Calcified Tissue International (2023) 112:403–421
https://doi.org/10.1007/s00223-022-01042-3

REVIEW ARTICLE

From Stem to Sternum: The Role of Shp2 in the Skeleton

Nathaniel R. Jensen[1] · Ryan R. Kelly[2,3] · Kirsten D. Kelly[2] · Stephanie K. Khoo[2] · Sara J. Sidles[2,3] · Amanda C. LaRue[2,3]

Received: 5 October 2022 / Accepted: 5 November 2022 / Published online: 24 November 2022
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Abstract
Src homology-2 domain-containing phosphatase 2 (SHP2) is a ubiquitously expressed phosphatase that is vital for skeletal development and maintenance of chondrocytes, osteoblasts, and osteoclasts. Study of SHP2 function in small animal models has led to insights in phenotypes observed in SHP2-mutant human disease, such as Noonan syndrome. In recent years, allosteric SHP2 inhibitors have been developed to specifically target the protein in neoplastic processes. These inhibitors are highly specific and have great potential for disease modulation in cancer and other pathologies, including bone disorders. In this review, we discuss the importance of SHP2 and related signaling pathways (e.g., Ras/MEK/ERK, JAK/STAT, PI3K/Akt) in skeletal development. We review rodent models of pathologic processes caused by germline mutations that activate SHP2 enzymatic activity, with a focus on the skeletal phenotype seen in these patients. Finally, we discuss SHP2 inhibitors in development and their potential for disease modulation in these genetic diseases, particularly as it relates to the skeleton.

Keywords SHP2 · Bone · SHP099 · Skeleton · Development

Introduction

The ubiquitously expressed Src Homology-2 domain-containing phosphatase 2 (SHP2), encoded by the *PTPN11* gene, participates in the development and physiological regulation of tissues derived from all three germ layers [1, 2]. Dysregulation of protein tyrosine phosphorylation, including SHP2, is a major contributor to many diseases, including cancer, autoimmune disorders, chronic respiratory diseases, and diabetes. The skeletal system is especially sensitive to alterations in SHP2 activity, as evidenced by well-documented phenotypes associated with mutant SHP2 proteins. With multiple allosteric SHP2 inhibitors currently in early clinical trials for cancer treatments, SHP2 inhibition may also be beneficial for treatment of skeletal diseases. In this review, we summarize the importance of SHP2 in controlling skeletal development and homeostasis, as well as direct SHP2-mediated signaling pathways that regulate osteoblasts and osteoclasts (Fig. 1). We will consider lessons learned from alterations in SHP2 activity in human diseases, murine models, and through targeted inhibition.

Nathaniel R. Jensen and Ryan R. Kelly have contributed equally to this work.

✉ Amanda C. LaRue
Rutha.Larue@va.gov

1 Department of Pediatrics, Washington University in St. Louis, St. Louis, MO, USA

2 Ralph H. Johnson VA Health Care System, Research Service, Charleston, SC, USA

3 Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, USA

SHP2 Structure and Function

Structure

The structure of SHP2 is tightly connected to its biochemical mechanism of action. SHP2 is a non-membrane associated tyrosine phosphatase containing two N-terminal Src Homology 2 (SH2) domains, a central phosphatase domain, and C-terminal regulatory tyrosine residues [3]. In the inactive conformation, the N-terminal SH2 domain obstructs and distorts the catalytic site [3, 4] (Fig. 2a). Binding of the SHP2 SH2 domains to phospho-tyrosine residues induces a conformational change that removes the steric hindrance to unfold the protein and expose the active site [5]. Maximal phosphatase activity is achieved when both SH2 domains bind two neighboring phospho-tyrosine residues on large scaffolds and activated receptors [6]. SHP2 interacts in this way with IGF1R and its scaffolds to enhance osteogenic

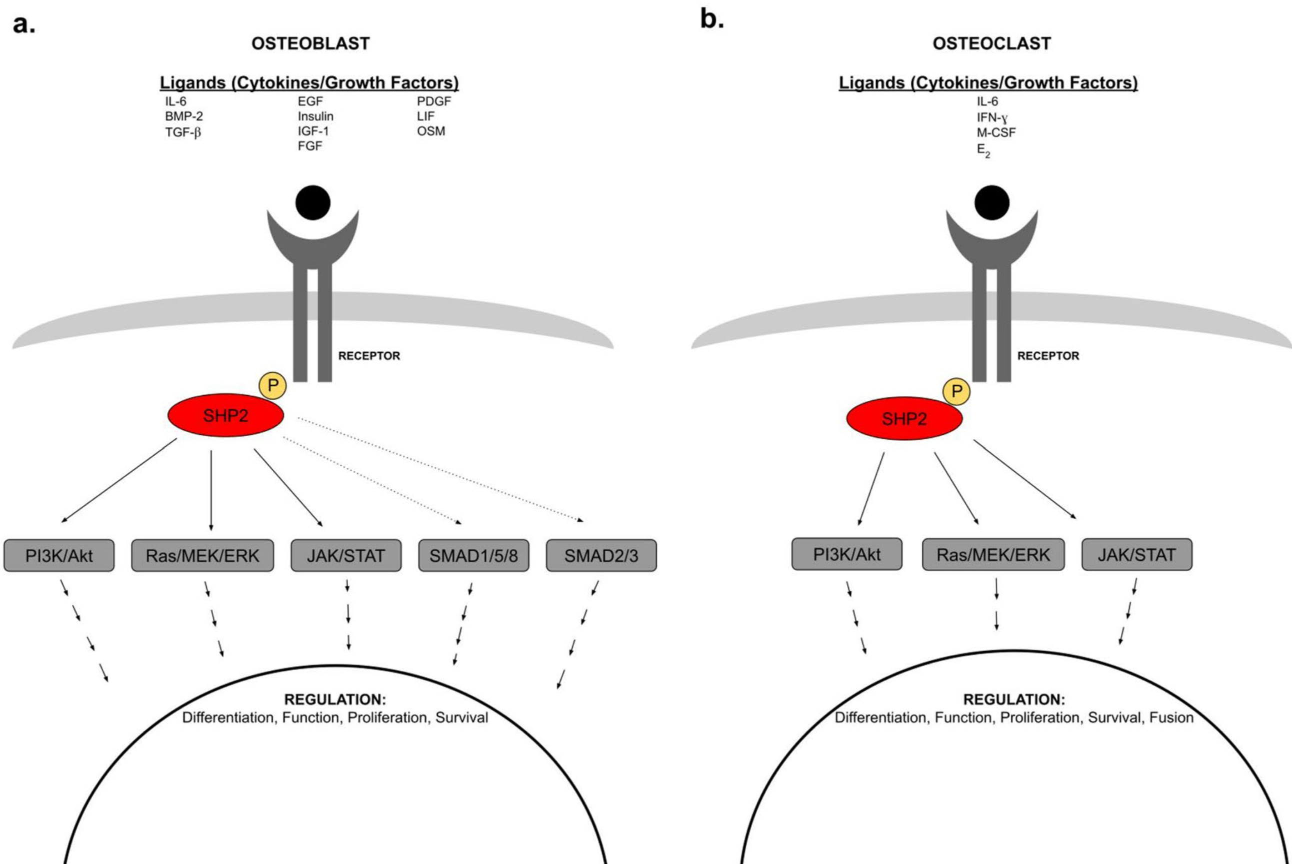

Fig. 1 Major SHP2-mediated signaling pathways regulating osteoblasts and osteoclasts

