SHP2 inhibitors:

Recent advancements and potential breakthroughs in cancer therapy

By

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A thesis submitted to School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy

Brac University

March 2022

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
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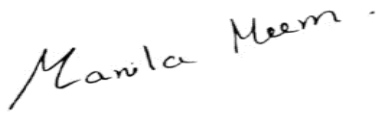

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Approval

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Ethics Statement

This study does not involve any human or animal trial.

Abstract

SHP2 (Src homology-2 domain-containing protein tyrosinephosphatase-2) works as a tyrosine phosphatase, to eliminate tyrosine phosphorylation in the non-receptor protein to link many oncogenic signaling pathways including RAS/RAF/MAPK and PI3K/AKT. Mutations and overexpression in SHP2 cause cancer and related abnormalities in the body including leukemia or solid tumors. Hence, SHP2 has piqued the interest of researchers as a target for inhibition. Several therapeutic compounds are undergoing clinical trials where SHP2 undergoes conformational modifications by binding either inside or outside the catalytic pocket of PTP. These compounds impact different types of cancer with varying efficiencies indicating their excellent chemotherapeutic potential. Therefore, this review has explored the function and structure of SHP2, its relationship with cancer, and strategies to target its catalytic pocket and allosteric regions as an effective cancer treatment option. Additionally, an insight into these compounds' prospects has portrayed their advancements and limitations in cancer treatment through modulation of SHP2.

Keywords: Cancer; SHP2 inhibitors; Targeted therapy; Catalytic inhibitor; Allosteric inhibitor.

Dedication

To cancer patients.

Acknowledgement

To start with, I would like to thank Almighty Allah, The most merciful and benefactor, for granting me life with infinite blessings. All praises to him for giving me patience and strength to continue my journey to complete this project.

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Last but not least, I want to express my gratitude to my parents and sisters for being my shelter. I cannot be more grateful for the unconditional love and courage they have given to me. Without them, I would not be able to walk this far.

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List of Acronyms

SHP2	Src homology-2 domain-containing protein phosphatases 2
PTK	Protein tyrosine kinases
PTP	Protein tyrosine phosphatase
NS	Noonan syndrome
LS	LEOPARD syndrome
JMML	Juvenile myelomonocytic leukemia
AML	Acute myelogenous leukemia
EMT	Epithelial-mesenchymal transition
TNBC	Triple-negative breast cancer
IL-6	Interleukin-6
PD-1	Programmed cell death-1
TAM	Tumor associated micro phase
RAS	Rat sarcoma
KRAS	Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
YAP	Yes-associated protein
MEK	Mitogen-activated protein kinase.
ERK	Extracellular signal-regulated kinase
JAK	Janus kinases

STAT	Signal transducer and activator of transcription
PI3K	Phosphatidylinositol 3-kinase
AKT	Ak strain transforming
MAPK	Mitogen-activated protein kinase
EGFR	Epidermal growth factor receptor
HNSCC	Head and neck squamous cell carcinomas
ITIM	Immune receptor tyrosine-based inhibitory motif
Th1	T helper cell 1
SCONP	Structural classification of natural products
BIOS	Biology oriented synthesis
PROTAC	Proteolysis Targeting Chimera
NSCLC	Non-small cell lung cancer
Dox	Doxycycline
MET	Mesenchymal Epithelial Transition
HER2	Human epidermal growth factor receptor 2
ER	Estrogen receptor
PDAC	Pancreatic ductal adenocarcinoma
HCC	Hepatocellular cancer

Chapter 1: Introduction

1.1 Cancer

Cancer emerges when a series of genes get altered that cause the activities of the cells to fluctuate to forge the cells to evolve uncontrollably. It can develop anyplace in the cells that work to make up the human body. Usually, human cells divide and proliferate to generate new cells as commanded by the body. Damaged cells are eliminated, and new cells substitute them for continuing the mechanisms of the particular zone of the body. This well-regulated process can sometimes make errors, leading to irregular or corrupted cells emerging and dividing when they shouldn't. It is widely assumed to be a multi-gene, as well as a multi-step condition that commences with a single defective cell with a clonal origin mutated DNA sequence. The mildly aberrant stage is triggered by the uncontrolled growth of these abnormal cells. Furthermore, it is proceeded by a second mutation. The emergence of tumor tissue results from repetitive cycles of mutation and proliferation of these cells (Hassanpour & Dehghani, 2017). Cancer has harmed eukaryotic living beings for hundreds of millions of years, and evidence of cancer in the forebears of modern humans' dates back well over thousands of years. Cancer is not produced by an organism that is foreign to our bodies, unlike bacterial infections, insects, and many environmental maladies. Human cells that have failed to maintain their reins and been recruited to some extent have altered into some pathological creatures or a fundamental component of cancer (Hausman, 2019). Cancer is generally characterized by impaired gene function and modified gene expression patterns. Several studies show that acquired epigenetic defects contribute to this dysregulation combined with gene mutations (Jones & Baylin, 2007).

1.2 Mutation and the development of cancer

There are many reasons for which mutation can take place in the body. For instance, environmental chemical compounds with cancerous qualities affect the cells in gene mutations. Additional carcinogens count viruses, bacteria, and UV rays. These account for almost 7% of all cancers. Furthermore, cancer destroys intercellular connections initiating all the essential genes. This interruption influences cycle, culminating in uncontrolled accretion. Under usual episodes, proto-oncogenes are necessary for multiplication and proliferation. Yet they mutate into oncogenes being the most detrimental for cell endurance. Additionally, the scarcity of tumor suppressor genes causes deregulated cell division. Chromosomal translocation, then point mutation, deletion, amplification, and insertion activation are examples of genetic events. An interchange of gene information across chromosomes 9 and 22 produces severe blood cancer. This initiates the synthesis of ph1 that can serve in the diagnosis of a biomarker (Hassanpour & Dehghani, 2017). According to studies, P53 is the most frequently detected mutated gene researched on human cancer. In general, p53 mutations (a missense mutation) are found in more than half of all cancers (Yue et al., 2017). The first epigenetic abnormality identified in human cancers was DNA hypo methylation. The new high-resolution genome-wide investigations demonstrate that DNA hypo methylation is almost always found with hyper methylation of the specific genome in cancer. Hypo methylation of some promoters is capable of inducing the production of oncogenes. For instance, in prostate and breast cancer, this circumstance happens for MASPIN (a tumor suppressor gene) (Ehrlich, 2009; Hassanpour & Dehghani, 2017).

1.3 Current scenario

Cancer is one of the significant public health issues that affect people worldwide, and it is the leading source of mortality in the US. In 2020, estimated new cases for cancer were 1,806,590, where 893,660 were male, and 912,930 were female based on gender and overall sites. Around

606,520 people died, where 321,160 were male, and 285,360 were female. If specific to sites for oral cavity & pharynx, 53,260 cases were recorded in the US where 38,380 were male, and 14,880 were female. The death number for oral cancer was around 20% as per the diagnosis; approximately 10,750 people died. Among them, 7,760 were male, and 2,990 were female. Based on cancers in the digestive systems that include stomach, esophagus, small intestine, colon, and others, the estimated number of cases was higher than oral cancer, 333,680 in number (187,620 male and 146,060 female). Among them, 167,790 people died. The number was even concerning for the respiratory system, having 247,270 total cases where 130,340 were male, and 116,930 were female. The death rate was around 50%, with an enormous number of approximately 140,730. There were many reports for skin cancer where 108,420 cases were reported (65,350 male and 43,070 females) with 11,480 estimated deaths. The registered number of breast cancer was very high, with 279,100 cases in total and 42,690 estimated deaths. In this type of cancer, the number of women was prevalent compared with the number of men, where only 2,620 cases were reported for men, and 276,480 cases were written for women. For the cancers in the genital system, 317,260 cases were reported, with 67,830 deaths in number. Lastly, 60,530 cases were reported for leukemia, with 23,100 deaths (Siegel et al., 2020).

