Spry4 Regulates Bone Angiogenesis and Osteogenesis: Investigating the Vascular–SSPC Link

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INTRODUCTION: Bone regeneration depends on the coordinated interplay between angiogenesis and skeletal stem/progenitor cell (SSPC) activity. In bone, a specialized subset of blood vessels known as Type H vessels is crucial for this process. These vessels form vascular niches that promote SSPC proliferation and osteogenic differentiation (Kusumbe et al., 2014). Sprouty4 (Spry4), a negative regulator of receptor tyrosine kinase signaling, has been shown to influence angiogenesis in other organs (Gong et al., 2013); however, its role in regulating bone vasculature and the SSPC niche has not been investigated. We hypothesize that Spry4 modulates bone formation by affecting endothelial proliferation and vessel density, particularly of Type H vessels, which may, in turn, influence SSPC osteogenic responses.

METHODS: To investigate this, we employed a Spry4 knockdown (Spry4KD) mouse model. Endothelial proliferation was assessed by EdU incorporation and quantified using flow cytometry. Total and type H endothelial cell populations were also analyzed by flow cytometry, and overall vessel density, including type H vessels, was evaluated using immunofluorescence. Bone architecture and mass were measured using micro-computed tomography (microCT). SSPC osteopriming was assessed by examining the expression of early osteogenic genes. Spry4 expression was evaluated in endothelial cells and SSPCs using flow cytometry and immunofluorescence. Single-cell RNA sequencing (scRNA-seq) is ongoing to characterize transcriptional changes and ligand–receptor interactions. All animal procedures were conducted in accordance with NYU Langone IACUC guidelines.

RESULTS SECTION: Spry4 was found to be expressed in nearly all endothelial cells and in a fraction of SSPCs (Fig 1A). Spry4KD significantly increased endothelial cell proliferation (n = 3-5 per group, $p < 0.05$) and elevated the percentage of total and type H endothelial cells (n=4, $p < 0.01$). Immunofluorescence analysis confirmed increased vascular density, including type H vessels (n = 5 per group, $p < 0.05$) (Fig 1B-C). MicroCT demonstrated enhanced trabecular bone volume and cortical thickness in Spry4KD mice (n = 7-10 per group, $p < 0.05$) (Fig 1D-F). SSPCs from Spry4KD mice exhibited increased osteopriming, as indicated by higher expression of early osteogenic markers (n=3, $p < 0.05$).

DISCUSSION: These findings indicate that Spry4 regulates both angiogenesis and SSPC osteogenic potential. The concurrent increases in endothelial proliferation, vessel density, and SSPC osteopriming suggest that enhanced angiogenesis may contribute to improved osteogenesis. However, because Spry4 is also expressed in a subset of SSPCs, cell-intrinsic effects cannot be excluded. Ongoing scRNA-seq studies will help to clarify whether the effects on SSPCs are primarily mediated through vascular changes or involve direct regulation by Spry4 within the progenitor compartment.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding whether angiogenesis directly drives Spry4-mediated SSPC osteogenic responses will provide critical insight into vascular–skeletal communication. This knowledge may guide therapeutic strategies aimed at enhancing bone regeneration

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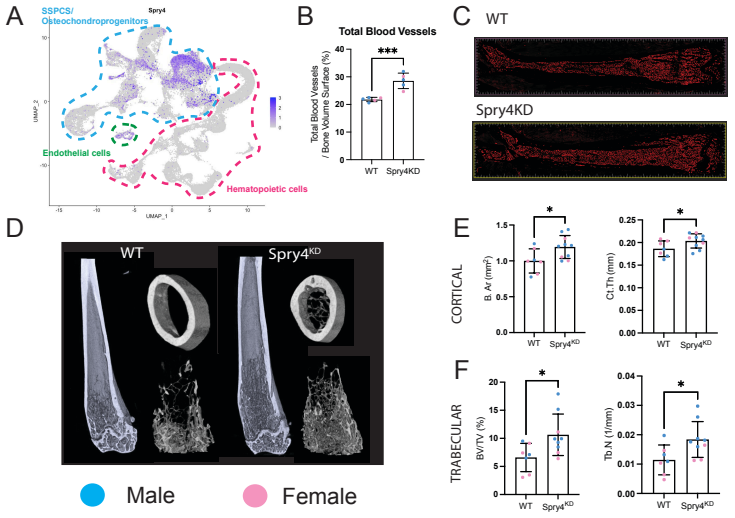

Figure 1: Spry4 knockdown increases bone structure and blood vessel density in mice

(A) UMAP visualization of digested bone and bone marrow cells showing Spry4 expression (purple) across skeletal stem/progenitor cells (SSPCs)/osteochondroprogenitors (outlined in blue), endothelial cells (outlined in green), and hematopoietic cells (outlined in red).
(B-C) Quantification and representative images of blood vessel density in WT and Spry4KD femurs. Endomucin staining was used to identify total blood vessels. (n = 5 per group; *** $p < 0.001$)
(D) 3D-rendered coronal microCT cross-sections of femurs from WT and Spry4KD mice (10–16 weeks).
(E) Quantification of cortical bone parameters, including bone area (B.Ar) and cortical thickness (Ct.Th). (n = 7–10 per group; *$p < 0.05$)
(F) Quantification of trabecular bone parameters throughout the marrow cavity, including bone volume/tissue volume (BV/TV) and trabecular number (Tb.N). (n = 7–10 per group; *$p < 0.05$)