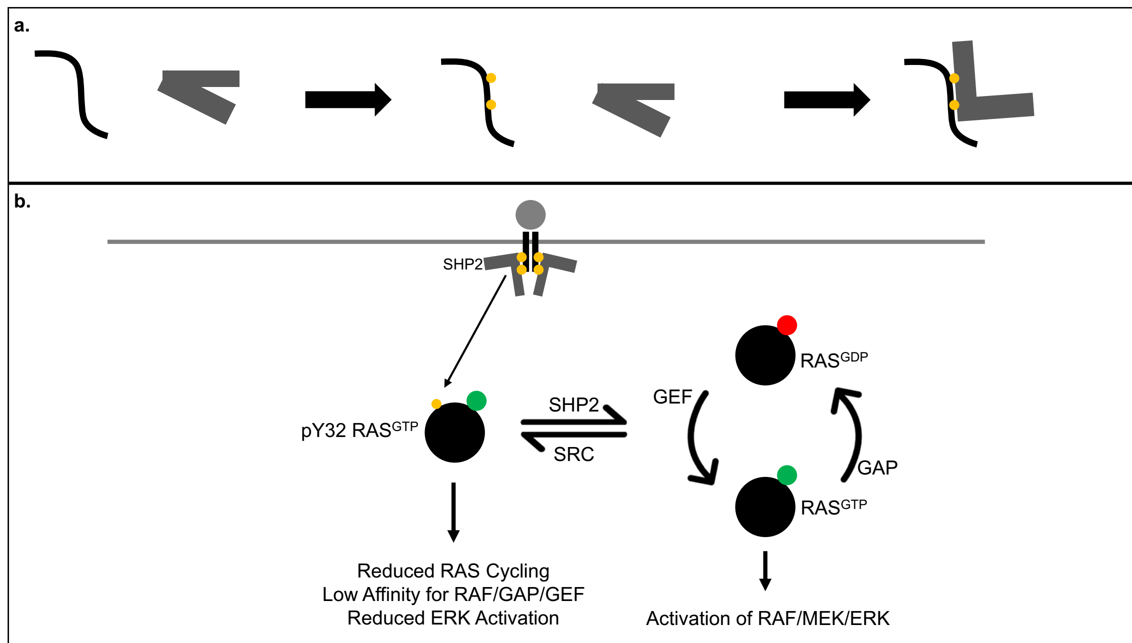

Fig. 2 Mechanism of SHP2 activation. **a** Interaction between SHP2 SH2 domains and phospho-tyrosine residues (●) induces a conformational change exposing the catalytic site and alleviating auto-inhibition. **b** Putative mechanisms of SHP2-mediated RAS activation

precursor proliferation and differentiation [7, 8]. This interaction has also been observed in response to a diverse array of receptors, ligands, and scaffolds critical to bone development and maintenance of osteoblasts and osteoclasts, including FGFR1 [9], PDGFR [10, 11], ErbB [12], DDR2 [13], cytokines [8], IL-6 [14, 15], M-CSF [7, 16], integrin [17], IGF-1 [18, 19], insulin [20, 21], RANK [22], FRS-2 [2, 23], SHC [24], GAB1 [8], GAB2 [25, 26], IFN-ɣ [27], BMP-2 [28], TGF-β [28], OSM [29], LIF [29], and E_2 [30]. Such diversity of interactions underlies a critical role for SHP2 in mediating growth factor and cytokine signaling (for review of other protein tyrosine phosphatases and their skeletal signaling, we refer the reader to [31]).

Function

The principal effect of SHP2 activation is to promote Ras signaling. Various mechanisms of SHP2-dependent Ras activation have been proposed [2, 32]. Recently, a model has emerged describing Ras-GDP as a direct substrate of SHP2 phosphatase [33, 34]. In this model, SHP2-mediated dephosphorylation of RAS Y32 relieves an inhibitory feedback mechanism to promote RAS-GTP cycling and enhanced pathway activation [34] (Fig. 2b). While Ras cycling occurs in the presence of Y32 phosphorylation, dephosphorylation of this residue accelerates the cycling rate. Thus, SHP2-mediated dephosphorylation of Ras provides a highly dynamic method of regulating Ras signaling. SHP2 may also potentiate Ras signaling through its association with the GRB2/SOS RasGEF complex. This noncatalytic scaffolding mechanism of action is supported by studies of phosphatase-dead SHP2 mutants that continue to influence intracellular signaling [32, 35–37].

While most studies have focused on SHP2-mediated ERK1/2 activation, SHP2 activation is also important for signaling through the collateral MAPK pathways, p38 [38], JNK [39], and ERK5 [40]. Regulation of NF-κB by SHP2 can proceed through both Ras-dependent [41, 42] and Ras-independent [43] mechanisms. Further, there is evidence for Ras-independent SHP2-mediated control of PI3K/AKT [44], JAK2/STAT3 [45], JAK2/STAT5 [46], SMAD [28], and NFAT [22] signaling.

Skeletal development is a highly dynamic process dependent upon the function of anabolic osteoblasts and catabolic osteoclasts. SHP2 is expressed in osteoblasts and their osteoprogenitors, and a detailed review of targeted disruption of SHP2 function in these cell types is provided below. The role of Ras-MAPK signaling in osteoblast development and function remains controversial [47–49], as interruption of Ras/MAPK signaling can either enhance or repress osteoblast function [50]. However, multiple studies have shown that MAPK pathway inhibition promotes osteogenic differentiation and function of MC3T3-E1 cells [51, 52]. Similarly, Ras-hyperactivation reduced bone mineral content in multiple models, most notably in children with neurofibromatosis type 1 [53]. Given the divergent data available from model organisms, high-quality clinical studies are needed to examine the secondary effects of pharmacologic Ras/MAPK inhibition on osteoblastic differentiation and function. Encouragingly, MAPK inhibition with selumetinib enhanced bone mineral density in a patient with neurofibromatosis, although it is unclear if this is due to the effects of pathway inhibition on the function of osteoblasts, osteoclasts, or both cell types [54].

The catalytic regulatory cells of bone, osteoclasts, are derived from hematopoietic stem cells (HSCs) through the myeloid lineage. Pharmacologic and genetic SHP2 inhibition lead to increased bone mineral density by reducing osteoclast number and function [22]. The effects of SHP2 inhibition on bone catabolism result from its vital functions during osteoclast differentiation, from regulation of HSC survival and myeloid differentiation to mature osteoclast bone resorption. SHP2 is essential for murine HSC maintenance by promoting c-Kit expression [55, 56]. In mice, inducible ablation of *Ptpn11* led to a significant reduction in bone marrow (BM) HSCs, myeloid progenitors, and peripheral leukocytes [55, 57]. Reduction in the HSC population was accompanied by departure from quiescence and increased apoptosis in *Ptpn11*-ablated BM [55]. The effects of SHP2 knockdown on adult HSCs were independently corroborated in isolated human BM-derived cells. SHP2 knockdown in vitro in human $CD34^+$ cells led to significantly increased apoptosis, diminished proliferative response to growth factor stimulation, and reduced myeloid differentiation [46]. In addition to regulating myeloid and osteoclastic differentiation, SHP2 is known to regulate osteoclast function through its control of RANK-induced *NFATc1* expression, a key regulatory transcription factor for osteoclastic resorption genes [22]. As reviewed below, pharmacologic or genetic inhibition of SHP2 dramatically reduces osteoclast number and function.

SHP2 in Human Skeletal Pathology

A variety of human skeletal pathologies have been linked to SHP2. The most well-characterized have been genetic disorders that arise from germline mutation of the *PTPN11* gene. SHP2 is also a bona fide oncogene in leukemia, with a growing body of evidence implicating its role in solid tumors as well. Here, we consider the skeletal pathologies associated with genetic syndromes and neoplasms driven by dysregulated SHP2 (Table 1).

