Review

Oncology

Oncology
DOI: 10.1159/000490106

Received: April 26, 2018
Accepted after revision: May 14, 2018
Published online: June 20, 2018

Targeting SHP-1, 2 and SHIP Pathways: A Novel Strategy for Cancer Treatment?

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Keywords
SHP-1, 2 · SHIP · Immunology · Inhibitors · Cancer treatment · Protein tyrosine phosphatases · Immunomodulation

Abstract
Well-balanced levels of tyrosine phosphorylation, maintained by the reversible and coordinated actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), are critical for a wide range of cellular processes including growth, differentiation, metabolism, migration, and survival. Aberrant tyrosine phosphorylation, as a result of a perturbed balance between the activities of PTKs and PTPs, is linked to the pathogenesis of numerous human diseases, including cancer, suggesting that PTPs may be innovative molecular targets for cancer treatment. Two PTPs that have an important inhibitory role in haematopoietic cells are SHP-1 and SHP-2. SHP-1, 2 promote cell growth and act by both upregulating positive signaling pathways and by downregulating negative signaling pathways. SHIP is another inhibitory phosphatase that is specific for the inositol phospholipid phosphatidylinositol-3,4,5-trisphosphate (PIP3). SHIP acts as a negative regulator of immune response by hydrolysing PIP3, and SHIP deficiency results in myeloproliferation and B-cell lymphoma in mice. The validation of SHP-1, 2 and SHIP as oncology targets has generated interest in the development of inhibitors as potential therapeutic agents for cancers; however, SHP-1, 2 and SHIP have proven to be an extremely difficult target for drug discovery, primarily due to the highly conserved and positively charged nature of their PTP active site, and many PTP inhibitors lack either appropriate selectivity or membrane permeability. To overcome these caveats, novel techniques have been employed to synthesise new inhibitors that specifically attenuate the PTP-dependent signaling inside the cell and amongst them; some are already in clinical development which are discussed in this review.

Introduction

Well-balanced levels of tyrosine phosphorylation, maintained by the reversible and coordinated actions of protein tyrosine kinases (PTKs) and protein tyrosine

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phosphatases (PTPs), are critical for a wide range of cellular processes including growth, differentiation, metabolism, migration, and survival [1, 2]. Aberrant tyrosine phosphorylation, as a result of a perturbed balance between the activities of PTKs and PTPs, however, is linked to the pathogenesis of numerous human diseases, including cancer [3, 4]. Consequently, signaling events driven by tyrosine phosphorylation may offer innovative molecular targets for therapeutic interventions [5, 6].

Inhibitory phosphatases are typically recruited to ITIMs (immunoreceptor tyrosine-based inhibitory motif) in the cytoplasmatic tails of inhibitory receptors that are themselves phosphorylated by tyrosine kinases induced during lymphocyte activation. These PTPs inhibit signal transduction by removing phosphate moieties from residues from key signaling molecules and thereby antagonise tyrosine kinases [7].

Two PTPs that have an important inhibitory role in lymphocytes and other haematopoietic cells are SHP-1 and SHP-2 (SH2 domain-containing phosphatases 1 and 2). Although a PTP was traditionally thought to inactivate kinases and to serve as a negative regulator of cell functions, SHP-1, 2 have been shown to promote cell growth and act by both upregulating positive signaling pathways [8] and by downregulating negative signaling pathways [9]. SHP-1 is expressed widely throughout the haematopoietic system and has been shown to impact a multitude of cell signaling pathways [10]. In addition, SHP-2 contributes to the progression of a number of cancer types including leukaemias as well as gastric and breast cancers. It also regulates T-cell activation by interacting with inhibitory checkpoint inhibitors such as programmed cell death-1 (PD-1) and the B- and T-lymphocyte attenuator (BTLA) [11].

Another inhibitory phosphatase that is rather specific for an inositol phospholipid is SHIP (SH2 domain-containing inositol phosphatase). Like SHP-1, 2, SHIP binds to phosphorylated ITIM sequences on specific inhibitory receptors and removes a phosphate group from phosphatidylinositol (3,4,5)-triphosphate (PIP3), a phospholipid in the inner leaflet of the plasma membrane which then leads to inhibition of PI3K (phosphoinositol-3 kinase) signaling in lymphocytes [12]. SHIP acts as a negative regulator of immune response by hydrolysing PIP3, and, as a result, a SHIP deficiency leads to myeloproliferation and B-cell lymphoma in mice [13].

Although the success of such targeted approaches has been well demonstrated by over 40 PTK inhibitors that are already approved for treatment, the therapeutic potential of modulating the PTPs is still underexplored despite the fact that several PTPs have also been identified as high-value targets [14].

Excessive tyrosine phosphorylation is a hallmark of cancer, usually caused by abnormal expression and/or activation of receptor PTKs. By catalysing the dephosphorylation of phosphotyrosine residues, PTPs are usually viewed as negative regulators of signal transduction and therefore perceived as products of tumour suppressor genes. Several PTPs, including phosphatase and tensin homolog deleted on chromosome ten (PTEN), have been identified as tumour suppressors [15], whereas others (e.g., SHP-1, 2, SHIP) have been shown to promote the malignant phenotype.

This strong validation of SHP-1, 2 and SHIP as oncology targets has generated considerable interest in the development of small molecule inhibitors as potential therapeutic agents for haematologic malignancies and solid tumours [12, 16–18]. Unfortunately, SHP-1, 2 and SHIP have proven to be an extremely difficult target for drug discovery, primarily due to the highly conserved and positively charged nature of its PTP active site [19], and PTP inhibitor development has sent many pharmaceutical companies to the graveyard in the last decade.

The majority of reported PTP inhibitors lack either appropriate selectivity or membrane permeability, limiting their capacity in modulating the activity of the intracellular PTPs [18]. In order to overcome these caveats, novel techniques have been employed to synthesise new inhibitors that specifically attenuate the PTP-dependent signaling inside the cell and, amongst them, some are already in clinical phase I development [11, 16, 19, 20].

