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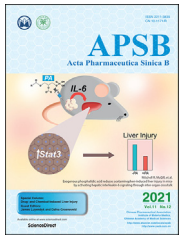

REVIEW

Strategies to overcome drug resistance using SHP2 inhibitors

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Abstract Encoded by *PTPN11*, the SHP2 (Src homology-2 domain-containing protein tyrosine phosphatase-2) is widely recognized as a carcinogenic phosphatase. As a promising anti-cancer drug target, SHP2 regulates many signaling pathways such as RAS-RAF-ERK, PI3K-AKT and JAK-STAT. Meanwhile, SHP2 plays a significant role in regulating immune cell function in the tumor microenvironment. Heretofore, five SHP2 allosteric inhibitors have been recruited in clinical studies for the treatment of cancer. Most recently, studies have proved the therapeutic potential of SHP2 inhibitor in overcoming drug resistance of kinase inhibitors and programmed cell death-1 (PD-1) blockade. Herein, we review the structure, function and small molecular inhibitors of SHP2, and highlight recent progress in overcoming drug resistance using SHP2 inhibitor. We hope this review would facilitate the future clinical development of SHP2 inhibitors.

1. Introduction

Tyrosine phosphorylation is a dynamic and reversible post-translational modification, which plays a vital role in a wide range of cellular functions, including cell proliferation, differentiation, survival or apoptosis, and oncogenic transformation[1,2]. This dynamic modification is mediated by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Tyrosine phosphorylation mediates the dynamic and critical regulatory processes of most intracellular signaling pathways, and signal disorders are recognized as the cause of the diseases[3,4]. At present, many drugs targeting PTKs have been approved by U.S. Food and Drug Administration (FDA)[5]. Due to the incomplete understanding of PTPs, the unacceptable selectivity of existing inhibitors, and poor pharmacokinetic properties, there are no PTP targeting drugs in clinical[6].

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The PTPs family consists of CX_5R motif, and the structural PTPs consists of a membrane domain (D1) and a proximal domain (D2)[7]. The D1 domain is mainly responsible for catalytic activity, while the D2 domain has almost no activity. According to the structure of the extracellular domain, PTPs can be divided into three subgroups: dual-specificity PTPs (DUSP-PTPs), low molecular weight PTPs (LMW-PTPs), and high molecular weight PTPs (HMW-PTPs)[8]. According to the location, PTPs can be divided into receptor-like transmembrane PTPs and non-receptor cytoplasmic PTPs[4]. The increased tyrosine phosphorylation activity caused by overactivation or overexpression of PTKs are a marker of many cancers[9], while PTPs are considered to be a negative regulator of signal pathways and tumor suppressor gene products by regulating dephosphorylation. In fact, abnormal PTPs activity can also lead to the occurrence and development of many human disorders such as cancer, metabolic and autoimmune diseases, infectious diseases and neurodegeneration[10].

The non-receptor protein tyrosine phosphatase Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2) plays a critical role in many cancer-related signaling pathways, such as RAS-RAF-ERK, PI3K-AKT and JAK-STAT[11]. Heretofore, five allosteric inhibitors have been recruited for clinical studies, suggesting SHP2 as a promising anti-cancer drug target[6,12–14]. Most recently, a number of studies have shown that SHP2 allosteric inhibitors can be combined with other protein inhibitors to overcome drug resistance. The combined therapies using SHP2 inhibitor has been proved to be more effective than monotherapy. Moreover, SHP2 is also the convergence node of multiple signal pathways in immune cells and cancer cells. In T cells, SHP2 participates in the downstream signal transduction of immunosuppressive receptor PD-1, which is the key immune checkpoint of cancer immunotherapy. Blocking PD-1 or SHP2 can induce T cell help (Th1) immunity, activate T cells and eliminate the immunosuppressive effect of cancer[15,16]. It should be mentioned that many oncogenic mutants in SHP2 may cause drug resistance and hamper the clinical development of SHP2 inhibitors. A novel approach based on dual allosteric inhibition may help to improve the inhibition rate of mutants and overcome drug resistance. In addition, it will be a new direction to chemically induce SHP2 degradation using proteolysis-targeting chimeras (PROTACs) technology.

Herein, we introduce the structure and functions of SHP2, and briefly review the development of SHP2 inhibitors. Importantly, we summarized the recent strategies to overcome drug resistance and synergistic tumor immunotherapy using SHP2 inhibitors. We also discuss the future clinical applications of SHP2 inhibitors, hoping to provide a certain reference for the future drug development.

2. Structure and self-inhibition of SHP2

As non-receptor protein tyrosine phosphatase, SHP2 is encoded by the *PTPN11* gene and contains 593 amino acid residues. The structure of SHP2 consists of two SH2 domains (N-SH2 and C-SH2), a PTP catalytic domain and a C-terminal with two tyrosine phosphorylation sites (Y542/Y580)[17,18]. The C-SH2 domain consists of 112−215 residues responsible for the binding energy but doesn't contribute to the activation of SHP2. On the contrary, the N-SH2 domain contains 2−104 amino acids and acts as a conformational switch in the activation of SHP2[19,20]. The PTP catalytic domain is composed of 220−525 residues, of which Cys459 is a highly active cysteine in the conserved characteristic motif of the PTP catalytic domain and has essential catalytic functions[19,21]. The C-terminal tail (Tyr542, Tyr580) can be phosphorylated during extracellular stimulation[22–24] (Fig. 1A). In the basal state, SHP2 maintains a self-inhibition state with low catalytic activity through the intramolecular interaction between the N-SH2 domain and the PTP domain[19]. Binding of growth factors or cytokine abolish the self-inhibition and activates SHP2[25,26] (Fig. 1B). Therefore, maintaining the self-inhibited conformation would effectively inhibit the activity of SHP2, which provide new mechanism for the design of SHP2 inhibitors[27].

At the cellular level, SHP2 located in the cytoplasm and plasma membrane. SHP2 plays a crucial role in different receptor signal pathways, mediating cell growth, cell cycle maintenance, differentiation, migration, adhesion and apoptosis[14]. At the same time, SHP2 is also involved in some events in the nucleus and mitochondria[28]. Mice carrying SHP2 deletion alleles are fatal in embryos[29], and some experimental results have shown that SHP2 is necessary for the early development of mice[30,31]. Studies on tissue-specific conditional gene knockout mice have revealed the various functions of SHP2[4].

3. SHP2 mutations and diseases

SHP2 mutations lead to dysregulated enzymatic activities, which lead to various diseases such as Noonan syndrome (NS), leopard syndrome (LS), juvenile myelomonocytic leukemia (JMML), acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS), and human malignant tumors[32] (Fig. 2). *PTPN11* gene

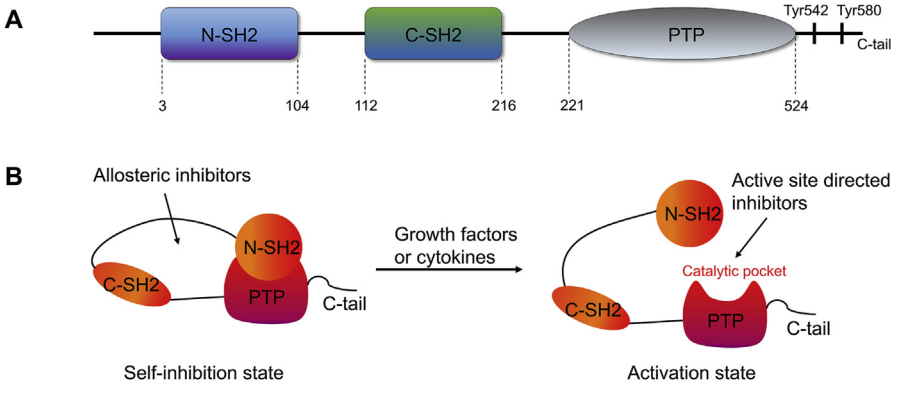

Figure 1 (A) SHP2 functional domains. (B) The self-inhibition state, activation state and inhibitors action sites of SHP2.

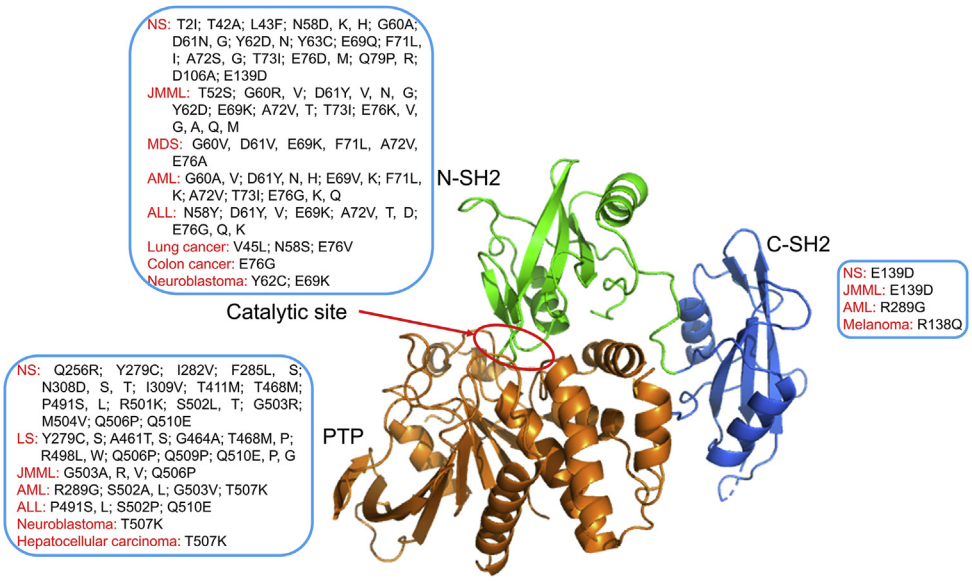

Figure 2 Schematic diagram of SHP2 mutations that occur in human diseases (PDB ID: 2SHP).

mutations occur at a low rate in various types of solid tumors, AML, and neuroblastoma[33]. $SHP2^{E76K}$ is one of the most common SHP2 mutations found in leukemia and solid tumors and exhibited 20-fold increase in the basal phosphatase activity[6,34]. NS is an autosomal dominant genetic disease. Germline mutations of *PTPN11* are found in about 40%−50% of NS patients. In the PTP domain, the SHP2 related mutant N308D, which accounts for 25% of cases, is 3-fold more active than wild-type SHP2; Asn308 is a mutation hotspot of NS; the other two SHP2 mutants N308S and Q506P show higher catalysis only under specific substrate induction active[4,35]. Other mutants T42A, D106A and E139D show relatively basal PTP activity, and low levels of p-Tyr peptide can effectively activate these SHP2 mutants. LS is a rare autosomal dominant genetic disease. Germline mutations in *PTPN11* have been found in at least 80% of LS. These mutations weaken the intramolecular interaction between the N-SH2 domain and the PTP domain, resulting in a conformational change in SHP2. Y279C and T468M are the most common mutants that can significantly activate the RAS/ERK signaling pathway[8,36]. About 10% of NS also develop into JMML, a relatively rare leukemia that affects approximately one in a million children with a poor prognosis and prone to relapse. The only treatment currently available is bone marrow transplantation[37,38]. Studies have shown that leukemia and solid tumor mutations (D61Y and E76K) have higher catalytic activity than NS-related mutations (N308D), which indicate that the PTP activity of leukemia should be higher than that of NS[39]. Therefore, we speculate that low levels of activated SHP2 cause abnormal diseases, while high levels of activated SHP2 can cause cancer. According to the position and function of mutations, it can be divided into six groups. Group I mutations located in the interaction between N-SH2 and PTP domains, which destroyed the self-inhibition conformation of SHP2. So far, 506 positions mutations in the N-SH2 and PTP domains have been reported and most of them are located at this interface[35,40]. For example, $SHP2^{E76Q}$ and $SHP2^{S502P}$ mutations resulted in the loss of Glu76-Ser502 hydrogen bond between N-SH2 and PTP domains[6]. Groups II and III of mutations include residues that are exposed to the surface of the PTP domain to stabilize inactive and catalytic conformations. Different from the functions of the first three groups of mutations, group IV mutations have a certain effect on maintaining the entire PTP structure or participating in the interaction of catalytic amino acids. For example, $SHP2^{F285S}$ destroys the hydrophobic B'C loop of the PTP domain, resulting in a closed conformation opening 2 Å[41]. Group V mutations are residues in the phosphor-peptide binding pockets of the two SH2 domains, destroying the effect of binding affinity and specificity. In the last group, the mutated residues are located in the junction region between the N-SH2 and C-SH2 domains, regulating the direction of SH2, and a few mutations are located in this region[32,36].

