Interactions between the cells corresponding to the vascular compartment with the rest of the cells were also analyzed in vitro under normoxia and hypoxia and a modulating effect on cell survival was observed. Studies on the effect of a hypoxic stress on vascular cells alone or co-cultured with neuron and glial cells in vitro were performed for the angiopoietins and VEGF systems by RT-PCR and Western Blots. Preliminary results on isolated vascular cells in vitro show an important difference on the transcription levels of the Angiopoietin-1 when compared to vascular cells co-cultured with neuron and glial cells. Our results could advantageously help to analyze the signaling networks involved in regulating the cortico-angiogenesis under hypoxia.

Program/Abstract # 130
Coordinated directional cell motility driving vertebrate limb bud morphogenesis
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The vertebrate limb bud is a classical model of organogenesis. Genetic analyses have revealed a complex forelimb gene regulatory network orchestrated by Tbx5 gene function, yet how forelimb formation is regulated at the cellular level is less well understood. Here we present a cellular model of early forelimb morphogenesis based on a single-cell resolution fate map and 4D cell tracking in zebrafish embryos. By following long-term fates of labeled single cells in the lateral plate mesoderm (LPM), we demonstrated that wild type forelimb precursors converge from a broad region of the body axis while maintaining their relative anteroposterior (AP) positions, suggesting coordinated cell migration during limb bud compaction. Using 4D confocal microscopy with a LPM-specific fluorescence reporter, we followed trajectories of individual wild type LPM cells during limb bud compaction, and confirmed that their nearest neighbor relationships remain largely undisrupted. Moreover, our 4D cell tracking revealed that anterior forelimb precursors migrate further along the AP axis than posterior precursors during limb bud compaction. To investigate how Tbx5 influences coordinated directional cell motility in forelimb precursors, we performed 4D cell tracking in Tbx5 loss-of-function embryos. Our results support a model that Tbx5 redirects coordinated motility in anterior forelimb precursors, deficiency in which may lead to failure of limb bud compaction. We are currently investigating downstream mechanisms that may supply directionality cues to different populations of forelimb precursors. (This work is partially funded by the Chicago Biomedical Consortium with support from The Searle Funds at The Chicago Community Trust.)

doi:10.1016/j.ydbio.2011.05.153

Program/Abstract # 132
Functional characterization of limb-specific enhancers in the mouse
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Although many mutant mouse limb phenotypes have been generated via gene targeting (e.g. Prx1, Shh) little research has exploited the use of murine functional genetics to explore the role of individual transcriptional enhancers during limb development. As regulatory regions, it is known that enhancers can regulate the timing, location and quantity of gene expression, but a general understanding of how individual enhancers contribute to development is lacking. To better understand the effects of altered gene regulation on mouse development we deleted two enhancers known to be active in the limb bud. The enhancers chosen for manipulation were drawn from the VISTA Enhancer Browser website. These enhancers were identified using chromatin immunoprecipitation coupled with sequencing (ChIP-seq) targeted at p300, a transcriptional activator, followed by the production of LacZ-expressing transgenic mice (Visel et al. Nature. 2009, 457:854). All Vista Browser enhancers are scored for positive LacZ regulation at E11.5 and transgenic mice are generated using human sequences. Vista enhancer 280 (V280) drives a wedge-shaped expression pattern in a region that will likely give rise to digits 2–4; enhancer V1442 drives a graded pattern with the highest intensity located in the limb’s posterior margins. We generated transgenic mice using the orthologous mouse enhancers. Preliminary results showed that the mouse enhancers also drive expression in the limbs, though with some possible differences that need to be explored further. We have several V280-targeted chimeric mice that we are breeding to establish knockout lines. We hypothesize that the targeted deletion of these enhancers will lead to aberrant limb development.

doi:10.1016/j.ydbio.2011.05.154

Program/Abstract # 133
Ectodermal inactivation of Smad4 causes limb deformity
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Vertebrate limb morphogenesis is coordinated by a complex network of signaling centers that influence growth, differentiation and movement of the cells in the developing limb field. Several members of signaling molecules of the Transforming Growth Factor beta (TGF-b) family are shown to be potent regulators of limb morphogenesis. Smad4 is a central mediator of the TGF-beta-related signaling in a variety of developmental contexts. Zygotic inactivation of Smad4 results in lethality at peri-gastrulation stage. We generated a conditional knockout