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Role of SHIP1 in bone biology

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Abstract

The bone marrow milieu comprised of both hematopoietic and non-hematopoietic lineages has a unique structural organization. Bone undergoes continuous remodeling in the body throughout life. This dynamic process involves a balance between bone-forming osteoblasts (OBs) derived from multipotent mesenchymal stem cells (MSCs) and bone-resorbing osteoclasts (OCs) derived from hematopoietic stem cells (HSCs). Src homology 2-domain-containing inositol 5'-phosphatase 1 (SHIP1) regulates cellular processes such as proliferation, differentiation, and survival via the PI3K/AKT signaling pathway initiated at the plasma membrane. SHIP1-deficient mice also exhibit profound osteoporosis that has been proposed to result from hyperresorptive activity by OCs. We have previously observed that SHIP1 is expressed in primary OBs, which display defective development in SHIP1-deficient mice. These findings led us to question whether SHIP1 plays a functional role in osteolineage development from MSC *in vivo*, which contributes to the osteoporotic phenotype in germline SHIP1 knockout mice. In this short review, we discuss our current understanding of inositol phospholipid signaling downstream of SHIP1 in bone biology.

Keywords

SHIP1; bone; osteoclast; osteoblast; PI3K; inositol phospholipid

Introduction

The Src homology 2-domain-containing inositol 5'-phosphatase 1 (SHIP1) regulates cellular processes such as proliferation, differentiation and survival via the PI3K/AKT signaling pathway initiated at the plasma membrane. SHIP1 dephosphorylates the product of PI3K, phosphatidylinositol (3,4,5) triphosphate (PI(3,4,5)P₃), to phosphatidylinositol (3,4)-bisphosphate (PI(3,4)P₂), which, like PI(3,4,5)P₃, can facilitate downstream activation of Akt.¹ The SHIP1 protein encoded by the INPP5D gene was simultaneously identified as an LPS-response gene in B cells, for its ability to bind the SH3 domain of Grb2 via its poly-proline motifs (PxxP) and for binding to the PTB domain of Shc via its NPXY motifs.¹ Furthermore, by virtue of its SH2-domain, this phosphatase can be recruited to receptor-associated signalling complexes and the plasma membrane directly or via adapter proteins

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such as Shc, Grb2, Dok3, or by scaffolding proteins such as Gab1/2. SHIP1 arrests DAP12 mediated activation of macrophages and osteoclasts by masking DAP12's ITAM via its SH2 domain, which prevents the recruitment of other SH2 domain-containing proteins, including p85 and Syk.² Regulation of SHIP protein expression occurs both at the transcriptional and post-transcriptional levels, while the SHIP1 protein can also be targeted for proteasomal degradation via ubiquitination.¹

SHIP1 in osteoclasts

Bone undergoes continuous remodeling in the body throughout life. This dynamic process involves a balance between bone-forming osteoblasts (OBs) derived from multipotent mesenchymal stem cells (MSCs) and bone-resorbing osteoclasts (OCs) derived from hematopoietic stem cells (HSCs).³ A balance of these immune and bone cells is required for maintaining normal bone homeostasis.⁴ In addition to developing severe mucosal inflammatory disease in the lungs and terminal ileum,⁵ SHIP^{-/-} mice exhibit profound osteoporosis that was proposed to result from hyper-resorptive behavior by OCs.⁶ The overall OC resorptive function resulting from increased monocyte proliferation coupled with OC differentiation and survival was attributed to increased PI3K/Akt activation downstream of TREM2,² increased D-type cyclins and down-regulation of p27 in response to M-CSF,⁷ and to the non-enzymatic functions of SHIP1 resulting in unopposed PI3K signaling at DAP12-associated receptors.²

SHIP1 in mesenchymal stem cells and osteoblasts

The bone marrow milieu, responsible for both hematopoietic and non-hematopoietic lineages, has a unique structural organization. Previously, we and others found that HSCs from SHIP-deficient mice demonstrate defective repopulating and self-renewal capacity upon transfer to SHIP-competent hosts.⁸ These findings suggested that SHIP-deficiency leads to an intrinsic defect in HSC function; however, this defect was not observed when HSCs were rendered SHIP-deficient in adult hosts where the BM milieu remained SHIP-competent.⁹ We therefore hypothesized that defective HSC function in germline SHIP-deficient mice might arise from disruption of niche cell components. Consistent with this hypothesis, primary osteoblasts were found to express the SH2 domain containing 145 and 150kD isoforms encoded by the SHIP1 locus.⁹ In addition, bone marrow derived SHIP1^{-/-} osteoblasts, grown *ex vivo* apart from SHIP^{-/-} OCs, expressed less alkaline phosphatase (ALP) activity, which is required for bone formation by OBs. This suggests that impaired OB development and function might be directly impaired by SHIP deficiency.⁹ However, the cellular components and molecular pathways that constitute the BM microenvironment altered by SHIP deficiency remained to be defined. Nonetheless, these above findings strongly suggest that inositol phospholipid signaling pathways are critical to the function of the BM microenvironment that supports HSCs.