4. Role of SHP2 in signaling pathways

SHP2 plays an essential role in various signaling pathways[8]. SHP2 regulates physiological and pathological processes through positive (signal enhancement) and/or negative (signal inhibition) of signal transduction pathways in various growth factors, cytokines and extracellular matrix receptors induced signaling pathways[17,42] (Fig. 3). Under the stimulation of cytokines (IL-3, IL-6, GM-CSF, CagA) and growth factors (PDGF, EGF, FGF), the PTP activity (dephosphorylation) of SHP2 is necessary for the complete activation of RAS/RAF/ERK signaling pathway[17,43]. As a key GTPase, RAS produces inactive RAS-GDP under the control of GTPase-activating proteins (GAP) (NF1, p120RASGAP), and produces active RAS-GTP under the control of guanine nucleotide exchange factors (GEF)[44], which cyclically transmits signals from outside the cell to the nucleus[6]. Obviously, GAP has an inhibitory effect on RAS activation. Located at the downstream of receptor tyrosine kinase (RTK), SHP2 dephosphorylate epidermal growth factor receptors (EGFR, EGFR2) and p120RASGAP to indirectly activate RAS[45,46]. On the other hand, SHP2 could indirectly induce RAS activation by activating Src family kinases. SHP2 dephosphorylates CSK, a negative regulator of Src or paxillin, which leads to the dissociation of CSK and promotes the activation of Src family kinases[47−49]. Moreover, SHP2 can promote dephosphorylation of sprouty, a negative regulator of RAS-MAPK. Dephosphorylated sprouty loss the ability to bind to

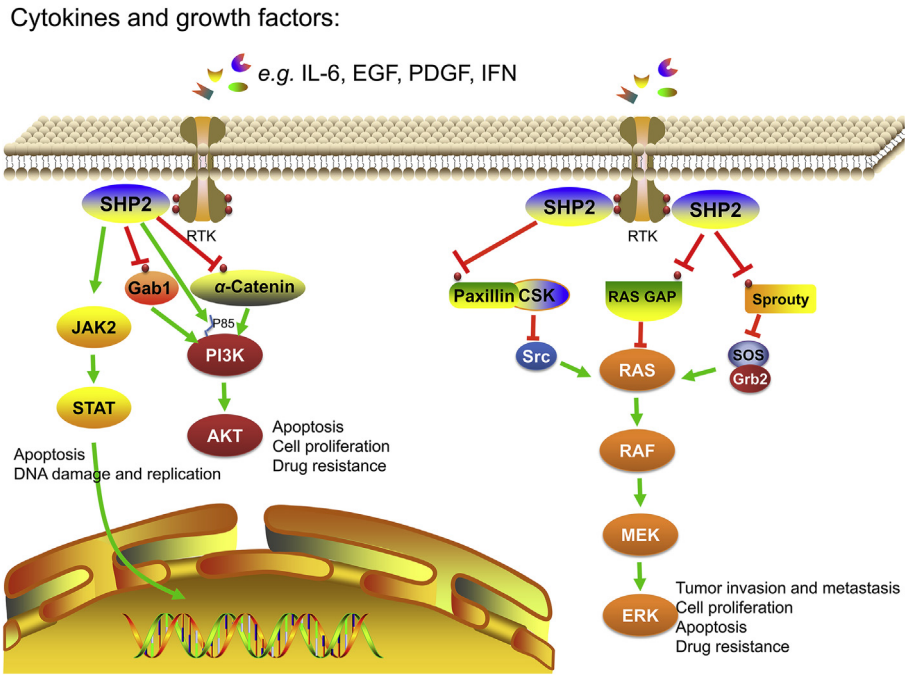

Figure 3 Schematic diagram of SHP2 related cytokines and growth factor dependent RAS-RAF-ERK, PI3K-AKT and JAK-STAT signaling pathways. SHP2 dephosphorylates negative regulators in RAS-RAF-ERK pathway through a variety of mechanisms, such as RAS-GAP, paxillin and sprouty, to regulate signal transduction, tumor invasion, cell proliferation, differentiation, apoptosis and survival; SHP2 inhibits the activation of PI3K-AKT pathway and regulates cell proliferation and apoptosis through dephosphorylation of α-catenin, Gab1 and P85 binding sites; SHP2 dually regulates the JAK-STAT signaling pathway, which is essential for regulating DNA damage, cell growth, differentiation, survival and death.

growth factor receptor-bound protein 2 (GRB2), which promotes the recruitment of GRB2/SOS complexes to fibroblast growth factor receptor substrate (FRS) and finally activate RAS[4,8,12,43,50]. Meanwhile, the dephosphorylation of sprouty-related-1 (Spred1) protein by SHP2 weakens the inhibitory effect of Spred1 protein on RAS-ERK pathway[51,52]. SHP2 not only can activate the RAS-RAF-ERK signaling pathway with PTP catalytic activity (dephosphorylation), but also act as a scaffolding adaptor that connects upstream and downstream signals to activate the RAS-RAF-ERK signaling pathway[32]. When stimulated by cytokines or growth factors, SHP2 recruits and binds GRB2 associated binding protein-1/2 (GAB1/2), GRB2, insulin receptor substrate 1 (IRS1), FRS2 and other proteins, resulting in ERK activation. Therefore, the scaffolding adaptor function of SHP2 is significant for the activation of ERK signaling pathway[53−56]. Based on current studies, SHP2 plays a positive role in RAS-RAF-ERK signaling pathway.

PI3K/AKT signal is an important signal pathway that regulates biological and pathophysiological responses such as cell growth, metabolism and survival[57,58]. SHP2 can dually regulate PI3K-AKT signal with PTP catalytic activity (PTP dependent) or scaffold function (PTP independent)[59,60]. For example, in structurally activated fibroblast growth factor receptor 3 (FGFR3) induced cells, PTP with catalytic activity promoted α-catenin dephosphorylation to activate PI3K/AKT pathway[32,61]; SHP2 selectively dephosphorylates platelet-derived growth factor receptor (PDGFR), shortens the binding time of PI3K and RASGAP with receptors and activates PI3K[62,63]. These indicate a positive regulation dependent on PTP activity. However, several studies have shown that SHP2 inhibits the activation of PI3K pathway induced by EGF through dephosphorylation of Gab1 and P85 binding sites, indicating a negative PTP dependent regulation[61,64,65]. In vascular endothelial growth factor receptor 2 (VEGFR2) mediated ATK signaling, SHP2 forms complexes with Gabs to promote the activation of PI3K pathway, which indicates that SHP2 may play a role as a scaffolding adaptor, leading to PI3K/AKT pathway activation in a manner independent of PTP catalytic activity[66−69].

STAT protein plays an essential role in the physiological functions of cells. The post-translational modification of dephosphorylation involved in SHP2 double-regulates the STAT signaling pathway[70]. SHP2 has a positive function in JAK/STAT pathway. For example, SHP2 indirectly activates STAT5 phosphorylation by activating JAK2–PrlR complexes and promotes STAT5 activation in mice mammary glands. SHP2 deletion can significantly inhibit STAT5 activity[6,71,72]; in SHP2 mutant cells, the JAK2/STAT5 signal stimulated by IL3 was impaired, and wild-type SHP2 could reactivate this signal. In SHP2 inactivated cells, JAK2 activity and STAT5 phosphorylation are decreased[36]; The Tyr1017 phosphorylation site of JAK forms a complex with SOCS, which prevents JAK from binding with STAT, resulting in the inhibition of JAK-STAT signal. SHP2 can dephosphorylate the tyrosine phosphorylation site of JAK and prevent JAK from binding with SOCS, thus reactivating STAT signaling pathway[8,73]. Meanwhile, SHP2 also has a negative regulatory effect on the JAK-STAT signaling pathway. Under IL-3 stimulation, the overexpression of SHP2 increased the dephosphorylation level of STAT5 in BaF3 cells and primary bone marrow hematopoietic progenitor cells, thereby inhibiting STAT5 activity[74−76]; STAT3 is a protein that plays a vital role in embryonic stem cell differentiation and hematopoiesis. SHP2 promotes the dephosphorylation of STAT3, thereby negatively regulating the STAT3 signaling pathway[77]; in fibroblasts, SHP2 can dephosphorylate activated STAT1 and down regulate the activity of JAK1−STAT1 signaling pathway induced by IFN[32,78]. In conclusion, SHP2 directly or

indirectly regulates JAK/STAT mediated signal transduction in a receptor specific or cell specific manners[6,32].

In addition to the mentioned signaling pathways, SHP2 also participates in the regulation of many other signaling pathways through dual regulation. For example, nuclear factor kappa-B (NF-κB), c-Jun N-terminal kinase (JNK), nuclear factor of activated T-cells (NFAT) signal pathways, etc[32,79].

5. Overview of small molecular SHP2 inhibitors

5.1. *Catalytic site inhibitors*

Since the PTP catalytic sites are positive charge in nature, the catalytic site inhibitors usually possess ionizable functional groups to facilitate interaction with the active-site. In this regard, based on the inhibitors structural characteristics can be divided into the following categories (Fig. 4).

Quinoline hydrazine derivatives NSC-87877 (**1**). Compound **1** is a SHP2 inhibitor identified for the first time through screening in 2006. It can effectively inhibit SHP2 (IC_{50} = 0.32 μmol/L) and has higher selectivity for SHP2 than other PTPs (PTP1B, DEP1, HEPTP, LAR, CD45), but no selectivity against SHP1 (IC_{50} = 0.36 μmol/L) *in vitro*. Compound **1** inhibits the PTP domain's catalytic activity in SHP2 and effectively blocks EGF-induced RAS/ERK1/2 activation[80,81].

Phenylhydrazonopyrazolone sulfonate derivatives PHPS1 (**2a**). In 2008, Hellmuth et al.[82] reported a potential phosphotyrosine inhibitor **2a** as a selective SHP2 inhibitor (SHP2 IC_{50} = 2.1 μmol/L) over SHP1 (SHP1 IC_{50} = 30 μmol/L) and PTP1B (PTP1B IC_{50} = 19 μmol/L). Compound **2a** is not toxic to normal epithelial cells and can prevent the anchorage-dependent growth of various tumor cells. Mechanism studies have shown that **2a** binds to the PTP domain of SHP2 and inhibits the SHP2-dependent RAS-MAPK pathway. Specially, the sulfonic acid group is a p-Tyr mimic and extends to the substrate binding pocket. By introducing different substituents in the 2,4-dihydro-3*H*-pyrazol-3-one scaffold, Grosskopf et al.[83] reported **2b** (SHP2 IC_{50} = 0.37 μmol/L), **2c** (SHP2 IC_{50} = 0.15 μmol/L) with improved activity. GS-493 (**2d**) exhibited the good SHP2 inhibitory activity (SHP2 IC_{50} = 0.07 μmol/L) and PTP selective (SHP1 IC_{50} = 2.08 μmol/L; PTP1B IC_{50} = 3.17 μmol/L).

Oxindole derivatives NSC-117199 (**3a**). Lawrence et al.[84] reported a potential selective oxindole SHP2 inhibitor **3a** (SHP2 IC_{50} = 46.8 μmol/L, SHP1 IC_{50} = 68 μmol/L, PTP1B K_i = 96.7 μmol/L) through virtual screening. The structure of **3a** was further optimized to obtain bis-carboxylic acid derivatives **3b** and **3c**. The activity of **3b** (SHP2 IC_{50} = 0.8 μmol/L) is higher than that of **3c** (SHP2 IC_{50} = 15.8 μmol/L), and **3b** also possess 20-fold selectivity against SHP1. These results indicated the importance of the position of the carboxyl group in the hydrazine aromatic ring. Using **3a** as a lead compound, the author identified **3d** with higher activity (SHP2 IC_{50} = 1 μmol/L) and selectivity (SHP1 IC_{50} = 18.3 μmol/L; PTP1B IC_{50} = 14.5 μmol/L). Although the introduction of sulfonamide improves the solubility of **3d**, it contains negatively charged carboxyl groups, resulting in poor membrane permeability and bioavailability.

Salicylic acid derivatives (**4** and **5**). Zhang et al.[85] found that p-Tyr mimics salicylic acid **4** inhibit SHP2 with IC_{50} value of 212 μmol/L. They developed a series of substituted salicylic acid derivatives through the click reaction. Among them, **5a** (SHP2 IC_{50} = 5.5 μmol/L) has moderate potency and moderate selectivity than other PTPs (SHP1 IC_{50} = 15.7 μmol/L; PTP1B IC_{50} = 14.3 μmol/L). In cellular assays, **5a** can block the activation of ERK1/2 stimulated by growth factors and inhibit the hyperproliferation of hematopoietic cells induced by the

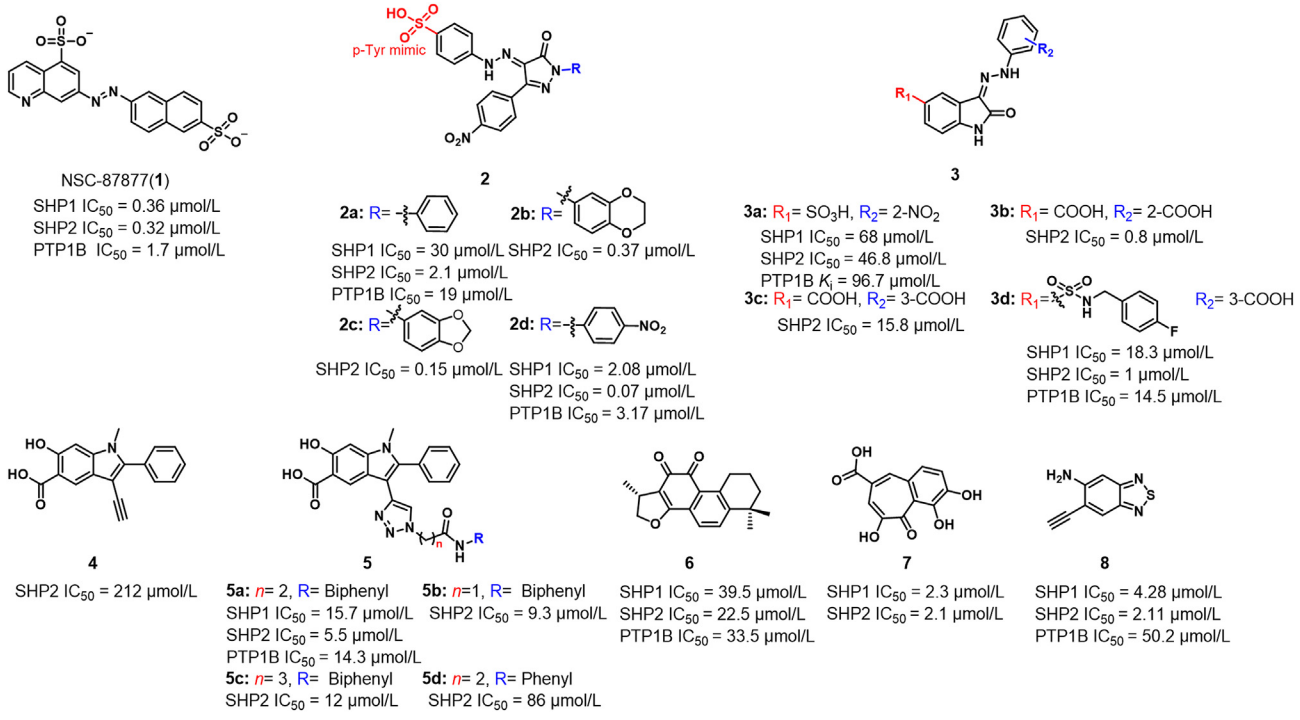

Figure 4 Structures and inhibitory activities of SHP2 catalytic site inhibitors.

granulocyte-macrophage colony-stimulating factor (GM-GSF) through SHP2 gain-of-function mutants[80,86].