1.4 The future of cancer treatment

Till now, there are different effective cancer treatment options. Cancer therapies often include surgery, chemotherapy, and radiation therapy. Surgery and radiation therapy are traditionally used to treat primary tumors and significant metastases. Chemotherapy is the mainstay of treatment for some metastatic tumors, such as breast, colorectal, and prostate cancer. These traditional anticancer chemotherapy drugs block mitosis and DNA replication. Also, platinum compounds, topoisomerase inhibitors, nucleoside analogs, and vinca alkaloids were among the first anticancer medications developed. Patients with childhood leukemia and testicular cancer have exhibited tremendous healing impact and marginally prolonged survival. They are not,

however, effective against all forms of cancer. In the context of chemotherapy's history and drawbacks, alternative treatment options have received considerable attention in recent years. Researchers centers their efforts on several new treatment techniques that target the disease. The action of focused treatment was linked to many biological targets and signaling pathways. Multiple mechanisms were involved in the anticancer benefits of this abovementioned targeted therapy. Following this, the development of efficiently targeted medicines is aided by a greater understanding of tumor immunology. Cancer molecular diagnostics has recently advanced at a rapid pace. The FDA has approved a higher number of targeted treatment medicines for various cancer subtypes in the last two decades. These powerful and safe medications offer new therapeutic options to individuals who have previously been unable to receive appropriate conventional chemotherapy. However, resistance to medicinal drugs is a significant issue in cancer treatment, and it is thought to affect the efficacy of targeted therapies. During cancer treatment, drug resistance is predictable, especially with new targeted regimens that target specific molecules. Hence, anticancer medicines with better molecular targeting have been investigated by researchers (Ke & Shen, 2017).

1.5 Rationale of the study

To defeat cancer, the efforts of scientists have been remarkable till today. However, not a single of these efforts has eradicated the disease completely. Recently, targeted therapy has opened the door for personalized therapies as each person's genetics differ. The study has suggested that SHP2 has a connection in different types of cancers exhibiting distinct pathophysiology and mechanisms. Hence, the focus to inhibit this to control cancer can be effective in a more in-depth focal point. In recent years, pharmaceuticals and researchers have been trying to design the most effective drug that would inhibit SHP2 as a treatment option for cancer. This review attempts to discuss the link between SHP2 and different types of cancer and its

inhibition process. Additionally, efficiency, potency, and a comprehensive analysis have been done to evaluate the inhibitors and their impact on different types of cancer.

1.6 Aim and objectives of the study

Aim:

This review aims to discuss the recent advancement of potential breakthroughs of SHP2 inhibitors as a targeted therapy in cancer treatment.

Objectives:

- To elaborate the role and significance of SHP2 in different types of cancers and solid tumors. Also, to visualize the contribution of SHP2 in signaling pathways.
- To evaluate the success and limitations of various SHP2 inhibitors' efficacy, selectivity, and other requirements.
- To analyze the outcome of the experimental data for SHP2 inhibition based on the types of cancers.

Chapter 2: Research Methodology

The focus of the study was to investigate the recent advances made with SHP2 inhibitors as a targeted therapy in cancer treatment. The material of the review paper was obtained from multiple primary sources, including Google Scholar, Research Gate, NCBI, Science Direct, Nature, Elsevier, Springer, etc. Secondary research publications are also used to gather information, such as PubMed. After scanning the articles for pertinent information, an outline was constructed to portray the information in a structured order. First and foremost, it was essential to explore the origins of cancer, mutations, development, and the prospects of cancer treatment based on SHP2 inhibition. An additional literature search was conducted to highlight the structure and function of SHP2, as well as its relationship to cancer prognosis and contribution to various signaling pathways. In addition, the inhibitory process was explained, with its mechanisms of action demonstrated. Finally, considering the regulatory pathways, several therapeutic compounds were discovered with their efficacy in various cancer types for the successful demolition of cancer. Also, reliable materials were gathered throughout the entire write-up, and a proper citation was prepared with careful consideration.

Chapter 3: Overview of SHP2

3.1 SHP2 and protein tyrosine phosphatase

Phosphorylation in tyrosine reside is in charge of regulating the cell signaling pathway. To be precise, it mainly regulates biotic activities (Song et al., 2022). This is handled by PTK (protein tyrosine kinases) and PTP (protein tyrosine phosphatases). They have a role in maintaining cellular homeostasis and cellular response. PTK operates to play the role of substrate phosphorylation, whereas PTP operates as substrate being dephosphorylated. Many diseases can occur in case of any undesired disruption in these two, including cancer, diabetics, and autoimmune disorders. That's why PTK and PTP are used in targeted cancer therapy because the proteins depend on various growth factor signals associated with tumors. Recently, several inhibitors of PTK have been utilized to boost cancer treatment. This includes c-Kit, Bcr-Abl, EGFR, and so on. However, in the case of PTP, targeted therapy has not been explored that much. Those are known to have shown unacceptable selectivity, potency, pharmacokinetic properties. Also, there is a lack of understanding for known PTP inhibitors explored so far. If the activity of tyrosine phosphorylation increases, it will indicate the emergence of cancer in the tumor-suppressing genes and signaling pathway of the dephosphorylation of pTyr residue (phospho-tyrosine). In that case, PTP is considered a negative regulator. SHP2, a part of the PTP family named PTPN11, has gained much attention because it is exceptional as an essential condition for amplifying the signal. SHP2 activation and alterations have been identified in various leukemia and other solid tumors (Guo et al., 2021; Yuan et al., 2020). SHP2 was once thought to be a target that cannot be used for drugs, but the allosteric site presents hopeful anticipation for developing novel drug targets. Besides, various effective SHP2 inhibitors were discovered by high throughput screening in recent decades (Song et al., 2022).

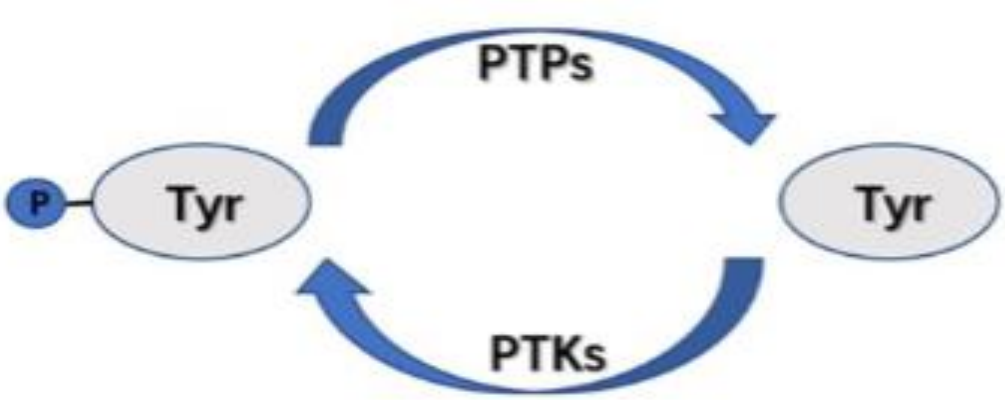

Figure 1: PTP and PTK regulating tyrosine phosphorylation (Guo et al., 2021).

3.2 Structure of SHP2

SHP2, a protein tyrosine phosphatase which stands for Src homology-2 domain-containing protein phosphatase-2, is a part of PTP encoded by PTPN11 proto-oncogene. It works on the growth and division of cells through the MAPK signaling pathway (Garcia Fortanet et al., 2016; Mostinski et al., 2020; Wang et al., 2020). SHP2 is organized into three domains: these include N-terminal as well as C-terminal and also a PTP catalytic domain (Mostinski et al., 2020). The human PTPN11 gene contains 593 amino acids, with the N-SH2 and C-SH2 domains containing 3–104 and 112–216 amino acid sequences, respectively. The PTP domain comprises amino acid sequences extending from 221 to 524. The C terminal amino acid sequence is 525–593, with two tyrosine sites at 542 and 580 phosphorylated. The PTP domain is a hybrid structure comprising nine alpha-helices and fourteen beta chains. Around the alpha E helix, the ten beta group is called a composite parallel/antiparallel beta pattern. N-SH2 regions are pressed into the PTP domain in the resting state to generate intramolecular interactions. As SHP2 is inactively auto inhibited, the active phosphatase region is directly blocked. The peptide chain connects the C-SH2 area to the neighboring domain. However, there is no apparent contact between the N-SH2 region and the PTP domain in the three-

dimensional structure (Guo et al., 2021).

