BACKGROUND: Both internal and external distraction devices have been used successfully in correcting midface hypoplasia. Although the indication for surgery and the osteotomy techniques for a Le Fort I and Le Fort III may be similar, deciding when to use an internal vs external device has not been well studied. We studied patient reported outcomes using the FACE Q Patient Reported Outcomes Instrument and functional surveys for internal and external devices for both Le Fort I and Le Fort III patients.

METHODS: Midface hypoplasia patients who underwent distraction advancement after Le Fort I and Le Fort III were surveyed using the FACE-Q survey and a functional survey. Equal groups of internal and external device patients were compared (n=64). Data recorded included: Sex, age, follow-up, diagnosis, OR time, EBL, Length of stay, distraction length, consolidation time, and complications. FACE Q scales included: Satisfaction with Facial Appearance, Social Function (confidence), Psychological Well-being, Early life impact (recovery), and Satisfaction with Outcome and Decision. Functional surveys included Airway/Breathing (correction of sleep apnea, removal of tracheostomy), Ocular/Vision (globes protection), Occlusion/Eating, and Speech/Articulation.

RESULTS: Internal and external device groups were similar with regards to patient demographics (Craniofacial diagnosis, mean age (14.4 years), sex, follow-up), operative time, EBL, distraction length (24.2 vs 25.1), and follow-up. Consolidation times differed (Internal=1.1 vs External=3.6 months). For FACE Q appearance appraisal (Overall Appearance, Cheeks, Nose, Upper lip/Smile), there were similarities in domain and scale. For Functional surveys (Airway/Breathing, Ocular Vision, Occlusion/Eating, Speech/Articulation), there were also similar scoring between groups. However, internal device patients had superior scores compared to external devices with FACE Q for Quality of Life including: Social Function (80.9 ± 19 vs 68.9 ± 18), Early Life Impact (92.9 ± 22 vs 62.4 ± 11), Dental anxiety (70.2 ± 17 vs 48.3 ± 10), and Psychological Well-being (87.8 ± 18 vs 68.6 ± 11). In addition, internal device patients were better with FACE Q Satisfaction Decision (81.2 ± 19 vs 56.9 ± 10) and Satisfaction Outcome (91.0 ± 22 vs 84.7 ± 18).

CONCLUSIONS: Le Fort I and Le Fort III distraction patients had similar patient reported outcomes for appearance and functional improvement while using internal or external devices; however, those with internal devices were more satisfied with their quality of life and decision to undergo the procedure.

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Loss Of TGF-beta Activated Kinase (TAK1) Activity Induces Cellular Proliferation And Diminishes Differentiation During Bone Healing

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PURPOSE: The ability to modulate signaling pathways at the site of injury represents a novel paradigm in wound healing with implications for regenerative medicine and tissue engineering. Transforming growth factor-beta (TGF-β) activating kinase 1 (TAK1) is a key regulator in the TGF-β and bone morphogenetic protein (BMP) signaling pathways with central roles in proliferation, differentiation, survival, and apoptosis of myriad tissues during development and in a range of pathologic processes. Here we examine the effect of TAK1 inhibition both in vitro and in the in vivo wound environment. We introduce a novel dual inducible Cre/loxP and Flp/FRT recombinase system to precisely control the expression of TAK1 during wound healing and identify a role for TAK1 as a molecular switch between mesenchymal cell proliferation and differentiation.

METHODS: We developed a novel dual recombinase system (Cre/loxP; Flp/FRT) allowing TAK1 to be specifically inactivated (Ad.Cre) and sequentially re-activated afterwards (Ad.Flp). We first evaluated the effect of this system in vitro mesenchymal cells harvested from sites of musculoskeletal injury. Osteogenic differentiation was assayed via alizarin red and alkaline phosphatase staining. SMAD protein signaling was analyzed directly via immunocytochemistry and western blot analysis. Proliferation was assayed directly via BrdU and cell counting. In vivo, critical sized-calvarial defects (4mm) were performed and mice received either: 1. Ad.LacZ (control) or 2. Ad.Cre (inactivation) or 3. Ten days Ad.Cre (inactivation) followed by Ad.FLP (reactivation) to modulate TAK1 expression. Calvarial tissue was harvested to assay for gene expression and cellular proliferation and differentiation were quantified histologically.
RESULTS: *In vitro* analysis of mesenchymal cells carrying the Ad.Flp/Ad.Cre construct demonstrated a significant loss in osteogenic potential (p<0.05) and pSMAD1/5 signaling in the absence of Tak1. Reactivation of Tak1 was sufficient to restore pSMAD1/5 signaling and osteogenic differentiation. Tak1 knockout demonstrated an opposite effect on cell proliferation with immediate and significant (p<0.05) increases in cell growth upon knockout and normalization of cell proliferation on gene reactivation. Loss of Tak1 in the calvarial wound environment resulted in increased presence of mesenchymal cells and increased expression of proliferative genes including Ccnd1, E2f1, and Ki67, an effect reversed by Ad.Flp reactivation of Tak1. Consistent with our *in vitro* data, loss of Tak1 in calvarial tissue led to diminished osteogenic differentiation genes including Bmp2, Tgfβ1, Col1, Ocn, and Runx2. Again this effect was reversed by Ad.Flp reactivation of Tak1.

CONCLUSION: We demonstrate that precise control of Tak1 can be used to modulate a switch between proliferation and osteogenic differentiation in mesenchymal cells. These findings are possible due to a novel dual-recombinase system with applications in other animal models studying TGF-B signaling and TAK1. Our *in vivo* data suggests that Tak1 can be used to modulate a switch between proliferation and osteogenic differentiation in mesenchymal cells.

METHODS: Mutant *irf6*–/– embryos were dispensed 10 embryos per well in 96-well plates and incubated in media containing propidium iodide (renders ruptured embryos fluorescent). The ICCB known-bioactives library containing FDA-approved drugs with well-characterized biological targets was screened. Mutant embryos treated with DMSO were used as solvent controls. Wildtype embryos treated with drugs were used as toxicity controls. Time-lapse images of the wells were captured by automated bright-field and fluorescence microscopy and analyzed with ImageJ. Molecular pathways associated with the positive hits were identified through the library index and analyzed using computational modeling programs.

RESULTS: 65 of the 480 small molecules screened reached statistical significance in delaying periderm rupture compared to DMSO-treated controls without causing developmental delays in wildtype embryos. The molecular targets of the small molecule hits were analyzed by Gene Ontology and revealed not only molecular pathways previously known to play important roles in palate development such as PDGF and FGF, but also novel pathway connections between *IRF6* and the retinoic acid, aryl hydrocarbon, and adenosine pathways among others. Furthermore, when these pathways were aberrantly modulated in wildtype zebrafish embryos, craniofacial defects were observed.

CONCLUSION: Zebrafish *irf6*–/– embryos represent a robust platform for high-throughput small molecule screens to identify modulators of *IRF6* capable of mitigating cleft pathogenesis. The results identified many critical developmental pathways, some of which have been previously reported as essential in palate development while others have not yet been extensively characterized. These pathways could represent unexplored regulatory mechanisms of palate development and novel nodes of pharmacological intervention for orofacial clefting.