Diterpenoid quinone derivatives (**6**). Liu et al.[87] screened a natural product database and identified cryptotanshinone **6**, which possess moderate SHP2 inhibitory activity (SHP2 IC_{50} = 22.5 μmol/L) and low selectivity (SHP1 IC_{50} = 39.5 μmol/L; PTP1B IC_{50} = 33.5 μmol/L).

Other inhibitors (**7** and **8**). Wu et al.[81] identified **7** as dual SHP1/2 inhibitor (SHP1 IC_{50} = 2.3 μmol/L; SHP2 IC_{50} = 2.1 μmol/L). Zhou et al.[88] reported a SHP2 inhibitor **8** (IC_{50} = 2.11 μmol/L) with weak selectivity against SHP1 (IC_{50} = 4.28 μmol/L) and good selectivity against PTP1B (IC_{50} = 50.2 μmol/L).

5.2. *Allosteric inhibitors*

Because of the highly conserved sequence of PTP catalytic domain, developing high selective SHP2 catalytic site inhibitors is still very difficult, which is one of the major challenges in future clinical development. In addition, due to the positive charge environment of PTP catalytic sites, the catalytic site inhibitors are required to possess multiple negative ionizable functional groups. These functional groups usually have low membrane permeability and oral bioavailability, which are factors that hinder the possibility of such inhibitors to become approved drugs[21].

SHP2 allosteric inhibitors are essential components of tumor therapeutic molecules with high therapeutic potential[89,90]. At present, four different allosteric binding sites have been reported in SHP2 protein, including tunnel-like site formed by N-SH2, C-SH2 and PTP domains[91], latch-like and groove-like sites located between the N-SH2 and PTP domains[92], non-conserved cysteine residue 333 (Cys333) site located in the PTP domain (Fig. 5). It should be mentioned that SHP2 allosteric inhibitors targeting the Groove-like site have not yet been reported. According to different binding sites, current SHP2 allosteric inhibitors can be divided into three categories (Fig. 6).

Through high-throughput screening, Novartis discovered a novel allosteric inhibitor SHP836 (**9**) (SHP2 IC_{50} = 12 μmol/L; SHP^{PTP} IC_{50} > 100 μmol/L) based on the aminopyrimidine scaffold. Crystal structure revealed that compound **9** binds to the tunnel-like region formed between the C-SH2, N-SH2 and PTP domains. Structure−activity relationship studies show that the chlorine in the benzene ring is essential for the activity against SHP2[81,93]. In 2016, Novartis[6,91] announced a novel SHP2 allosteric inhibitor SHP099 (**10**) (SHP2 IC_{50} = 0.07 μmol/L; SHP^{PTP} IC_{50} > 100 μmol/L; SHP1 IC_{50} > 100 μmol/L; PTP1B IC_{50} > 100 μmol/L) with *in vivo* activity, highly selectivity, high orally bioavailability, illuminating to the fact that allosteric inhibition can serve as a promising direction for the development of SHP2 inhibitors. Novartis also reported another series of pyrazolopyrimidinone derivatives, of which **11a** is a very effective SHP2 inhibitor (IC_{50} = 6 nmol/L). Unfortunately, the development of **11a** was terminated due to high human ether-a-go-go-related gene (hERG) inhibition (hERG IC_{50} = 4 nmol/L). Finally, through structural optimization, SHP389 (**11b**) with similar potency and acceptable hERG inhibition (IC_{50} = 17,000 nmol/L) was produced[94]. In 2019, Novartis[95] reported the aminopyrimidinone derivative SHP394 (**12**) which showed higher SHP2 activity (IC_{50} = 23 nmol/L), better hERG selectivity (hERG IC_{50} > 30 μmol/L) and pharmacokinetic properties in the Detroit-562 xenograft model, and also resulted in a dose-dependent decrease in tumor growth. The **13** (SHP2 IC_{50} = 3 nmol/L) identified by Novartis[96] showed stronger

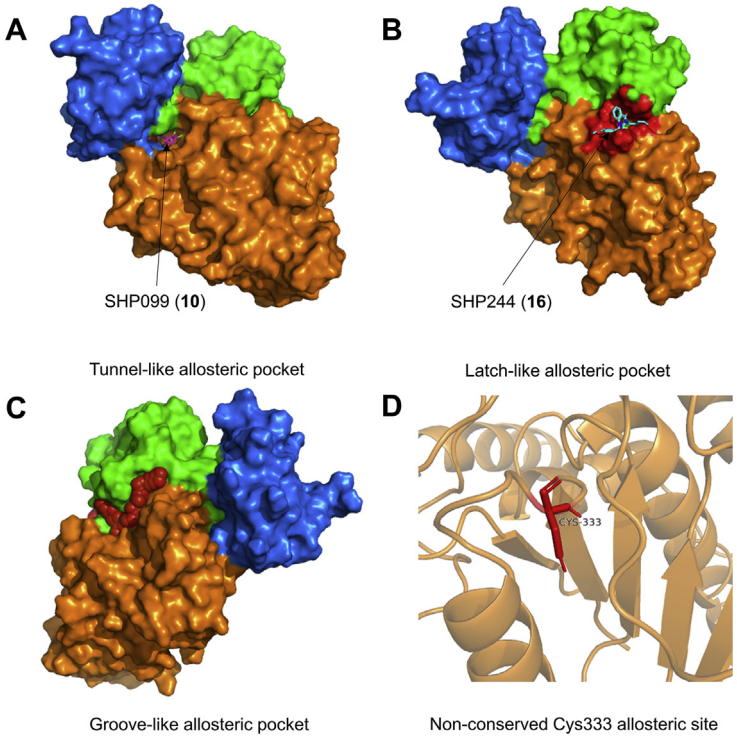

Figure 5 Allosteric pockets of SHP2 self-inhibitory conformation. PTP domain is colored in orange, N-SH2 in green, C-SH2 in marine, allosteric site in red. (A) Tunnel-like allosteric pocket with allosteric inhibitor SHP099 (**10**) (PDB ID: 5HER). (B) Latch-like allosteric pocket with allosteric inhibitor SHP244 (**16**) (PDB ID: 6MBR). (C) Groove-like allosteric pocket (PDB ID: 6MBR). (D) Non-conserved Cys333 allosteric site (PDB ID: 3B7O).

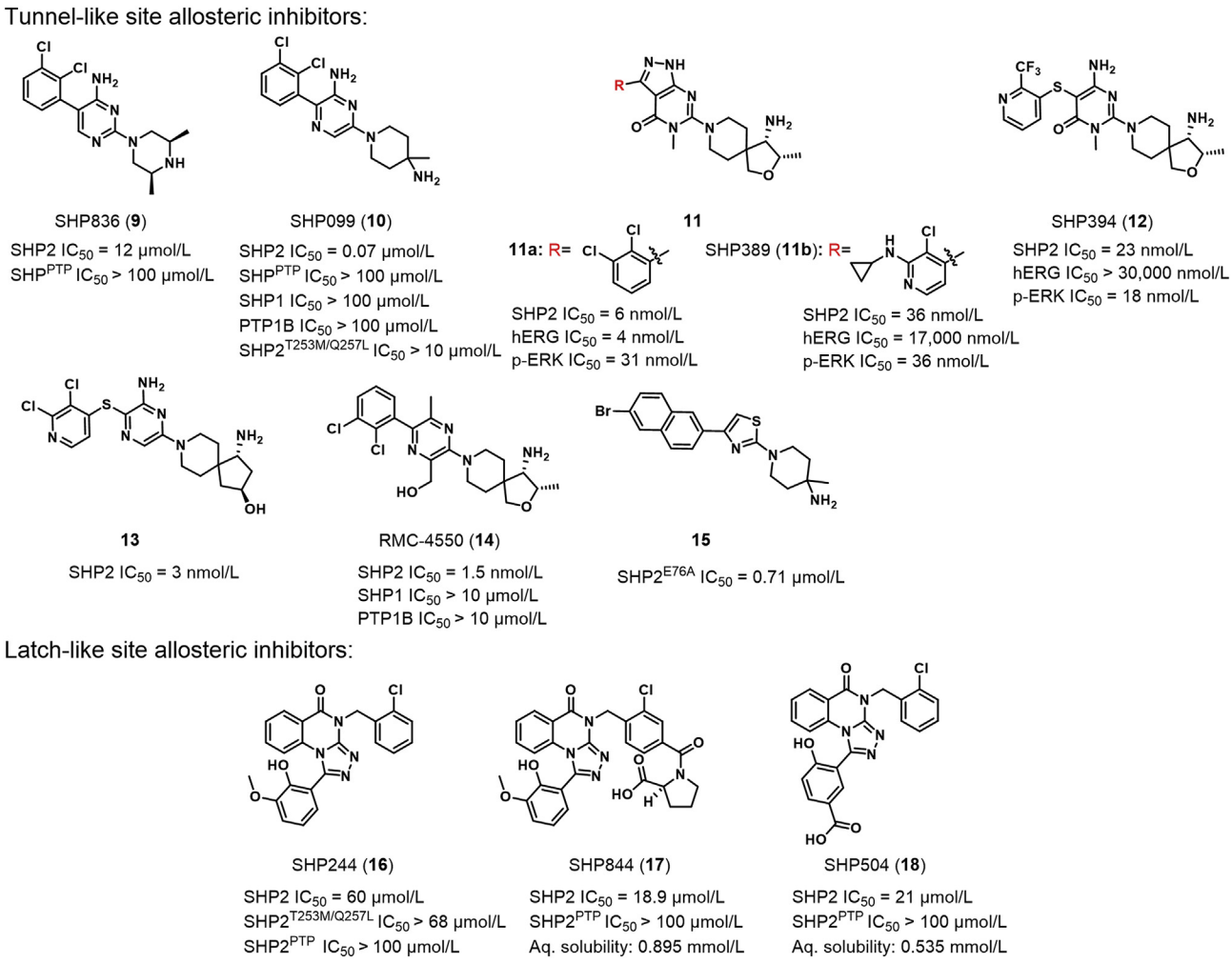

Figure 6 Structures and IC_{50} values of representative SHP2 allosteric inhibitors.

inhibitory activity *in vitro*, inhibited the growth of ALK rearranged non-small cell lung cancer (NSCLC) cells, and inhibited tumor growth of MGH049 and MGH045-2A xenograft models *in vivo*. Nichols et al.[97] reported that RMC-4550 (**14**) is a more potent small molecule SHP2 allosteric inhibitor (SHP2 IC_{50} = 1.5 nmol/L; SHP1 IC_{50} > 10 μmol/L; PTP1B IC_{50} > 10 μmol/L), which stabilizes the self-inhibitory conformation of SHP2, but has no inhibitory activity on the mutant proteins ($SHP2^{E76K}$, $SHP2^{T253M}$, $SHP2^{Q257L}$). Specially, compound **14** can prevent the excessive activation of RAS-ERK signal and inhibit tumor growth by inhibiting the activity of RAS protein.

The interference of the N-SH2/PTP interface leads to the instability of the self-inhibition conformation, which is the primary mechanism of resistance to SHP2 allosteric inhibitors[98]. Moreover, some mutations destroy the integrity of the self-inhibition interaction, and current SHP2 allosteric inhibitors exhibited low activity against specific oncogenic SHP2 mutant proteins, such as $SHP2^{E76A}$, $SHP2^{G60V}$, $SHP2^{S502P}$, etc.[99]. Also, the $SHP2^{E76K/T253M/Q257L}$ and $SHP2^{T253M/Q257L}$ mutations reduce the inhibitory activity of **10** against SHP2[100,101]. These mutations lead to the inherent instability of the self-inhibitory conformation, whereas the binding of **10** and similar type of allosteric inhibitors required a stable self-inhibitory conformation of SHP2[99]. Through structure-based drug design, discovered an effective allosteric inhibitor **15** for the mutant protein $SHP2^{E76A}$ (IC_{50} = 0.71 μmol/L) by targeting the tunnel-like site.