Table 1 Germline mutations in PTPN11 result in human skeletal pathologies

Syndrome	Predominant *PTPN11* mutation	Common phenotype
Noonan Syndrome (NS)	Dominant activating mutations of *PTPN11* that tend to occur in N-terminal SH2 domain, destabilizing auto-inhibited conformation and leading to chronic low-level activation of SHP2	Bony dysmorphisms (short stature, pectus carinatum, pectus excavatum, kyphosis, and scoliosis) Hypertelorism, ptosis, low-set ears, broad or webbed neck Heart defects Learning disability Growth hormone insensitivity, reduced serum IGF-1 Increased incidence of cancer
Noonan Syndrome with Multiple Lentigines (NSML)	> 85% have germline heterozygous missense mutation of *PTPN11* thought to produce dominant negative SHP2 mutant, with mutations occurring in catalytic phosphatase site	Multiple lentigines, ECG abnormalities, short statute, sternal defects, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, growth retardation, deafness Hypertelorism and other facial dysmorphisms Hypertrophic cardiomyopathy Increased incidence of cancer
Metachondromatosis	Second-hit or loss of functional copy of SHP2 in chondrocytic precursor	Multiple cartilage-capped bony outgrowths, exostoses, ectopic intracortical cartilage

Germline Mutation of PTPN11

Noonan Syndrome

Noonan syndrome (NS; OMIM #163950) is an autosomal dominant genetic syndrome with an estimated incidence of 1 in 1000–2500 births. Bony dysmorphisms, including short stature, pectus carinatum, pectus excavatum, kyphosis, and scoliosis, are common. Typical facies display hypertelorism, ptosis, low-set ears, and a broad or webbed neck. Significant clinical features, such as congenital heart defects, hypertrophic cardiomyopathy, intellectual disability, and myeloid leukemia, are also common [58–60]. One of the most prevailing phenotypes of NS is short stature. In a study of 73 adults with NS, > 50% women and 38% of men were below the 3rd percentile for height [61]. Growth hormone insensitivity and a concomitant reduced serum IGF-1 are typical in NS patients [62, 63].

Dominant activating mutations of *PTPN11* are the most common cause of NS [64]. While a variety of *PTPN11* mutations in NS have been described, they tend to occur in the N-terminal SH2 domain, destabilizing the auto-inhibited conformation and leading to chronic low-level activation of SHP2 [18, 58, 65, 66]. NS mutations show growth factor-independent Ras activation and chronic activation of MAPK [67]. Chronic Ras activation in a mouse model of NS with mutant SHP2 reduced serum IGF-1 levels and impaired linear growth through direct effects on chondrocyte differentiation and growth plate size [68]. SHP2 is also an important regulator of calcium oscillation in response to growth factor stimulation in primary murine fibroblasts and cardiomyocytes. SHP2-mediated control of calcium signaling alters NFAT activity and has important implications for the cardiac and skeletal phenotypes of NS [69]. Thus, the dysregulation of SHP2 activity in NS leads to skeletal phenotypes through both endocrine effects and local defects in bone cells.

Noonan Syndrome with Multiple Lentigines

Noonan Syndrome with Multiple Lentigines (NSML; OMIM #151100) was originally named LEOPARD syndrome for a clinical constellation of multiple Lentigines, ECG abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, growth Retardation, and Deafness. The condition is rare, with about 200 cases reported [70]. Hypertelorism and other facial dysmorphisms are common features of NSML. Hypertrophic cardiomyopathy is the most common and life-threatening cardiac defect in these patients [71]. Lentigines, similar to café-au-lait spots, appear in childhood and increase through puberty, numbering into the thousands [70]. Patients display short stature with 25% of cases below the 3rd percentile. Sternal defects are also common in NSML, with one study demonstrating the presence of pectus carinatum or pectus excavatum in half of the NSML patients [71].

Over 85% of NSML patients have a germline heterozygous missense mutation of *PTPN11* thought to produce a dominant negative SHP2 mutant [72]. The mutations described in NSML occur in the catalytic phosphatase site. Biochemical assays confirm that these mutations abrogate phosphatase activity [72]. A variety of features, including short stature, skeletal dysmorphisms, cardiac defects, and increased incidence of cancer, are shared among patients with NS and NSML. How the activating *PTPN11* mutations of NS and the inactivating *PTPN11* mutations of NSML give rise to an overlapping clinical picture remain unknown.

Metachrondromatosis

Metachondromatosis (OMIM #156250) is a rare inherited autosomal dominant tumor syndrome [73]. Patients develop multiple cartilage-capped bony outgrowths, exostoses, and ectopic intracortical cartilage, termed endochondromas [74]. At least 50% of metachondromatosis patients inherit one defective copy of *PTPN11*. Consistent with classical inherited tumor syndromes of tumor suppressors, development of metachondromatosis is dependent on a second-hit or loss of the functional copy of SHP2 in a chondrocytic precursor [75]. Loss of SHP2 in paracrine regulators of chondrocytes likely contributes to the pathology of metachondromatosis [76]. The discovery of the role of SHP2 in the pathogenesis of metachondromatosis has been further facilitated through multiple murine models (reviewed below). These studies have greatly enhanced our understanding of this previously orphan disease and suggest novel therapeutic strategies [77, 78].

Wildtype PTPN11

Neoplasia

In contrast to the hematological malignancies associated with NS, epithelial neoplasms typically express wildtype SHP2 [79, 80]. Although mutation and amplification of SHP2 in solid tumors are rare, cancers that activate Ras through RTK-amplification [81] or NF1 loss [82] rely on SHP2 function. Wildtype SHP2 is emerging as a critical node that can be targeted in a variety of solid tumors dependent on Ras activation [81–83]. SHP2 is also thought to play a critical role in the observed resistance to pharmacologic inhibitors of carcinomas with oncogenes in the Ras pathway [83]. While this remains an area of active research, studies have demonstrated a role for SHP2 in the intrinsic resistance of BRAF-mutant colon cancer [84] and acquired resistance in KRas-mutant pancreatic and lung cancers [85, 86]. Our understanding of the role of wildtype SHP2 in neoplastic disease is rapidly expanding, as targeted agents facilitate more rigorous study. For a more complete consideration of the role of SHP2 in these processes, the reader is referred to recent reviews [87–89]. Future studies are needed to better understand the role of wildtype SHP2 in the pathogenesis of non-neoplastic bone disease.

Mouse Models of SHP2 Mutants

Mouse models have contributed significantly to our understanding of the importance of SHP2 in bone development and pathology. Here, we discuss the skeletal findings of murine models with alteration of SHP2 in mesodermal tissues, comprising the mesenchymal and hematopoietic systems. Recent reviews summarizing the extra-skeletal findings of these models are also available [18, 90]. We begin with whole-body knockout and progress through time- and tissue-specific knockouts. SHP2 knockouts of all the major cell types encompassed in bone development have been characterized, including mesenchymal stem cells, hypertrophic chondrocytes, and osteoclast precursors. The scope of this work reflects the significance of SHP2 in healthy bone development and homeostasis (Figs. 3, 4).

Whole Organism

The first murine knockout of Shp2 demonstrated that homozygous deletion of residues 46–110 resulted in death by gestational day 10. Shp2 null embryos displayed notable deficiencies in gastrulation, mesodermal patterning, and anterior–posterior axis formation [91, 92]. Primary Shp2-mutant fibroblasts showed significantly reduced ERK1/2 activation by FGF, a critical embryonic growth factor [91]. Chimeric SHP2-mutant embryos demonstrated the requirement of SHP2 for limb development [9]. The progress zone of budding limbs required SHP2, phenocopying FGFR1 mutants characterized earlier and suggesting the importance of SHP2 in transmitting the cytoplasmic signal from the activated FGF receptor. These early knockout studies demonstrated that ubiquitously expressed SHP2 was active in growth factor signaling and required for embryonic development.

While chimeric analyses enabled study of SHP2 deletion post-gestational day 10, it was impossible to observe the effects of SHP2 knockout in adult tissues derived from embryonic structures that required wildtype SHP2. Thus, subsequent work developed multiple inducible SHP2 mutants, including a mutant with loxP-flanking of the catalytic phosphatase on exon 11 [93] and loxP-flanking of exon 4, which deletes residues 111–176 and introduces a frameshift and premature stop codon [94].

