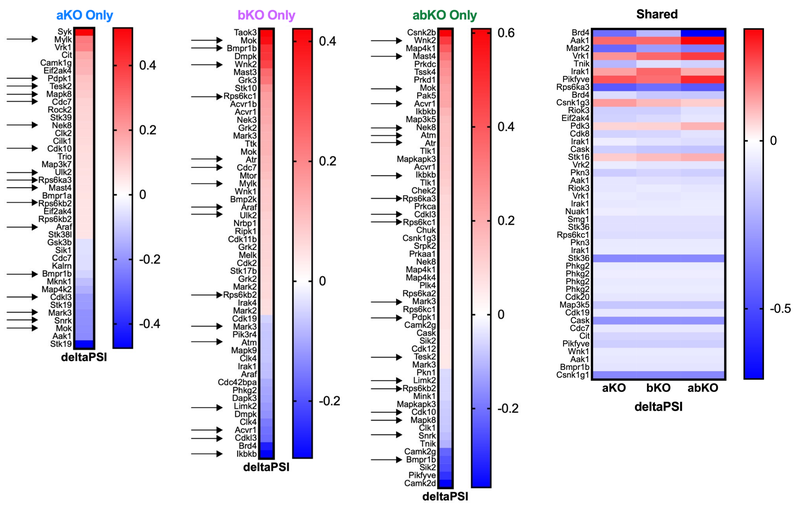


Figure 5. Heatmaps depicting deltaPSI (compared to WT) for skipped exon events involving protein serine/threonine kinases unique to *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) or *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) samples, or common to all three genotypes. Arrows indicate transcripts subject to alternative splicing in multiple genotypes at distinct exons.

Protein kinase domains are commonly lost or gained upon loss of *Pdgfra* and/or *Pdgfrb*

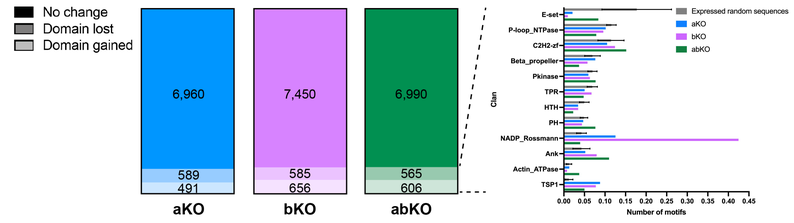


Figure 6. Bar graphs depicting numbers of unchanged, lost or gained domains following skipped exon events in *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) and *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) samples (left) and the most-commonly affected Pfam clans normalized to protein length (right).

Validation of alternative splicing changes in transcripts encoding protein serine/threonine kinases upon loss of *Pdgfra* and/or *Pdgfrb*