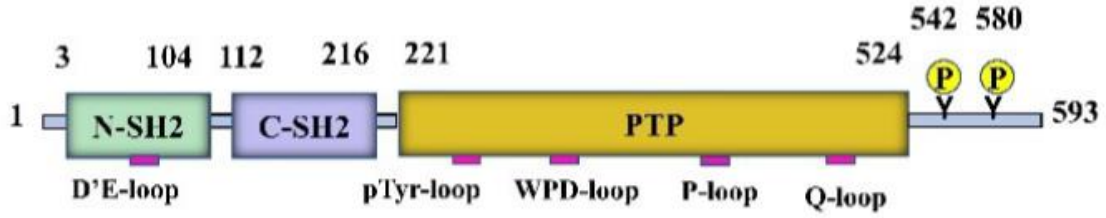

Figure 2: Structure of SHP2 in full length and color labeled (Guo et al., 2021).

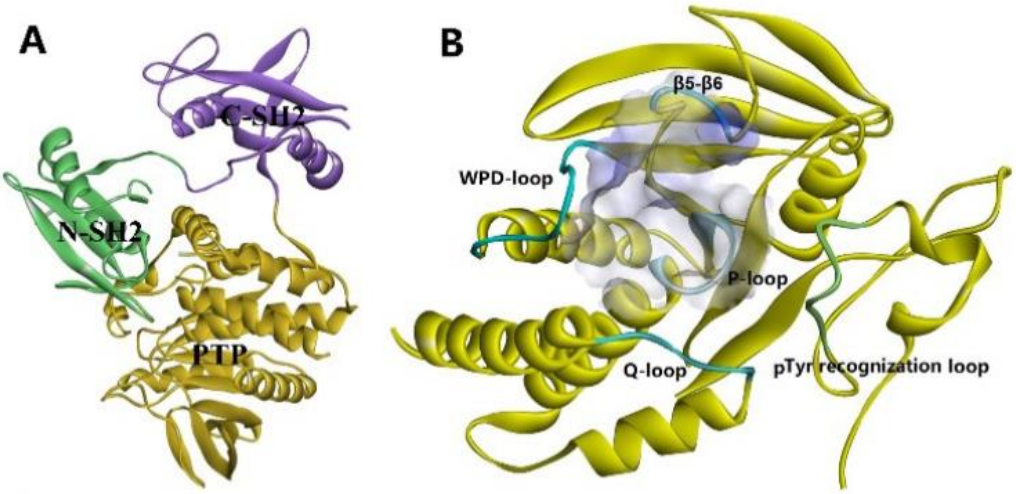

Figure 3: PTP domain's structure along with the catalytic site (Guo et al., 2021).

SHP2 has five loops surrounding its catalytic site, including the WPD, Beta 5- Beta 6, P, Q, and pTyr loops. In the auto inhibitory structure of SHP2, pTyr that forms bonds with the two domains of SHP2 will be dissipated further. There are two conformations in the pTyr and N-SH2 domain binding sites that portray the peptide bond state that has been activated and the inactive state of the PTP. In this dormant state, the interaction between N-SH2 and PTP domains occurs to initiate the collaboration to block the pTyr peptide to bind. Whenever the pTyr peptide interacts with N-SH2. As a result, N-SH2 conformation will be activated from the inactive state. Due to the activation, the auto inhibitory interaction occurring between N-SH2 and PTP will be distorted. Consequently, the catalytic pocket will be accessible to the substrate that contains pTyr residues for binding. The C-SH2 domain creates a small number

of interactions where N-SH2 and PTP domains will interact without any inhibitory outcome on the PTP domain. If N-SH2 alone interacts with pTyr peptide, basal activity can be managed by the activated SHP2. Again, when it is N-SH2 and C-SH2 binds with pTyr peptides, higher dephosphorylation activity will be observed by SHP2 (10-folds). It has been seen that tandem domains of SHP2 exert more selectivity than the single SHP2 domain. Furthermore, hyperactive catalytic acts can be seen for PTP caused by PTPN11 via GOP mutations. This is because of the undermined SHP2 auto inhibitory confirmation. Consequently, the intramolecular interaction for SHP2 will be damaged, resulting in the partial opening of the conformation to increase accessibility to substrate into the catalytic site (Yuan et al., 2020).

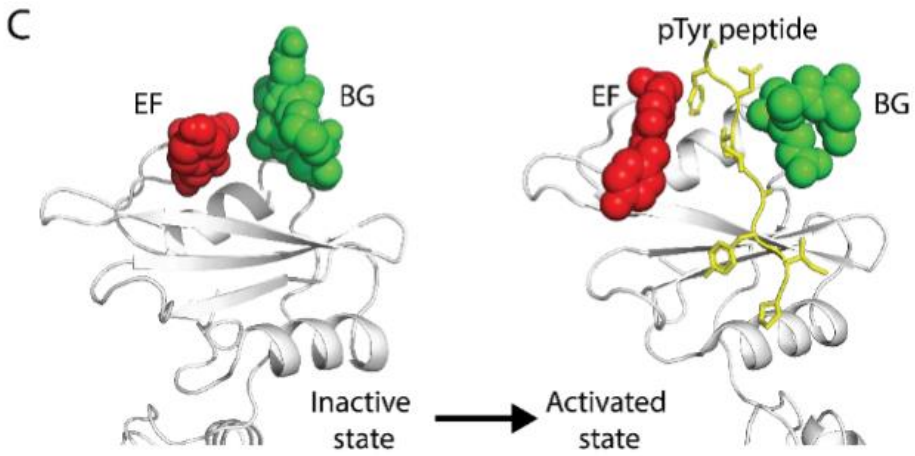

Figure 4: Transformation of pTyr from an inactive to active state (Yuan et al., 2020).

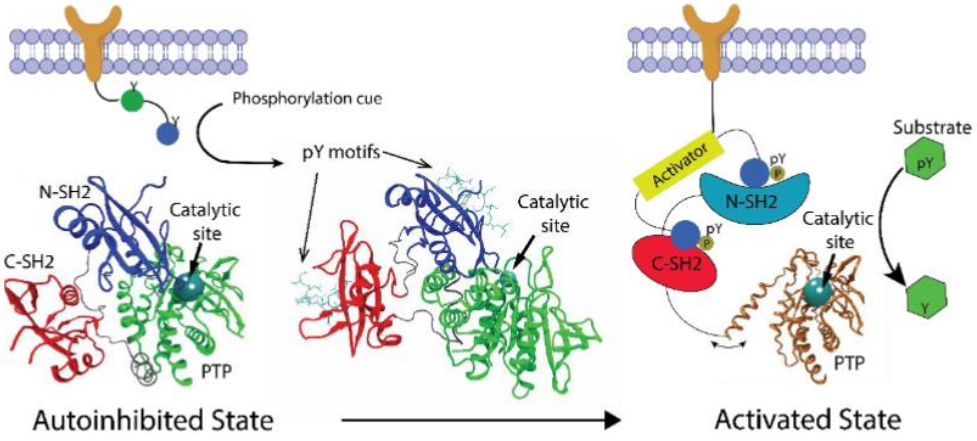

Figure 5: N-SH2, C-SH2, and PTP domains in their auto inhibited and activated state (Yuan et al., 2020).

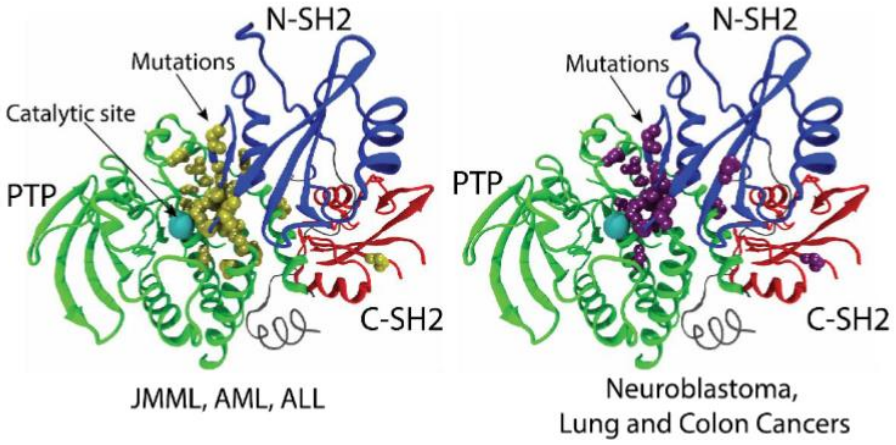

Figure 6: The reported mutations in the SHP2 structure (Yuan et al., 2020).