Ubiquitous expression of inducible ert2-Cre was used to model global Shp2 knockout in the adult mouse. Global Shp2 knockout in 6–8-week-old mice resulted in rapid weight-loss and early mortality, with 50% mortality at 4 weeks post-tamoxifen-mediated induction [16]. Adult Shp2 knockout mice were notable for hematopoietic and skeletal abnormalities. Mice displayed substantial reductions in common lymphoid progenitors and mature B and T cells. As early as two weeks post-induction, adult Shp2-mutant mice developed skeletal abnormalities, including kyphosis and scoliosis, characteristic of NS and NSML. Increased bone mineral density of the vertebrae, ribs, and humeral and femoral metaphyses were noted by x-ray and μCT analyses and confirmed histologically. Disorganization of cartilaginous growth plates of Shp2-mutant adult mice was

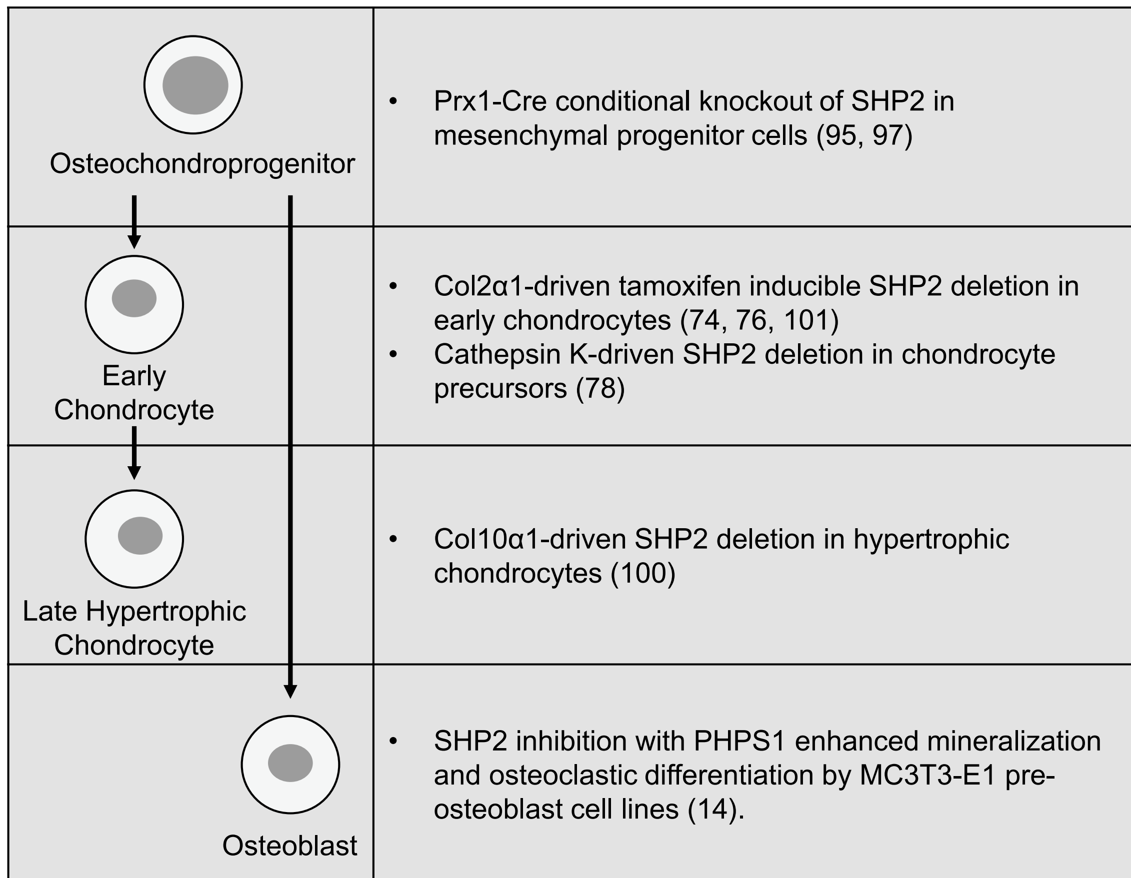

Fig. 3 Studies of SHP2 knockout and targeted inhibition in osteoblast development

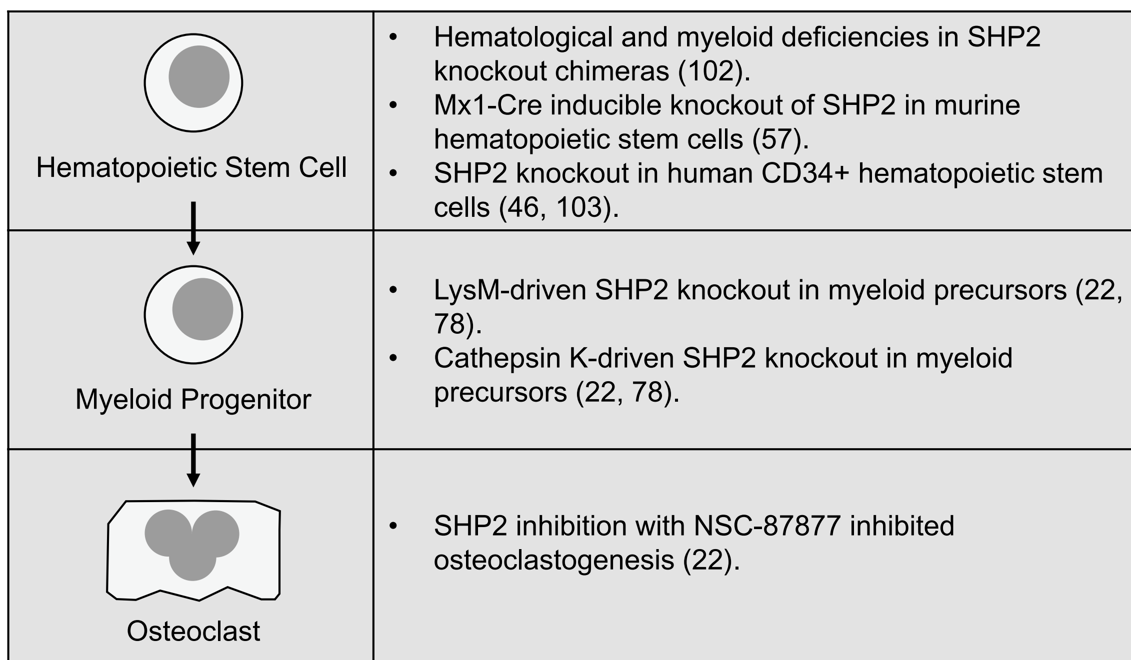

Fig. 4 Studies of SHP2 knockout and targeted inhibition in osteoclast development

extensive and likely contributed to the skeletal abnormalities of these growing mice. The osteopetrotic phenotype is at least partially due to a notable reduction in osteoclastogenesis in adult Shp2-deficient mice. These studies provided an important framework for understanding the roles of SHP2 in adult skeletal physiology and contributed important tools for interrogation of SHP2 deletion in the specific tissues that develop into and regulate the skeletal system.

Mesenchymal-Specific

The paired-related homeobox gene-1 (Prx1) promoter is utilized by mesenchymal tissues and was used to knock out Shp2 in osteocytic, adipogenic, and cartilaginous cell types [95]. These Prx1-induced Shp2 knockout mice showed absence of subcutaneous fat, reduced mature osteocytes, and increased chondrocytes, particularly hypertrophic chondrocytes. Molecular analyses indicated osteoblastic differentiation was interrupted at a late stage, as no differences in early osteoblast markers *Col1a1*, *Runx2*, *Osterix* or *Atf4* were noted between knockout and wildtype samples, but a dramatic reduction in the mature marker, *Osteocalcin*, was observed. These alterations of mature mesenchymal cell types led to dramatic phenotypic skeletal changes, including drastically shortened forelimbs and hindlimbs and chest deformities characteristic of defects in endochondral ossification. Furthermore, calvarial bone defects indicated impaired intramembranous ossification. The skeletons of the Prx1-induced Shp2 knockout mice displayed reduced cortical mineral density, likely resulting from impaired osteoblast function.

