Citation: BoneKEy Reports **3**, Article number: 572 (2014) | doi:10.1038/bonekey.2014.67

www.nature.com/bonekey

COMMENTARY

Osteoblasts as leukemia-initiating cells

Benjamin J Frisch[1,2,3] and Laura M Calvi[1,2,3,4]

[1]Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester NY, USA. [2]Center for Musculoskeletal Research, University of Rochester School of Medicine and Dentistry, Rochester NY, USA. [3]Wilmot Cancer Institute, University of Rochester School of Medicine and Dentistry, Rochester NY, USA. [4]Endocrine Division, University of Rochester School of Medicine and Dentistry, Rochester NY, USA.

BoneKEy Reports **3**, Article number: 572 (2014) | doi:10.1038/bonekey.2014.67; published online 3 September 2014

Commentary on: Kode A, Manavalan JS, Mosialou I, Bhagat G, Rathinam CV, Luo N, Khiabanian H, Lee A, Murty VV, Friedman R, Brum A, Park D, Galili N, Mukherjee S, Teruya-Feldstein J, Raza A, Rabadan R, Berman E, Kousteni S. Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts. *Nature* 2014;**506**(7487):240–244.

Hematopoietic stem and progenitor cells (HSPCs) rely on a supportive microenvironment, or niche, within the bone marrow for the regulation of fate choices.[1] A variety of bone marrow cell types have been implicated as HSPC niche components, and osteolineage cells have emerged as one of the best-established HSPC niche cells. Leukemia is widely considered to be a stem cell disease, with a malignant stem or progenitor cell as the leukemia-initiating cell.[2] As such, leukemia cells, particularly leukemia stem cells, also engage in interactions with cells of the bone marrow microenvironment, including osteoblasts and other members of the HSPC niche.[3] Previous studies have shown that alteration of the bone marrow microenvironment can induce hematopoietic dysfunction[4,5] and that genetic alteration of osteolineage cells can lead to acute myelogenous leukemia (AML).[6] These findings suggest that the bone marrow microenvironment, including osteolineage cells, may have a role in leukemogenesis.

In a recent letter to *Nature,* Kode *et al.*[7] present studies to show that genetic alteration of osteoblasts in the bone marrow microenvironment can induce an aggressive AML. In this report mice are genetically altered to express a constitutively active mutant allele of β-catenin specifically in osteoblastic cells using a 2.3 kb fragment of the α1(I) collagen promoter (*Ctnnb1*^{CAosb} mice). Thus, *Ctnnb1*^{CAosb} mice will express constitutively active β-catenin in osteolineage cells, starting at pre-osteoblast stage, through downstream maturation states of mature osteoblasts, to osteocytes. Remarkably, between 2 and 3.5 weeks of age all *Ctnnb1*^{CAosb} mice develop hematopoietic malignancy that meets the Bethesda criteria for AML in mice.[8] This is the first model AML to demonstrate 100% penetrance following a primary genetic alteration to the bone marrow microenvironment. The authors also demonstrated that the AML in *Ctnnb1*^{CAosb} mice is transplantable into WT recipients, and that the leukemia-initiating cell is a long-term HSC (LT-HSC). The transplantability of this AML suggests a permanent alteration within the LT-HSCs of the *Ctnnb1*^{CAosb} mice. Upon investigation, the authors discovered that hematopoietic cells of all *Ctnnb1*^{CAosb} mice had somatic mutations and/or recurrent chromosomal alterations that allowed progression of AML independent of the original constitutive activation of β-catenin in osteoblastic cells. Several of these genetic abnormalities have been associated with human AML and myelodysplastic syndromes (MDS), including mutations in the gene *Crb1*, as well as both gains and losses within the mouse ortholog of human chromosome 7q, a mutation commonly observed in human AML. These findings demonstrate for the first time that genetic alteration of mature osteolineage cells can lead to AML in mice with no germline mutation in the hematopoietic system.

