A Stem Cell Basis for Spine Fusion

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INTRODUCTION: Spine fusion is a common orthopedic procedure, and multiple cellular adjuncts have been studied for their ability to improve fusion outcomes with limited efficacy. The specific stem cell mediating spine fusion remains unknown. We aim to identify the specific skeletal stem cell responsible for generating the osteoblasts mediating spine fusion.

METHODS: The study was approved by the institutional IACUC. Our group identified a distinct murine vertebral skeletal stem cell (vSSC) and a *Zic1-cre* targeting this cell was created. Functional studies confirmed the stemness of this vSSC and its physiologic contribution to vertebral mineralization. A murine posterolateral spine fusion model was validated utilizing using either iliac crest bone graft (ICBG) or demineralized bone matrix (DBM) (**Fig 1**). Bone volume (BV) formed was measured with microCT and lineage tracing was performed with confocal microscopy. Flow cytometry was used to isolate vSSCs.

RESULTS SECTION: Lineage tracing within the fusion mass confirmed the contribution of the *Zic1*+ vSSC lineage to osteoblasts in the fusion mass with both ICBG and DBM (**Fig 1**). Inducing a gain-of-function in *Zic1*+ VSSCs via conditional deletion of the bone formation inhibitor, *Shn3*, triggered an increase in fusion BV (**Fig 2**). Similarly, inducing a loss of function in *Zic1*+ VSC via conditional deletion of the positive regulation of bone osteoblast formation, *Stat3*, resulted in a decrease in fusion BV (**Fig 2**). Taken together, this confirms that *Zic1* stem cells functionally contribute to fusion mass formation. FACS identified that induction of fusion triggers egress of *Zic1*+ vSSCs from adjacent host bone. *Zic1*+ vSSCs present in the fusion mass were reisolated and implanted into secondary hosts, demonstrating their ability to form bone organoids. This demonstrates that the *Zic1*+vSSCs present in the fusion mass not only have the markers of *Zic1*+vSSCs, but moreover have osteogenic capacity and provides functional evidence that *Zic1*+vSSCs are the osteogenic population responsible for forming the fusion mass. As *Zic1-cre* labels the posterior spinous musculature in addition to vertebral cells, *Pax7-cre mTmG* reporter mice labeling muscle underwent spine fusion, finding that muscle cells did not contribute to the fusion mass formation. Lastly, we observed ventral and dorsal differences in *Zic1* cell concentration and identified a second stem cell distinct to the anterior body, labeled by *Tbx1-cre*. An anterior interbody fusion model was further developed showing that *Tbx1*-lineage cells are responsible for anterior fusion but not posterior fusion. Further studies to delineate the differences between *Zic1*-lineage and *Tbx1*-lineage cells in spine fusion are ongoing.

DISCUSSION: A novel vertebral skeletal stem cell marked by *Zic1-cre* and other markers is the cell of origin for the fusion mass in posterolateral spine fusion. Fusion mass generation involves egress of this *Zic1*+ vSSC from vertebral bone and into the fusion mass where it retains its fusion promoting capacity. We also identify that the cells mediating posterolateral and anterior spine fusion are distinct, non-overlapping populations, uncovering a previously unappreciated difference in the biologic basis for these two procedures.

SIGNIFICANCE/CLINICAL RELEVANCE: By identifying this candidate *Zic1*+ VSC, we have identified a novel cellular basis for the formation of the fusion mass, finding that this is derived from the *Zic1*+ VSC. Identification of this cell will enable subsequent efforts to identify druggable targets and pathways specific to this stem cell to maximize the fusion mass and thereby prevent pseudarthrosis.

REFERENCES: Debnath S, Yallowitz AR, McCormick J et al. Discovery of a Periosteal Stem Cell Mediating Intramembranous Bone Formation. Nature. 2018 Oct; 562(7725):133-139.

IMAGES AND TABLES:

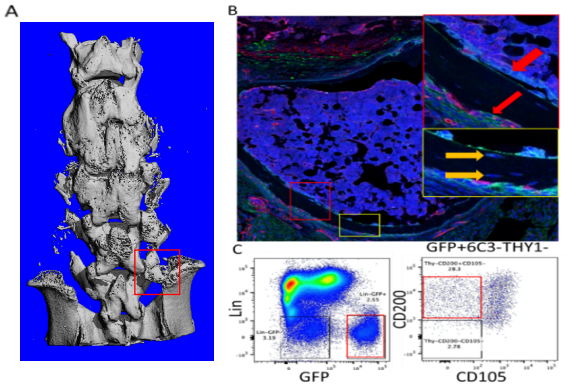

Fig 1. The Zic1 vSSC-lineage contributes to the fusion bone mass. 6-week-old *Zic1-cre mTmG* mice underwent L4-6 spine fusion as described in the text, here with an iliac crest graft. 6 weeks later, fusion was assessed by µCT (A) and histology (B), with the region of histologic sampling indicated with a red box on the µCT reconstruction. High power inserts of the fusion bone mass are shown in (B), with the corresponding regions indicated with color matched boxes. mGFP+ *Zic1* vSSC-lineage morphologic osteoblasts (red arrows) and osteocytes (orange arrows) are indicated. (C) Flow cytometry of the fusion mass 2 weeks after surgery showing the presence of mGFP+ *Zic1* vSSC-lineage cells, including CD200+CD105-6C3-THY1-*Zic1* vSSCs themselves (red box, right plot). Cells in the right plot are all gated to be 6C3-THY1-EMB-. Results representative of n=8.

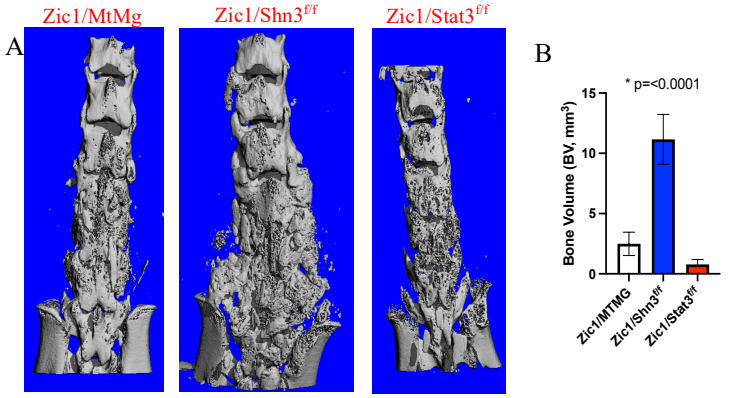

Fig 2. vSSCs functionally contribute to formation of the fusion mass. 6-week-old male *Shn3*$^{fl/fl}$ *Zic1-cre*, Stat3$^{fl/fl}$ *Zic1-Cre*, or *Zic1-cre* controls underwent the spine fusion model. 6 weeks later, bone mass in the fusion was determined by µCT as shown in a 3D reconstruction (A) and by quantitative determination of the volume of bone in the fusion mass outside of the underlying vertebrae (B). n=6/group. P<0.0001 by ANOVA.