By screening against the $SHP2^{T253M/Q257L}$ double mutant, which is a defective mutant for SHP099 (**10**), Fodor et al.[92] identified a novel triazole-quinazolinone molecule SHP244 (**16**) (SHP2 IC_{50} = 60 μmol/L; $SHP2^{T253M/Q257L}$ IC_{50} = 68 μmol/L; SHP^{PTP} IC_{50} > 100 μmol/L; aqueous solubility = 0.047 mmol/L) targeting the latch-like allosteric pocket (Fig. 6B). In order to further improve the activity and aqueous solubility of **16**, the authors performed structural optimization and obtained new allosteric inhibitors SHP844 (**17**) (SHP2 IC_{50} = 18.9 μmol/L; SHP^{PTP} IC_{50} > 100 μmol/L; aqueous solubility = 0.895 mmol/L) and SHP504 (**18**) (SHP2 IC_{50} = 21 μmol/L; SHP^{PTP} IC_{50} > 100 μmol/L; aqueous solubility = 0.535 mmol/L) (Fig. 6). These inhibitors bound with SHP2 in a similar manner to **16**, and both show higher activity to SHP2 and possess improved aqueous solubility. Through X-ray structure analysis, it can be observed that the tunnel-like allosteric pocket is not interfered by the binding of **17** or **18** to the latch-like allosteric pocket, indicating that both allosteric sites may be double-occupied. Then, the authors determined the crystal structure of SHP2 complexed with both tunnel-like site binder (**10**) and latch-like site binders (**16**−**18**), confirmed the simultaneous binding hypothesis.

Moreover, a dose-dependent decrease in SHP2 activity and a modest enhancement of IC_{50} for **10** was observed with increasing concentrations of **18**, indicating possible cooperativity between the two binding modes[92]. Furthermore, in KYSE-520 cells, **10**, **17** and **18** significantly reduced DUSP6 level (a downstream marker of MAPK pathway)[92]. Meanwhile, the combination treatment of KYSE-520 cells with **18** (30 μmol/L) and **10** (0.2 μmol/L) improved DUSP6 downregulation compared to either of the single agents. The results showed that combining two different but compatible SHP2 inhibitors (dual allosteric inhibitors) improved the inhibition rate of SHP2 and may overcome drug resistance[92].

5.3. *Covalent inhibitor*

The non-conserved Cys333 allosteric site on the PTP catalytic domain can also be used as a target for selective SHP2 inhibition[102]. The covalent binding of Cys333 with **19** could significantly inhibit the activity of wild-type SHP2 (IC_{50} = 35 μmol/L)[102] (Fig. 7). **19** showed weak but still significant time-dependent inhibition of the mutant protein $SHP2^{C333A103}$. Therefore, targeting non-conserved Cys333 with covalent inhibitors may be a meaningful new way for developing more effective allosteric inhibitors to block SHP2 activity or overcome drug resistance[36,102].

6. Combine use of SHP2 inhibitors to overcome drug resistance

The rapid emergence of drug resistance by tyrosine kinase inhibitors largely limits the efficacy of targeted tumor therapy. Studies have demonstrated that patients who are resistant to most kinase inhibitors have identified as point mutations in the kinase domain of the corresponding target kinase. Gene amplification, overexpression and changes in protein expression levels are the other two main mechanisms for oncogenic activation or signal pathway modification to produce drug resistance. In contrast to secondary drug resistance, primary drug resistance can be caused by multiple mechanisms that prevent or reduce kinase inhibitors and their kinase targets in the cytoplasm. The intracellular drug concentration depends on the expression of transporters that mediate the influx of kinase inhibitors into or out of plasma. In tumor cell lines, multidrug resistance is usually associated with decreased accumulation of ATP-dependent cellular drugs, which is attributed to the overexpression of certain ATP-binding cassette (ABC) transporters. In general, the overexpression of the ABC transporter protects tumor cells from kinase inhibitor inhibition, that is, the chem-immune system appears to recognize kinase-targeting drugs as xenobiotics at the membrane and tissue barriers. In the case of active efflux, it protects intracellular

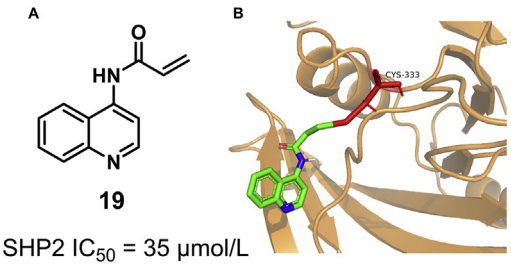

Figure 7 (A) Structure and IC_{50} value of **19**. (B) X-ray crystallography structure of **19** with SHP2 (PDB ID: 3B7O). **19** is shown in green sticks and Cys333 is depicted as red sticks.

targets from kinase inhibitors[103–105]. Overexpression of drug transporters may confer more potential drug resistance. In addition, cancer stem cells (CSCs) are responsible for tumor initiation and possess hyperproliferative potential and are insensitive to periodic chemotherapy drugs, which become a major obstacle to cancer therapy. In the treatment of leukemia, kinase inhibitors can effectively target proliferating mature cells, but failed to eliminate leukemia stem cells. In breast CSCs, cells with high CD44 and low CD24 expression ($CD44^{high}CD24^{low}$ cells) has been shown to be enriched. Knockout of SHP2 reduces the $CD44^{high}CD24^{low}$ cell population in MCF10A-HER2/3 and SUM159 breast cancer cells, indicating SHP2 is a potential biomarker and reasonable therapeutic target for breast cancer stem cells. Also, immune checkpoint inhibitors have been used to treat advanced NSCLC, but about 80% of patients are resistant to immunosuppressants alone[106,107].

The combined therapy of SHP2 inhibitors with existing kinase targeting drugs or immune checkpoint inhibitors to improve efficacy and/or combat drug resistance can be hot area of research.

6.1. *Combination of RTK inhibitors with SHP2 inhibitors*

Several compelling evidences have shown that cancer cells acquire drug resistance through a series of signal pathways activated by RTK. For example, EGFR is a kind of RTK whose gene mutation (especially the secondary mutation T790M) and protein overexpression abnormally activate downstream pathways to induce drug resistance. At the same time, the simultaneous activation of redundant kinases can induce drug resistance by activating by-pass pathways[103]. In this sense, inhibiting RTK activity can play a vital role in tumor treatment[108]. As an important part of RTK signal, SHP2 is the downstream effector of many RTK activation signal cascades. Studies have shown that SHP2 is significantly up-regulated when RTK is activated to acquire adaptive resistance[108].

In v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutant tumor cells, the combination of RTK inhibitor and SHP2 inhibitor **10** can enhance the inhibitory effect on KRAS mutant tumor cells[109]. This indicates that cancer with KRAS mutation depends on the upstream signal of RTK and SHP2 and provides a new direction for RTK inhibitors and SHP2 inhibitors to treat cancer with KRAS mutation[93,109]. Inhibition of SHP2 by **10**, a selective allosteric inhibitor, has revealed the therapeutic prospect of RTK dependent cancers. Experimental evidence suggests that inhibition of SHP2 and RTK is useful in treating various KRAS mutant cancers that depend on upstream growth factor signaling, including $KRAS^{G13D}$ and $KRAS^{Q61H}$ mutations[93,97,109].

Several genetic and biochemical evidences show that SHP2 is an integral part of RTK signals, including FGFR, VEGFR, PDGFR, and EGFR signals, leading to the complete activation of ERK and AKT pathways[47,110]. Sorafenib is a multi-kinase inhibitor, including RTK, which explicitly targets multiple growth factor pathways to block tumor cell proliferation and resist angiogenesis. Under the treatment of sorafenib, hepatoma cells obtain adaptive drug resistance by reactivation of RAS-MEK-ERK and AKT pathways. Combined use of SHP2 inhibitor (**10**) with sorafenib can block the reactivation of MEK/ERK and AKT signaling pathways, thus overcoming the adaptive resistance to sorafenib (Fig. 8). The combination of **10** and sorafenib can maximize tumor growth inhibition and significantly improve the survival rate, which may be a new treatment strategy against hepatocellular carcinoma (HCC)[108].

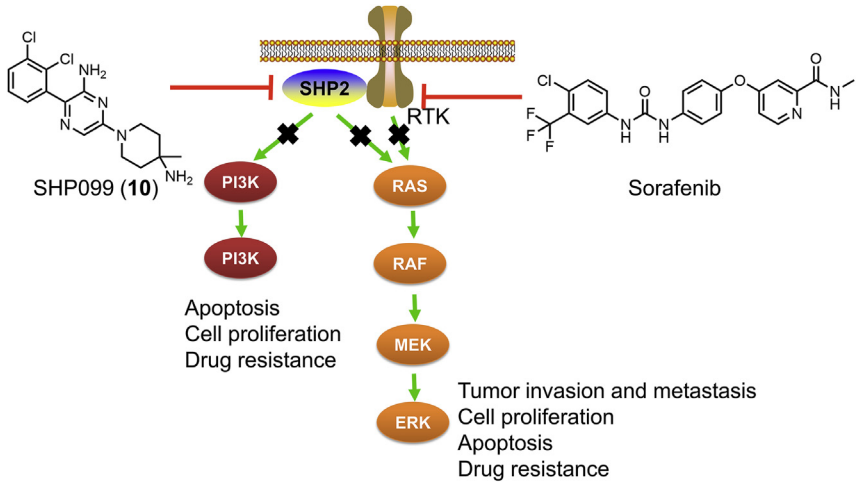

Figure 8 The combination of **10** and sorafenib blocks the reactivation of MEK/ERK and AKT signaling pathways.

6.2. *Combination of MEK inhibitors with SHP2 inhibitors*

RAS protein is a crucial driver of cancer, and RAS gene is easily mutated in human malignant tumors. In tumor cells, KRAS, a member of the Ras family, is the most prone to mutation[111]. MEK inhibitors have been widely used to treat cancers with RAS mutations. However, mutations in MEK lead to excessive activation of MEK or prevent the inhibitor from binding to MEK, resulting in drug resistance. Most cancers that are resistant to MEK inhibitors reactivate multiple RTKs upstream of the MAPK pathway, thereby initiating a signal cascade and eventually leading to excessive cell proliferation[112]. Therefore, the emergence of adaptive drug resistance limited future clinical use of MEK inhibitors[111]. Interestingly, recent study revealed that SHP2 inhibitors can prevent adaptive drug resistance of MEK inhibitors. Therefore, the combination of MEK and SHP2 inhibitors could be a new strategy for treating RAS-driven cancer[90]. In KRAS mutant lung cancer cell lines, SHP2 inhibitors alone have almost no effect on cell proliferation. In contrast, the combination of SHP2 and MEK inhibitors show a strong synergistic anti-proliferation effect[113]. However, inhibition of SHP2 in the KRAS mutant NSCLC *in vivo* can induce a senescence response, which is aggravated by MEK inhibition[113]. The combination of MEK inhibitors and SHP2 inhibitors also overcome the adaptive drug resistance of wild-type RAS tumor cells that are difficult to treat, such as gastric cancer, triple-negative breast cancer (TNBC) and high-grade serous ovarian cancer[111,114]. In some tumors, the deletion or inhibition of SHP2 can delay tumor growth, but the effects are not enough to achieve tumor regression[115]. The combination of low-dose SHP2 inhibitor RMC-4630 and MEK inhibitor cobimetinib showed synergistic effects in xenograft model of $KRAS^{G12C}$ NCI-H358 cells and prevented tumor growth[6]. In KE39 and CAT12 tumor cells, the combination of SHP2 inhibitor **10** and MEK inhibitor trametinib (GSK1120212) can significantly inhibit tumor growth and induce regression[114]. When MEK inhibitor selumetinib (AZD6244) is used in combined therapy with SHP2 inhibitors, pancreatic cancer cell lines and colon cancer cell lines show higher sensitivity to selumetinib[113] (Fig. 9). In general, the combined inhibition of SHP2 and MEK activity has strong synergistic effects in KRAS mutant tumors, especially in NSCLC[113–115].

6.3. *Combination of ERK signal suppression and SHP2 inhibitors*

ERK activation is one of the main signals of SHP2 functional gain mutation[116]. In a variety of ERK dependent tumor environments, the remission of negative feedback of RAF or MEK inhibitors promotes the upregulation of various RTK, while in turn RTK activates RAS, which leads to the rebound of ERK activity and tumor adaptive resistance to inhibitors[117]. Simultaneously manipulating ERK signaling and SHP2 activity can effectively overcome the adaptive resistance of specific ERK-dependent tumors to RAF and MEK inhibitors. In order to evaluate the efficacy of combined inhibition of ERK signal and SHP2 *in vivo*, the combination of dabrafenib, Trametinib and SHP2 allosteric inhibitor **10** significantly suppressed p(Y542) SHP2 and ERK signals in mice carrying RKO xenografts while showed no significant effect on body weight. It is more effective than the combination of dabrafenib and trametinib to suppress ERK signal (Fig. 10).

