PSRC Abstract Supplement

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PURPOSE: Heterotopic ossification (HO) is a painful, debilitating formation of ectopic bone, often found after severe polytrauma, burn and spinal cord injury. Nerve growth factor (NGF) is a well-documented neurotrophic factor that has been correlated with phenotypes of chronic inflammation and pain. Recent literature has implicated neurotrophic signals including NGF as a crucial signal in normal bone development and fracture healing. Given the similar progression through endochondral ossification between fracture and post-traumatic heterotopic ossification, we hypothesized that neurogenic/neurotrophic signaling via sensory innervation is crucial for pathologic stem cell differentiation and HO.

METHODS: Microarray data of human ligament cells harvested from excisions of ossification of the posterior longitudinal ligament (GEO dataset GSE5464) were analyzed using linear modeling with empirical Bayes method for differential expression of genes. Using a proven mouse burn/tenotomy (B/T) HO model with bisection of Achilles tendon and concomitant 30% TBSA dorsal burn, mRNA was harvested from injured and uninjured tendon (n=3/group) and hybridized to Affymetrix microarray (1 week). To assess the requirement for TrkA+ nerves in HO genesis, TrkAF592A mice were used which are homozygous for knockin alleles rendering TrkA activity sensitive to specific inhibition by the membrane-permeable, small molecule 1NMPP1. Analysis of HO formation was performed by in TrkAF592A mice or littermate controls via µCT at 9 weeks (n=5–14/group). Imaging of nerve fibers was examined by confocal microscopy at 1 and 3 weeks using Thy1-YFP reporter sections.

RESULTS: Human spinal ligament cells subjected to cyclic strain exhibited robust upregulation of NGF and BRDF; a loading shown to be associated with ossification of connective tissues. Mouse BT induced upregulation of characteristic genes of inflammation (Il6, Ptg2, Piger1, Il1a, Tacr1) vs. uninjured tendon at 1 week, as well as NGF. Immunolabeling of the injury site 1 week after surgery demonstrated NGF expression in close proximity to the native tendon, with regions of overlap with PDGFRα+ mesenchymal progenitor cells vs. the uninjured contralateral tendon. Additionally, immunofluorescent labels on endogenous Thy1 reporters showed nerve fibers in close proximity to F4/80+ macrophages and PDGFRα+ progenitors, a cellular niche that facilitates HO formation. Interruption of TrkA signaling via the introduction of 1NMPP1 drug to homozygous F592A mutant mice demonstrated a significant reduction in the volume of floating, intramuscular HO.

CONCLUSIONS: HO induces upregulation of NGF, concordant with upregulation seen in strained human cells from patients with aberrant ossification of soft tissues. HO tissue demonstrated robust labeling of NGF at the injury site, exhibiting regions of colocalization with infiltrating mesenchymal progenitor cells. These signals are found in similar regions where Thy1+ nerves are present, whose TrkA receptors facilitate NGF signaling. Selective interruption of NGF-TrkA coupling attenuated ectopic bone volume suggesting neural signals modulate aberrant wound healing and the mesenchymal cell programming heralding HO. These data suggest that inhibition of neurogenic signaling through NGF-TrkA represents a potential therapeutic target to prevent post-traumatic HO.

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ALX1 Regulates PAX3 to Enable Cranial Neural Crest Migration During Craniofacial Development

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PURPOSE: Migration and fusion of the frontonasal and paired maxillary prominences are crucial to the formation
of the midface. Disruption of this developmental process leads to severe craniofacial anomalies, such as frontonasal dysplasia (FND). Using induced pluripotent stem cells (iPSCs) derived from FND subjects with a heritable \textit{ALX1} L165F gene variant, we leveraged a novel method of cranial neural crest cell (CNCC) differentiation to study \textit{ALX1} function and interaction with \textit{PAX3}, delineating a transcriptional regulatory pathway crucial to CNCC differentiation.