Nevertheless, if the sequence of SHP2 interacts with the motif of phosphorylated tyrosine, it will detach from the PTP domain. It removes the auto inhibitory state exposing PTP's active region followed by the activation of SHP2. Subsequently, dephosphorylation will occur when the phosphorylated tyrosine is binding to the anchors of the SHP2 domain. Besides, pathogenic mutation takes place for PTPN11 which leads to modification of the inhibitory state by weakening the interaction, followed by abnormal activation of phosphatase (Guo et al., 2021).

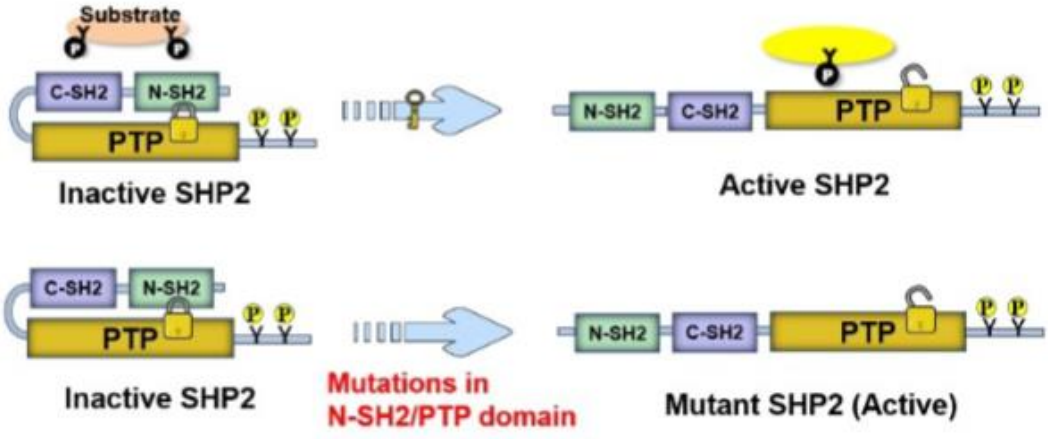

Figure 7: SHP2 changing from auto inhibitory to activation due to phosphotyrosine motif and N-SH2/PTP domain mutation (Guo et al., 2021).

One of N-terminal SHP2 hinders PTP domain interaction in the inactive state. The SH2 domain will engage in specific phospho-tyrosine sites on the receptor protein or a ligand-receptor if the correct ligand connects to the receptor. This arrests the inhibition of the SH2 domain. For instance, a novel interacting protein called hook 1 interacts with SHP2, which interacts directly with the PTP domain and N-SHP2. Hook 1, a microtubule-binding protein, works as a regulator to control the SHP activation as a negative endogenous regulator (He et al., 2019).

3.3 SHP2 and its relationship with cancer

PTPN11 was the first proto-oncogene that was identified for encoding tyrosine phosphatase. The mutation of SHP2 is closely related to cancer. PTPN11 mutation leads to the activation or inactivation of the phosphate of SHP2 depending on the cases, e.g., Noonan syndrome (NS), leukemia, and LEOPARD syndrome (LS) (Guo et al., 2021). Noonan syndrome (NS) is a genetic disorder showing the symptoms of facial dysmorphic, heart defects, low height, chest deformity, skeletal malfunction, and bleeding (Yuan et al., 2020).

NS has been linked to roughly 50% of mutations in PTPN11. The majority of the mutation will affect the N-SH2/PTP domain resulting in the damage of auto inhibitory conformation. As a

result, hyperactivation phosphatase will take place. The rest of the mutations will work in the affinity of the domain of SHP2 to initiate phosphor-tyrosine. This incidence can initiate the over-activation of RAS-MAPK where no mutation will be taken place for PTPN11. The same occurs for the mutations in RAF1, KRAS, and SOS1 genes. A germline mutation is also a factor for LS (90% cases), where they share many symptoms with NS. Mutations in PTPN11 in LS will inactivate the SHP2 by modifying the residues of the domain of PTP.

Nevertheless, there is a contradictory finding where a reversed effect is observed on phosphatase activity of SHP2, which ends up causing phenotypic diseases. NS patients also tend to develop JMML, which stands for Juvenile myelomonocytic Leukemia. This is a clone induced through myelodysplastic disorder causing hematopoietic diseases. Similar to NS, SHP2 mutations occur in JMML where N-SH2 residues and domains of PTP will be affected, resulting in the expanded activity of the phosphate. No PTPN11 mutations will be observed for this incidence nor the mutations in NF1, KRAS, and NRAS with RAS/MAPK pathway. In addition, a mutation in PTPN11 has been detected for acute myelogenous leukemia (AML), myelomonorate leukemia (CMML), and B-acute lymphoblastic leukemia (B-ALL). Over-expression of SHP2 is also responsible for solid tumors, e.g., breast and gastric cancer. Moreover, upregulation of SHP2 is found in lung, oral, laryngeal cancers. On the opposite, SHP2 works as a promoter in the case of tumorigenesis and maintenance of cancer (Guo et al., 2021).

Patients suffering from LEOPARD syndrome have also reported two recurring mutations. These include Tyr279Cys and Thr468Met. Gln506Pro is a mutation in the PTP domain, SHP2 is mainly activated. Such mutations would promote cell proliferation by extending signal flux throughout the Erk2/MAPK1 pathway. And this process necessitates docking through Grb2-associated binder-1 (Gab1). PTPN11 mutations are observed in a small number of human cancers, including acute myelogenous leukemia (AML) and neuroblastoma. One of the most

prevalent and active PTPN11 mutations that have been reported in leukemia and solid tumors is SHP2-E76K. An inhibitor of SHP2 named PHPS1 works to inhibit the activation of ERK1/2 via SHP2-E76K and subsequently block the growth of many independent cell lines responsible for the tumor growth (Zhang et al., 2015).

3.4 Function of SHP2

3.4.1 Tumor invasion and associated metastasis

Epithelial-mesenchymal transition (EMT) in breast cancer cells is mediated and upregulated by SHP2. Depleting SHP2 avoids invasion *in vivo,* along with knockdown of SHP2. It is established in breast tumors, inhibiting the maturation process and impeding metastasis. In other cancer cases, SHP2 overexpression is linked to advanced clinical stages of cancer and lymph node metastases *ex vivo*. SHP2 also increases oral cancer cell invasion and metastasis (Zhang et al., 2015).

3.4.2 Apoptosis

SHP2's role in apoptosis was first recognized in several myeloma cells. SHP2 is implicated in the activity by which interleukin-6 (IL-6) inhibits cell death produced. SHP2 expression suppression also induces apoptosis and reduces the development in leukemic cells. Additionally, SHP2, a tyrosine phosphatase that activates Erk, averts apoptosis in tumor stem cells. The apoptotic pathway is suppressed by the SHP2-E76K mutation, which is the most prevalent PTPN11 mutation seen in leukemia and solid tumors. Again, by reducing apoptosis in hematopoietic stem cells, SHP2 demonstrates an essential role in their durability. SHP2-deficient mice have a significant decrease in surfactant proteins, as well as enhanced alveolar epithelial apoptosis (Zhang et al., 2015).

3.4.3 Tumor cell proliferation

SHP2 is also a cell proliferation-promoting factor. SHP2 modulates multimodal signaling regulation in glioma cells to limit expansion in cancer. SHP2, a tyrosine phosphatase, also increases breast cancer proliferation. These findings pave the way for further research into SHP2's role in the cell cycle. For example, SHP2 suppresses cellular senescence by allowing glioblastoma cells to proliferate. SHP2 is also engaged in radio resistance in nasopharyngeal cancer cells via regulating cell cycle distribution. SHP2 depletion triggers checkpoint-mediated cell death in the HeLa cell line. These findings demonstrated SHP2's importance in checkpoint management and showed a new relationship between SHP2 and the cell cycle checkpoint (Zhang et al., 2015).

