Stem cell niche-specific Ebf3 maintains the bone marrow cavity

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Bone marrow is the tissue filling the space between bone surfaces. Hematopoietic stem cells (HSCs) are maintained by special microenvironments known as niches within bone marrow cavities. Mesenchymal cells, termed CXC chemokine ligand 12 (CXCL12)-abundant reticular (CAR) cells or leptin receptor-positive (LepR$^+$) cells, are a major cellular component of HSC niches that gives rise to osteoblasts in bone marrow. However, it remains unclear how osteogenesis is prevented in most CAR/LepR$^+$ cells to maintain HSC niches and marrow cavities. Here, using lineage tracing, we found that the transcription factor early B-cell factor 3 (Ebf3) is preferentially expressed in CAR/LepR$^+$ cells and that Ebf3-expressing cells are self-renewing mesenchymal stem cells in adult marrow. When *Ebf3* is deleted in CAR/LepR$^+$ cells, HSC niche function is severely impaired, and bone marrow is osteosclerotic with increased bone in aged mice. In mice lacking *Ebf1* and *Ebf3*, CAR/LepR$^+$ cells exhibiting a normal morphology are abundantly present, but their niche function is markedly impaired with depleted HSCs in infant marrow. Subsequently, the mutants become progressively more osteosclerotic, leading to the complete occlusion of marrow cavities in early adulthood. CAR/LepR$^+$ cells differentiate into bone-producing cells with reduced HSC niche factor expression in the absence of *Ebf1/Ebf3*. Thus, HSC cellular niches express Ebf3 that is required to create HSC niches, to inhibit their osteoblast differentiation, and to maintain spaces for HSCs.

[*Keywords*: Ebf3; stem cell niche; hematopoietic stem cell; mesenchymal stem cell; bone]

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Bone marrow is the tissue filling the space between bone surfaces. Hematopoietic stem cells (HSCs), which give rise to all blood cells, including immune cells, are maintained and regulated by special microenvironments known as niches in the bone marrow cavity (Li and Clevers 2010; Ehninger and Trumpp 2011; Nagasawa et al. 2011; Mercier et al. 2012; Morrison and Scadden 2014; Boulais and Frenette 2015). The identity of HSC niches has been a subject of long-standing debate, and recent studies have identified diverse candidate cells that may constitute a niche for HSCs. Rare cell populations, including osteoblasts lining the bone surface (Calvi et al. 2003; Zhang et al. 2003), periarteriolar nestin$^+$NG2$^+$ mesenchymal stem cells (Méndez-Ferrer et al. 2010; Kunisaki et al. 2013; Itkin et al. 2016; Kusumbe et al. 2016), CD45$^-$ lineage marker-negative (Lin$^-$)platelet-derived growth factor receptor α-positive (PDGFRα$^+$)Sca-1$^+$ (PαS) cells (Greenbaum et al. 2013), and nonmyelinating Schwann cells (Yamazaki et al. 2011) as well as macrophages expressing α-smooth muscle actin (α-SMA) (Ludin et al. 2012) and megakaryocytes (Bruns et al. 2014; Zhao et al. 2014; Nakamura-Ishizu et al. 2015) have been reported to create a niche for HSCs. On the other hand, more abundant populations of nonhematopoietic cells, including sinusoidal endothelial cells (Kiel et al. 2005; Butler et al. 2010; Ding et al. 2012; Ding and Morrison 2013) and the adipo–osteogenic progenitors, called CXC chemokine ligand 12 (CXCL12)-abundant reticular (CAR) cells, which strongly overlap with leptin receptor-positive (LepR$^+$) cells (Sugiyama et al. 2006; Omatsu et al. 2010, 2014; Ding et al. 2012; Ding and Morrison 2013), have been shown to create a niche for HSCs. CAR cells express markedly higher levels of CXCL12, stem cell factor (SCF), Lepr, and the transcription factor Foxc1 as compared with other bone marrow cell populations (Sugiyama et al. 2006; Omatsu et al. 2010, 2014; Ding et al. 2012). Additionally, HSCs are significantly more likely to be close to CAR cells rather than placed randomly (Sugiyama

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