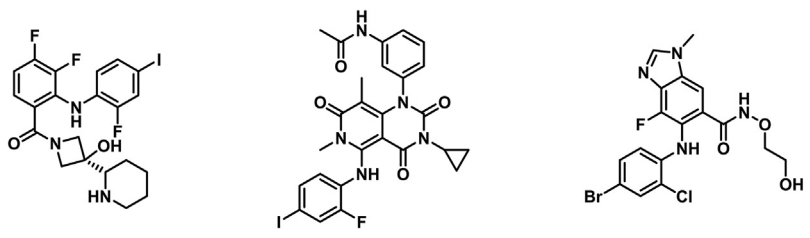

Figure 9 Structures of MEK inhibitors used in combination with SHP2 inhibitors.

However, the use of dabrafenib or trametinib or **10** alone had little effect on the tumor growth or ERK signal transduction of xenografts. It is further proved that ERK signal and SHP2 suppression may be effective methods for treating BRAF (V600E) colorectal tumors. The current findings establish a combination of ERK signal transduction and SHP2 inhibition, which can effectively overcome the adaptive resistance of ERK-dependent tumors to RAF and MEK inhibitors[117].

6.4. *Combination of ALK inhibitors with **10***

Under normal circumstances, anaplastic lymphoma kinase (ALK) activates cell growth after ligand binding. However, when EML4 on DNA is fused with ALK, the ALK kinase region is abnormally activated, which has carcinogenic activity. ALK inhibitors initially restrain most NSCLC with ALK rearrangement, but SHP2 provides a parallel survival input downstream of multiple tyrosine kinases that promote resistance to ALK inhibitors. Recently, it has been found that SHP2 inhibitor **10** has little effect on cell proliferation of several tumor cells, however, when used in combination with ALK tyrosine kinase inhibitor ceritinib, it can prohibit the growth of drug resistant patient-derived cells by preventing the reactivation of RAS and ERK1/2 (Fig. 11). These findings suggest that the combined inhibition of ALK and SHP2 might provide a promising strategy for drug-resistant cancer therapy. Moreover, short-term or long-term use of **10** alone doesn't reduce the activity of RAS in any patient-derived tumor cells, while short-term combined use of ceritinib and **10** could reduce the level of GTP-RAS in all models[118].

Similarly, treatment of ceritinib in MGH049-1A and MGH073-2B xenografts produced mild and transient responses, while MGH045-2A xenografts were completely resistant. However, the combination of SHP2 inhibitor **10** and ceritinib resulted in a deep regression of MGH049-1A and MGH073-2B xenografts and moderately inhibited the growth of MGH045-2A tumors, consistent with the significantly reduced DUSP6 mRNA level. In the MGH073-2B and MGH049-1A models, when the combination of **10** and ceritinib stopped, the tumor cells grow again, and these tumor cells are still sensitive when the drugs are used once more. The combination of **10** and ceritinib show mild toxicity at the initial stage and are alleviated during treatment. In conclusion, inhibition of ALK and SHP2 activity may provide a broad therapeutic strategy for overcoming the ALK-independent mechanism of acquired drug resistance in NSCLC patients[118].

6.5. *Combination of PD-1 blockade with SHP2 inhibitors*

Two groups of costimulatory receptors are expressed on the surface of T cells: costimulatory receptors and co-inhibitory receptors. PD-1 is a co-suppressor receptor expressed on T cells[119]. It is highly expressed in tumor-infiltrating lymphocytes (TIL) and inhibits T cell activation. PD-1 deficient mice showed immunoglobulin production disorder under the background of C57BL/6, and autoimmune cardiomyopathy occurred under the background of BALB/c. This might provide a solid evidence of the vital role of PD-1 as a negative regulator of T cell activation[120,121]. PD-1 is a crucial immune checkpoint in cancer immunotherapy[122]. When PD-1 binds to programmed death ligand-1 (PD-L1), T cell receptor (TCR) targeting gene and Th1 cytokines significantly inhibited and transmit inhibitory signals[16]. Inhibition of the interaction between PD-1 and PD-L1 can enhance T cell response and mediate preclinical antitumor activity[123]. Although anti-PD-1/PDL-1 treatment has achieved great success, many patients with solid tumors still exhibited primary and acquired drug resistance. Under treatment of PD-1/PDL-1, tumors can form tumor microenvironment (TME) to block the anti-tumor effect of T cells. This may be due to insufficient antigen immunogenicity, disfunction of antigen presentation, irreversible T cell exhaustion, resistance to IFN-γ signaling and immunosuppression. Some patients will eventually develop resistance or relapse after PD-1/PDL-1 treatment. In the presence of PD-1/PDL-1 inhibitors, through tumor immune editing, tumor cells that escape anti-tumor immunity gradually dominate. In addition, activation of PD-1/PDL-1 independent inhibitory pathways and re-depletion of activated T cells can once again cause the loss of T cell function[124].

Hui et al.[119] demonstrated that the co-receptor CD28 is more suitable as the target of SHP2 phosphatase dephosphorylation recruited by PD-1 in relative to TCR. Dephosphorylated and

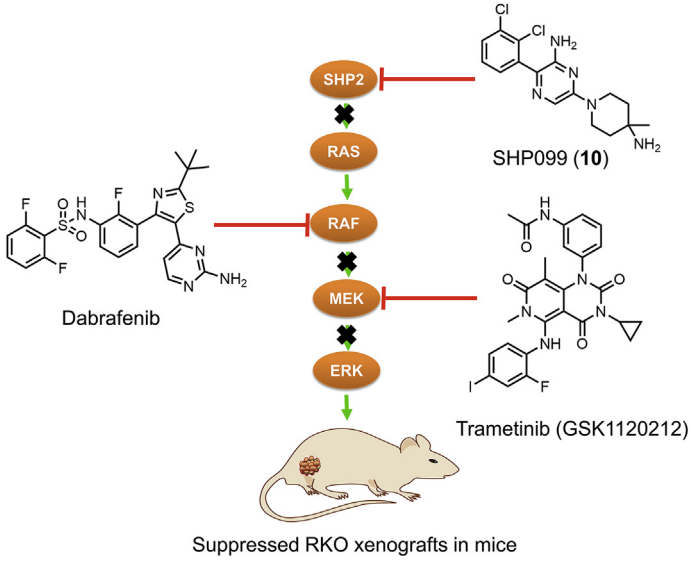

Figure 10 The combination of ERK signal suppression and SHP2 inhibitor overcomes the adaptive resistance of tumors to inhibitors.

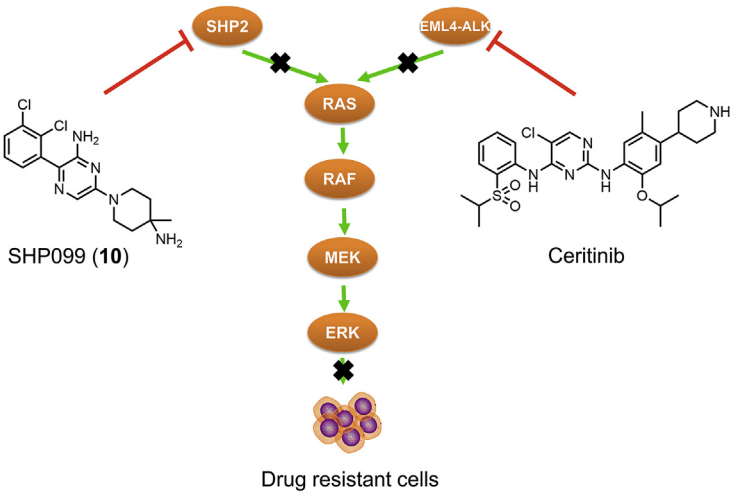

Figure 11 The combination of ALK inhibitor and SHP2 inhibitor prevents the growth of resistant cells.

inactivated CD28 inhibits T cell function by restraining the activation of the PI3K-AKT signaling pathway and reducing the activation of transcription factors, indicating that SHP2 mediates PD-1 inhibition of T cell function by inactivating CD28 signal[15,125] (Fig. 12). Therefore, SHP2 is considered to be the key mediator of PD-1 signal inhibition[16,126,127]. Although SHP2 is commonly expressed in T cells, the level of SHP-2 in TIL is significantly higher compared with peripheral blood lymphocytes (PBL). The expression level of SHP2 in TIL of head and neck squamous cell carcinoma (HNSCC) patients was positively correlated with the expression of PD-1[16]. Blockade of PD-1 or SHP2 is sufficient to restore strong Th1 immunity and T cell activation, thus reversing immunosuppression in tumor microenvironment[16]. SHP2 can bind to a variety of immunosuppressive receptors to inhibit the activation of immune cells, which explains the powerful tumor-killing effect of SHP2 inhibitors. PD-1 antagonists, as immunotherapeutic agents, are being actively explored in clinical trials and have shown clinical efficacy in several solid tumors. Sun et al.[128] reported that the combination of 10 and anti-PD-1 antibody showed a higher efficacy than monotherapy in inhibiting tumor growth. Therefore, the development of a specific SHP2 inhibitor combined with PD-1 antagonists will be a promising strategy for tumor immunotherapy in the future[16].

6.6. *Combination of other inhibitors with SHP2 inhibitors*

Preclinical studies have shown that, compared with a single drug, combination therapy is more effective, overcomes drug resistance[6], and solves the over activation of signaling pathway caused by a single drug. The combination of SHP2 inhibitors and other inhibitors has attracted more attention. For example, in the treatment of liver cancer, multiple myeloma and chondrosarcoma, the use of SHP2 inhibitors can activate an essential cancer-promoting factor STAT3, thus SHP2 inhibitors should be used carefully in these tumors. It also suggests that close attention should be paid to the phosphorylation level of STAT3 when using SHP2 inhibitors to treat related tumors. The combination of SHP2 inhibitors and STAT3 inhibitors may be a new treatment strategy[15]. Besides, SHP2 inhibitor TNO-155 is combined with PD-1 antibody spartalizumab or CDK inhibitor ribociclib in the treatment of advanced solid tumors and combined with $KRAS^{G12C}$ inhibitor MRTX849 in the treatment of $KRAS^{G12C}$ solid tumors. TNO-155 can also be combined with BRAF inhibitor dabrafenib, ERK inhibitor LTT462, MEK inhibitor trametinib and RAF inhibitor LXH254 in the treatment of advanced/metastatic *BRAF* V600 colorectal cancer[6] (Fig. 13).

7. Future clinical development of SHP2 inhibitor

At present, there are five SHP2 allosteric inhibitors in clinical research, as summarized in Table 1[6,80]. In January 2018, JAB-3068, a small molecule oral anticancer drug independently designed and developed by Jacobio with global intellectual property rights, was officially approved by FDA to enter clinical trials, mainly for the treatment of adult advanced solid tumors. JAB-3068 is currently in phase II clinical research stage. Studies have shown that JAB-3068 alone can promote the antitumor function of $CD8^+$ T cells and can also be combined with PD1/PD-L1 antibody. Therefore, JAB-3068 can be used in the treatment of non-responsive tumors with PD-1/PD-L1 antibody. In August 2019, JAB-3312, the second original antineoplastic drug

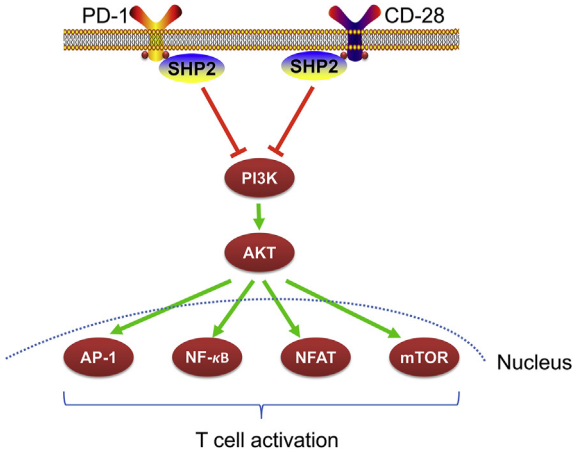

Figure 12 SHP2-mediated direct inhibition of the downstream PI3K-AKT signaling pathway to prevent T cell activation.

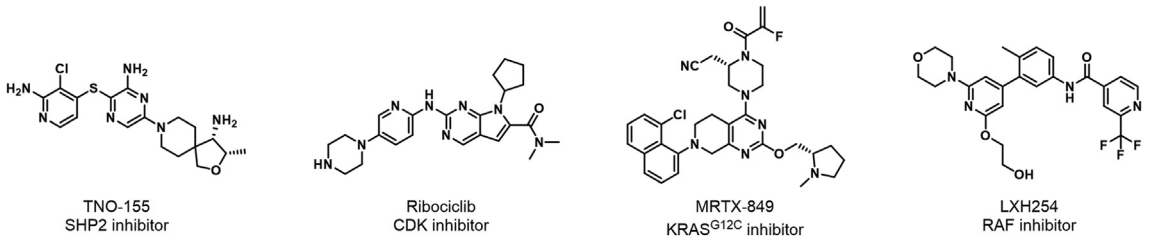

Figure 13 Structures of SHP2 inhibitor and other inhibitors.

Table 1 Clinical trials of SHP2 allosteric inhibitors[6,80].

