Developmental Genetics

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Signal Integration by Gene Regulatory Landscapes during Mouse Limb Bud Organogenesis – Robustness and Evolutionary Diversification

We are interested in understanding the molecular mechanisms underlying the regulatory circuits that govern vertebrate organogenesis. To this aim we study how gene regulatory landscapes integrate multiple signaling inputs into robust but highly dynamic transcriptional outputs. We are taking advantage of our in-depth knowledge of the self-regulatory signaling systems that control vertebrate limb bud organogenesis. In particular, we are identifying the functionally relevant *cis*/*trans*-regulatory interactions that control the establishment and propagation of this self-regulatory signaling system during initiation and progression of mouse limb bud organogenesis. Often, gene expression is regulated by multiple *cis*-regulatory modules (CRMs) that are scattered in large genomic landscapes, but how these landscapes integrate transcriptional inputs is still largely unknown. Therefore, we profile chromatin architecture, epigenetic marks, interaction of transcriptional regulators with CRMs and transcriptomes to identify the relevant CRMs, which are functionally analysed by CRISPR/Cas9 genome editing (Fig. 1). Experimental analysis is combined with bioinformatics and *in silico* simulations to reveal gene regulatory networks (GRNs) and interaction kinetics. A key node in the self-regulatory limb bud signaling system is the BMP antagonist *Grem1,* whose expression is controlled by a large genomic landscape integrating inputs from all major signaling pathways. Within the *Grem1* landscape, we have identified the region required for limb bud expression, which encodes three CRMs regulated by key transcriptional regulators in limb buds. Currently, we are studying these CRMs that appear to regulate *Grem1* expression in a cooperative manner, possibly as part of a super enhancer. As modern tetrapod limbs display amazing evolutionary diversity, a second fascinating aspect of your research are insights into how this robust and self-regulatory signaling system evolved and diversified. Recently, we showed that one of the molecular alterations underlying evolution of the streamlined limb skeletons characteristic of artiodactyls is the inability of limb bud mesenchymal cells to sense the morphogenetic SHH signal. This is due to functional degeneration of a CRM controlling up-regulation of the SHH receptor *Ptch1* in the limb bud mesenchyme.

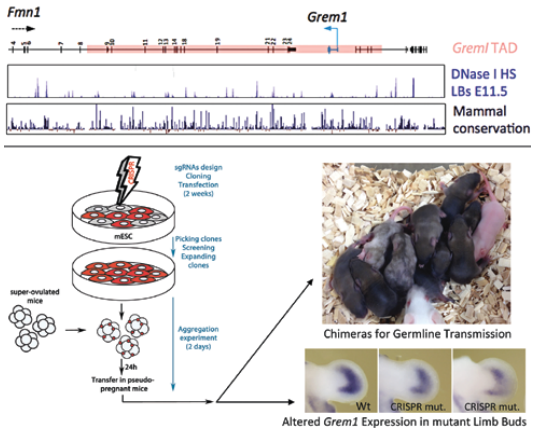

Fig. 1: CRISPR/Cas9-based functional analysis of candidate CRMs in genomic landscapes