In investigating the mechanism by which hematopoietic malignancy is induced, the authors discovered that the Notch ligand jagged 1, a β-catenin target gene, was upregulated in the osteoblastic cells of *Ctnnb1*^{CAosb} mice. Jagged 1 expression on osteoblasts has previously been described to play a significant role in the regulation of HSCs.[9,10] The authors also found that Notch signaling was only increased in LT-HSCs, and not in any of the more mature hematopoietic progenitors in *Ctnnb1*^{CAosb} mice, suggesting that osteoblastic cells, at or beyond the differentiation stage at which the 2.3 kb fragment of the α1(I) collagen promoter is activated, participate in the regulation of the most immature HSCs. This confirms many previous findings on the role of osteoblastic cells in the LT-HSC niche.[9,11,12] The removal of one allele of the jagged 1 gene in the same osteoblastic population that expresses constitutively active β-catenin results in a complete loss of AML development. This demonstrates a remarkable dependence on osteoblastic jagged 1 for the development of AML in this model. In addition, the authors treated 2-week-old *Ctnnb1*^{CAosb} mice with a γ-secretase inhibitor to block all Notch signaling, rescuing the hematopoietic dysfunction observed in *Ctnnb1*^{CAosb} mice. Therefore, following the development of AML, Notch signaling is required for the maintenance of hematologic malignancy, suggesting that Notch activation in leukemic cells is critical not just for AML development but also for disease progression. In conditions of osteoblastic jagged 1 hemizygosity and γ-secretase inhibitor treatment, severe osteopetrosis is maintained. Thus, an overabundance of bone and osteoblastic

cells is not sufficient to induce hematopoietic dysfunction; rather, specific signals from osteoblastic cells are required for appropriate regulation of LT-HSCs.

Finally, the authors conclude by investigating the activation of β-catenin and the Notch-signaling pathway in human patients with MDS, classically considered a pre-leukemic state, or AML. Bone biopsies were obtained from these patients as well as from healthy controls, and activation of β-catenin was observed in osteoblastic cells for 38.3% of the 107 combined MDS and AML patients studied. None of the healthy controls showed nuclear localization of β-catenin. Also correlating with the mouse model, increased expression of the Notch ligands *JAG1* and *DLL1* in osteoblastic cells and increased expression of Notch target genes in hematopoietic cells of AML and MDS patients with demonstrated nuclear localization of β-catenin were observed. Importantly, these results suggest a relevance of this mouse model to human disease, and highlight potential therapeutic targets. In addition, one could envision nuclear localization of β-catenin as an additional tool to predict transition of MDS to AML.

The many exciting findings from these studies suggest some intriguing research directions. For example, activation of Notch signaling is a well-known event in T-cell leukemias;[13] however, Notch activation does not yet enjoy a widely recognized role in the development or progression of myeloid malignancies. This could have exciting implications, as γ-secretase inhibitors are currently in clinical trials, making for a potentially rapid translation for the treatment of myeloid leukemia.[13] Moreover, *Ctnnb1*^{CAosb} mice develop marked osteopetrosis, something that is never reported in human AML and MDS despite the finding that a significant proportion of patients will have activation of β-catenin in osteolineage cells. In mouse models, bone loss and osteoblastic inhibition can be consequences of myeloid malignancies.[14,15] Therefore, it is possible that leukemia-induced osteoblastic defects may counterbalance and prevent the osteopetrotic phenotype in the subset of AML and MDS patients with osteoblastic activation of β-catenin.

The results of this study also raise concerns about the use of an anti-sclerostin antibody (Romosozumab) for the treatment of low bone mineral density.[16] Sclerostin is produced by osteocytes and inhibits the Wnt and bone morphogenetic protein signaling pathways, thereby reducing osteoblastic bone formation. Therefore, antagonism of sclerostin results in increased bone formation through the activation of β-catenin in osteoblastic cells. To date, there are no reports of sclerostin inhibition resulting in hematopoietic malignancy. In addition, the autosomal-recessive disorder sclerosteosis results from an inactivating mutation in the gene *SOST* that encodes for the sclerostin protein. The disorder is very rare, however; a 38-year study was conducted that describes only one case of hematologic malignancy, Hodgkin's lymphoma, in a cohort of 63 patients with sclerosteosis.[17] Thus, though there is no evidence yet that the use of anti-sclerostin antibody will result in increased risk for hematologic malignancies; in the context of this report, additional studies may be warranted.