Drug	Company	Phase	Indication	NCT identifier
JAB-3068	Jacobio	Phase I/II	Advanced solid tumors	NCT03518554 NCT03565003
JAB-3312	Jacobio	Phase I/II	Advanced solid tumors	NCT04121286 NCT04045496
TNO-155	Novartis	Phase I/Ib	Advanced solid tumors	NCT03114319 NCT04000529
RLY-1971	Relay Therapeutics	Phase I	Advanced or metastatic solid tumors	NCT03989115
RMC-4630	Revolution Medicines	Phase I/II	Relapsed or refractory solid tumors	NCT03634982 NCT03989115

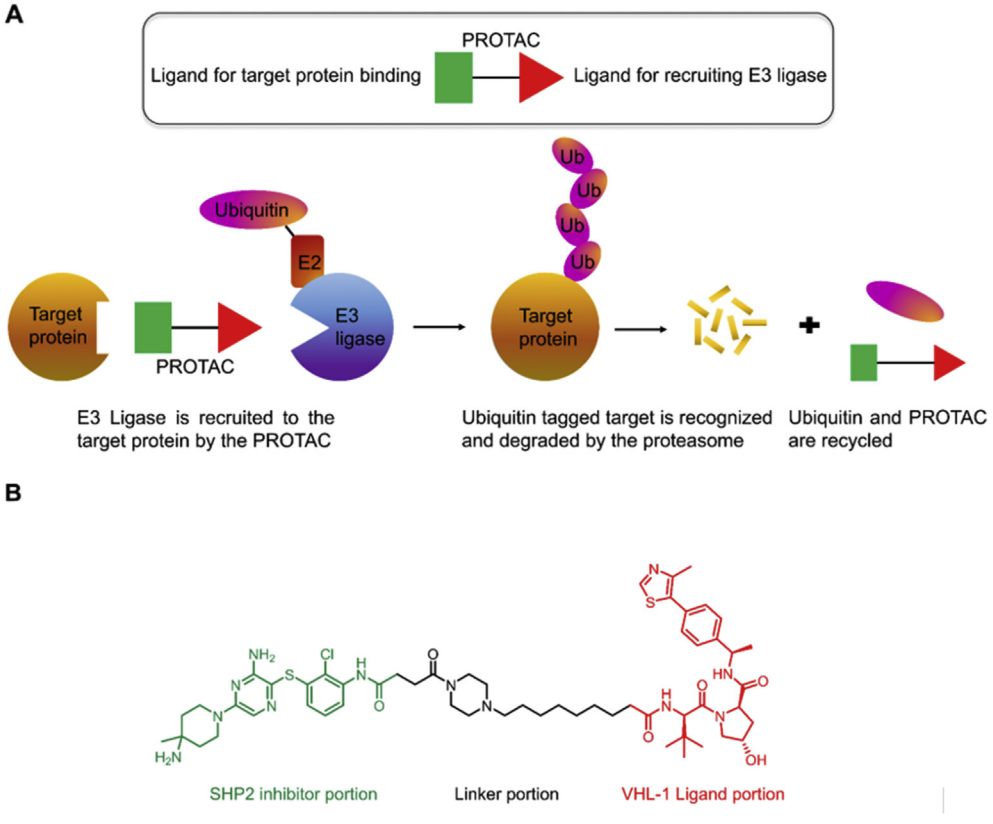

Figure 14 (A) The mechanism of PROTAC. (B) Structure of SHP2 degrader SHP2-D26.

independently developed by Jacobio, launched phase I clinical trials in HealthONE Clinic Oncology Center, USA. JAB-3312 can block the PD-1 pathway of T cells and the KRAS pathway of tumor cells by inhibiting SHP2, thereby having dual roles of tumor immunity and tumor targeting. JAB-3312 is used to treat solid tumors such as NSCLC, colorectal cancer, and pancreatic cancer. It can also relieve the tumor immunosuppressive microenvironment and enhance the efficacy of existing tumor

immunotherapy. Novartis' TNO-155 is a practical, selective, and orally active SHP2 allosteric inhibitor (IC_{50} = 0.011 μmol/L). TNO-155 has potential in treating RTK-dependent malignant tumors, especially advanced solid tumors, and is currently in phase I clinical trials. RLY-1971 developed by Relay Therapeutics is used for the treatment of solid tumors. A phase I dose escalation study is being conducted in patients with advanced or metastatic solid tumors to evaluate the safety and tolerability of RLY-1971. Revolution Medicines' RMC-4630 is a practical, sensitive, oral SHP2 allosteric inhibitor, which can inhibit SHP2-dependent RAS signal mutations (such as $KRAS^{G12C}$, NF1, BRAF, KRAS amplification, etc.). The results of the phase I clinical study clarified that RMC-4630 showed reasonable tolerability and preliminary clinical activity in KRAS mutant NSCLC patients, especially $KRAS^{G12C}$ mutant patients. Also, the tolerability of intermittent administration was improved compared with daily administration. For patients with other mutations in the RAS pathway and patients with disease progression after receiving $KRAS^{G12C}$ inhibitors, a study of the use of RMC-4630 in combination with the MEK inhibitor cobimetinib is ongoing (NCT03989115).

Preclinical studies have demonstrated that combination therapy is more effective than monotherapy and is an effective way to overcome resistance to a single drug[6]. SHP2 inhibitors combined with other kinase inhibitors are more effective than single therapy and are less likely to develop drug resistance. At the same time, development of multi-target inhibitors is also worth studying in the future. In addition, immunochemotherapy can not only inhibit the proliferation of tumor cells, but also activate the immune response of T cells to tumor cells, which is an important research direction. The current research data provides a strong theoretical basis for the clinical combination strategy of SHP2 inhibitors and drugs that directly target the immune system[129]. Fodor et al.[92] reported a rare case of dual, simultaneously occupying both tunnel-like and latch-like allosteric pockets of SHP2 protein. Studies have shown that dual inhibition prevents the emergence of resistance to each drug in preclinical animal models[130]. Therefore, exploring dual allosteric inhibitors may help improve the inhibitory activity of SHP2 and overcome drug resistance caused by mutations in SHP2. The discovery of irreversible inhibitors for PTP non-conservative Cys333 also provides a new direction for the development of SHP2 allosteric inhibitors. The inhibitors screened based on Cys333 may be a new tool for PTP targeted drug discovery[102]. Several *in vivo* and *in vitro* studies on different target proteins have shown that the combination of allosteric and orthomorphic inhibitors can keep the protein conformation stable, thus delaying the emergence of drug-resistant mutations of target proteins[98]. This suggests that the combined use of SHP2 PTP catalytic site inhibitors and allosteric inhibitors may be a helpful direction for clinical development. Future research should focus on deciphering the new molecular mechanism targeting SHP2 and accelerating the development of selective SHP2 inhibitors[32].

In the past few years, the proteolysis-targeting chimeras (PROTACs) technology has become another hot spot in drug discovery. This strategy recruits the target protein to E3 ligase system and induces the degradation of targeted protein through ubiquitin proteasome system[36,131] (Fig. 14A). PROTACs strategy to achieve endogenous degradation of target proteins has been increasingly reported[132]. Most recently, the PROTACs technology has been successfully applied to the design of small molecule SHP2 degraders, opening a new field for targeting SHP2 degradation into medicines[6,133]. Small molecular degrader SHP2-D26 was synthesized by linking the known SHP2 inhibitor and VHL-1 ligand and exhibited low DC_{50} values in KYSE520 and MV4; 11 cells (Fig. 14B). Importantly, SHP2-D26 is significantly better than classical SHP2 allosteric inhibitors in inhibiting ERK phosphorylation as well as proliferation in KYSE520 and MV4; 11 cells. Thus, this study proved for the first time that targeted degradation of SHP2 is a very effective strategy to inhibit SHP2 activity[133].

8. Conclusions and outlook

In the past two decades, we have gained a great understanding of the molecular structure, functional characteristics, and signal regulation of SHP2. SHP2 gene abnormalities, including mutations (GOF and LOF) and abnormal expression (upregulated and down-regulated), are closely related to leukemia and solid tumors[36,40]. The function of SHP2 protein is different in distinct environments. For example, overexpression of SHP2 is associated with breast cancer, gastric cancer and lung cancer. Knockout or inhibition of SHP2 can significantly prevent the growth of cancer cells and exert anticancer activity. However, SHP2 may has an inhibitory effect in other cancers such as liver cancer and osteosarcoma[36]. With the progress in studying the mechanism of action, SHP2 become an important biomarker and a promising therapeutic target for tumor therapy. It is of great significance to clarify these problems for understanding the relationship between SHP2 function and related diseases[48].

Due to the high conservation and positive charged PTP catalytic domain, the development of catalytic site SHP2 inhibitors meets lot of difficulties such as low selectivity and cell permeability, which severely limits the clinical use of SHP2 catalytic site inhibitors[81]. This once put the research and development of SHP2 inhibitors in a dilemma. Until 2016, Novartis developed a novel allosteric inhibitor **10**, which can stabilize SHP2 in the self-inhibitory conformation by acting as a "molecular glue". Hereafter, the development of new SHP2 allosteric inhibitors has aroused great enthusiasm. With the discovery of several other allosteric pockets, a number of new allosteric inhibitors have been reported. These SHP2 allosteric inhibitors exhibited high selectivity, water solubility, cell permeability, oral availability, and *in vivo* activity. Currently, the clinical trials of five SHP2 allosteric inhibitors in various solid tumors are also in progress, which further promote an in-depth study of SHP2 in tumor diseases.

The development of SHP2 inhibitors have been systematically reviewed[6,36,80,81] elsewhere. In this review, we focused on the recent development of combined therapy using SHP2 inhibitors, which showed a significant advantage than single-drug therapy and have the potential to overcome drug resistance. Drug resistance is one of the major challenges in current cancer treatment. The mechanism of drug resistance determines the proliferation and metastasis of tumors and ultimately leads to the death of patients. Therefore, developing new strategies to overcome drug resistance is an important direction for future cancer treatment. Recent studies have already revealed that the signal pathways involved in the regulation of SHP2 play an important role in the drug resistance mechanism of kinase inhibitors. Therefore, the development of efficient, highly selective SHP2 inhibitor and the combination of SHP2 inhibitor with kinase inhibitors will become the main direction of SHP2 inhibitor research. Meanwhile, the development of immunochemotherapy, dual target inhibitors, dual allosteric inhibitors, covalent inhibitors, and the combined use of

allosteric inhibitors and orthosteric inhibitors, PROTACs technology to degrade mutant proteins provide new ideas for overcoming drug resistance using SHP2 inhibitor. We hope this review would be helpful for the future development of SHP2 inhibitors.