The fate of the osteochondroprogenitor cell is tightly controlled by the transcription factor Sox9 [96]. Prx1-induced SHP2 deletion led to increased phosphorylation and SUMOylation of Sox9, leading to enhanced chondrogenesis [97]. Mice displayed increase cartilage mass at normal and ectopic sites, including the bone cortex and marrow. Appendicular ossification was concomitantly decreased. Studies of SHP2 knockout in mesenchymal precursor tissues display skewing toward chondrocytic differentiation over osteoblastic differentiation. These studies are remarkably consistent with the work in NS models in which activating SHP2 mutations display reductions in chondrocyte differentiation, especially hypertrophic chondrocytes [68]. These independent lines of inquiry demonstrate the necessity of SHP2 function for osteoblastic differentiation.

Chondrocyte-Specific

Given its role in determining the fate of the osteochondroprogenitor, it is not surprising that knock out of SHP2 has been pursued in progressively more differentiated cell types. To date, only one recent study has reported SHP2 deletion in *Bglap*$^+$ mouse bone cells, which leads to osteopenia, failure of bone cell maturation, and enhanced osteoclast activity [98]. However, many studies have targeted SHP2 deletion in mature chondrocytes and are discussed below.

Shp2 deletion in chondrocytes delayed terminal differentiation in vitro, prolonging the hypertrophic stage [76]. Tamoxifen-inducible deletion of Shp2 driven by the Col2α1 promoter of early chondrocytes produced osteopenia, kyphoscoliosis, chest wall deformities, and lesions reminiscent of metachondromatosis in adult mice [74, 77, 99, 100], similar to those reported in Cathepsin K-driven loss of Shp2 in chondrocyte precursors [78]. Seven weeks post-induction, the mice displayed disorganized and enlarged vertebral growth plates with accompanying ectopic ossification and endochondromas [76]. Importantly, tamoxifen-induced Shp2 deletion was incomplete, and the endochondral lesions were composed of Shp2 mutant and wildtype chondrocytes, indicating the importance of paracrine effects of Shp2-mutant cells in promoting cartilage disorganization. Shp2 knockout of *Fsp1*-positive, bone-lining fibroblasts was sufficient to induce cartilaginous outgrowths of the metacarpals, phalanges, fibula, tibia, and femur, confirming the role of Shp2 in paracrine chondrocyte regulation. While FGF2 protein expression was increased by Shp2 deletion in the developing rib and paw, activation of Erk was noticeably impaired [74]. The role of SHP2 in chondrocyte differentiation involves a MAPK-dependent regulation of Indian hedgehog [78]. Shp2 function is also important in orofacial cartilage, as its depletion leads to mandibular condyle deformity [101]. In addition, development of primary cilia, a critical signaling organelle, was disrupted in the absence of Shp2. Taken together, Shp2 mutation in early chondrocytes promotes cartilaginous expansion and recapitulates the phenotype of metachondromatosis.

While it was clear that SHP2 is vital for proper cartilage differentiation, the dramatic effects of its knockout in early chondrocytes prevented researchers from assessing its role in the later stages of endochondral ossification. Col10α1 is not produced until the final stages of endochondral ossification when hypertrophic chondrocytes undergo terminal differentiation and secrete this matrix component for mineralization. Col10α1-driven Shp2 knockout in hypertrophic chondrocytes did not produce metachondromatosis, confirming the importance of Shp2-loss in a proliferative progenitor population for the pathology of this disease [100]. Shp2 knockout in hypertrophic chondrocytes resulted in significant osteopenia, promoted maintenance of chondrocytic master transcriptional regulator *Sox9*, and reduced expression of osteogenic genes. It is possible that SHP2 controls the transdifferentiation of a small pool of hypertrophic chondrocytes to an osteogenic cell type. Lineage tracing studies demonstrated that Col10α1-expressing cells downregulated Col2α1 and upregulated osteogenic genes, *Ibsp, Mmp13, Runx2* and

Ctnnb1, and that this switch was impaired with Shp2 deletion. While the phenomenon of osteogenic trans-differentiation remains controversial, it is an enticing prospect given the role of SHP2 in regulating SOX9 expression and ultimate cell fate of the osteochondrogenic precursor cell [97].

Hematopoietic-Specific

Early chimeric studies indicated a critical role for SHP2 in hematopoiesis and regulation of myeloid cell development [102]. Inducible Shp2 knockout in murine HSCs utilizing Mx1-Cre confirmed the essential role of Shp2 in HSC maintenance and survival [57]. Loss of Shp2 led to BM aplasia and reduced lifespan to a median of 5 weeks. HSCs lacking Shp2 displayed impaired Erk activation after stem cell factor stimulation and increased Noxa-mediated apoptosis. Restoration of Erk signaling by expression of mutant Kras rescued survival in Shp2-deficient HSCs. Similar results were obtained when SHP2 function was studied in human $CD34^+$ HSCs [46, 103]. Knockdown reduced $CD34^+$ cell proliferation, survival, and colony-forming units and inhibited myeloid differentiation [46]. Thus, SHP2 is necessary for HSC maintenance by promoting ERK signaling.

Myeloid-specific Shp2 knockout mouse models have facilitated precise studies that show SHP2 functions at multiple points in myeloid differentiation to promote osteoclast development, a critical element of skeletal physiology. The Lysozyme M (LysM) promoter has been extensively used to drive specific knockout in monocytes, macrophages, and osteoclasts [104–106]. LysM-driven Shp2 knockout resulted in mild osteopetrosis [78]. Cathepsin K (*Ctsk*) promoter-driven Shp2 deletion produced osteopetrosis, in addition to scoliosis and metaphyseal exostoses. Transplant of *Ctsk*-driven Shp2 knockout BM into lethally irradiated recipient mice confirmed that the osteopetrosis arose from Shp2 knockout in osteoclasts. The metaphyseal exostoses, however, arose from a previously unknown population of *Ctsk*-expressing chondroprogenitors and phenotypically recapitulate the observation of SHP2 loss in metachondromatosis. The osteopetrosis caused by Shp2 deletion in osteoclasts was later characterized in greater detail [22]. Shp2 knockout in these cells led to a 42.8% increase in bone volume to trabecular volume ratio, increased trabeculae number, and decreased trabecular spacing. Mice had fewer osteoclasts, while in vitro cultured BM produced fewer osteoclasts when stimulated with M-CSF and RANKL. Shp2-deficient pre-osteoclasts demonstrated impaired RANK-dependent preosteoclast fusion. The knockout pre-osteoclasts failed to upregulate Nfatc1, an essential osteoclast transcription factor that drives expression of the fusion protein, DC-STAMP. Analysis of the few Shp2-deficient osteoclasts that did develop demonstrated functional impairment with reduced pit resorption. Together, these studies showed that SHP2 is essential for osteoclast development and activity and further demonstrate its importance in skeletal development and homeostasis.