What, then, is the primary event in human myeloid malignancies: leukemia initiation or microenvironmental alteration? This report, as well as others, has successfully demonstrated that osteolineage cells can induce hematologic dysfunction and malignancy in genetically altered mouse models.[6,7] However, it is also well established that leukemic disease can alter the bone marrow microenvironment, including osteolineage cells.[14,15,18] This paper, in the context of recent data illuminating leukemia-initiated changes in the osteoblastic lineage, suggests complex and potentially reiterative interactions of leukemia within its bone marrow microenvironment, which could not only explain disease resistance to treatment but also suggest additional therapeutic targets. Whether osteolineage cells can alter HSPCs to initiate leukemia, or, alternatively, the leukemic environment alters the microenvironment remains an open question in human patients. The answers to these questions will further develop our understanding of the origins of myeloid malignancies, and provide the potential for new avenues of therapy.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work is supported by the National Institutes of Health (NIDDK: DK081843; NIAID: AI091036 and AI107276; NCI: CA166280; NIA: AG046293) and the Department of Defense (BM110106) to LMC. BJF is supported by the Gregory R. Mundy Fellowship from the International Bone and Mineral Society.

References

1. Smith JN, Calvi LM. Concise review: Current concepts in bone marrow microenvironmental regulation of hematopoietic stem and progenitor cells. *Stem Cells* 2013;**31**:1044–1050.
2. Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. *Blood* 2009;**114**:1150–1157.
3. Tabe Y, Konopleva M. Advances in understanding the leukaemia microenvironment. *Br J Haematol* 2014;**164**:767–778.
4. Walkley CR, Olsen GH, Dworkin S, Fabb SA, Swann J, McArthur GA *et al.* A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. *Cell* 2007;**129**:1097–1110.
5. Walkley CR, Shea JM, Sims NA, Purton LE, Orkin SH. Rb regulates interactions between hematopoietic stem cells and their bone marrow microenvironment. *Cell* 2007;**129**:1081–1095.
6. Raaijmakers MH, Mukherjee S, Guo S, Zhang S, Kobayashi T, Schoonmaker JA *et al.* Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature* 2010;**464**:852–857.
7. Kode A, Manavalan JS, Mosialou I, Bhagat G, Rathinam CV, Luo N *et al.* Leukaemogenesis induced by an activating beta-catenin mutation in osteoblasts. *Nature* 2014;**506**:240–244.
8. Kogan SC, Ward JM, Anver MR, Berman JJ, Brayton C, Cardiff RD *et al.* Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. *Blood* 2002;**100**:238–245.
9. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 2003;**425**:841–846.
10. Weber JM, Forsythe SR, Christianson CA, Frisch BJ, Gigliotti BJ, Jordan CT *et al.* Parathyroid hormone stimulates expression of the Notch ligand Jagged1 in osteoblastic cells. *Bone* 2006;**39**:485–493.
11. Calvi LM, Bromberg O, Rhee Y, Weber JM, Smith JN, Basil MJ *et al.* Osteoblastic expansion induced by parathyroid hormone receptor signaling in murine osteocytes is not sufficient to increase hematopoietic stem cells. *Blood* 2012;**119**:2489–2499.
12. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature* 2014;**505**:327–334.
13. Andersson ER, Lendahl U. Therapeutic modulation of Notch signalling—are we there yet? *Nat Rev Drug Discov* 2014;**13**:357–378.
14. Frisch BJ, Ashton JM, Xing L, Becker MW, Jordan CT, Calvi LM. Functional inhibition of osteoblastic cells in an *in vivo* mouse model of myeloid leukemia. *Blood* 2012;**119**:540–550.
15. Schepers K, Pietras EM, Reynaud D, Flach J, Binnewies M, Garg T *et al.* Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. *Cell Stem Cell* 2013;**13**:285–299.
16. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A *et al.* Romosozumab in postmenopausal women with low bone mineral density. *N Engl J Med* 2014;**370**:412–420.
17. Hamersma H, Gardner J, Beighton P. The natural history of sclerosteosis. *Clin Genet* 2003;**63**:192–197.
18. Lane SW, Wang YJ, Lo Celso C, Ragu C, Bullinger L, Sykes SM *et al.* Differential niche and Wnt requirements during acute myeloid leukemia progression. *Blood* 2011;**118**:2849–2856.