RESULTS: Combination of T cell depletion and CTLA4-Ig plus short course of Rapamycin increased VCA survival significantly while untreated controls rejected their allografts (MST 105 days; Untreated, MST 9 days; CTLA4 Ig only, MST 17 days, Rapamycin, MST 20 days; T cell depletion, 20 days; p<0.01). Mixed chimerism was detected in recipients receiving this combined treatment protocol with 5.013±1.23 % of CD11b+ cells being donor-derived on POD 55. Vβ - TCR staining profiles in recipients after full treatment showed 1.570±0.3700 % of Vβ5+CD4+ T cells, while naïve C57BL/6 express 3.567±0.3690 % of Vβ5+CD4+ T cells, suggesting the actuation of central deletion of developing donor-reactive T cells. In order to further prolong allograft survival, one week expanded Tregs were then included in the combination therapy. The suppressive activity of the CD4+CD25+ Tregs was confirmed with in vitro suppression assays. The addition of ex vivo expanded regulatory T cells further increased VCA survival to >200 days and induced long-term stable mixed chimerism with 16.7±1.5 % of CD11b cells being donor-derived on POD 55 after administration of expanded Treg cells.

CONCLUSION: The combination of T cell depletion, costimulation blockade, and a short-course of Rapamycin prevents VCA rejection and significantly prolongs graft survival without the need for myeloablative conditioning or maintenance therapy. Moreover, regulatory T cells added in the early post-transplant period further optimize immune regulation by inducing sustained mixed chimerism.

4

Single Cell Transcriptome Analysis of Epithelial vs Chondrogenic Cell Types in Palate Morphogenesis

Claudio Macias-Trevino, MD-PhD Candidate1,2, Shannon H. Carroll, PhD1,2, Nora Alhazmi, DDS1,2, Edward B. Li, PhD1,2, Shawn A. Hallett, BA1,2, Dawn Truong, BA1,2, Eric C. Liao, MD/PhD1,2

PURPOSE: IRF6 is the most frequently mutated gene in cleft lip and/or palate (CL/P) cohorts across all major populations. We hypothesized that interactions between epithelial cells and chondrocytes are essential for palate formation and craniofacial morphogenesis. We utilized advanced methods in transgenesis coupled with enhancer profiling to define the transcriptional landscape in epithelial and neural crest derived populations. Modern techniques such as single-cell RNA-sequencing enable us to probe transcriptional landscapes with high dimensionality, and are critical in defining developmental processes that can be targeted therapeutically in patients at risk for CL/P.

METHODS: One important IRF6 gene variant associated with isolated cleft-lip has been mapped to a multiple-species conserved sequence roughly 9.7kb (MCS9.7) upstream of the IRF6 gene. We cloned this MCS9.7 sequence upstream of an eGFP reporter gene and recombined the construct into the zebrafish genome by co-injecting the construct and the Tol2 transposase system into wild-type zebrafish embryos. We generated a dual-reporter irf6:eGFP;sox10;mCherry line. This transgenic reporter line will be characterized by live-imaging using two-photon confocal microscopy. In addition, we generated an epithelial-specific wntless (wls) line and a chondrocyte specific col2a1 line that enable us to tag and purify specific cranial neural crest cellular populations during embryogenesis. Specific lineage cell types will be isolated using FACS to generate purified cellular populations that will be analyzed using single cell RNA-sequencing to reveal dynamic lineage specific gene expressions during craniofacial morphogenesis.

RESULTS: We have generated the irf6 reporter construct using the MCS9.7 sequence and were successful in achieving germline transmission of the transgene after crossing the founder fish into a sox10;mCherry background. Imaging of developing embryos of the dual reporter line revealed expression of irf6 restricted to the ectoderm, and sox10 expression in chondrocytes of the zebrafish palate. We also delineated epithelial expression of wls in a similar wls:eGFP;sox10;mCherry line. We are using FACS to isolated oral epithelial cells and cranial neural crest cells for single-cell RNA sequencing of purified subpopulations.