Targeted Modulation of Shp2

Given the causal role of SHP2 in genetic syndromes and neoplastic disease, it is not surprising that it has long been the subject of intense pharmacologic discovery programs. However, developing targeted tyrosine phosphatase inhibitors has proven more challenging than targeting the tyrosine kinase domain [107]. Targeting the human phosphatase active site has been difficult, partially due to its highly conserved nature [108, 109]. Another difficulty arises from the strongly positive nature of the catalytic site, which is necessary for interacting with the phosphate group [108, 110]. Strong polar charges of potential active site inhibitors reduce their bioavailability and clinical development [109, 110]. Despite these challenges, a variety of SHP2 competitive inhibitors have been described, although none has progressed to clinical studies. Recently, a new class of allosteric SHP2 inhibitors has emerged and progressed rapidly into clinical trials. Here, we consider the inhibitors that have been used in vivo and in clinical studies, with an emphasis on their application in musculoskeletal and other non-neoplastic pathologies (Table 2). For reviews focusing on SHP2 inhibition in cancer, the reader is referred to other sources [87, 90].

Competitive Inhibitors

NSC-87877

The first competitive SHP2 inhibitor to be reported showed similar activity against SHP1 and SHP2. The specificity of NSC-87877 continues to be of concern, as a recent report demonstrated that NSC-87877 failed to prevent PDGF-BB or EGF-induced MEK1/2 or ERK1/2 activation, which is highly dependent on SHP2 [10]. This study underlies the inherent difficulty of understanding protein function with small molecule inhibitors. The best studies of function should combine genetic and pharmacological methodologies.

The effect of NSC-87877 on osteoclast development has been reported [22]. Differentiation of osteoclasts from BM precursor cells in the presence of NSC-87877 was significantly reduced. The authors demonstrated a reduction in the total number of osteoclasts and in expression of osteoclastic differentiation markers, including *Trap*, *Cathepsin K*, *DC-Stamp*, and *Nfatc1*, with Shp2 inhibition. As NFATc1 is thought to be the master transcription factor of osteoclastogenesis, the authors conclude that SHP2-mediated

Table 2 Targets and effects of SHP2 inhibitors

Agent	Target	Notable effects	Concerns
NSC-87877	Cell-permeable competitive SHP2 inhibitor with similar activity against SHP1 and SHP2	Reduced osteoclast differentiation, likely through decreased NFATc1 expression [22]	Poor specificity (also targets SHP1) and failed to prevent PDGF-BB or EGF-induced MEK1/2 or ERK1/2 activation [10]
		Reduced ERK activation and viability of breast cancer cells [112]	
		Inhibited cardiac cell activity and survival [23]	
		Ameliorated TGF-β-induced dermal- and bleomycin-induced pulmonary fibrosis [45]	
		Potential impacts on long-term potentiation and contextual fear conditioning response [113]	
PHPS1	Cell-permeable, phosphotyrosine mimetic, competitive SHP2 inhibitor	Increased osteoblast differentiation and activity in MC3T3-E1 cells [14]	Reduced potency against SHP2 compared to NSC-87877 but greater SHP2 specificity
		Delayed endothelial barrier recovery after thrombin treatment in HUVECs [117]	
		Enhanced IL-4 directed M2 polarization in fibrosis models [45, 118]	
		Attenuated immune response and skewed macrophages toward M2 phenotype [119]	
		Reduced cigarette smoke-induced IL-8 and inflammatory infiltration [120]	
		Delayed tumor formation in vivo and suppressed estradiol-dependent transcription of cyclin D1 [121]	
II-B08	Cell-permeable SHP2 inhibitor that targets SHP2 catalytic domain	Inhibited long-term ERK activation in HEK293 [124]	No bone-related studies to date
		Inhibited stem cell factor (SCF)-induced chemotaxis and enhanced apoptosis of mast cells [125]	
		Reduced Kit + leukemia cell viability and colony formation [127]	Unknown SHP2 specificity [10]
		Inhibited GM-CSF-dependent proliferation of BM cells [124]	
11a-1	Developed through library approach to improve on II-B08 with at least fivefold selectivity over 20 related PTPs	Ameliorated TGF-β-induced dermal and bleomycin-induced pulmonary fibrosis [45]	No bone-specific studies to date
		Reduced arthritis severity in serum transfer model [129]	
		Reduced systemic lupus erythematosus pathology [130]	
GS-493	Most potent and selective competitive SHP2 inhibitor to date [131]	Inhibited in vivo proliferation of postnatal myogenic progenitors [132]	May have off-target effects [10]
		Extensively studied in cancer [84, 85, 131, 133]	No bone-specific studies to date

Table 2 (continued)

Agent	Target	Notable effects	Concerns
Allosteric SHP2 inhibitors (SHP099, TNO-155, RMC4550)	Allosteric site at interface of SHP2 N and C terminals that stabilizes the closed, inactive conformation of SHP2	Extensively studied in cancer and used in clinical trials [87, 135]	Limited study in non-cancer pathologies
		SHP099 delayed insulin receptor endocytosis and improved insulin sensitivity in mice [139]	
	Highly potent in targeting mutant SHP2	SHP099 reduced in vivo model liver fibrosis [142]	
		SHP099 injection into rat intervertebral disc had therapeutic effects in model of degenerative disc disease [143]	

inhibition of osteoclast differentiation is predominantly the result of decreased NFATc1 expression. Consistent with this conclusion, overexpression of NFATc1 rescued the effect of NSC-87877 treatment on osteoclast differentiation. Functionally, osteoclasts cultured in the presence of NSC-87877 or with SHP2 knockout showed defective pit resorption. Interestingly, removal of NSC-87877 led to a rebound in osteoclast numbers, suggesting Shp2 inhibition prevented the maturation of osteoclasts but did not kill the progenitor population. This observation has profound implications for targeted inhibition of SHP2 in skeletal pathologies. To date, anti-osteoclastic therapies, such as bisphosphonates, produce an irreversible reduction in osteoclast numbers [111]. Thus, SHP2 inhibition may provide a novel strategy for inhibiting osteoclastogenesis in a reversible manner.

NSC-87877 has been used to inhibit SHP2 in a variety of non-skeletal models. This SHP2 inhibitor reduced ERK activation and viability of breast cancer cell lines [112]. SHP2 was required for survival of proliferating cardiac progenitor cells and for upregulation of cardiac proteins, such as myosin heavy chain and the cardiac master transcriptional regulator, Nkx2.5, which was inhibited upon treatment with NSC-87877 [23]. The effect of NSC-87877 was specific to its inhibition of SHP2 by rescue with constitutively active SHP2, an important control given the similar potency of this compound in inhibiting SHP1. Treatment with NSC-87877 ameliorated TGF-β-induced dermal- and bleomycin-induced pulmonary fibrosis [45]. The role of SHP2 in hippocampal synaptic plasticity and memory was examined in a model of contextual fear conditioning [113]. Long-term potentiation (LTP) of glutamatergic synapses results from increased postsynaptic expression of AMPA receptors and has been shown to be Ras-dependent [114, 115]. Fear conditioning with foot shock recruited SHP2 and the AMPA subunit GluA1 to spines, indicating LTP [113]. Importantly, NSC-87877 treatment prevented GluA1 recruitment.

PHPS1

The next competitive inhibitor to become available was PHPS1 [116]. PHPS1 demonstrated reduced potency but greater SHP2 specificity compared to NSC-87877. PHPS1 was used to inhibit SHP2 activation in the murine MC3T3-E1 pre-osteoblast cell line and primary murine calvarial osteoblasts [14]. Markers of osteoblast differentiation, including Runx2, osterix, osteocalcin and alkaline phosphatase, increased with SHP2 inhibition in MC3T3-E1 cells. Extracellular matrix mineralization by MC3T3-E1 cells increased with SHP2 inhibition. PHPS1 partially inhibited the IL-6-dependent activation of ERK and AKT; however, complete AKT or ERK inhibition had no effect on MC3T3 mineralization. The authors conclude that SHP2 inhibition prevents the negative effects of IL-6 on osteoblast

differentiation, but it is difficult to explain how partial inhibition by PHPS1 has such a dramatic effect that was not observed with specific MEK or AKT inhibition.