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Author contributions

Prof. Hao Fang and Prof. Xuben Hou provided the writing ideas and guided the revision of manuscript content. Meng Liu summarized the literature and wrote the manuscript. Shan Gao provided ideas for figures and revised the manuscript. Reham M. Elhassan edited the language of the manuscript. All authors gave approved to submit the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Jiang ZX, Zhang ZY. Targeting PTPs with small molecule inhibitors in cancer treatment. *Cancer Metastasis Rev* 2008;**27**:263−72.
2. Cohen P. The regulation of protein function by multisite phosphorylation-a 25 year update. *Trends Biochem Sci* 2000;**25**: 596−601.
3. Frankson R, Yu ZH, Bai YP, Li QL, Zhang RY, Zhang ZY. Therapeutic targeting of oncogenic tyrosine phosphatases. *Cancer Res* 2017;**77**:5701−5.
4. Butterworth S, Overduin M, Barr AJ. Targeting protein tyrosine phosphatase SHP2 for therapeutic intervention. *Future Med Chem* 2014;**6**:1423−37.
5. Drake JM, Lee JK, Witte ON. Clinical targeting of mutated and wild-type protein tyrosine kinases in cancer. *Mol Cell Biol* 2014;**34**: 1722−32.
6. Yuan XR, Bu H, Zhou JP, Yang CY, Zhang HB. Recent advances of SHP2 inhibitors in cancer therapy: current development and clinical application. *J Med Chem* 2020;**63**:11368−96.
7. Gutch MJ, Flint AJ, Keller J, Tonks NK, Hengartner MO. The caenorhabditis elegans SH2 domain-containing protein tyrosine phosphatase PTP-2 participates in signal transduction during oogenesis and vulval development. *Genes Dev* 1998;**12**:571−85.
8. Somani RR, Madan DP, Rai PR. Protein tyrosine phosphatase SHP-2 as drug target. *Mini-Reviews Org Chem* 2016;**13**:410−20.
9. Krause DS, Etten RAV. Tyrosine kinases as targets for cancer therapy. *N Engl J Med* 2005;**353**:172−87.
10. Julien SG, Dube N, Hardy S, Tremblay ML. Inside the human cancer tyrosine phosphatome. *Nat Rev Cancer* 2011;**11**:35−49.
11. Song YH, Zhao M, Wu YH, Yu B, Liu HM. A multifunctional cross-validation high-throughput screening protocol enabling the discovery of new SHP2 inhibitors. *Acta Pharm Sin B* 2020;**11**:750−62.
12. Chan G, Kalaitzidis D, Neel BG. The tyrosine phosphatase Shp2 (PTPN11) in cancer. *Cancer Metastasis Rev* 2008;**27**:179−92.
13. Zhou X, Coad J, Ducatman B, Agazie YM. SHP2 is up-regulated in breast cancer cells and in infiltrating ductal carcinoma of the breast, implying its involvement in breast oncogenesis. *Histopathology* 2008;**53**:389−402.
14. Zhang J, Zhang F, Niu R. Functions of Shp2 in cancer. *J Cell Mol Med* 2015;**19**:2075−83.
15. Liu QQ, Qu J, Zhao MX, Xu Q, Sun Y. Targeting SHP2 as a promising strategy for cancer immunotherapy. *Pharmacol Res* 2020; **152**:104595.
16. Li J, Jie HB, Lei Y, Gildener-Leapman N, Trivedi S, Green T, et al. PD-1/SHP-2 inhibits Tc1/Th1 phenotypic responses and the activation of T cells in the tumor microenvironment. *Cancer Res* 2015;**75**: 508−18.
17. Neel BG, Gu H, Pao L. The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. *Trends Biochem Sci* 2003;**28**: 284−93.
18. Feng GS, Hui CC, Pawson T. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. *Science* 1993; **259**:1607−11.
19. Hof P, Pluskey S, Dhe-Paganon S, Eck MJ, Shoelson SE. Crystal structure of the tyrosine phosphatase SHP-2. *Cell* 1998;**92**:441−50.
20. Ostman A, Hellberg C, Bohmer FD. Protein-tyrosine phosphatases and cancer. *Nat Rev Cancer* 2006;**6**:307−20.
21. Scott LM, Lawrence HR, Sebti SM, Lawrence NJ, Wu J. Targeting protein tyrosine phosphatases for anticancer drug discovery. *Curr Pharmaceut Des* 2010;**16**:1843−62.
22. Zhang B, Lu W. Src homology 2 domain-containing phosphotyrosine phosphatase 2 (Shp2) controls surface GluA1 protein in synaptic homeostasis. *J Biol Chem* 2017;**292**:15481−8.
23. Miura K, Wakayama Y, Tanino M, Orba Y, Sawa H, Hatakeyama M, et al. Involvement of EphA2-mediated tyrosine phosphorylation of Shp2 in Shp2-regulated activation of extracellular signal-regulated kinase. *Oncogene* 2013;**32**:5292−301.
24. Mohi MG, Neel BG. The role of Shp2 (PTPN11) in cancer. *Curr Opin Genet Dev* 2007;**17**:23−30.
25. Xie J, Si X, Gu S, Wang M, Shen J, Li H, et al. Allosteric inhibitors of SHP2 with therapeutic potential for cancer treatment. *J Med Chem* 2017;**60**:10205−19.
26. Oishi K, Zhang H, Gault WJ, Wang CJ, Tan CC, Kim IK, et al. Phosphatase-defective leopard syndrome mutations in PTPN11 gene have gain-of-function effects during drosophila development. *Hum Mol Genet* 2009;**18**:193−201.
27. Wang RR, Liu WS, Zhou L, Ma Y, Wang RL. Probing the acting mode and advantages of RMC-4550 as an src-homology 2 domain-containing protein tyrosine phosphatase (SHP2) inhibitor at molecular level through molecular docking and molecular dynamics. *J Biomol Struct Dyn* 2020;**38**:1525−38.
28. Zheng H, Alter S, Qu CK. SHP-2 tyrosine phosphatase in human diseases. *Int J Clin Exp Med* 2009;**2**:17−25.
29. Saxton TM, Henkemeyer M, Gasca S, Shen R, Rossi DJ, Shalaby F, et al. Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2. *EMBO J* 1997;**16**:2352−64.
30. Wu D, Pang Y, Ke Y, Yu J, He Z, Tautz L, et al. A conserved mechanism for control of human and mouse embryonic stem cell pluripotency and differentiation by shp2 tyrosine phosphatase. *PLoS One* 2009;**4**:e4914.
31. Yang W, Klaman LD, Chen B, Araki T, Harada H, Thomas SM, et al. An Shp2/SFK/Ras/Erk signaling pathway controls trophoblast stem cell survival. *Dev Cell* 2006;**10**:317−27.
32. Huang WQ, Lin Q, Zhuang X, Cai LL, Ruan RS, Lu ZX, et al. Structure, function, and pathogenesis of SHP2 in developmental disorders and tumorigenesis. *Curr Cancer Drug Targets* 2014;**14**: 567−88.
33. Bentires-Alj M, Paez JG, David FS, Keilhack H, Halmos B, Naoki K, et al. Activating mutations of the noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. *Cancer Res* 2004;**64**:8816−20.
34. Araki T, Mohi MG, Ismat FA, Bronson RT, Williams IR, Kutok JL, et al. Mouse model of noonan syndrome reveals cell type- and gene dosage-dependent effects of PTPN11 mutation. *Nat Med* 2004;**10**: 849−57.

35. Keilhack H, David FS, McGregor M, Cantley LC, Neel BG. Diverse biochemical properties of Shp2 mutants. Implications for disease phenotypes. *J Biol Chem* 2005;**280**:30984−93.
36. Shen D, Chen W, Zhu J, Wu G, Shen R, Xi M, et al. Therapeutic potential of targeting SHP2 in human developmental disorders and cancers. *Eur J Med Chem* 2020;**190**:112117.
37. Choong K, Freedman MH, Chitayat D, Kelly EN, Taylor G, Zipursky A. Juvenile myelomonocytic leukemia and noonan syndrome. *J Pediat Hematol Onc* 1999;**21**:523−7.
38. Passmore SJ, Chessells JM, Kempski H, Hann LM, Brownbill PA, Stiller CA. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia in the UK: a population-based study of incidence and survival. *Br J Haematol* 2003;**121**:758−67.
39. Tartaglia M, Gelb BD. Germ-line and somatic PTPN11 mutations in human disease. *Eur J Med Genet* 2005;**48**:81−96.
40. Rehman AU, Rahman MU, Khan MT, Saud S, Liu H, Song D, et al. The landscape of protein tyrosine phosphatase (Shp2) and cancer. *Curr Pharmaceut Des* 2018;**24**:3767−77.
41. LaRochelle JR, Fodor M, Xu X, Durzynska I, Fan L, Stams T, et al. Structural and functional consequences of three cancer-associated mutations of the oncogenic phosphatase SHP2. *Biochem* 2016;**55**:2269−77.
42. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. *Biochim Biophys Acta* 2010;**1802**:396−405.
43. Matozaki T, Murata Y, Saito Y, Okazawa H, Ohnishi H. Protein tyrosine phosphatase SHP-2: a proto-oncogene product that promotes Ras activation. *Cancer Sci* 2009;**100**:1786−93.
44. Karachaliou N, Cardona AF, Bracht JWP, Aldeguer E, Drozdowskyj A, Fernandez-Bruno M, et al. Integrin-linked kinase (ILK) and src homology 2 domain-containing phosphatase 2 (SHP2): novel targets in EGFR-mutation positive non-small cell lung cancer (NSCLC). *EBioMedicine* 2019;**39**:207−14.
45. Montagner A, Yart A, Dance M, Perret B, Salles JP, Raynal P. A novel role for Gab1 and SHP2 in epidermal growth factor-induced Ras activation. *J Biol Chem* 2005;**280**:5350−60.
46. Zhou X, Agazie YM. Molecular mechanism for SHP2 in promoting HER2-induced signaling and transformation. *J Biol Chem* 2009;**284**:12226−34.
47. Zhang SQ, Yang WT, Kontaridis MI, Bivona TG, Wen GY, Araki T, et al. Shp2 regulates Src family kinase activity and Ras/Erk activation by controlling csk recruitment. *Mol Cell* 2004;**13**:341−55.
48. Li SM. The biological function of SHP2 in human disease. *Mol Biol* 2016;**50**:27−33.
49. Ren Y, Meng SS, Mei L, Zhao ZJ, Jove R, Wu J. Roles of Gab1 and SHP2 in paxillin tyrosine dephosphorylation and Src activation in response to epidermal growth factor. *J Biol Chem* 2004;**279**:8497−505.
50. Hanafusa H, Torii S, Yasunaga T, Matsumoto K, Nishida E. Shp2, an SH2-containing protein-tyrosine phosphatase, positively regulates receptor tyrosine kinase signaling by dephosphorylating and inactivating the inhibitor sprouty. *J Biol Chem* 2004;**279**:22992−5.
51. Nonami A, Kato R, Taniguchi K, Yoshiga D, Taketomi T, Fukuyama S, et al. Spred-1 negatively regulates interleukin-3-mediated Erk/mitogen-activated protein (MAP) kinase activation in hematopoietic cells. *J Biol Chem* 2004;**279**:52543−51.
52. Wakioka T, Sasaki A, Kato R, Shouda T, Matsumoto A, Miyoshi K, et al. Spred is a sprouty-related suppressor of Ras signalling. *Nature* 2001;**412**:647−51.
53. Ning Z, Wang A, Liang JX, Liu JW, Zhou T, Yan Q, et al. Abnormal expression of Nek2 in pancreatic ductal adenocarcinoma: a novel marker for prognosis. *Int J Clin Exp Pathol* 2014;**7**:2462−9.
54. Cunnick JM, Mei L, Doupnik CA, Wu J. Phosphotyrosines 627 and 659 of Gab1 constitute a bisphosphoryl tyrosine-based activation motif (BTAM) conferring binding and activation of SHP2. *J Biol Chem* 2001;**276**:24380−7.
55. Kuhne MR, Pawsontgll T, Lienhardll GE, Feng GS. The insulin receptor substrate 1 associates with the SH2-containing phosphotyrosine phosphatase Syp. *J Biol Chem* 1993;**268**:11479−81.
56. Cai T, Nishida K, Hirano T, Khavari PA. Gab1 and SHP-2 promote Ras/MAPK regulation of epidermal growth and differentiation. *J Cell Biol* 2002;**159**:103−12.
57. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009;**9**:550−62.
58. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2010;**141**:1117−34.
59. Kwon M, Ling Y, Maile LA, Badley-Clark J, Clemmons DR. Recruitment of the tyrosine phosphatase Src homology 2 domain tyrosine phosphatase-2 to the P85 subunit of phosphatidylinositol-3 (PI-3) kinase is required for insulin-like growth factor-I-dependent PI-3 kinase activation in smooth muscle cells. *Endocrinology* 2006;**147**:1458−65.
60. Zito CI, Kontaridis MI, Fornaro M, Feng GS, Bennett AM. SHP-2 regulates the phosphatidylinositide 3′-kinase/Akt pathway and suppresses caspase 3-mediated apoptosis. *J Cell Physiol* 2004;**199**:227−36.
61. Burks J, Agazie YM. Modulation of alpha-catenin tyr phosphorylation by SHP2 positively effects cell transformation induced by the constitutively active FGFR3. *Oncogene* 2006;**25**:7166−79.
62. Li W, Nishimura R, Kashishian A, Batzer AG, Kim WJ, Cooper JA, et al. A new function for a phosphotyrosine phosphatase: linking Grb2-Sos to a receptor tyrosine kinase. *Mol Cell Biol* 1994;**14**:509−17.
63. Mattoon DR, Lamothe B, Lax I, Schlessinger J. The docking protein Gab1 is the primary mediator of EGF-stimulated activation of the PI-3K/Akt cell survival pathway. *BMC Biol* 2004;**2**:669−72.
64. Breitkopf SB, Yang X, Begley MJ, Kulkarni M, Chiu YH, Turke AB, et al. A cross-species study of PI3K protein–protein interactions reveals the direct interaction of P85 and SHP2. *Sci Rep* 2016;**6**:20471.
65. Agazie YM, Movilla N, Ischenko I, Hayman MJ. The phosphotyrosine phosphatase SHP2 is a critical mediator of transformation induced by the oncogenic fibroblast growth factor receptor 3. *Oncogene* 2003;**22**:6909−18.
66. Tan WH, Popel AS, Gabhann FM. Computational model of Gab1/2-dependent VEGFR2 pathway to Akt activation. *PLoS One* 2013;**8**:e67438.
67. Gu HH, Neel BG. The 'Gab' in signal transduction. *Trends Cell Biol* 2003;**13**:122−30.
68. Laramee M, Chabot C, Cloutier M, Stenne R, Holgado-Madruga M, Wong AJ, et al. The scaffolding adapter Gab1 mediates vascular endothelial growth factor signaling and is required for endothelial cell migration and capillary formation. *J Biol Chem* 2007;**282**:7758−69.
69. Stewart RA, Sanda T, Widlund HR, Zhu S, Swanson KD, Hurley AD, et al. Phosphatase-dependent and independent functions of Shp2 in neural crest cells underlie leopard syndrome pathogenesis. *Dev Cell* 2010;**18**:750−62.
70. Kim M, Morales LD, Jang IS, Cho YY, Kim DJ. Protein tyrosine phosphatases as potential regulators of STAT3 signaling. *Int J Mol Sci* 2018;**19**:2708.
71. Ali S, Nouhi Z, Chughtai N, Ali S. SHP-2 regulates SOCS-1-mediated Janus kinase-2 ubiquitination/degradation downstream of the prolactin receptor. *J Biol Chem* 2003;**278**:52021−31.
72. Ke Y, Lesperance J, Zhang EE, Bard-Chapeau EA, Oshima RG, Muller WJ, et al. Conditional deletion of Shp2 in the mammary gland leads to impaired lobulo-alveolar outgrowth and attenuated STAT5 activation. *J Biol Chem* 2006;**281**:34374−80.
73. Levy DE, Darnell Jr JE. Stats: transcriptional control and biological impact. *Nat Rev Mol Cell Biol* 2002;**3**:651−62.
74. Shuai K, Liu B. Regulation of JAK-STAT signalling in the immune system. *Nat Rev Immunol* 2003;**3**:900−11.
75. Chen J, Yu WM, Bunting KD, Qu CK. A negative role of SHP-2 tyrosine phosphatase in growth factor-dependent hematopoietic cell survival. *Oncogene* 2004;**23**:3659−69.
76. Xu D, Qu CK. Protein tyrosine phosphatases in the JAK/STAT pathway. *Front Biol* 2008;**13**:4925−32.