In this paper, we will review the mechanisms of action and the clinical development of newly available SHP-1, 2 and SHIP inhibitors and activators and discuss the major issues facing this rapidly evolving field.

SHP-1, 2 Pathways: Molecular Biology

SHP-1

SHP-1 (encoded by the PTPN6 gene) is a widely expressed inhibitory PTP and a negative regulator of antigen-dependent activation and proliferation. It is a cytosolic protein and therefore not amenable to antibody-mediated therapies, but its role in activation and proliferation could make it an attractive target for genetic manipulation in adoptive transfer strategies, such as chimeric antigen receptor (CAR) T cells [18].

SHP-1 is expressed by all mature haematopoietic lineages and at low levels (different isoforms) by endothelial

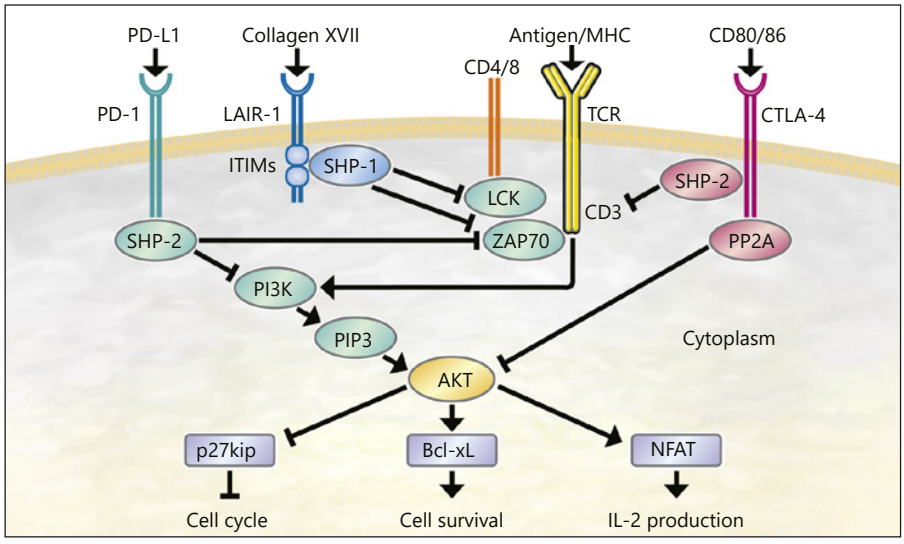

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Fig. 1. PD-1 and CTLA-4 target different molecules to inhibit T-cell activation. Upon T-cell conjugates with an antigen-presenting cell, PD-1 is located in the immune synapse interface and recruits SHP-2 to inhibit TCR-induced activation of the PI3K-Akt and Ras-MEK/ERK pathways. PD-1 also suppresses transcription of S-phase kinase-associated protein 2 (SKP2) to result in accumulation of p27kip1, which is an inhibitor of cyclin-dependent kinases to block cell cycle and proliferation. Ligation of CTLA-4 dephosphorylates signaling molecules including ZAP-70 and src kinase FYN. CTLA-4 inhibits AKT phosphorylation and activation by recruiting PP2A to its cytoplasmic tail. Ligation of CTLA-4 phosphorylates the pro-apoptotic factor BAD and enhances bcl-xL activity to prevent T-cell apoptosis. SHP-1 is constitutively associated with the inhibitory receptor LAIR-1, which is phosphorylated by LCK, although SHP-1 may also be activated by other ITIM-containing inhibitory receptors. Activation of SHP-1 leads to the inhibition of antigen-induced TCR signaling either through direct dephosphorylation of the TCR-zeta chain or by dephosphorylation of downstream proteins such as LCK and ZAP-70. PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte antigen-4; SHP, SH2 domain-containing phosphatase; LCK, lymphocyte-specific protein tyrosine kinase; ZAP-70, zeta-chain-associated protein 70; PP2A, protein phosphatase 2A; PI3K, phosphoinositol-3 kinase; PIP3, phosphatidylinositol triphosphate; AKT, protein kinase B; NFAT, nuclear factor of activated T cells; Bcl-xL, B-cell lymphoma extra large; MHC, major histocompatibility complex; p27kip, protein 27 kinase inhibitory protein; LAIR-1, leucocyte-associated Ig-like receptor-1; ITIM, immunoreceptor tyrosine-based inhibitory motif.

cells [10, 21]. SHP-1 consists of 3 domains; the N-terminal Src homology-2 (SH2) domain, the C-terminal SH2 domain, and the C-terminal catalytic PTP domain [22], and maximal phosphatase activity is achieved only when both SH2 domains are engaged. It has been shown that SHP-1 constitutively interacts with ITIM containing leucocyte-associated Ig-like receptor 1 (LAIR-1, ligand: collagen XVII) [23]. LAIR-1 is a member of the Ig superfamily, which is expressed on the majority of PBMCs and thymocytes. Antibody-induced cross-linking of the receptor in vitro provides a potent inhibitory signal that is capable of inhibiting cellular functions of NK cells, effector T cells, B cells, and dendritic cell precursors [24]. This inhibitory signal is dependent on phosphorylation of tyrosine residues located in ITIMs present in the cytoplasmic tail of LAIR-1 [25]. Recently, Lebbink et al. [26] have demonstrated that collagens (e.g., collagen XVII) are ligands for LAIR-1 which appears to be a novel mechanism of peripheral immune regulation by extracellular matrix proteins. Little is known about other SHP-1-binding partners in human cells; however, there is some evidence that zeta-chain-associated protein kinase 70 (ZAP-70) [27], lymphocyte-specific protein tyrosine kinase (LCK) [28], phosphoinositol-3 kinase (PI3K) [29], Vav [30], and T-cell receptor (TCR)-zeta [31] are also strongly implicated (Fig. 1, 2).