CONCLUSION: This work establishes an irf6 reporter line in zebrafish that will be critical in studying developmental
processes. A major strength of the zebrafish model system is the ability to image live embryos and visualize multiple stages of early development in vivo. Fate-mapping of irf6-expressing cells at different time points in zebrafish embryos using the dual reporter line will be critical in studying the interactions of epithelial and chondrocyte cells during palate morphogenesis. As new developments in gene and cellular therapies are pursued, understanding the basic transcriptional circuits is essential in designing genomic targets for gene therapy, or in the engineering of bioactive implantable materials for optimal surgical outcomes.

5

Inhibition of Mechanotransduction Yields Regenerative Wound Healing by Engrailed1-negative Fibroblasts

Shamik Mascharak, Alessandra L. Moore, Heather E. desJardins-Park, Maria R. Borrelli, Bryan Duoto, Malini Chinta, Deshka S. Foster, Hermann P. Lorenz, Michael T. Longaker

Stanford University, Stanford, CA, USA

PURPOSE: Skin scarring poses a significant medical burden for tens of millions of patients every year. Recently, Engrailed1-positive fibroblasts (EPFs) were shown to be responsible for the majority of scarring on the dorsal skin after embryonic day (e)18.5 in mice. However, comparatively little is known about the postnatal function of Engrailed1-negative fibroblasts (ENFs), which are present in all layers of the dermis and are putatively non-scarring. We sought to characterize the ENF lineage and assess if ENF-mediated wound healing leads to more ordered repair of skin with regeneration of dermal appendages.

METHODS: Experiments were performed in En1Cre;R26mTmG (En1mTmG) and En1Cre;Ai6 (En1 Ai6) mice, with Engrailed1-positive fibroblasts (ENFs), which are present in all layers of the dermis and are putatively non-scarring. We sought to characterize the ENF lineage and assess if ENF-mediated wound healing leads to more ordered repair of skin with regeneration of dermal appendages.

RESULTS: FACS-isolated ENFs from p1 mice activate Engrailed1 after 7 days of culture on plastic. However, ENFs do not activate Engrailed1 in vitro after inhibition of stiffness sensing (ROCK inhibitor Y-27632) or culture in soft three-dimensional collagen hydrogels, suggesting a mechanotransduction-mediated mechanism for postnatal Engrailed1 expression. Postnatal ENFs transplanted into dorsal skin also show activation of Engrailed1 after wounding. Transcriptomic analysis by RNA-sequencing reveals that postnatal ENF to EPF transition is accompanied by expression of genes related to fibrosis (e.g., WNT/TGFβ) and mechanotransduction signaling, including several target genes of Yes-associated protein (YAP). Accordingly, wounds treated with a single administration of YAP inhibitor Verteporfin yield scars with markedly fewer EPFs, reduced fibrosis, and greater ENF presence at 2 weeks. After 4 weeks, Verteporfin-treated wounds show sustained presence of ENFs, as well as regeneration of dermal appendages.

CONCLUSIONS: Postnatal ENFs activate Engrailed1 in vitro by canonical mechanotransduction signaling and take on a fibrotic phenotype; a similar process occurs in the in vivo wound environment. We demonstrate that inhibition of YAP signaling promotes ENF-mediated wound healing with reduced fibrosis and regeneration of secondary elements. Our findings suggest that ENFs may play a critical role in scarring by activating Engrailed1 in response to mechanical cues within the wound bed. Considering that the Engrailed1-negative lineage represents several distinct subpopulations of cells, we aim to assess postnatal Engrailed1 activation in the papillary, reticular, and hypodermal layers to identify a specific ENF population that contributes to scarring.

6

Toward an Objective Outcome in Facial Reanimation Surgery: An Eyetracking Study

Thanapoom M. Boonipat¹, Amjed Abu-Ghname, MD¹, Ali Charaffadine, MD¹, Kevin D. Fleming, PhD², Mitchell Stotland, MD³, Samir Mardini, MD¹

¹Mayo Clinic, ROCHESTER, MN, USA, ²Norwich University, Northfield, VT, USA, ³Sidra Medical, Doha, Qatar