PURPOSE: The human hand plays an essential role in our normal daily activities. The anatomical complexity of the hand, and critical functions of each of its component tissues, highlights the need to understand and effectively treat injuries to this vital multi-tissue structure. Surgical repair is often complicated by the scarcity of local soft tissue and loss of functional capacity. Reports of “salamander-like” spontaneous regeneration in human fingertips and recent advances using mouse models of digit tip regeneration raise the possibility of restorative/regenerative rather than reparative approaches for traumatic hand injuries. These mouse studies demonstrate the various embryonic origins of mammalian limb tissue, including ectoderm, mesoderm, and neural crest, the role of lineage-restricted stem and progenitor cells in mammalian digit regeneration and some of the signaling pathways involved. The discrete signaling mechanisms governing this regenerative process, however, remain poorly understood.

METHODS: Tissue harvested from murine digit tips 7 days after amputation at the regenerative plane (distal to the germinal matrix) or non-regenerative plane (proximal to the germinal matrix) was compared using microarray analysis. This comparison detected only genes differentially expressed in regeneration, and allowed exclusion of genes related to typical non-regenerative, fibrotic wound healing. Viral knockdown of candidate genes identified by microarray analysis was used to confirm a functional role for these signaling networks in vivo. Tissue specific expression and response patterns of target genes were assessed using gene specific reporter mice.

RESULTS: Microarray analysis identified 287 genes differentially regulated in regenerating v. non-regenerating digit tips. Ingenuity pathway analysis identified the top associated gene networks, the highest ranked of which were related to typical non-regenerative, fibrotic wound healing. Viral knockdown of candidate genes identified by microarray analysis was used to confirm a functional role for these signaling networks in vivo. Tissue specific expression and response patterns of target genes were assessed using gene specific reporter mice.

CONCLUSION: Unbiased microarray analysis combined with in vivo knockdown studies identify a central role for Wnt and HH signaling in digit tip regeneration. Ectodermal Wnt signaling targeting the ectoderm interacts with ectodermal Hedgehog signaling targeting the mesoderm to critically regulate regeneration of the mouse digit tip. Understanding and targeting the cross-talk between these pathways could enable regeneration in normally non-regenerative extremity injuries.

L-Type Voltage Gated Calcium Channels and Sodium Phosphate Symporters are Required for Osteogenic Differentiation Induced by Nanoparticulate Mineralized Collagen Materials

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PURPOSE: The increasing understanding that modulation of extracellular matrix (ECM) components can instruct differential cell fate determination has resulted in significant interest in the synthesis of ECM-inspired materials for regeneration. Previously we described a nanoparticulate mineralized collagen glycosaminoglycan (MC-GAG) material that efficiently induced osteogenic differentiation of human mesenchymal stem cells (hMSCs) and calvarial bone healing without exogenous growth factors or progenitor cell expansion via an autogenous activation of the bone morphogenetic protein receptor (BMPR) signaling pathway. Such activities were not induced in non-mineralized collagen glycosaminoglycan (Col-GAG) materials, suggesting that the upstream trigger is related to the inorganic content of the material. In this work, we evaluated
the contributions of inorganic ion signaling to MC-GAG-induced osteogenic differentiation.

METHODS: Primary hMSCs were osteogenically differentiated on collagen glycosaminoglycan materials in the absence or presence of inhibitors to the L-type voltage gated calcium channels (L-VGCC), calcium sensing receptor (CaSR), and the sodium phosphate symporters (PiT-1/2) using nifedipine, NPS2143, and phosphonoformic acid (PFA), respectively. Western blots were performed for phosphorylated and total Smad1/5, ERK1/2, Akt, p38, JNK1/2, total Runx2, and actin. Expression of BMPs were assessed with immunofluorescent staining of scaffold sections. Mineralization was evaluated by Alizarin red staining and micro-computed tomographic analyses. Analyses of variance with posthoc comparisons were performed.

RESULTS: PFA diminished phosphorylated Smad1/5, BMP-2, and Runx2 expression as well as mineralization for both Col-GAG and MC-GAG scaffolds. While NPS2143 diminished Runx2 and mineralization on Col-GAG, no effects on osteogenic protein expression, BMP-2 expression, or mineralization were evident on MC-GAG. In contrast, no effects were mediated by nifedipine on Col-GAG, whereas nifedipine inhibited phosphorylated Smad1/5, phosphorylated ERK1/2, Runx2, BMP-2 staining, and mineralization on MC-GAG.

CONCLUSION: In combination, these data suggested that the autogenous activation of the BMPR signaling pathway induced by MC-GAGs is first triggered by calcium and phosphate influx via L-VGCCs and the sodium and phosphate symporters.

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High-Resolution Gene Expression Analysis of RNA Splicing Regulators Esrp1 and Esrp2 In Palate Development

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PURPOSE: Alternative RNA splicing is a fundamental process that amplifies protein diversity and fine-tunes cell communications. Epithelial splicing regulatory proteins 1 and 2 (Esrp1 and Esrp2) are the only known tissue-specific regulators of RNA splicing. Disruption of Esrp1/2 results in cleft lip and palate in the mouse. Recently, human cohorts with ESRP2 gene variants were identified to be associated with cleft lip and palate. In order to gain mechanistic understanding of how epithelial cells regulate morphological changes during palate development, we applied a new gene expression detection method, RNAscope, to analyze the spatiotemporal expression of Esrp1 and Esrp2 during mouse and zebrafish palate development.

METHODS: We applied a novel RNA in situ technology (RNAscope) to carry out detailed gene expression analysis of Esrp1 and Esrp2 in mouse and zebrafish craniofacial structures with unprecedented single cell resolution.

RESULTS: Esrp1 and Esrp2 mRNA are found to be highly co-expressed in the periderm and basal epithelia of mouse as well as zebrafish epithelium during early embryogenesis. In the mouse, this included the epithelial layers surrounding the palatal shelves prior to and during fusion. With this enhanced RNA expression detection technique, we have identified new expression profiles for Esrp1/2 as well as other cleft lip/palate associated genes. Furthermore, we can differentiate between the expressions of protein isoforms (i.e. Esrp1 and 2), which were not previously possible with immunofluorescence.

CONCLUSION: Detailed gene expression data of Esrp1 and Esrp2 resolved at the single-cell level supports a role for epithelial-specific alternative RNA splicing in the regulation of craniofacial and palate development. We are now applying RNAscope to delineate spatiotemporal changes in Esrp1 and Esrp2 gene expressions during impaired palatogenesis in various orofacial mutant mouse and zebrafish models. Investigating Esrp1/2 expression during craniofacial development will expand our understanding of cleft lip/palate etiology, specifically the role of the embryonic epithelium in cleft pathogenesis.

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Investigating The Role Of R-spondin3 In Craniofacial Morphogenesis And Orofacial Cleft Pathogenesis