Mechanistically, SHP-1 and PD-1 were found to act independently to inhibit T-cell activation; with PD-1 preferentially inhibiting T cells with the highest-affinity TCRs, while SHP-1-mediated inhibition increased incrementally as TCR affinity increased [32]. Of particular interest, however, is that SHP-1 was found to be inhibitory to T regulatory cells (T_{regs}) [33] suggesting that inhibition of SHP-1 in T_{regs} may lead to increased suppressor function. As a result, this effect might be attributed to increases in TCR-APC conjugate formation and dura-

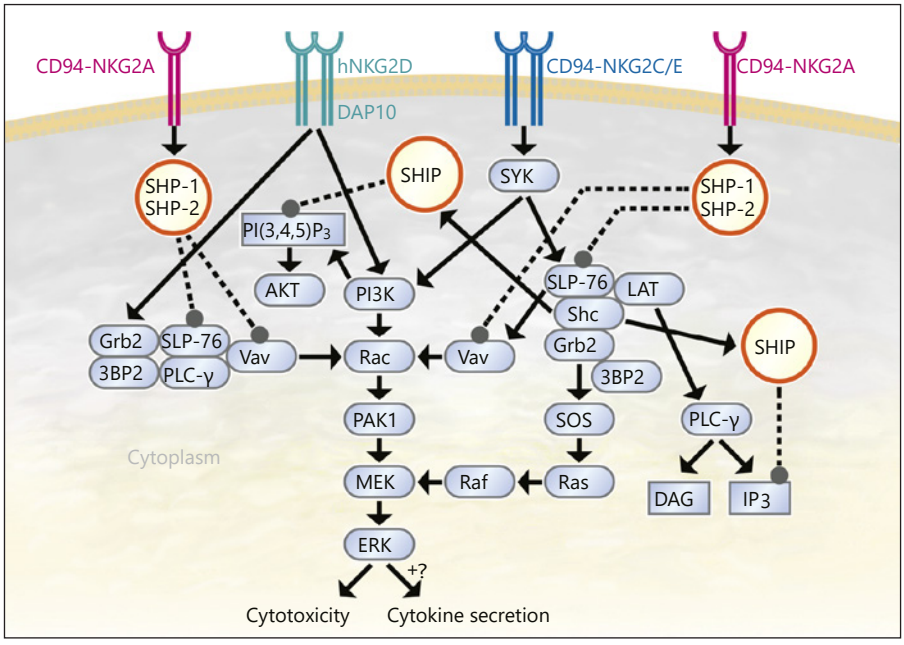

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Fig. 2. Oligomeric activating receptors, which include ITAM-bearing molecules as well as inhibitory MHC class I-specific receptors, are depicted. KIR-S are activating killer cell Ig-like receptors with a short intracytoplasmic domain and no intrinsic signaling properties, whereas KIR-L are inhibitory receptors with an intracytoplasmic ITIM. In the mouse, but not in humans, two alternative spliced forms of NKG2D coexist. The link between SHP-2 and SLP-76 is not fully characterised but could occur by way of Grb2. The signaling pathways leading to cytokine secretion appear to be strictly dependent on ITAM-bearing receptors, but their precise delineation remains to be completed. The substrates for SHP-1 and SHP-2 tyrosine phosphatases downstream of ITIM-bearing molecules include Vav1. SHP, SH2 domain-containing phosphatase; AKT, protein kinase B; SYK, spleen tyrosine kinase; PI(n)P, phosphoinositol(n)phosphate; Grb2, growth factor receptor bound-2; SLP-76, SH2 domain-containing linker of 76 kD; 3BP2, c-abl src homology 3 domain-binding protein-2 (adaptor protein); PLC-γ, phospholipase-Cgamma; Vav, GDP exchange protein (95 kD) (Vav is named after the 6th letter of the Hebrew alphabet); PI3K, phosphoinositol-3 kinase; Rac, ras-related C3 botulinum toxin substrate; PAK1, p21 protein-activated kinase 1; MEK, mitogen-activated protein kinase kinase; ERK, extracellular-signal-regulated kinase; Raf, rapidly accelerated fibrosarcoma kinase; Ras, rat sarcoma; SOS, son of sevenless; Shc, Src homology 2 domain-containing; LAT, linker for activated T cells; DAG, diacylglycerol; IP_3, inositol (1,4,5)-triphosphate; SHIP, SH2 domain-containing inositol phosphatase.

tion. Moreover, inhibition/deletion of SHP-1 in all CD4-positive T cells in mice demonstrated a key role for SHP-1 in negatively regulating the responsiveness of CD4-positive T cells to interleukin-4 signaling, and thus might be of importance for the maintenance of a T_{h1} phenotype [34].

Several SHP-1 inhibitors have been developed (e.g., NSC-87877, sodium stibogluconate [SSG], tyrosine phosphatase inhibitor 1 [TPI-1], suramin, and others); however, only a few of them have shown activity in experimental tumour models [18]. Among them, SSG (approved treatment for leishmaniasis) has been studied in phase I trials in patients with malignant melanoma; however, results were disappointing (see below for details).

The determination of which cell types contribute to the different aspects of the phenotype caused by SHP-1 loss or mutation and which pathways within these cells are regulated by SHP-1 is therefore important to enhance our understanding of the immune system regulation which then could form the basis for the development of novel SHP-1 inhibitors for cancer treatment.

SHP-2

SHP-2 (encoded by the PTPN11 gene) provides essential physiological functions in organism development and homeostasis maintenance by regulating fundamental intracellular signaling pathways in response to a wide range of growth factors and hormones. SHP-2 participates in myriad signaling cascades, including the pleiotropic Ras/mitogen-activated protein kinase (MAPK), the JAK-STAT, and the PI3K/AKT cascades, and positively contributes to multiple cellular functions including prolifera-