METHODS: Wildtype and \textit{ALX1-/-} iPSCs were differentiated into the CNCC lineage through the addition of epithelial growth factor into DMEM F12 medium. Subsequent cells were assayed for their CNCC properties and ability to multi-lineage differentiate into adipocytes, Schwann cells, chondrocytes, and osteoblasts. We then accessed surface marker expression, and sensitivity to apoptosis between wildtype and \textit{ALX1-/-} CNCCs using FACS and migration assay. Differential gene expression in iPSC and \textit{alx1-/-} zebrafish embryos was analyzed using qPCR. For \textit{in vivo} analysis of CNCC migration, wild-type, \textit{alx1-/-} and \textit{pax3} injected zebrafish embryos were analyzed using \textit{sox10}:KAEDE transgenic line.

RESULTS: Through analysis of CNCC markers CD90, CD73, CD105, CD57, cellular morphology, gene expression data, we demonstrate that our protocol was able to differentiate iPSCs into CNCCs. Both \textit{ALX1-/-} and wild-type cells were capable of differentiating into adipocytes, Schwann cells, chondrocytes, and osteoblasts. However, after CNCC differentiation, while wildtype cells were able to further engage in the CNCC lineage through CD57 down-regulation, \textit{ALX1-/-} NCCs maintained a high level of CD57. \textit{ALX1-/-} neural crest cells were also more sensitive to apoptotic stress and experienced migratory impairment. qPCR analysis of \textit{ALX1-/-} iPSCs and \textit{alx1-/-} zebrafish embryos revealed an overexpression of \textit{PAX3} during CNCC differentiation and embryonic development. Conversely, overexpression of \textit{PAX3} into control CNCCs impaired migration in iPSC model. Overexpression of \textit{pax3a/b} mRNA in zebrafish embryos phenocopied the craniofacial anomalies seen in \textit{alx1-/-} mutants, and injection of a dominant-negative \textit{alx1} (\textit{alx1DN}) variant impaired migration of CNCCs to the central midface.

CONCLUSION: Studies of FND using complementary iPSC and zebrafish models revealed that \textit{ALX1} down-regulates \textit{PAX3} to modulate CNCC migration and cell maturation. Disruption of \textit{ALX1} resulted in unsuppressed \textit{PAX3} expression, which caused NCCs to be unable to persist in a progenitor state, more sensitive to apoptosis, and unable to properly migrate. Discordant neural crest cell differentiation and migration resulted in a decreased contribution of NCC to the frontonasal prominences and midface deformities. These studies revealed requirement of \textit{ALX1} in transcriptional regulation of midface development. This study elucidated the molecular and cellular basis of FND pathogenesis, advancing craniofacial malformations from description of affected anatomy to fundamental understanding.

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Near-Infrared Tissue Oximetry Predicts Flap Necrosis in a Rat Dorsal Skin Flap


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PURPOSE: The application of technology to predict and prevent perfusion related complications promises to revolutionize plastic surgery by improving patient care and preventing the downstream sequelae of flap necrosis. Although a myriad of devices have been developed toward this goal, their widespread application has been limited by cost, the need for potentially unwieldy machinery, the use of intravenous dyes, and questionable efficacy in human trials. The objectives of this study are to assess the potential efficacy of a novel, handheld, dye-less device utilizing near-infrared spectroscopy in quantifying tissue oxygenation, predicting the risk for flap necrosis, and preventing perfusion-related complications.

METHODS: Twenty-four Sprague-Dawley rats underwent elevation of a dorsal, 10cm x 3cm cranially-based random pattern skin flap using the modified McFarlane technique. Rats were divided into 1 of 3 treatment groups: control, single-dose topical nitroglycerin, and two-dose topical nitroglycerin applied immediately post-operatively and again twelve hours later. Tissue oxygenation was measured intra-operatively following flap elevation and at 24-hours post-operatively. On post-operative day seven, the animals were euthanized, and flap survival was ascertained clinically and histologically. The Pearson product-moment correlation coefficient was used to correlate tissue oxygenation to distance from flap pedicle. Statistical analyses were