3.4.4 Damage on DNA and its replication in cancer

SHP2 must conserve checkpoints following DNA damage produced through cisplatin and radiation in HeLa cells. Subsequently. SHP2 depletion impairs checkpoint-mediated DNA repair and checkpoint kinase 1 activation (Zhang et al., 2015).

3.4.5 Immune cell

SHP2 has a significant role in the immune cell, including macrophages and T-cells. The PD-1 (programmed cell death-1) is via different mechanisms for T cells. SHP2 converts CSF1R signaling, known as the colony-stimulating factor 1 receptor. Hence, it can be used as a checkpoint inhibitor in immunotherapy to treat cancer (Liu et al., 2020).

3.5 Contribution of SHP2 in the signaling pathway

HRAS, NRAS, and KRAS are the three human RAS genes most frequently mutated in cancer. About 188-189 amino acid proteins serve as molecular switches as membrane-bound binding proteins for GDP/GTP. They link the tyrosine kinase inhibition, both receptor and non-

receptor, to downstream the cytoplasmic and nuclear signaling events responsible for cell proliferation, division, and apoptosis (Bunda et al., 2015). SHP2 is implicated in several signaling pathways, including RAS-ERK, RAS- MAPK, JAK-STAT, mTOR, PI3K-AKT, and NF-B (Guo et al., 2021). SHP2 has also been implicated in the governance of YAP oncogene transcriptional activities, albeit it is unclear if this regulation is dependent on the phosphatase activity of SHP2 (Xie et al., 2017). SHP2 exhibits dual roles in cell proliferation, differentiating, metabolic conditioning, anti-apoptosis, and immunomodulation. SHP2 can have a positive role in the RAS-RAF-MEK-ERK pathway when stimulated by growth factors. Still, it can also promote or be an antagonist in the PI3K-AKT and JAK-STAT signaling pathways depending on the specificity (Song et al., 2022).

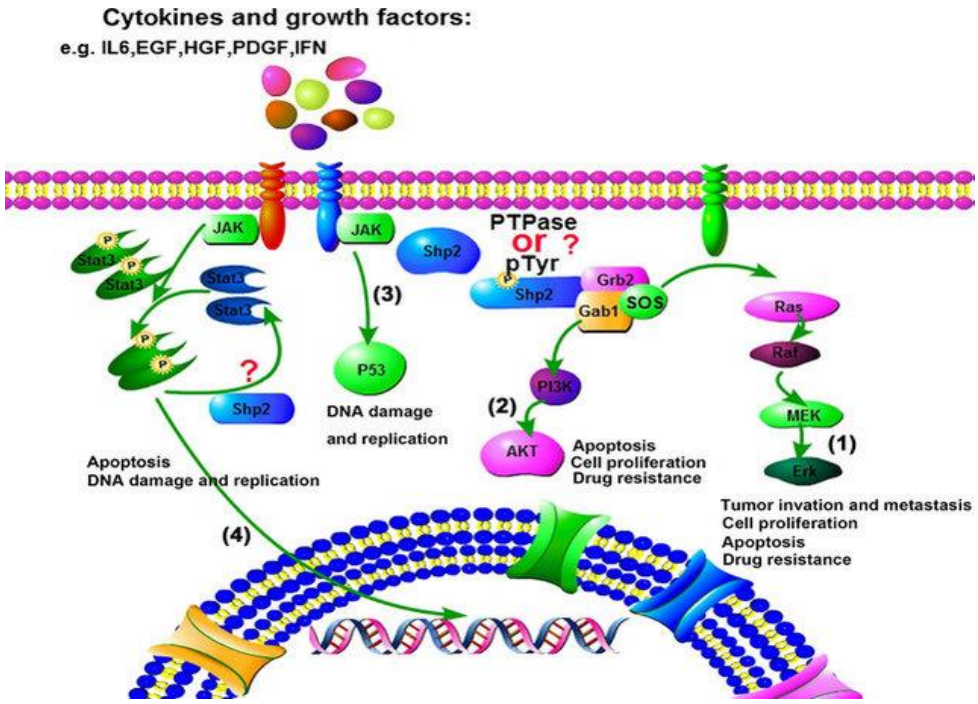

Figure 8: Functions and signaling of SHP2 (Zhang et al., 2015).

3.5.1 RAS/MAPK pathway

RAS/MAPK has a distinctive role in regulating cell proliferation, transfer, division, and other functions where SHP2 is accountable for the RAS activation in the pathway (Guo et al., 2021). For the RAS-MAPK cycle, KRAS, HRAS, and NRAS are rat sarcoma (RAS) oncogenes that

code for GTPase proteins that control cell proliferation, division, as well as lifespan. RAS proteins regulate the activation of proto-oncogene serine/threonine kinase (RAF), extracellular-signal-regulated kinases (ERK), and mitogen-activating protein kinase (MEK) by orbiting between their GTP-bound active and GDP-bound inactive states. More precisely, RAS is activated by the regeneration of GDP to GTP. GTP-bound RAS has a high affinity for effecter molecules like RAF. It triggers the cell proliferation pathway mitogen-activated protein kinase (MAPK). RAS is then turned off when the bound GTP is hydrolyzed to GDP by its GTPase activity, aided by GTPase-activating proteins (GAPs). RAS-activating mutations are detected in 20-40% of adult-onset cancers in hotspot codons 12, 13, and 61 following the conversation of RAS to oncoprotein. It is very active through weakening the GTPase activity. Tyrosine phosphatase SHP2 is a RAS activator that enhances RAS dephosphorylation and activates the RAS-MAPK pathway by increasing RAS binding to RAF. SHP2 also works as an effective regulator upstream in the RAS-ERK pathway, promoting the signaling transduction of RAS-RAF-ERK kinase. As a result of SHP2 inhibition, ERK is dephosphorylated. The pro-oncogenic-based function of the RAS-RAF-ERK pathway is repressed, leading to cell inhibitory effects and apoptosis in cancerous cells (Bunda et al., 2015; Valencia-Sama et al., 2020; Wang et al., 2020).

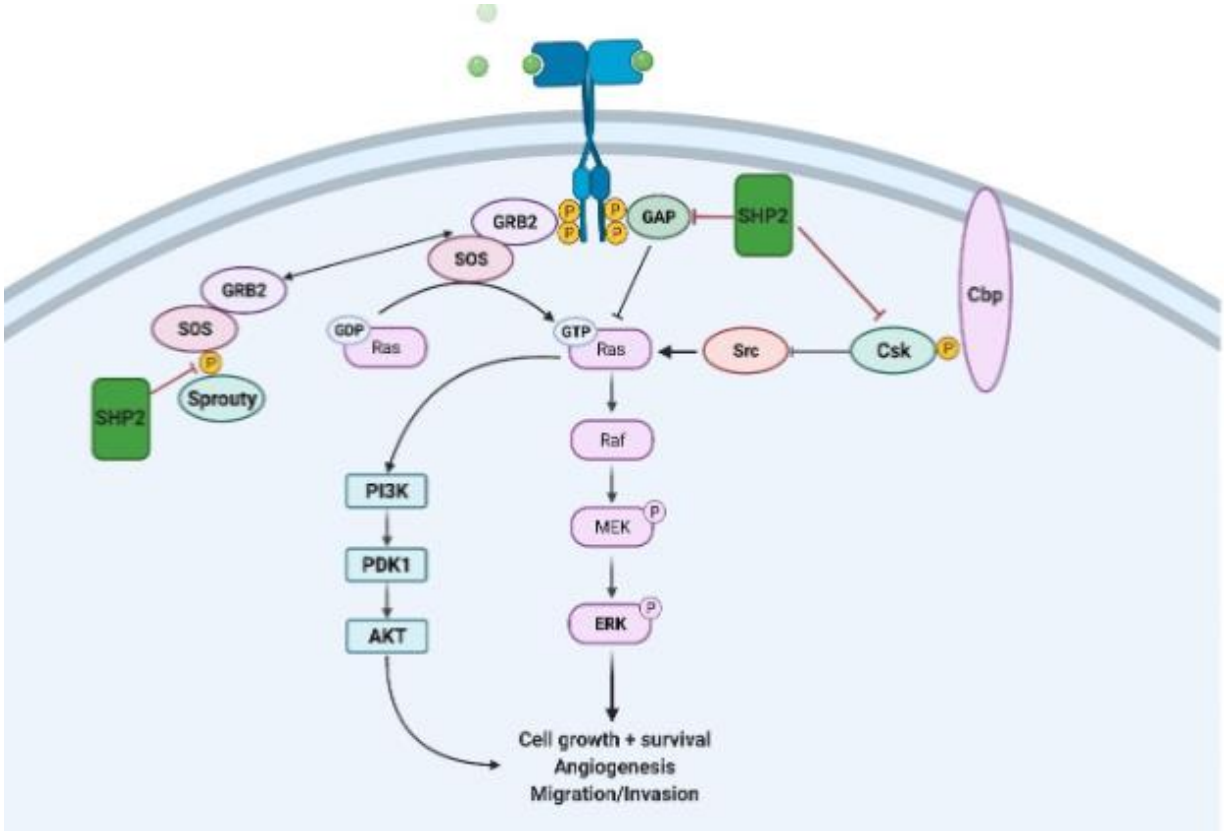

Figure 9: Regulation of different signaling pathways by SHP2 (Guo et al., 2021).