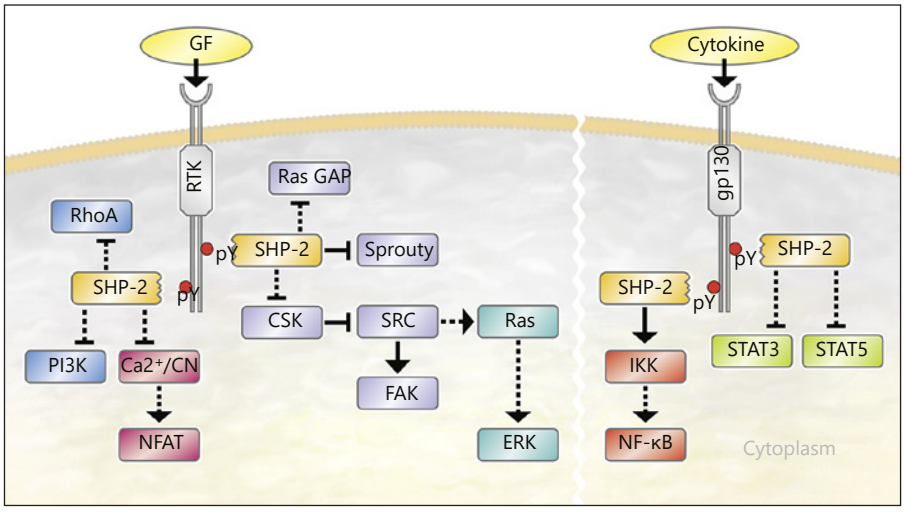

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Fig. 3. The major signaling pathway activated by SHP-2 downstream of RTKs and GFs is the ras/ERK MAP kinase cascade. SHP-2 activates ras/ERK through different mechanisms: these include dephosphorylation of rasGAP binding sites on specific receptors and adaptor proteins or dephosphorylation of the negative ras/ERK regulator, Sprouty. Alternatively, regulation of src kinase activity by SHP-2 through either direct dephosphorylation of src or by indirect regulation of the src inhibitor CSK can enhance activation of the ERK pathway. SHP-2 also regulates PI3K, FAK, and RhoA, as well as Ca^{2+}/calcineurin/NFAT signaling. In addition to RTK signaling, SHP-2 has been implicated downstream of cytokine signaling in the regulation of STAT signaling pathways and in the activation of NF-κB. Dashed lines: indirect interactions; solid lines: direct interactions. GF, growth factor; RTK, receptor tyrosine kinase; RhoA, Ras homolog gene family, member A; SHP, SH2 domain-containing phosphatase; PI3K, phosphoinositol-3 kinase; CN, calcineurin; NFAT, nuclear factor of activated T cells (transcription factor for IL-2 and IL-4); RAS GAP, Ras GTPase activating protein; SRC, Rous sarcoma virus; CSK, c-terminal src kinase; FAK, focal adhesion kinase; ERK, extracellular-signal-regulated kinase; IKK, IkappaB kinase; NF-κB, nuclear factor kappaB; STAT, signal transducer and activator of transcription; pY, phosphorylated Y-720.

tion, differentiation, cell cycle maintenance, and migration [35–37].

Moreover, somatic activating mutations in SHP-2 are associated with juvenile myelomonocytic leukaemia, acute myeloid leukaemia (AML), myelodysplastic syndrome, and acute lymphoid leukaemia [35]. Given the critical role of SHP-2 in haematopoietic stem cell function and in human haematopoiesis, it is not surprising that dysregulated SHP-2 is commonly found in human myeloid leukaemias [35]. Although SHP-2 *mutations* are rare in adult acute leukaemias, SHP-2 has been shown to be overexpressed at both the protein and RNA levels in several human AML cell lines and primary samples [38].

Several types of solid tumours including lung adenocarcinoma, colon cancer, neuroblastoma, glioblastoma, melanoma, hepatocellular carcinoma, prostate cancer, and triple-negative and HER-2-positive breast cancer have also been shown to harbour SHP-2 mutations [39, 40].

SHP-2 contains two SH2 domains (N-SH2/CSH2), a catalytic (PTP) domain, and a C-terminal tail with 2 tyrosine phosphorylation sites [17]. SHP-2 binding sites are found in receptor tyrosine kinases (RTKs) and scaffolding adapter proteins, thus this "molecular switch" ensures that SHP-2 is activated only at specific cellular compartments. In growth factor and cytokine signaling, SHP-2 acts upstream of Ras to dephosphorylate and to enable full activation of the ERK/MAP kinase pathway (Fig. 2, 3). In addition, the C-terminal tyrosines of SHP-2 undergo phosphorylation in response to most agonists. Tyrosyl-phosphorylated SHP-2 recruits Grb2/SOS, contributing to Ras activation [41]. Furthermore, SHP-2 binds immune-inhibitory receptors, including PD-1 [42], and, often in concert with SHP-1, inhibits signaling from activating immunoreceptors (e.g., TCR). A summary of SHP-1-modulated functions is given in Table 1.

Due to its pronounced role in tumours, various SHP-2 inhibitors have been discovered to target SHP-2 for cancer treatments. Inhibitors of SHP-2 have been widely studied because of its broad role in a bundle of different cancers. For instance, cryptotanshinone has been devel-

Table 1. Functions of SHP-2 in cancer [35, 38, 43–52]

Function	Role of SHP-2	Relevant references
Tumour invasion and metastasis	Increased epithelial mesenchymal transition Metastasis is promoted via angiogenesis Association with advanced tumour stage	Zhou and Agazie [43], 2008 Tang et al. [44], 2013 Xie et al. [45], 2014
Tumour apoptosis	Prevents apoptosis and blocks apoptosis in cancer stem cells Enhanced leukaemic cell clonogenic growth Controls survival of HSCs	Yang et al. [46], 2006 Xu et al. [38], 2005 Nabinger and Chan [35], 2012
Tumour cell proliferation and cell cycle	Regulates various signaling pathways to control proliferation Involved in radioresistance by controlling cell cycle distribution Controlling of cell cycle checkpoints	Furcht et al. [47], 2014 Peng et al. [48], 2014 Tsang et al. [49], 2012
DNA damage and replication	Depletion impairs checkpoint kinase 1 activation Involved in checkpoint-mediated DNA repair	Tsang et al. [49], 2012 Kathpalia et al. [50], 2006
Tumour drug resistance	Resistance to EGFR inhibitors Mediates IFN-γ resistance	Yu et al. [51], 2013 Tseng et al. [52], 2012

IFN, interferon; EGFR, epidermal growth factor receptor; HSCs, haematopoietic stem cells

oped to treat PTPN11-associated malignancies [53]. Moreover, mouse myeloid progenitors and leukaemic cells caused by E76K mutation are sensitive to this inhibitor [54]. Another molecule, II-B08, can inhibit SHP-2 and strongly bind to the receptor [55] and thereby enhances the effects of dasatinib on human and mouse mastocytoma cells.