The use of PHPS1 for SHP2 inhibition in extra-skeletal systems has focused on modulation of the immune response and application to cancer. SHP2 was involved in the recovery of the endothelial barrier after thrombin-mediated inflammation by direct phosphatase action on β-catenin [117]. SHP2 inhibition with PHPS1 was similar to SHP2 silencing in delaying endothelial barrier recovery after thrombin treatment. SHP2 inhibition with PHPS1 or conditional Shp2 knockout enhanced IL-4-directed M2 polarization, which may play a role in promoting the pathogenesis of bleomycin-induced pulmonary fibrosis [45, 118]. Similarly, treatment with PHPS1 attenuated the immune response and skewed macrophages toward an M2 phenotype when activated by bacterial infection with *Haemophilus influenzae* [119]. SHP2 activation of the M1 phenotype was independent of ERK and, instead, required NF-kB. SHP2 may also be involved in mediating the pro-inflammatory response to cigarette smoke that results in chronic obstructive pulmonary disease [120]. Treatment with PHPS1 reduced cigarette smoke-induced IL-8 and inflammatory infiltration. The role of SHP2 in regulating signaling by the estrogen receptor (ER) in breast cancer cell lines was examined, and SHP2 mRNA and protein expression were found to be increased by treatment with estradiol [121]. Further, PHPS1-mediated SHP2 inhibition delayed tumor formation in vivo, and PHPS1 and NSC-87877 suppressed estradiol-dependent transcription of Cyclin D1 and proliferation.

A recent study examined the role of SHP2 in the pathophysiology of high cholesterol-induced atherosclerosis [122]. LDL receptor-deficient mice were fed a high cholesterol diet for 4 weeks. During the final week of the high cholesterol diet, the mice received daily intraperitoneal injection with PHPS1 or vehicle. PHPS1 treatment significantly reduced the total number, size, and smooth muscle cell content of atherosclerotic plaques. In vitro studies with vascular smooth muscle cells (VSMCs) revealed that PHPS1 treatment reduced ERK phosphorylation and VSMC proliferation induced by oxidized LDL. However, PHPS1 did not reduce the proliferation of inactivated VSMCs. Thus, activated VSMCs, which drive the pathology of atherosclerosis, may be selectively sensitive to SHP2 inhibition.

II-B08

II-B08 was developed from a screen designed to find bidentate SHP2 competitive inhibitors with affinity for the conserved PTP active site and the less conserved flanking residues, which are also important for substrate binding [123]. II-B08 inhibited long-term ERK activation approximately 60 min after EGF treatment in HEK293. Early EGF-dependent ERK activation, however, was not affected by SHP2 inhibition with II-B08 [124]. The lack of early EGF-dependent ERK activation and suppression of PGDFRβ phosphorylation, which is upstream of and not thought to be regulated by SHP2 [10], raise concerns regarding the specificity of II-B08.

II-B08 was used to interrogate the role of SHP2 in mast cell function and mast cell proliferative diseases. II-B08 inhibited SCF-induced chemotaxis of BM-derived mast cells [125] and enhanced apoptosis following growth factor withdrawal in mast cells, indicating the importance of SHP2 in regulating the activity of apoptotic effectors, such as Bim, downstream of survival signals [83, 126]. II-B08 reduced Kit^+ leukemia cell viability and colony formation in vitro [127] and reduced proliferation and increased survival in vivo [128]. II-B08 inhibited GM-CSF-dependent proliferation of BM mononuclear cells expressing wildtype or NS-mutant SHP2 [124]. As demonstrated by these studies, II-B08 has primarily been used to inhibit SHP2 in models of RTK- and SHP2-driven cancers.

11a-1

The compound, 11a-1, was developed through a structure-guided and fragment-based library approach to improve on the published inhibitor, II-B08 [108]. The SHP2 IC_{50} of 11a-1 is 0.20 μM, with at least fivefold selectivity over 20 related protein tyrosine phosphatases. Treatment with 11a-1 ameliorated TGF-β-induced dermal and bleomycin-induced pulmonary fibrosis [45]. The role of SHP2 in the pathogenesis of rheumatoid arthritis was also examined [129]. SHP2 inhibition with 11a-1 reduced severity of arthritis, as indicated by reductions in inflammation, bone and cartilage erosion, and ankle swelling in a serum transfer model of rheumatoid arthritis. In vitro, 11a-1 treatment reduced migration and TNF- or IL-1β-induced upregulation of matrix metalloproteinases by rheumatoid arthritis fibroblast-like synoviocytes. Systemic lupus erythematosus (SLE) is another common autoinflammatory pathology that may benefit from SHP2 inhibition [130]. SHP2 inhibition with 11a-1 increased survival, ameliorated the common SLE-related renal pathology crescentic glomerulonephritis, and reduced skin lesions in an SLE murine model.

GS-493

The most potent and selective competitive SHP2 inhibitor reported to date is GS-493 [131]. While GS-493 showed enhanced SHP2 selectively in phosphatase assays, follow-up studies demonstrated its ability to directly dephosphorylate PDGFRβ, raising concerns of off-target effects that have continually plagued the competitive SHP2 inhibitors [10].

GS-493 was used to probe the role of SHP2 in a musculoskeletal mesodermal population [132]. Proliferation of postnatal myogenic progenitor cells was inhibited by SHP2 knockdown or targeted inhibition by GS-493 in vivo. The effect of SHP2 inhibition was thought to be due to the dramatic inhibition of phosphorylated ERK, as proliferation was rescued by constitutively active MEK. To date, there have been no reports assessing GS-493 in osteocytic populations. The development of GS-493 has provided a competitive SHP2 inhibitor with dramatically improved potency and selectivity. It has been used extensively in cancer research [84, 85, 131, 133] and will likely continue to facilitate a variety of new studies.

Allosteric Inhibitors

Recently a novel class of SHP2 allosteric inhibitors has been developed [81]. These compounds were developed utilizing a screen that selected small molecules with efficacy against full-length SHP2 activated by a biphosphoryl peptide but excluded compounds with efficacy against the catalytic subunit of SHP2. This simple but effective experimental design identified a class of inhibitors with allosteric activity. Structural studies demonstrated that the allosteric site is at the interface of SHP2 N and C terminals and that the interaction with the inhibitor stabilizes the closed, inactive conformation of SHP2. The structural work predicted the importance of two residues, T253 and Q257, which interacted with the inhibitor to stabilize the closed confirmation. Indeed, mutation of these residues had little effect on SHP2 phosphatase activity but reduced the potency of the inhibitor by 1000-fold, confirming the allosteric nature of the inhibitor. In comparison to the active site inhibitors, published studies with multiple allosteric inhibitors, SHP099 [81], TNO-155 [134], and RMC4550 [82], demonstrated their striking specificity for SHP2 over other human phosphatases.

Despite their recent introduction, the allosteric SHP2 inhibitors have already been extensively studied in cancer [87, 135]. The speed with which these agents have entered early-stage clinical trials is a testament to the promise they hold for treating cancer patients. These studies have led to an important observation on the potency of these inhibitors in targeting mutant SHP2. As described above, the activating mutation of NS predisposes individuals to leukemias. These mutations tend to destabilize the closed conformation of SHP2, in direct opposition to the action of the allosteric SHP2 inhibitors. Investigation revealed that some of the common mutant SHP2 proteins in NS are resistant to allosteric inhibition [136]. However, allosteric inhibitors targeting mutant leukemogenic SHP2 mutants have already been developed [137]. The application of these agents to human genetic disease continues to be explored [138].