3.5.2 JAK/STAT pathway

In the primary bone marrow hematopoietic progenitor cells, overexpression of SHP2 can lead to the dephosphorylation of STAT5. It occurs in response to IL-3 stimulation. SHP2 also inhibits STAT3, a key player in hematopoietic cell cytokine responses (Yuan et al., 2020). JAK/STAT works to migrate signals from the membrane receptors to the nucleus, building the immune system, mammary glands, and other cellular events. In this pathway, SHP2 exhibits a dual role where it has the ability to inhibit and activate the pathway, unlike the RAS/MAPK pathway. As for the mechanism, SHP2 will interact with STAT5 and STAT1 in the cytoplasm and nucleus respectively to dephosphorylate them through a negative regulation system. SHP2 can also activate the pathway where the origination of the JAK2-Socs1 complex will be nullified, subsequently relieving the inhibition of JAK2. As a result, prolactin receptor (PRLR) will be recruited in the pathway (Guo et al., 2021).

3.5.3 PI/3 K/Akt pathway

Governance of PI/3 K/Akt pathway is either cell or receptor-specific or can be both in some instances. In several RTK pathways, the pathway can be required growth factors by SHP2 for activation. SHP2 can dephosphorylate PI3K binding sites and inhibit the activation of PI3K/AKT (Yuan et al., 2020). SHP2 can inactivate PI3 K activation by forming GAB2-SHP2-p85 complex via dephosphorylation of phosphorylation reaction of p85 Tyrosine on Gab1 (Guo et al., 2021).

3.5.4 PD-1/PD-L1 pathway

SHP2 can be expressed in any place. However, the scale of SHP2 is high in lymphocytes compared with peripheral blood lymphocytes for HNSCC patients, which stands for head and neck squamous cell carcinoma. For HNSCC patients, SHP2 expression in tumor invading T cells is strongly correlated with PD-1 expression (Yuan et al., 2020). SHP2 is known as the essential effector for PD-1 for signal transduction. It helps immune evasion by activating the programmed cell death pathway (PD-1/PD-L1) and inhibiting T cell activation. The cytoplasmic domain of PD1 has two tyrosine residues; one is immune receptor tyrosine-based inhibitory motif (ITIM), and the other is immune receptor tyrosine-based switching motif (ITSM). Both of them will be phosphorylated when the PD-L1/PD-L2 ligands are stimulated, followed by the recruitment of SHP2. ITSM engages PD-1 to SHP2 as it shows a high affinity for C-SH2. Whereas N-SH2 will bind with phosphorylated ITIM, auto inhibition will occur in the PTP domain. Consequently, T-cells will be inhibited (Guo et al., 2021). Blocking PD-1/SHP2 binding has the ability to retrieve the activation of T cells. Interaction in PD-1 and SHP2 can initiate the inhibition of PD-1 in the T helper type 1 cells or Th1 cells. Moreover, inhibition of SHP2 can retrieve robust immunity and cell activation of Th1. Although evidence links SHP2 to the demolition of T cells, the regulation of the exhaustion of T cells by SHP2 observed in the SHP2-deficient mice has been a question. Due to the hopeful anticipation,

evaluating the efficacy of SHP2 inhibitor using as a combination with an immune checkpoint inhibitor could open the door for a better treatment option for cancer patients as cancer immunotherapy (Yuan et al., 2020).

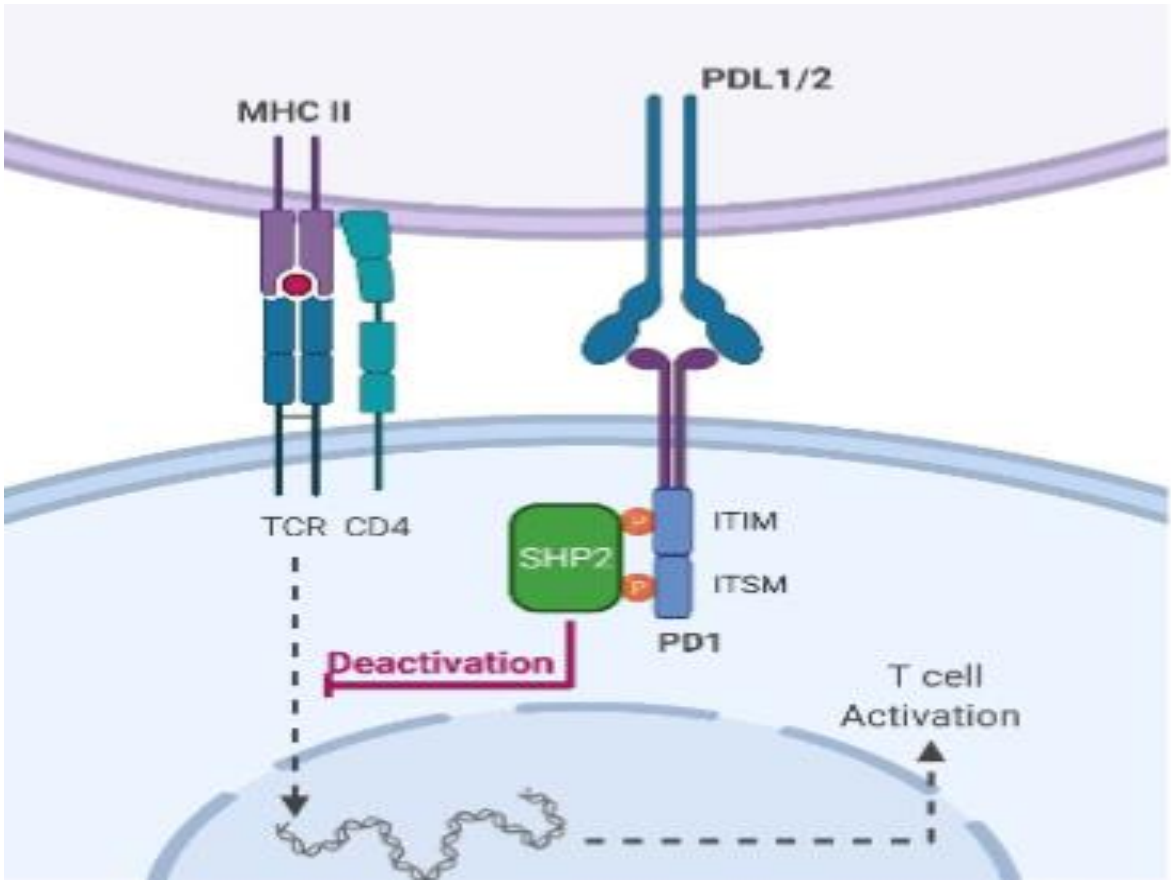

Figure 10: Inhibition of T cell activation by SHP2 (Guo et al., 2021)

Chapter 4: Inhibition of SHP2

4.1 Process of SHP2 inhibition

The SHP2 domains encapsulate the catalytic cleft of the PTP site once the enzyme is in a basal auto-inhibited condition. SHP2 undergoes conformational modifications after being stimulated by phosphorylated tyrosine-carrying proteins and peptides. It destroys the auto inhibitory connection of the SHP2 domain with the PTP site and lets the substrate-binding catalytic site be uncovered. However, though many of them are observed to have a promising notion, not all of them could pass the trials so far. Lysine and arginine's active site makes the environment highly conserved and polar (Mostinski et al., 2020).