Although these SHP-2 inhibitors have been reported to have substantial in vitro potency, PTP selectivity, and beneficial effects in animal models, collectively these molecules have poor bioavailability and/or troublesome pharmacophores for further drug development. In addition, none of them have been profiled extensively for off-target effects against other enzyme families. Furthermore, where in vivo efficacy has been reported, on-target activity has not been demonstrated convincingly [17].

Most recently, the development of allosteric SHP-2 inhibitors has been employed to circumvent these problems using computer-aided drug designs to discover SHP-2 inhibitors [11, 19, 20, 56]. Recently, the medical chemistry of the SHP-2 inhibitor SHP099 has been reported [20]. SHP099 is a very potent inhibitor (IC_{50} = 71 nM) and has no significant activity against a panel of other PTPs (including SHP-1) and kinases. In addition, SHP099 has almost minimal activity against other enzyme systems typically associated with toxicity. Chen et al. [20] also screened 250 well-annotated cancer cell lines with a deep-coverage shRNA library. Cell lines with activated RTKs/PTK fusions were found to be preferentially sensitive to SHP-2 depletion, while cells bearing Ras or B-Raf mutations were resistant. Most importantly, SHP099, administered orally, showed efficacy against an EGFR-driven cancer cell line xenograft and a FLT3-ITD-positive AML patient-derived xenograft. Remarkably, treated mice also had no evidence of toxicity.

Further evidence has been provided earlier by Zeng et al. [19] who identified the SHP-2 inhibitor 11a-1 with an IC_{50} value of 200 nM and more than 5-fold selectivity against 20 mammalian PTPs. The compound was found to block growth factor-mediated extracellular-signal-regulated kinases 1 and 2 (ERK1, 2) and protein kinase B (AKT) activation and exhibited excellent antiproliferative activity in lung cancer and breast cancer as well as leukaemia cell lines [19]. In addition, SHP-2 inhibition also abrogated/prevented emergence of resistance to B-Raf and MEK inhibitors, which is often caused by RTK activation [57] and may therefore provide a path to the clinic.

From being considered as an "undruggable" target [58], recent development of allosteric inhibitors [11] has made it possible to specifically target SHP-2 in RTK-driven malignancies. In this regard, SHP-2 has now emerged as an attractive target for therapeutic targeting in haematological malignancies. However, a better understanding of the role of SHP-2 in different haematopoietic lineages and its crosstalk with signaling pathways activated by other genetic lesions is required before the promise is realised in the clinic.

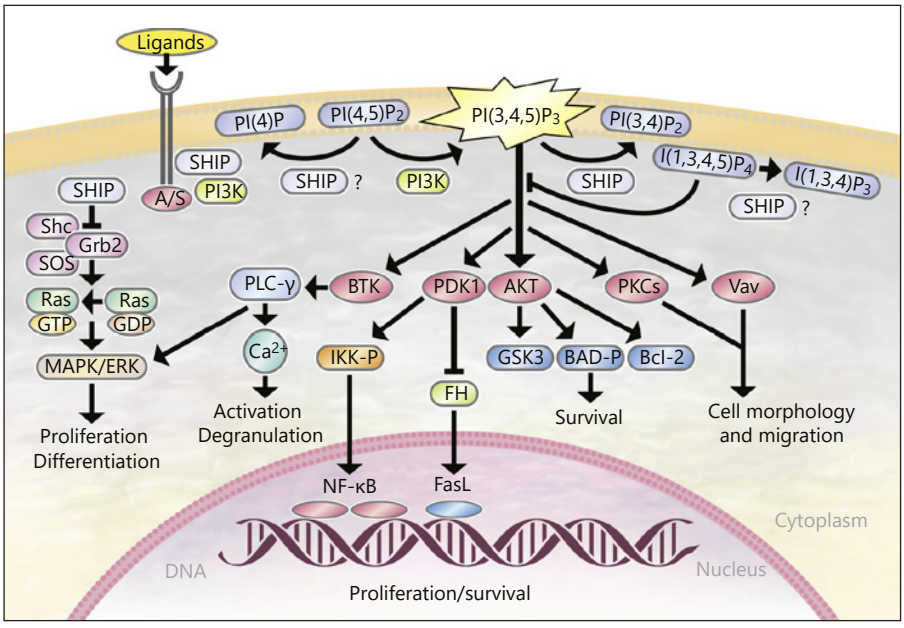

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Fig. 4. In haematolymphoid cells, SHIP can be recruited to a wide variety of receptor complexes including growth factor receptors and immune receptors. SHIP is recruited to receptor-associated signaling complexes via adaptors (e.g., Shc, Grb2), scaffold proteins like Gab1 (GrB-associated binding protein 1) or directly via its SH2 domain. After recruitment to the plasma membrane, SHIP can then hydrolyse PIP3. Hydrolysis of PIP3 inhibits recruitment of PH domain-containing kinases like AKT, BTK, and PLC-γ to the plasma membrane and thus limits the activity of several different PI3K effectors that promote cell survival, migration, differentiation, or proliferation. These include distal kinases like MAP/ERK and key transcription factors such as NF-κB and NFAT. Ras proteins have been shown to associate with the plasma membrane by binding to negatively charged PIP3 and PI(4,5)P_2. SHIP could also potentially inhibit this process. SHIP, SH2 domain-containing inositol phosphatase; Shc, Src homology 2 domain-containing; Grb2, growth factor receptor-bound 2; SOS, son of sevenless; Ras, rat sarcoma; MAPK/ERK, mitogen-activated protein kinase/extracellular-signal-regulated kinases; PI3K, phosphoinositol-3 kinase; BTK, Burton tyrosine kinase; PLC-γ, phospholipase-Cgamma; AKT, protein kinase B; PDK1, pyruvate dehydrogenase lipoamide kinase isozyme 1; IKK-P, IkappaB kinase phosphoprotein; FasL, first apoptosis signal receptor ligand (CD178); NF-κB, nuclear factor kappaB; GSK3, glycogen synthase kinase-3; BAD-P, bcl-2-associated death promoter; Bcl-2, B-cell lymphoma 2; PKCs, protein kinases C; Vav, GDP exchange protein (95 kD) (Vav is named after the 6th letter of the Hebrew alphabet); PI(n)P, phosphatidylinositol-(n)-phosphate; I(n)P, inositol-(n)-phosphate; A/S, adaptor/scaffold protein; FH, Forkhead transcription factor.