To date, evaluation of the utility of SHP2 allosteric inhibitors in non-cancer pathologies has been limited. The allosteric agent, SHP099, was used to probe the importance of SHP2 in insulin signaling, a critical pathway in skeletal development [139–141]. Inhibition of SHP2 delayed insulin receptor endocytosis and improved insulin sensitivity in mice [139]. One group reported efficacy of SHP099 in two in vivo models of liver fibrosis [142]. SHP2 inhibition reduced liver fibrosis through its activity in platelet-derived growth factor signaling. Importantly, the dose of SHP099 used in vivo was much lower than that reported for cancer studies, an important consideration for pathologies that may require long-term treatment. Despite the long-held known role for SHP2 in bone development and maintenance, the effect of SHP2 inhibition on bone in vivo has been understudied, to date. However, a recent report demonstrates the potential for this new class of targeted inhibitor to modulate musculoskeletal pathology [143]. In this report, investigators showed that SHP2 is highly expressed in intervertebral discs where it acts in the role described above to inhibit chondrocytic differentiation and gene expression. Injection of SHP2 inhibitor, SHP099, into the intervertebral disc promoted cartilaginous protein expression and had a therapeutic effect in a rat model of degenerative disc disease.

Conclusion

A significant effort has been made to understand the importance of SHP2 in development and disease through a global, concerted effort combining genetic and pharmacologic methodologies. Together, these studies demonstrate the important role of SHP2 in skeletal development and maintenance through actions on osteoblasts and osteoclasts. Osteoblasts arise from mesenchymal cells with the potential to differentiate into alternative mesenchymal tissues, including adipocytes and chondrocytes. The lack of SHP2 activity in mesenchymal tissues, either by genetic dysfunction or targeted inhibition, prevents osteoblastic in favor of chondrocytic differentiation. These findings align with the known role of ERK1/2 in osteoblastic differentiation through phosphorylation of RUNX2 [48, 144, 145]. Failure of SHP2-mutant mesenchymal cells to activate ERK1/2 may prevent activation of RUNX2 and osteoblast differentiation, and promote Sox9 expression and stability, resulting in chondrocyte differentiation (Fig. 5).

These studies also established an important role for SHP2 in osteoclast development. SHP2 is necessary for hematopoietic stem cell renewal, survival, and myeloid differentiation. It is required for expression and activity [93] of the osteoclastogenic master transcriptional regulator, NFATc1. In addition to its regulation of ERK1/2 activation by M-CSF and RANKL, there is evidence of a role for SHP2

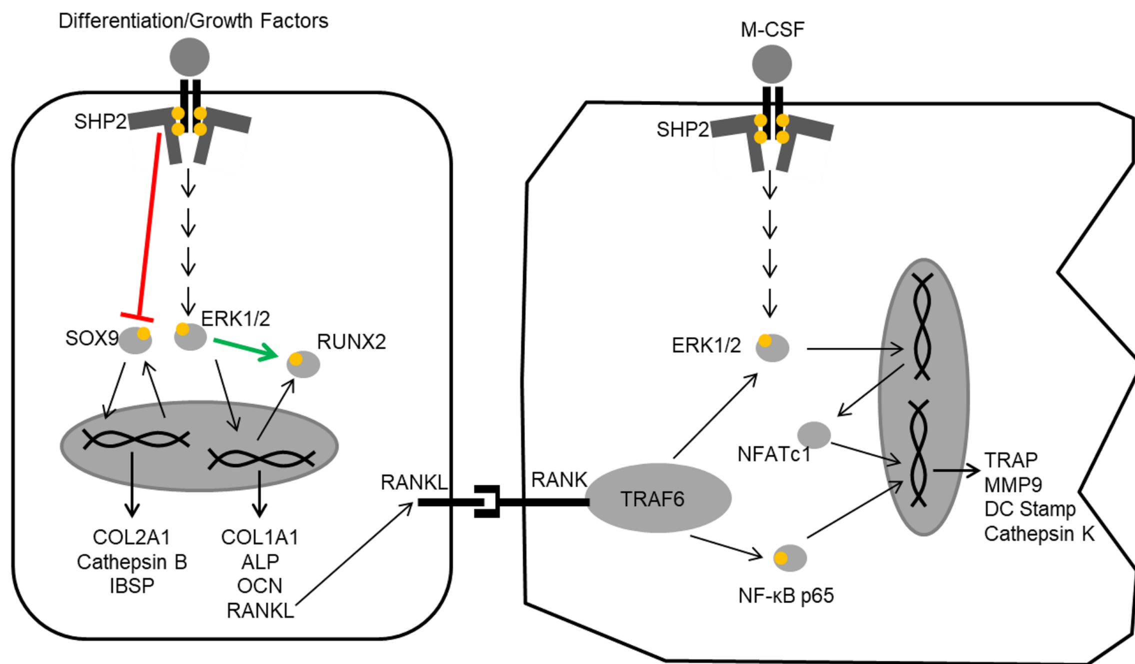

Fig. 5 SHP2 regulates differentiation of osteoblasts and osteoclasts

in controlling growth factor-mediated calcium oscillations, a key regulator of NFATc1 activity [69]. Future studies should also investigate the potential role of nuclear SHP2 in osteoblast, osteoclast, chondrocyte, and osteochondroprogenitor maintenance and survival. Nuclear SHP2 has been shown to be involved in regulating STAT5 signaling, as well as cell proliferation [146]. Interactions between YAP1 and SHP2 have also been shown to promote translocation of SHP2 to the nucleus, leading to activation of the Wnt pathway in tumors from patients with non-small cell lung cancer [147]. As Wnt signaling is also an important signaling pathway in bone maintenance, nuclear SHP2 may be an interesting target of further study in bone cells.

SHP2 is essential for proper function of chondrocytes, osteoblasts, and osteoclasts, all the cell types regulating endochondral ossification and skeletal maintenance. Targeted loss of SHP2 in tissues that promote endochondral ossification, such as *Bglap*$^{+}$ bone cells and early and late hypertrophic chondrocytes, produces significant osteopenia. In contrast, SHP2 knockout in osteoclasts results in osteopetrosis. While SHP2 functions in promoting both bone formation and resorption, it is important to note that, in murine models with ubiquitous SHP2 knockout, the mice developed rapid and progressive osteopetrosis, indicating a predominant effect on osteoclasts and the importance of tightly regulated balance for bone homeostasis.

Insights gained from study of SHP2 function in model organisms help to explain the phenotypes seen in SHP2-mutant human disease [148]. Germline mutations in SHP2 are among the most common rasopathies. The clinical syndromes demonstrate a spectrum of manifestations that result from aberrations in SHP2-mediated signaling. While this spectrum is broad, many of the prominent features relate to the role of SHP2 in mesenchymal development and skeletal homeostasis. These features have dramatic consequences for the patients born with these conditions, which may require surgical intervention and lifelong neoplastic surveillance.

Highly specific SHP2 inhibitors have been developed and are now in clinical trials. While these agents were initially developed for neoplastic conditions, they may represent a new tool in modulating SHP2-mediated genetic disease. Recent clinical successes have demonstrated that targeted agents can be useful in genetic syndromes, such as neurofibromatosis and achondroplasia. Decades of research and development have provided deep mechanistic understanding of the pathophysiology of clinical syndromes that result from SHP2 mutation and small molecules capable of modulating its function. Additionally, many of the major signaling pathways influenced by alterations in SHP2 activity are involved in multiple pathological conditions. Therefore, SHP2 inhibitors may have clinical benefit in other diseases, not just cancer, including diabetes [149, 150], autoimmune disease [130], chronic respiratory diseases [151], and musculoskeletal disorders [148]. It is important to be mindful of what was learned in the model systems and genetic syndromes as these agents move forward in clinical development. This foundation will help evaluate for adverse effects and expand therapeutic applications.

Acknowledgements This work was supported by the Biomedical Laboratory Research and Development Program of the Department of Veterans Affairs (VA Merit Award to A.C.L., BX000333 and BX005168).

Author Contributions All authors read and approved of the final manuscript.

Declarations

Competing interests Nathaniel R. Jensen, Ryan R. Kelly, Kirsten D. Kelly, Stephanie K. Khoo, Sara J. Sidles and Amanda C. LaRue declare no competing interests.

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