Mesenchymal stem cells (MSCs) are part the BM niche that supports HSCs.¹⁰ In addition, these multipotent stem cells can differentiate into various cell types that include HSC-supportive osteoblasts as well as chondrocytes, adipocytes, or myocytes, depending on signals derived from the cellular environment. Bone morphogenetic proteins (BMP) and transforming growth factor- β (TGF- β) play opposing roles in regulating MSC differentiation to OBs. For instance, BMP signaling stimulates osteoblast differentiation while TGF- β suppresses osteoblast differentiation and maturation.^{11,12} Alliston *et al.* demonstrated that the transcriptional repression mechanism of core-binding factor α 1 (Cbfa1), a key regulator of OB function, proliferation, and differentiation,¹³ is mediated by SMAD3 and SMAD4 in a TGF- β dependent manner (Fig. 1). Furthermore, canonical Wnt/ β -catenin signaling regulates different stages of osteoblast differentiation and evidence suggests that TGF- β

regulates β -catenin signaling via PI3K during osteoblastogenesis of human MSC (Fig. 1).¹⁴ Canonical Wnt/ β -catenin signaling has been shown to be required for skeletal development;¹⁵ however, some evidence suggests that in specific contexts Wnt/ β -catenin pathway activation can also suppress osteoblast differentiation and terminal differentiation.¹⁶

Our previous findings that SHIP1 expression occurs in primary OBs, and that OBs development is defective in SHIP1-deficient mice,⁹ led us to question whether SHIP1 plays a functional role during *in vivo* osteolineage development from MSCs that contributes to the osteoporotic phenotype of SHIP1-deficient mice.⁶ We hypothesized that osteolineage expression of SHIP1 is required for efficient development of OBs such that normal body growth, bone formation, and mineralization might be impaired in mice that lack osteolineage expression of SHIP1. Osteoblasts are key producers of factors, such as M-CSF and RANKL, that modulate osteoclastogenesis.¹⁷ Deletion of SHIP1 from osteoblasts, or MSC-producing OBs, might therefore have an effect on osteoclast differentiation. In addition, osteoblastic cells are important regulatory components of the hematopoietic stem cell microenvironment.¹⁸ Thus it will be interesting to elucidate the role of SHIP1 in the osteoblastic niche that is thought to maintain long-term HSCs and support their self-renewal.¹⁸

We speculate that SHIP1 may limit signaling pathways in MSCs that promote their proliferation and/or survival, and potentially facilitate MSC differentiation toward an osteoblast fate. Over-expression of the Id2 (inhibitor of differentiation 2) transcription factor has been shown to promote MSC proliferation while selectively blocking their osteolineage differentiation.¹⁹ Moreover, coordinate over-expression of the deubiquitinase USP1 has been shown to be necessary for preventing proteasomal degradation and sustaining expression of Id2 at levels sufficient to promote MSC proliferation and blockade of osteoblast differentiation.¹⁹ In HSCs, up-regulation of Id2 by β -catenin also suppresses myeloid differentiation and expands the hematopoietic precursor population.²⁰ We suspect that SHIP1 limits Id2 levels in MSCs, thus facilitating osteoblast development, potentially by sequestering USP1 and thereby preventing its interaction with its target Id2 (Fig. 1). In support of this hypothesis, we have observed significantly increased Id2 expression in SHIP1-deficient MSCs both prior to and upon osteogenic induction (Iyer, Margulies, and Kerr, unpublished observation).

Bone morphogenic proteins (BMPs) that promote either MSC cycling or osteolineage differentiation do so by activating SMAD family transcription factors, including SMAD4.²¹ BMPs have also been shown to induce expression of Ids in a SMAD4-dependent fashion.²¹ As induction of SMAD family transcription factors by TGF- β and LPS can induce SHIP1 expression,²² we propose that SMAD4-mediated induction of SHIP1, downstream of multiple osteogenic factors, could serve as a molecular switch to promote osteolineage commitment by MSCs (Fig. 1). SMAD4 induction of SHIP1 in MSCs/osteoprogenitors would then provide a negative feedback loop for the effects of BMPs on MSCs by repressing USP1/Id2 in order to limit MSC cycling, thus promoting their differentiation to osteoblasts. Therefore, SHIP1 may regulate a switch that controls the USP1/Id2 axis and would thus influence stem versus lineage commitment of MSC. If this model can be supported by additional experimental evidence, it would represent the first molecularly defined role for SHIP1 in the control of stem cell fate and proliferation.

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Empire Scholar of the State University of NY, and a Senior Scholar of the Crohn's and Colitis Foundation of America. W.G.K. has patents, pending and issued, relating to the analysis and targeting of SHIP1 in disease.

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Figure 1.

Role of SHIP1 in bone biology. TGF-β and IGF signaling can be mediated via the PI3K pathway where AKT is regulated by both the SHIP1 substrate PI(3,4,5)P₃ and product PI(3,4)P₂. AKT signalling leads to GSK3β inhibition, which increases β-catenin levels, and subsequently increases Id2 levels. The Smad pathway is also activated by the TGF-β receptor complex, which represses *cbfa1* and *osteocalcin*, and thus inhibits osteoblast differentiation. The Smad complex can upregulate SHIP1, which binds to USP1 and blocks Id2. In the absence of SHIP1, USP1 deubiquitinates Id2 and prevents its degradation leading to increased MSC proliferation and less differentiation to OB.