SHIP Pathway: Molecular Biology

The central signaling PI3K pathway regulates cell growth, proliferation, differentiation, cytokine production, and survival [59]. Its activation by growth factors, cytokines, or chemokines leads to phosphorylation of phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2) to generate phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P3 or PIP3). PIP3 acts as a second messenger resulting in the recruitment of several proteins containing a pleckstrin homology (PH) domain. The best characterised one is the serine/threonine kinase AKT which has a huge range of substrates involved in survival, proliferation, and metabolism. The others are the tyrosine kinase BTK, involved in Ca^{2+} signaling with effects on MAPK and NFκB via the BTK/PLCγ signalosome, Rac/ERK via Bam32, involved in proliferation and cytoskeleton regulation, and Vav, also involved in regulation of the cytoskeleton [60, 61] (Fig. 4).

SHIP is a negative regulator of immune cell activation and stimulation, because it dephosphorylates the PI3K product and lipid second messenger PIP3 to produce phosphatidylinositol-3,4-bisphosphate (PI-3,4-P2). SHIP and the ubiquitously expressed tumor suppressor PTEN which hydrolyses PIP3 to PI-4,5-P2, are the gatekeepers of the PI3K signaling pathway [59]. SHIP is also able to hydrolyse I-1,3,4,5-P4 (IP4). IP4 as a soluble phosphoinositide is markedly involved in Ca^{2+} signaling in lymphocytes [12].

Several splice variants of SHIP have also been detected. The two major SHIP isoforms encoded in mammalian genomes are the 145 kD form SHIP-1 (encoded by the INPP5D gene) and the 142 kD form SHIP-2 (encoded by the INPPL1 gene) [60]. Expression of SHIP-1 is primarily confined to all cells of the haematopoietic lineage such as T, B, and NK cells, granulocytes, platelets, dendritic cells, monocytes/macrophages, and mast cells, but it is also expressed on progenitor and stem cells [12, 61]. Through its SH2 domain, SHIP binds to the tyrosine phosphorylated forms of Shc, SHP-2, Dok-3, ITIM receptors like FcγRIIB, CD94, Ly49 and KIR (killer cell immunoglobulin-like receptor), as well as immunoreceptor tyrosine-based activation motif (ITAM) receptors such as FcγRIIA, FcγRI-associated zeta-chain, TCR zeta chain, CD28, FcεRI, and the BCR (Igα/β) [61, 62]. SHIP possesses a centrally located phosphatase domain that specifically hydrolyses the 5′-phosphate from PIP3 by generating PI-3,4-P2. C-terminal to the phosphatase domain, a C2 domain has been identified as an allosteric activating site when bound by PI-3,4-P2. The proline-rich C-terminus of SHIP binds proteins with a phosphotyrosine binding (Dok-1, Dok-2) or an SH2 domain (e.g., SHIP-2) and can also bind SH3-containing proteins such as Grb2, Src, Lyn, Abl, and PLCγ-1 when phosphorylated [61, 62]. SHIP-1 functions as a negative regulator in immunoreceptor signaling and haematopoietic progenitor cell proliferation/survival, and as an inducer of cellular apoptosis. SHIP-1 has also been implicated both as a haematopoietic tumour suppressor and activator [11, 63].

SHIP-2 is ubiquitously expressed in all cell and tissue types in rodents and humans, with especially high levels of SHIP-2 being found in the heart, liver, brain, skeletal muscle, and the placenta [16, 59]. Like SHIP-1, SHIP-2 dephosphorylates PIP3 into PI-3,4-P2. Although SHIP-1 and SHIP-2 share a high rate of amino acid conservation, they differ significantly in their cellular expression and receptor recruitment. It was reported that SHIP-1 binds to the SH3 domains of Grb2 and Src, whereas SHIP-2 binds to the SH3 domain of Abl, but not to Grb2 [62]. SHIP-2 is considered as a negative regulator of glucose homeostasis [59] and is involved in the maturation and activation of mast cells as well as in phagocytosis directed by Fc receptors for IgG, thus regulating allergic reactions and antibacterial defense [60].

SHIP as a cytoplasmic protein has to be recruited to the plasma membrane after direct binding of SHIP with ITIM/ITAM receptor chains through its SH2 domain and appears to be associated with adapter proteins (e.g., Shc, Grb2, Dok-3) or scaffold proteins (e.g., Gab1, 2). In B cells, SHIP directly binds phosphorylated FcγRIIB via its SH2 domain. Current data indicate that BCR activates Lyn which phosphorylates the ITIM motif in FcγRIIB, and Grb2 might stabilise SHIP binding to FcγRIIB. Pauls and Marshall [61] presented a model in which BCR stimulation without involvement of FcγRIIB leads to the recruitment of SHIP to the phosphorylated ITAM of BCR dependent on SYK. The SH2 domain of SHIP as well as a complex of proteins including Grb2, Dok-2, and Shc mediate this ITAM recruitment [61].