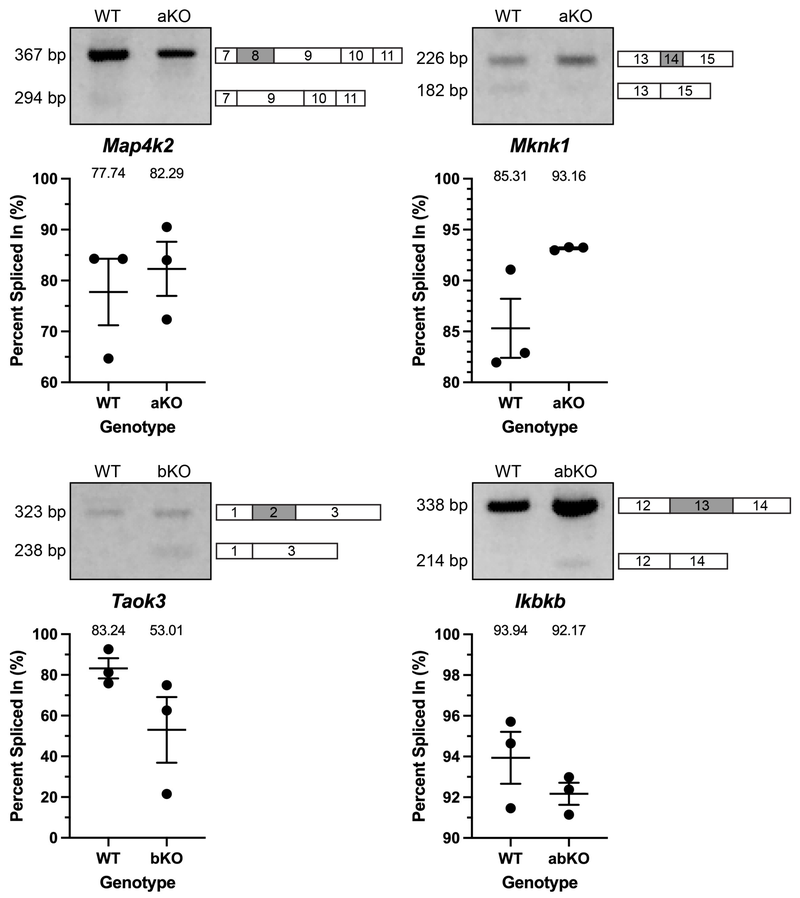


Figure 7. Representative qPCR gels with depictions of differentially alternatively spliced exon (gray), upstream and downstream sequences that were assessed by qPCR, as well as scatter dot plots depicting percent spliced in as assessed by qPCR for transcripts encoding protein serine/threonine kinases functioning within the MAPK and/or PI3K pathways. Data are mean +/- s.e.m.

Alternative splicing frequently leads to changes in NMD or protein domains for protein serine/threonine kinases functioning within the MAPK and/or PI3K pathways

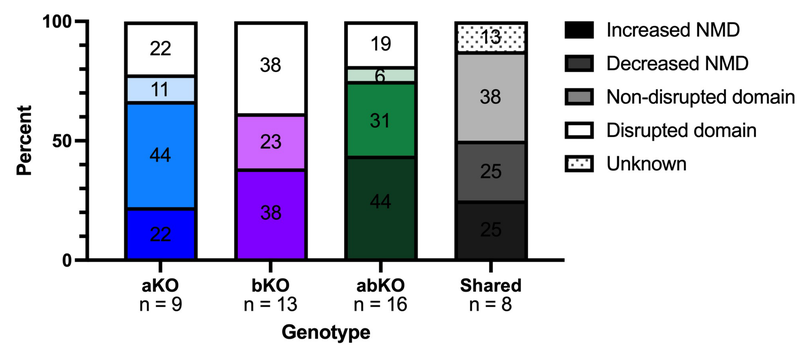


Figure 8. Bar graphs depicting percent of events leading to increased nonsense-mediated mRNA decay (NMD), decreased NMD and disrupted protein domains following skipped exon events in *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) and *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) samples, or common to all three genotypes.

FUTURE DIRECTIONS

- Confirm predicted effects of alternative splicing of transcripts encoding protein serine/threonine kinases on protein level and/or protein size
- Examine changes in substrate phosphorylation resulting from alternative splicing of transcripts encoding protein serine/threonine kinases
- Assess levels of PI3K and MAPK signaling in *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{+/+}$;*Wnt1-Cre*$^{+/Tg}$ (aKO), *Pdgfra*$^{+/+}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (bKO) or *Pdgfra*$^{fl/fl}$;*Pdgfrb*$^{fl/fl}$;*Wnt1-Cre*$^{+/Tg}$ (abKO) maxillary process lysates

ACKNOWLEDGEMENTS

We are grateful to Jessica Johnston and Erin Binne for technical assistance. RNA-seq experiments were performed at the Genomics Shared Resource at the University of Colorado Anschutz Medical Campus. This work is supported by funding from the School of Dental Medicine at the University of Colorado Anschutz Medical Campus and NIDCR grants R01DE027689 and K02DE028572 (to K.A.F.).