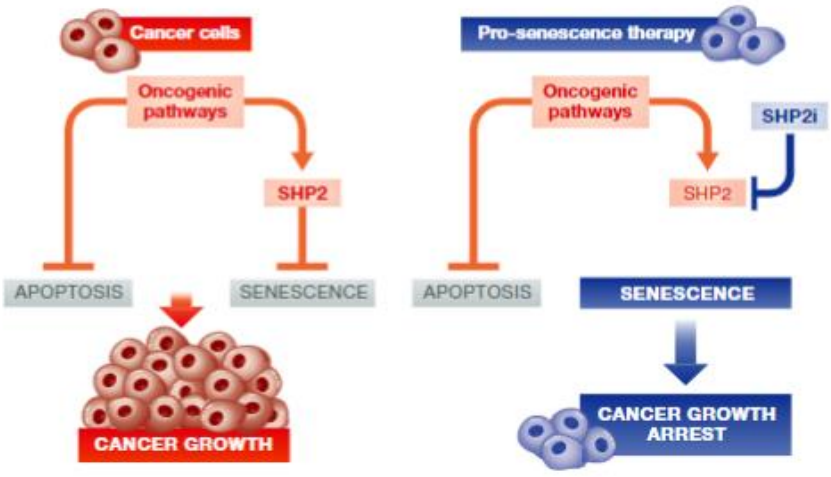

Figure 11: SHP2 is essential for cancer cells to resist senescence, and SHP2 inhibitors can induce senescence (Serrano, 2015).

There is a connection between SHP2 and senescence. Mainly, three effectors contribute to canceling senescence for initiating cancer. The effectors include: lowering the amount of the p27 owing to the upregulation of an E3 ubiquitin ligase SKP2, upregulating AKURA (mitotic kinase) leading to p53 inhibition, upregulating DLL1 (Notch receptor-ligand) contributing to suppress p53. As p27 and p53 increase and SHP2 is inhibited, it causes a significant induction of senescence (Serrano, 2015).

4.2 Types of SHP2 inhibitors

4.2.1 Catalytic site (Type I) inhibitor

The majority of the catalytic site inhibitors include PHPS1 and GS-493, an optimized version of PHPS1. The amino acid sequence has highly conversed. Based on this, small molecules were designed for the targeted inhibition of SHP2. NSC-87877 (8-hydroxy-7-(6-sulfonaphthalen-2-yl) diazenyl-quinoline-5-sulfonic acid) created in early 2006 was a potential inhibitor for SHP2 where the value of IC_{50} was observed 0.32 μM. It was revealed that NSC-87877 tends to be more selective. However, no data proved any notable difference in SHP1 and SHP2 inhibition *in vitro*, where for SHP1, the IC_{50} value was 0.36 μM, and for SHP2, the IC_{50} value was found 0.32 μM. To clarify more, SHP1 and SHP2 have different specificities for substrate despite having 60% shared sequence overall and 75% shared with the domain of PTP. This indicates that they are different in a substantial manner in many aspects. As NCS-87877 lacked the selectivity for SHP1, a new compound was chosen named NCS-117199 because of its effectiveness and more manageable modification scope as a hit. NCS-117199 most likely contributes to the ligand's selectivity for SHP2 (Guo et al., 2021).

Besides, phenylhydrazonopyrazolonesulfonate (PHPS) shows great inhibitory activity for SHP2. For example, PHPS1, PHPS4 (Guo et al., 2021). PHPS1 is a cell-permeable, non-cytotoxic protein only activated by SHP2-dependent signaling (Hellmuth et al., 2008). PHPS1 was detected as a hit compound exhibiting selective inhibition using virtual high-throughput screening. It works as an active-site competitive inhibitor in which a synergistic activity occurs. The efficacy of the mentioned synergistic activity with BRAF and MEK inhibitor is very promising. As a result, repression of tumor cell proliferation is seen. However, though it has a high potency, it has some limitations, including antipathetic liabilities and functionalities. This prevents its further optimization as a lead compound. The main concern is the presence of a

hydrazone fragment that is chemically unstable. The element releases toxic metabolites by attaching it with the pyrazolone scaffold *in vivo*. Again, in the compounds, the nitro structure contributes to cytotoxicity *in vivo*, and sulfonic acid happens to modify the inhibitor's cell permeability. Replacement of both of them is very hard as they have a crucial role in binding with the catalytic site of SHP2 (Mostinski et al., 2020)

For PHPS1, the sulfonic acid group was stretched to the substrate-binding groove and hypothesized to act as a pTyr mimic. Titration experiments revealed that PHPS1 inhibited SHP2 implied a competitive inhibitor. In addition, the carboxylic ethyl ester group was added to PHPS1 and exerted a 3-fold increase in potency. The addition of the group resulted in the production of PHPS4. PHPS4 showed an IC_{50} value of 0.63 μM for SHP2. For other PTPs, the IC_{50} values were for SHP1= 1.8 μM, PTP1B = 2.1 μM. PHPS1 had improved selectivity for SHP2. PHPS1 derivatives, which are 4-trifluoromethyl and 4-chloro, were accepted equally well. 4-methyl and 4-methoxy substitutions in PHPS1 lead to a 4-fold enhancement in potency. A 4-nitro group was added to GS-493. SHP1 showed the IC_{50} value of 2.08 μM; for SHP2, the IC_{50} value was 71 nM; for PTP1B, the IC_{50} value was 3.17 μM. All of the compounds favorably inhibited SHP2. GS-493 was the most powerful Type I SHP2 inhibitor, with superior selectivity against SHP2 than PHPS1. Arg362 that exists in SHP2 may interact with GS-493, while the equivalent residue for SHP1 is Lys356. However, the underlying selectivity of GSP-493 between SHP1 and SHP2 remains unknown (Yuan et al., 2020).

To add more to this, some more concepts and strategies are being developed with natural product-based catalytic inhibitors. For instance, SCONP and BIOS are investigated for structural classification of natural products and biology-oriented synthesis. SHP2 inhibitors were designed based on O-heterocyclic scaffold using furanodictin A from the compound library. The name of the discovered compound was NAT6-297775, and it was proven to be effective for excellent selectivity concerning PTP1B (Guo et al., 2021).

Another class of cinnamic acid derivatives was reported to attach to the retinoid acid receptor gamma (RAR). Following that, it causes cell destruction in various cancer cell types, including AML, where one shows an IC_{50} value of around 2.1 μM while the other shows an IC_{50} value of approximately 0.45 μM. However, their selectivity is yet to be known. Besides, one of them contains the group of 3-chloro-cinnamic acid from which beta carbon might interact with the cysteine found in the protein. However, this compound's inhibition mode is yet to be evaluated (Yuan et al., 2020).

Again, cryptotanshinone was discovered to inhibit both SHP1 and SHP2. For SHP1, the value of IC_{50} was found at 39.5 μM, and for SHP2, the value of IC_{50} was found at 22.5 μM. In traditional Chinese medicine, the roots of saliviamiltiorrhiza bunge contain cryptotanshinone working as a main active component. Cryptotanshinone has been shown to operate as a radical oxygen producer and suppress the phosphorylation of Tyr705 in STAT3 based on previous research. However, it is still unclear whether cryptotanshinone is opportunistic with SHP1 and SHP2. Additional optimization led to the identification of derivatives Tanshinone I, Tanshinone IIA, and Dihydrotanshinone I, which improved the potency profile of SHP1 and SHP2 by approximately 6- to 9-fold. (Yuan et al., 2020).

Oxindole derivatives named NSC-117199 can also be another Type I inhibitor that shows selective inhibition for SHP2. In the study, for SHP1, the value of IC_{50} was found at 68 μM; for SHP2, the value of IC_{50} was found 46.8 μM; for PTP1B, the value of Ki was found 96.7 μM. In the model, there occurs a bond between this group and SHP2. This initiated the imitation of the phosphate substrate by the nitro group, followed by hydrogen bonds with Lys366 and Arg362 by a sulfonic group. Also, the model suggests that several polar groups can swap sulfonic acid and hydrazine. For example, for bis-carboxylic acid derivatives, the potency was increased significantly (58 fold) instead of NSC-117199. Here, for SHP1, the value of IC_{50} was 15.4 μM; for SHP2, the value of IC_{50} was 0.8 μM; for PTP1B, the value of IC_{50} = 1.5 μM.

