A Stem Cell Basis for Spine Fusion

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Disclosures: Kyle W. Morse (Sustain Surgical, Inc, Johnson & Johnson, GE Health), Jun Sun (N), Seoyeon Bok (N), Shawon Debnath (N), Michelle Cung (N), Alisha Yallowitz (N), Kathleen N. Meyers (N), Adrian Tan (N), Jason McCormick (N), Srvaisht Iyer (Globus Medical Inc., Elliquence, LLC, Innovasis, Inc, Healthgrades, Inc), Matthew Greenblatt (N).

INTRODUCTION: Spine fusion is a common orthopedic procedure, and multiple cellular adjacents 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, Shh, 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 cells functionally contribute to fusion mass formation. Flow cytometry identified 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 spinal 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.


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 Shh3+/– Zic1-cre, Stat3+/– Zic1-cre, or Zic1-cre controls underwent the spine fusion model. 6 weeks later, bone mass in the fusion was determined by μCT reconstruction 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.