SHIP-1 negatively regulates immune cell signaling by phosphatase-dependent activity by effecting inhibitory ITIM receptors, but also by phosphatase-independent functions/activity via protein-protein interactions called "intrinsic brake" [61]. Dephosphorylation and therefore reduction of PIP3 by SHIP interrupts the recruitment of PIP3-binding effector proteins, including BTK, AKT, and Vav. SHIP is required for FcγRIIB-mediated inhibition of intracellular Ca^{2+} responses by blocking BTK membrane recruitment; the latter regulates PLCγ-2 phosphorylation and Ca^{2+} fluxes. As a consequence, the activation of Ca^{2+} signaling-dependent downstream effectors such as MAPK and NFκB is blocked.

In phosphatase-independent functions, the SHIP–Shc interaction after BCR ligation is suggested to be involved in Shc binding to Grb2 and prevents the activation of the Ras/MAPK pathway, as shown in murine B-cell lines. In addition, binding of Dok-1 to SHIP after interaction with FcγRIIB negatively regulates Ras-ERK signaling, thus dampening migration. In macrophages treated with lipopolysaccharide, SHIP disrupts the interaction between TLR-4 (toll-like receptor-4) and the adaptor protein MyD88 and also blocks the nucleotide-binding oligomerisation domain-like receptor 2 (NOD2) signaling, resulting in the inhibition of downstream activation of MAPK/NFκB pathways (summarised in [61]). Therefore, SHIP is also involved in lipopolysaccharide-induced activation of monocytes/macrophages.

There are many studies using SHIP KO mice to investigate the influence of SHIP to different types of immune cells. By investigating the effect of SHIP-1 deficiency on T cells, SHIP-1 is strongly suggested to regulate CD4+ T cells differentiation towards T_{h1} and T_{h17} cells, but downregulates T_{reg} cells [59–61]. However, one has to keep in mind that SHIP-2 may compensate the absence of SHIP-1, but its role in those SHIP-1 deficiency models has to be clarified.

The control of SHIP expression levels seems to be different in haematopoietic cell lineages (myeloid and NK

cells), which results in the distinct functions of each of the cell lineages. The regulation of several gene expression levels contributing to differential expression of SHIP protein is caused by SMAD family transcription factors (SMAD, small body size *Drosophila*), post-transcriptional by microRNA-mediated degradation of transcripts, and post-translational processes through proteasomal degradation and ubiquitination [12, 61]. MiR-155, a microRNA, is expressed in haematopoietic cells and has been identified as a SHIP-1 repressor. It could be shown that over-expression of miR-155, also found in patients with B-cell lymphoma and AML, and SHIP-1 deficiency in SHIP-1 KO mice resulted in the same phenotype. These observations indicate that overexpression of miR-155 and reduced expression of SHIP-1 might induce such types of cancer [59, 60].

The importance of SHIP as negative regulator of the PI3K pathway is emphasised by observations that SHIP is mutated or markedly decreased in many leukaemias and lymphomas [62]. Due to diminished SHIP expression in these cancer types, levels of T_{h2} and T_{reg} cells may predominantly increase, and SHIP therefore may dampen the immune system to attack cancers, an observation that is in line with the finding that solid tumours grow more rapidly in SHIP KO mice [62].

Since PI3K is involved in inflammation and autoimmunity, small molecules as agonists for SHIP-1 had been found to mediate an anti-inflammatory effect in blood cells [64]. SHIP-1 has been reported as a haematopoietic tumour suppressor and activator, and SHIP inhibition has been shown to be effective in killing cancer cells [12, 16, 63]. However, although a potential therapeutic benefit of SHIP-1/2 agonists or inhibitors in immunomodulation or with an antitumour effect is evident, the complete switching-on/off of several signaling pathways may result in unexpected side effects; therefore, strategies to select mechanisms for modulating SHIP and the PI3K pathways would be desired [60]. In addition, small molecules activating (e.g., analogs of pelorol such as AQX-1125) or inhibiting SHIP-1/2 (e.g., quinoline small molecules such as NSC13480 and NSC305787 or 3-α-aminocholestane) had also been described [12, 16, 63, 65], but only one, AQX-1125, is currently being studied in clinical trials.

3-α-Aminocholestane (3AC) is a selective inhibitor of SHIP-1 (EC_{50} = 10 μM) and shows no inhibition of the other isoform, SHIP-2, at concentrations up to 1 mM. 3AC promotes apoptosis of SHIP-1-expressing leukaemia cells (KG-1) and multiple myeloma cells (OPM) suggesting SHIP-1 inhibition is a potential drug target for blood cancers. Mice treated with 3AC showed increased numbers of MIR cells in the spleen and lymph nodes and increased numbers of granulocytes [66]. Fuhler et al. [76] investigated the biochemical consequences of 3AC treatment in multiple myeloma (MM) cells, and demonstrated that SHIP-1 inhibition arrests MM cell lines in either G0/G1 or G2/M stages of the cell cycle, leading to activation of caspases and subsequently to apoptosis. In addition, they have shown that in vivo growth of MM cells is blocked by treatment of mice with 3AC. Furthermore, this group of researchers has also demonstrated that pan-SHIP-1/2 inhibitors are also capable of killing MM cells through G2/M arrest, caspase activation, and apoptosis induction. Interestingly, in SHIP-2-expressing breast cancer cells, pan-SHIP-1/2 inhibition also reduced viable cell numbers, which could be rescued by addition of exogenous PI(3,4)P2. These findings therefore add weight to the proposal that inhibition of SHIP-1 and SHIP-2 may have broad clinical application in the treatment of multiple tumours.

Clinical Development

To date, clinical trials of small-molecule SHP-1 inhibitors such as sodium stibogluconate (SSG) in cancer patients remain restricted to phase I studies, and therefore antitumour effects, although measured, were not the primary objective of the studies. No clinically measurable antitumour effects were observed in either study [67, 68] which appears to be disappointing and does bring into question the effectiveness of SSG administration as an anticancer strategy. No phase II studies of small-molecule SHP-1 inhibition have been conducted so far. Evaluation of toxicity of SSG was somewhat limited in both studies due to the combination of SSG with interferon and/or chemotherapy, and therefore severe and/or life threatening adverse effects were observed (in up to 68% of patients). Dose-limiting toxicities observed included pancreatitis, bone marrow suppression, fatigue, lipase elevation, and gastrointestinal upset (Table 2). Interestingly, especially when considering global SHP-1 inhibition with agents such as SSG, SHP-1 expression is altered in a range of malignancies; upregulated in breast and ovarian cancers [69, 70] and gene-silenced in T-cell lymphomas, leukaemias, and colorectal cancers [54, 71, 72] which might, at least in part, contribute to the disappointing results observed so far.