77. Chan RJ, Johnson SA, Li Y, Yoder MC, Feng GS. A definitive role of Shp-2 tyrosine phosphatase in mediating embryonic stem cell differentiation and hematopoiesis. *Blood* 2003;**102**:2074−80.
78. Wu TR, Hong YK, Wang XD, Ling MY, Dragoi AM, Chung AS, et al. SHP-2 is a dual-specificity phosphatase involved in stat1 dephosphorylation at both tyrosine and serine residues in nuclei. *J Biol Chem* 2002;**277**:47572−80.
79. Fukunaga K, Noguchi T, Takeda H, Matozaki T, Hayashi Y, Itoh H, et al. Requirement for protein-tyrosine phosphatase SHP-2 in insulin-induced activation of c-Jun NH_2-terminal kinase. *J Biol Chem* 2000;**275**:5208−13.
80. Tang K, Jia YN, Yu B, Liu HM. Medicinal chemistry strategies for the development of protein tyrosine phosphatase SHP2 inhibitors and PROTAC degraders. *Eur J Med Chem* 2020;**204**:112657.
81. Song ZD, Wang MJ, Ge Y, Chen XP, Xu ZY, Sun Y, et al. Tyrosine phosphatase SHP2 inhibitors in tumor-targeted therapies. *Acta Pharm Sin B* 2020;**11**:13−29.
82. Hellmuth K, Grosskopf S, Lum CT, Wurtele M, Roder N, Kries JP, et al. Specific inhibitors of the protein tyrosine phosphatase Shp2 identified by high-throughput docking. *Proc Natl Acad Sci U S A* 2008;**105**:7275−80.
83. Grosskopf S, Eckert C, Arkona C, Radetzki S, Bohm K, Heinemann U, et al. Selective inhibitors of the protein tyrosine phosphatase SHP2 block cellular motility and growth of cancer cells *in vitro* and *in vivo*. *ChemMedChem* 2015;**10**:815−26.
84. Lawrence HR, Pireddu R, Chen LW, Luo YT, Sung SS, Szymanski AM, et al. Inhibitors of Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2) based on oxindole scaffolds. *J Med Chem* 2008;**51**:4948−56.
85. Sarmiento M, Wu L, Keng YF, Song L, Luo Z, Huang Z, et al. Structure-based discovery of small molecule inhibitors targeted to protein tyrosine phosphatase 1B. *J Med Chem* 2000;**43**:146−55.
86. Zhang X, He Y, Liu S, Yu Z, Jiang ZX, Yang Z, et al. Salicylic acid based small molecule inhibitor for the oncogenic Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2). *J Med Chem* 2010;**53**:2482−93.
87. Liu W, Yu B, Xu G, Xu WR, Loh ML, Tang LD, et al. Identification of cryptotanshinone as an inhibitor of oncogenic protein tyrosine phosphatase SHP2 (PTPN11). *J Med Chem* 2013;**56**:7212−21.
88. Wang WL, Chen XY, Gao Y, Gao LX, Sheng L, Zhu J, et al. Benzo[*c*][1,2,5]thiadiazole derivatives: a new class of potent Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2) inhibitors. *Bioorg Med Chem Lett* 2017;**27**:5154−7.
89. Chen X, Zou F, Hu Z, Du G, Yu P, Wang W, et al. PCC0208023, a potent SHP2 allosteric inhibitor, imparts an antitumor effect against KRAS mutant colorectal cancer. *Toxicol Appl Pharmacol* 2020;**398**:115019.
90. Fedele C, Ran H, Diskin B, Wei W, Jen J, Geer MJ, et al. SHP2 inhibition prevents adaptive resistance to MEK inhibitors in multiple cancer models. *Cancer Discov* 2018;**8**:1237−49.
91. Fortanet JG, Chen CH, Chen YN, Chen Z, Deng Z, Firestone B, et al. Allosteric inhibition of SHP2: identification of a potent, selective, and orally efficacious phosphatase inhibitor. *J Med Chem* 2016;**59**:7773−82.
92. Fodor M, Price E, Wang P, Lu H, Argintaru A, Chen Z, et al. Dual allosteric inhibition of SHP2 phosphatase. *ACS Chem Biol* 2018;**13**:647−56.
93. Chen YN, LaMarche MJ, Chan HM, Fekkes P, Garcia-Fortanet J, Acker MG, et al. Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. *Nature* 2016;**535**:148−52.
94. Bagdanoff JT, Chen Z, Dore M, Fortanet JG, Kato M, Lamarche MJ, et al., Novartis AG, assignee. Compounds and compositions for inhibiting the activity of SHP2. 2016 Dec 22. WO Patent WO2016203404 A1.
95. Sarver P, Acker M, Bagdanoff JT, Chen Z, Chen YN, Chan H, et al. 6-Amino-3-methylpyrimidinones as potent, selective, and orally efficacious SHP2 inhibitors. *J Med Chem* 2019;**62**:1793−802.
96. Hao HX, Li F, Lamarche MJ, Wang HQ, Dardal AL, Engelman JA, inventors, Novartis AG, assignee. Pharmaceutical combination comprising an ALK inhibitor and a SHP2 inhibitor. 19 Jul, 2018. WO Patent WO2018130928 A1.
97. Nichols RJ, Haderk F, Stahlhut C, Schulze CJ, Hemmati G, Wildes D, et al. RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. *Nat Cell Biol* 2018;**20**:1064−73.
98. Lu SY, Qiu YR, Ni D, He XH, Pu J, Zhang J. Emergence of allosteric drug-resistance mutations: new challenges for allosteric drug discovery. *Drug Discov Today* 2020;**25**:177−84.
99. Romero C, Lambert LJ, Sheffler DJ, De Backer LJS, Raveendra-Panickar D, Celeridad M, et al. A cellular target engagement assay for the characterization of SHP2 (PTPN11) phosphatase inhibitors. *J Biol Chem* 2020;**295**:2601−13.
100. LaRochelle JR, Fodor M, Vemulapalli V, Mohseni M, Wang P, Stams T, et al. Structural reorganization of SHP2 by oncogenic mutations and implications for oncoprotein resistance to allosteric inhibition. *Nat Commun* 2018;**9**:179−92.
101. Padua RAP, Sun Y, Marko I, Pitsawong W, Stiller JB, Otten R, et al. Mechanism of activating mutations and allosteric drug inhibition of the phosphatase SHP2. *Nat Commun* 2018;**9**:4507.
102. Marsh-Armstrong B, Fajnzylber JM, Korntner S, Plaman BA, Bishop AC. The allosteric site on SHP2's protein tyrosine phosphatase domain is targetable with druglike small molecules. *ACS Omega* 2018;**3**:15763−70.
103. Huang L, Fu L. Mechanisms of resistance to EGFR tyrosine kinase inhibitors. *Acta Pharm Sin B* 2015;**5**:390−401.
104. Chen YF, Fu LW. Mechanisms of acquired resistance to tyrosine kinase inhibitors. *Acta Pharm Sin B* 2011;**1**:197−207.
105. Lamontanara AJ, Gencer EB, Kuzyk O, Hantschel O. Mechanisms of resistance to BCR-ABL and other kinase inhibitors. *Biochim Biophys Acta* 2013;**1834**:1449−59.
106. Aceto N, Sausgruber N, Brinkhaus H, Gaidatzis D, Martiny-Baron G, Mazzarol G, et al. Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells *via* activation of key transcription factors and a positive feedback signaling loop. *Nat Med* 2012;**18**:529−37.
107. Gu W, Prasadam I, Yu M, Zhang F, Ling P, Xiao Y, et al. Gamma tocotrienol targets tyrosine phosphatase SHP2 in mammospheres resulting in cell death through RAS/ERK pathway. *BMC Cancer* 2015;**15**:609.
108. Leung CON, Tong M, Chung KPS, Zhou L, Che N, Tang KH, et al. Overriding adaptive resistance to sorafenib through combination therapy with Src homology 2 domain-containing phosphatase 2 blockade in hepatocellular carcinoma. *Hepatology* 2020;**72**:155−68.
109. Hao HX, Wang H, Liu C, Kovats S, Velazquez R, Lu H, et al. Tumor intrinsic efficacy by SHP2 and RTK inhibitors in KRAS-mutant cancers. *Mol Cancer Therapeut* 2019;**18**:2368−80.
110. Yang XM, Tang CL, Luo H, Wang HJ, Zhou XD. Shp2 confers cisplatin resistance in small cell lung cancer *via* an AKT-mediated increase in CA916798. *Oncotarget* 2017;**8**:23664−74.
111. Torres-Ayuso P, Brognard J. Shipping out MEK inhibitor resistance with SHP2 inhibitors. *Cancer Discov* 2018;**8**:1210−2.
112. Kun E, Tsang YTM, Ng CW, Gershenson DM, Wong KK. MEK inhibitor resistance mechanisms and recent developments in combination trials. *Cancer Treat Rev* 2021;**92**:102137.
113. Mainardi S, Mulero-Sanchez A, Prahallad A, Germano G, Bosma A, Krimpenfort P, et al. SHP2 is required for growth of KRAS-mutant non-small-cell lung cancer *in vivo*. *Nat Med* 2018;**24**:961−7.
114. Wong GS, Zhou J, Liu JB, Wu Z, Xu X, Li T, et al. Targeting wild-type KRAS-amplified gastroesophageal cancer through combined MEK and SHP2 inhibition. *Nat Med* 2018;**24**:968−77.

115. Ruess DA, Heynen GJ, Ciecielski KJ, Ai J, Berninger A, Kabacaoglu D, et al. Mutant KRAS-driven cancers depend on PTPN11/SHP2 phosphatase. *Nat Med* 2018;**24**:954−60.
116. Tien SC, Chang ZF. Oncogenic Shp2 disturbs microtubule regulation to cause HDAC6-dependent ERK hyperactivation. *Oncogene* 2014; **33**:2938−46.
117. Ahmed TA, Adamopoulos C, Karoulia Z, Wu X, Sachidanandam R, Aaronson SA, et al. SHP2 drives adaptive resistance to ERK signaling inhibition in molecularly defined subsets of ERK-dependent tumors. *Cell Rep* 2019;**26**:65−78.e5.
118. Dardaei L, Wang HQ, Singh M, Fordjour P, Shaw KX, Yoda S, et al. SHP2 inhibition restores sensitivity in ALK-rearranged non-small-cell lung cancer resistant to ALK inhibitors. *Nat Med* 2018;**24**: 512−7.
119. Hui E, Cheung J, Zhu J, Su XL, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Immunotherapy* 2017;**355**:1428−33.
120. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001;**291**:319−22.
121. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J Immunol* 2004; **173**:945−54.
122. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;**12**:252−64.
123. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;**366**:2443−54.
124. Lei QY, Wang D, Sun K, Wang LP, Zhang Y. Resistance mechanisms of anti-PD1/PDL1 therapy in solid tumors. *Front Cell Dev Biol* 2020; **8**:672.
125. Hoff H, Brunner-Weinzierl MC. The tyrosine phosphatase SHP-2 regulates differentiation and apoptosis of individual primary T lymphocytes. *Eur J Immunol* 2007;**37**:1072−86.
126. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J Exp Med* 2012;**209**:1201−17.
127. Chen DW, Barsoumian HB, Yang LP, Younes AI, Verma V, Hu Y, et al. SHP-2 and PD-L1 inhibition combined with radiotherapy enhances systemic antitumor effects in an anti-PD-1-resistant model of non-small cell lung cancer. *Cancer Immunol Res* 2020;**8**:883−94.
128. Zhao MX, Guo WJ, Wu YY, Yang CX, Zhong L, Deng GL, et al. SHP2 inhibition triggers anti-tumor immunity and synergizes with PD-1 blockade. *Acta Pharm Sin B* 2019;**9**:304−15.
129. Quintana E, Schulze CJ, Myers DR, Choy TJ, Mordec K, Wildes D, et al. Allosteric inhibition of SHP2 stimulates antitumor immunity by transforming the immunosuppressive environment. *Cancer Res* 2020; **80**:2889−902.
130. Wylie A, Schoepfer J, Berellini G, Cai H, Caravatti G, Cotesta S, et al. ABL001, a potent allosteric inhibitor of BCR-ABL, prevents emergence of resistant disease when administered in combination with nilotinib in an *in vivo* murine model of chronic myeloid leukemia. *Blood* 2014;**124**:398.98.
131. Schapira M, Calabrese MF, Bullock AN, Crews CM. Targeted protein degradation: expanding the toolbox. *Nat Rev Drug Discov* 2019; **18**:949−63.
132. Sun YH, Ding N, Song YQ, Yang ZM, Liu WL, Zhu J, et al. Degradation of bruton's tyrosine kinase mutants by PROTACs for potential treatment of ibrutinib-resistant non-hodgkin lymphomas. *Leukemia* 2019;**33**:2105−10.
133. Wang ML, Lu JF, Wang M, Yang CY, Wang SM. Discovery of SHP2-D26 as a first, potent, and effective PROTAC degrader of SHP2 protein. *J Med Chem* 2020;**63**:7510−28.