CROSSTALK SYMPOSIUM
Plasticity of Cell Fate in Musculoskeletal Tissues
Organized by: ORS Women's Leadership Forum

Organizers:
Alice Huang, PhD

Speakers:
Kathryn Cheah, PhD
Benjamin Levi, MD
Jessica Lehoczky, PhD
Skeletal lineage plasticity in development and disease

Endochondral long bone development occurs by the initial establishment of a cartilaginous template that is then replaced with bone. Growth is fueled by a specialized structure, the growth plate, which is composed of highly organized chondrocytes undergoing proliferation, maturation, and hypertrophy. The fate of hypertrophic chondrocytes has long been debated – does the hypertrophic stage represent terminal differentiation followed by cell death or is it simply a transitional stage toward the osteogenic fate? The established dogma held that the transition to bone is driven by replacement of chondrocytes by osteoblasts; however, emerging evidence in the last few years now challenge this assumption. Robust lineage tracing studies demonstrate that hypertrophic chondrocytes can survive and undergo a cell fate switch toward the osteogenic lineage. Further, these cells persist into adulthood and contribute to both osteoblasts and osteocytes. Identifying the mechanisms regulating growth plate dynamics (on the cell, signaling, and transcriptional levels) has critical implications in skeletal development, disease, and repair.

Supplemental Reading:


Cell plasticity in musculoskeletal trauma and heterotopic ossification

Extremity trauma stimulates migration of inflammatory cells to the injury site where they interact with resident progenitor cells which can participate in repair or undergo aberrant differentiation. Data suggest that the predominant source of HO is generally from local stromal / mesenchymal cells within the connective tissue of skeletal muscle, fascia and/or subcutis. These resident stromal / mesenchymal cells have been marked using Prx1-Cre(1, 2), Scx-Cre and Scx-CreERT2(1, 3), Mx1-Cre(3), NFATc1-Cre(4), and Glast-CreERT(5) strains that all have overlapping domains of distribution within reporter animals. Other cell types that may contribute to HO formation include endoneurial cells highlighted by Wnt1-CreERT(6), pericytes and other perivascular cells also shown in Glasc-ERT reporter animals(5). Additionally, studies suggest that circulating mesenchymal cell types may contribute a small proportion of HO cells(8-11), which are likely non-hematopoietic (11). Cells that have been shown to likely not participate include degenerating skeletal muscle fibers, vascular smooth muscle, and chronic inflammatory cells(12, 13). Given the diversity of murine HO models, it is important to delineate which cell types directly contribute to HO bone and cartilage, and which cell types represent requisite 'niche' factors for HO genesis. HO is a diverse pathologic process, with different etiologies, anatomic sites, mechanisms of ossification (endochondral vs. intramembranous), and putative cells of origin. These cells do not undergo aberrant cell fate at baseline but require interaction with inflammatory and niche cells. Elucidating this interaction between myeloid lineage, niche and local progenitor cells is necessary to understand tissue repair, regeneration and abnormal cell fate.

**Table 1:** Summary of local cell types that have been observed to contribute to murine HO using transgenic reporter mice.

<table>
<thead>
<tr>
<th>Cre driver</th>
<th>Cell source</th>
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<tbody>
<tr>
<td>Prx1-Cre</td>
<td>Mesenchyme(1)</td>
</tr>
<tr>
<td>Nfatc1-Cre</td>
<td>Mesenchyme(14)</td>
</tr>
<tr>
<td>Dermo1-Cre</td>
<td>Mesenchyme(15)</td>
</tr>
<tr>
<td>Scx-Cre; Scx-CreERT2</td>
<td>Tendon / periosteum / fascia(1, 3)</td>
</tr>
<tr>
<td>Mx1-Cre</td>
<td>Skeletal muscle interstitium / bone marrow(3)</td>
</tr>
<tr>
<td>Gli1-CreER</td>
<td>Institial / perivascular cells(16)</td>
</tr>
<tr>
<td>Glasc-ERT</td>
<td>Pericyte / adipocyte / connective tissue interstitium(5)</td>
</tr>
<tr>
<td>Wnt1-CreERT</td>
<td>Endoneurium(6)</td>
</tr>
<tr>
<td>Tie2-Cre / VE-Cadherin-Cre</td>
<td>Endothelium / muscle satellite cell(12, 17)</td>
</tr>
</tbody>
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**References/ Supplemental reading**


Lineage restriction during mouse digit tip regeneration

Large scale coordinated composite tissue regeneration—such as regeneration of an entire limb—does not innately occur in mammals, though this ability does exist in select invertebrates and lower vertebrates. Intriguingly however, several mammals (including mice and humans) are capable of regenerating the distal digit tip following amputation. While the digit tip is on a smaller anatomical scale than the entirety of the limb, the tissue composition has clear parallels (skin, bone, connective tissue, tendon, nerves, fat, vasculature, etc). The mammalian digit tip regenerates by a stereotypic process termed epimorphic regeneration which is mediated by a “blastema”: a population of proliferative progenitor cells that gives rise to the regenerate tissues. Classical research in non-mammalian epimorphic regenerative models led to a hypothesis that the blastema cells are homogeneous and capable of transdifferentiation. Over the past decade, the composition and plasticity of the blastema has been re-addressed with genetic tools. Our studies used mouse genetic lineage analyses and found no evidence of transdifferentiation between the blastema cells and the overlying epithelium, indicating the blastema is not pluripotent. Moreover, we find that the blastema is celluarly heterogeneous and of the lineages we evaluated (Sp7, Lgr6, Msx1) these sub-populations of cells are lineage-restricted, for example Sp7 or Lgr6-expressing osteoprogenitors only give rise to bone in the regenerated digit tip, not other connective tissue. This model of blastema lineage restriction meshes well with data from other groups, both in mouse digit tip and other epimorphic regenerative model organisms. In our present work we have performed single cell RNA sequencing of mouse blastema cells which not only confirms the heterogeneity of the cells, but gives a new found resolution to the cell types participating in digit tip regeneration. Moving forward, much remains to be determined about the origin and lineage of all the blastema cell sub-populations, and ultimately how these findings can impact broader composite tissue regeneration.

Supplemental Reading:


Khedgikar V and Lehoczky JA, Evidence for Lgr6 as a novel marker of osteoblastic progenitors in mice. JBMRplus https://doi.org/10.1002/jbm4.10075