Table 2. Selected clinical trials with SHP-1, 2 and SHIP inhibitors

Trial number	Compound	Target	Disease	Status
NCT00629200	Sodium stibogluconate	SHP-1 antagonist	Malignant melanoma	Phase I completed, no objective response, life-threatening events in up to 68% of patients
NCT00498979	Sodium stibogluconate	SHP-1 antagonist	Malignant melanoma	Phase I completed, no objective response, DLTs include pancreatitis, bone marrow suppression, nausea
NCT03114319	TNO155	SHP-2 antagonist	Solid tumours (NSCLC, H and N, etc.)	Phase I ongoing
NCT02858453	AQX-1125 (rosiptor)	SHIP-1 agonist	Inflammatory diseases	Phase III ongoing

DLT, dose-limiting toxicity.

In terms of potential for cancer therapy, SHP-2 arguably represents a more attractive molecular target than SHP-1. Activating mutations of SHP-2 are found in 10% of patients with AML [73] and are frequently present in juvenile myelomonocytic leukaemia, particularly in patients with Noonan's syndrome where missense mutations of SHP-2 are well recognised. SHP-2 mutations have also been reported in numerous other malignancies including neuroblastoma, melanoma, and lung, breast, and colorectal cancers confirming its potential as a proto-oncogene [39]. Despite this, only one SHP-2 small molecule inhibitor is currently going through clinical testing. TNO155 is an allosteric inhibitor of SHP-2 that has been developed by Novartis (Basel, Switzerland) from the tool compound SHP099. It is orally bioavailable and is currently being studied in a dose-finding study in adult patients with advanced solid tumours. This is a phase I dose escalation and expansion trial involving predominantly EGFR-mutant non-small cell lung cancer and head and neck squamous cell cancers to establish safety and tolerability. No efficacy data are yet available on TNO155 from this study which aims to recruit 105 patients over 3 years. In vitro analysis has established that the compound prevents SHP-2-mediated signaling, inhibits MAPK signals, and prevents growth of SHP-2 expressing tumour cells through blockade of the Ras-RAF-ERK pathway. It also appears to modulate immune checkpoints, via regulation of PD-1-mediated signal transduction, suggesting the antitumoural action may be generated through immune mechanisms as well as antiproliferative activity [20].

Rosiptor (AQX-1125) is the only SHIP-1 activator currently in clinical trials [65, 74]. Overall, approximately 395 patients have received rosiptor in 8 completed clinical trials. Results have demonstrated that rosiptor has desirable pharmacokinetic, absorption, and excretion properties that make it suitable for once-daily oral administration. It also has anti-inflammatory and antipain properties consistent with those exhibited in preclinical studies; and it is generally well tolerated, exhibiting mild to moderate adverse events primarily related to gastrointestinal upset that resolve without treatment or long-term effects. In addition, rosiptor has also been shown to inhibit bleomycin-induced pulmonary fibrosis by SHIP-1 activation in mice [75]. Rosiptor is an activator of SHIP-1, which reduces the activity of the PI3K cellular signaling pathway. Over-activity of the PI3K pathway can cause immune cells to produce an abundance of proinflammatory signaling molecules and increase their migration to and concentration in tissues, resulting in excessive or chronic inflammation. By activating SHIP-1, rosiptor is believed to decrease the inflammatory process, thereby reducing inflammation and inflammatory pain [65, 76]. Clinical trials with cancer patients are also planned.

Finally, although there are no inhibitors of SHIP-1 in clinical studies, in vitro experiments using SHIP-1 deficient mice have shown profoundly diminished SDF1/CXCL12 expression in the bone marrow suggesting that SHIP-1 promotes the homing of haematopoietic stem cells to the bone marrow niche. Consistent with this finding is the observation that SHIP-1 inhibitors can markedly increase granulocyte production in vivo in mice and increase neutrophil and platelet recovery in myelosuppressed hosts [66]. This suggests that SHIP-1 inhibition might represent an attractive way of promoting bone marrow recovery after chemotherapy or following allogeneic transplantation, aside from any antitumoural activity, that might justify future clinical development.

Conclusion

The recent elucidation of the roles of SHP-1, 2 and SHIP pathways in cancer biology gives considerable justification to the further development of small molecule inhibitors and activators of these phosphatase proteins. The SHP-1 inhibitor SSG is furthest through clinical development, although arguably it is the SHP-2 inhibitor TNO155 that looks more promising as a potential antitumoural agent given the frequency of activating mutations of SHP-2 in many cancers, notably AML. Targeted studies have revealed that a combination of inhibitors may be required to effectively block a given function in cancer research. Studies that broaden our understanding of the functions of SHP-2 could lead to a re-evaluation of the role in determining clinical outcome. However, future studies of the clinical importance should be carefully designed to explain conflicting viewpoints. Drugs should be used with caution as a result of the different functions of SHP-2 in various signaling pathways and cancer types. Ultimately, future studies should focus on confirming the effects of SHP-2 on tumours in different tumour microenvironments, as well as the signaling pathway, including the substrate of SHP-2 phosphatase activity. In the case of the bone marrow microenvironment, SHIP-1 appears to be a major determinant of the haematopoietic niche and stem cell homing. The next few years are likely to see further exploration of inhibitors and activators of all these phosphatases with the promise of clinical development in the field of cancer treatment and other disorders.

Acknowledgement

The authors are indebted to Alwin Hierl (Munich, Germany) for preparing the artwork.

Disclosure Statement

The authors declare that they have no conflict of interest.

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