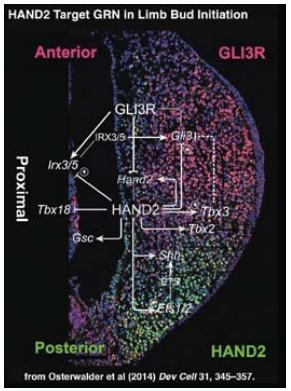

Fig. 2

Another key process is the establishment of the signaling centers controlling proliferation and patterning of progenitor cells. In limb buds, the SHH signaling center is established in the nascent mesenchyme as the limb bud forms. We have shown that the HAND2 transcription factor is essential to this process and polarization of the nascent mesenchyme. ChIP-Seq in combination with genetic analysis revealed the gene regulatory logic by which the HAND2-target GRN establishes proximal, anterior and posterior compartments and activates *Shh* expression in early limb buds (Fig. 2). Recently, we have shown that HAND2 controls similar GRNs during early heart development. Our studies begin to reveal the similarities in the HAND2-target GRNs controlling both limb and heart organogenesis, which points to a possible shared origin of these two structures.
Finally, we have shown that SMAD4, which is part of the transcriptional complexes mediating response to canonical BMP signaling, is essential for normal limb bud development. We have used ChIP-Seq and comparative transcriptome analysis to identify the genes and GRNs that are regulated by SMAD4 chromatin complexes. Our analysis provides insight into the functionally relevant cis-regulatory dynamics of SMAD4-mediated BMP signal transduction during limb bud organogenesis and chondrogenic differentiation. With respect to the latter, we are performing an in depth analysis of the functions of the BMP antagonists Grem1 and *Noggin* and SMAD4-mediated BMP/TGFß signal transduction during chondrogenesis and digit ray formation. Understanding BMP functions in the switch from proliferating mesenchymal to differentiating chondrogenic progenitors is directly relevant to development-inspired engineering of cartilage (joint SNF project with I. Martin).

Selected Publications

Zuniga A. (2015) Next generation limb development and evolution: old questions, new perspectives. Development 142, 3810–3820

Vaillant C, Valdivieso P, Nuciforo S, Kool M, Schwarzentruber-Schauerte A, Méreau H, Cabuy E, Lobrinus JA, Pfister S, Zuniga A, Frank S, Zeller R. (2015) Serpine2/PN-1 is required for Proliferative Expansion of Pre-Neoplastic Lesions and Malignant Progression to Medulloblastoma. PLoS One 10, e0124870 doi: 10.1371/journal.pone.0124870

Van Dusen NJ, Casanovas J, Vincentz JW, Firulli BA, Osterwalder M, Lopez-Rios J, Zeller R , Zhou B, Grego-Bessa J, De La Pompa JL, Shou W, Firulli AB. (2014) Hand2 is an Essential Regulator for Two Notch-Dependent Functions within the Embryonic Endocardium. Cell Reports 9 , 2071–2083

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Lopez-Rios J, Duchesne A, Speziale D, Andrey G, Peterson KA, Germann P, Ünal E, Liu J, Floriot S, Barbey S, Gallard Y, Müller-Gerbl M, Courtney AD, Klopp C, Rodriguez S, Ivanek R, Beisel C, Wicking C, Iber D, Robert B, McMahon AP, Duboule D, Zeller R. (2014) Attenuated sensing of SHH by Ptch1 underlies evolution of bovine limbs. Nature 511 , 46–51. (Article)

Connection to Clinical Practice

Prof. Ivan Martin
DBM and USB

Development – inspired engineering of cartilage
(Joint project Zeller/Martin groups)

Mesenchymal stromal/stem cells (MSC) are give rise to various tissue types under lineage specific differentiation conditions, which includes cartilage, the main focus of our research. Using MSCs for generating cartilage remains challenging due to (1) the heterogenous nature of MSC populations, which likely only contain a small and variable fraction of "stem" cells and/or early progenitors; (2) their compromised potency after *in vitro* expansion and (3) inefficient cartilage differentiation. We have done an indepth characterization of a rare population of adult mouse endosteum derived MSC-type cells as Sca-1+ PDGFR-α+ population (PαS; previously described by Matsuzaki an co-workers) as these cells posses a very robust tri-lineage differentiation potential. We have been able to characterize four subpopulations of PαS cells by FACS analysis. In particular, ontogenic analysis revealed the progressive appearance of the four PαS subpopulations during mouse embryonic limb development. A development-inspired culture protocol was used to generate stable cartilage templates from the PαS subpopulations and their endochondral bone forming potential was assessed by engraftment of cartilage constructs into nude mice. This analysis revealed the distinct potential of one of the PαS subpopulations for endochondral bone formation and support of host-derived hematopoiesis. We have initiated transcriptome analysis to enable in depth characterisation of these four PαS subpopulations.

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