Another derivative of 3-carboxylic exhibited weaker activity, indicating that the carboxylic group's position should be at the place of the hydrazine aromatic ring. For the second derivative, for SHP1, the value of IC_{50} was found 72.5 μM; for SHP2, the value of IC_{50} was found 15.8 μM; for PTP1B, the value of IC_{50} was found 38.2 μM. Moreover, to improve the inhibition and selectivity, another compound was derived. For SHP1, the value of IC_{50} was found 18.3 μM; for SHP2, the value of IC_{50} was found 1 μM; for PTP1B, the value of IC_{50} was 14.5 μM. A new compound's better solubility profile was found upon introducing a sulfonamide group (Yuan et al., 2020).

Nonetheless, despite these compounds having the potential to be a good mimic for pTyr, none of them are worthy of being a drug. Because they exert a weak bioavailability, low affinity, and weaker membrane permeability, this is why a better design was necessary. Hence, a hydroxyl indole carboxyl derivatives course was designed to act as SHP2 inhibitors to meet the requirements. Here, for SHP1, the value of IC_{50} was found 15.7 μM; for SHP2, the value of IC_{50} was found 5.5 μM; for PTP1B, the value of IC_{50} was found 14.3 μM. Thus, a salicylic derivative was selected as a potential candidate II-B08 with satisfactory selectivity and inhibitory effect on SHP2. Even though the salicylic acid scaffold was shown to have outstanding cellular activity and pTyr mimic ability, the selectivity of the biphenyl derivatives compound is inadequate and insufficient for future research. Therefore, a new class of salicylic acid was found in 2014 produced from the mentioned derivative through functional modifications. However, these inhibitors, NSC-87877 and II-B08, might not show cellular activity to inhibit SHP2. This can be due to the clinical failure of poor selectivity or the PTP domain being too homologous (Guo et al., 2021; Yuan et al., 2020).

To recover the problems with poor bioavailability, cefsulodin was identified as a beta-lactam antibiotic showing an IC_{50} value of 16.8 μM for SHP2. The compound exhibited more selectivity (10 fold) than other PTPs, excluding SHP1. With further study, a hydrolyzed

combination of cefsulodin was discovered. Through additional screening, another derivative was identified with better selectivity and activity. For SHP1, the value of IC_{50} was found at 7.3 μM; for SHP2, the value of IC_{50} was found 1.5 μM; for PTP1B, the value of IC_{50} was found 9.3 μM (Yuan et al., 2020).

In 2015, fumisorinone was introduced as a selective inhibitor of SHP2, which shows the IC_{50} value of 6.3 μM for SHP2. In 2017, another chain of 1H-2, 3-dihydroperimidine derivatives (MW=175) was discovered to inhibit SHP2. It has weak inhibition activity and selectivity. Here, for SHP1, the value of IC_{50} was found at 4.28 μM; for SHP2, the value of IC_{50} was seen at 2.11 μM; for PTP1B, the value of IC_{50} was found 50.2 μM. It has a thiol-reactive chemotype that is promiscuous in its behavior (Yuan et al., 2020). Fumos exhibits variable degrees of cytotoxicity in different cell lines. For instance, 5FU is an inhibitor of DNA synthesis that blocks thymidine synthetase. It is widely used in cancer patients' combination chemotherapy treatments. The clinical usage of 5FU is still constrained by drug toxicity and resistance. Fumos as an inhibitor works in tandem with 5FU to prevent cell proliferation with an IC_{50} of 6.31 μM. This is an innovative strategy for increasing 5FU sensitivity (Chen et al., 2015, 2018).

4.2.2 Allosteric inhibition of SHP2

Allosteric inhibitors (Type II inhibitors) attach to an area outwards the catalytic pocket of PTP. For an effective interaction, Type I inhibitors must include numerous functional groups which can be negatively ionized. Type I SHP2 inhibitors often does not show appropriate selectivity and drug-worthy characteristics in preclinical trials to investigate the role of SHP2 in carcinogenic signaling networks. These inhibitors are in various phases of development, varying from preclinical laboratory tests to clinical trials. These include IFB-088 allosteric inhibitor (Phase I), LB-100 catalytic inhibitor (Phase I and II), and AKB-9778 catalytic inhibitor (Phase II), PRL3-zumab monoclonal antibody (Phase I), and other allosteric inhibitors

in phase I named MSI-1436, TNO155, and RLY-1971 (Yuan et al., 2020). The allosteric approach regulates SHP2's auto-inhibited conformation by binding to all the domains and altering from active to inactive. Specific inhibitors that serve as "molecular glue" to attach and act as SHP2 inhibitors have been documented, i.e., SHP099. SHP244, SHP836, and SHP389. These inhibitors prevent interactions directly with the catalytic site of PTP (Guo et al., 2021; Mostinski et al., 2020).

Novartis Company was the first to discover allosteric inhibitors in this quest. They announced a novel class of allosteric SHP2 inhibitors in 2015. SHP836 was revealed to be a weak allosteric SHP2 inhibitor. However, it failed to bind with the catalytic pocket. Hence, SHP099 was discovered through SAR analysis by replacing dimethyl piperazine with a pyrimidine or 4-amino-4-methyl piperidine. For SHP2, the value of IC_{50} was found at 0.07 μM. It was the first potent, bioavailable, and highly selective as an allosteric inhibitor of SHP2 (Yuan et al., 2020). SHP099, also known as 6-(4-amino-4-methyl piperidine-1-yl)-3-(2, 3-dichlorophenyl) pyrazin-2-amine, is an efficient inhibitor that was a significant achievement for Novartis scientists (Garcia Fortanet et al., 2016; Wang et al., 2020). It works more of a "chemical null" while inhibiting SHP2 (Ran et al., 2016). Additional derivatives of SHP099 have been explored for better optimization by Novartis. For example, derivatives in the amino pyrazine ring have shown better activity when incorporated with thioether linker. In 2019, SHP099 with amino-pyrimidinone derivatives was declared by Novartis, which exhibits a tremendous cellular potency profile. Further optimization with cyclization of spirocyclic ether has shown higher potency although showing unexpected hERG inhibition. Novartis also introduced another course of pyrazolopyrimidinone derivatives for SHP099, showing higher potency (Yuan et al., 2020). However, particular research has found that allosteric modulators possess minimal hERG selectivity and have a shockingly weak efficacy against various carcinogenic SHP2 variations in advanced biological studies. This shows the vulnerability to a mutation in the

mode of binding, which does not make it useful in therapeutic settings (Mostinski et al., 2020). However, the combination with SHP099 and MEK inhibitors as the direct inhibition of the MEK is clinically challenging and yet to be proven fully effective. The challenge arises due to the reactivation of the MAPK pathway for negative feedback variation. Hence, by using SHP099, the reactivation of the RTK can be examined in the RAS-driven system, leading to the suppression of the MAPK pathway in KRAS mutant cells and increased anti-proliferation activity. A downsizing of the combination therapy is the visible weight loss of mice experimented with SHP099 in its highest tolerable dose (100mpk/day) and trametinib. To avoid this low dose of SHP099 was used in 50/25mpk/every other day with 0.3mpk/day dose of trametinib that showed better tolerability in mice (Lu et al., 2019). Conformational selection, based on which SHP099 binds only to the closed compartment of SHP2, might be the possible binding mechanism for SHP099. Measuring the flux can determine if the conformational change occurs before or after the binding. According to the conformational selection model, SHP099 has a substantially weaker affinity for FL-E76K than FL-WTs (Pádua et al., 2018).

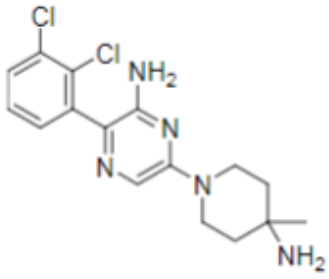

Figure 12: Structure of SHP099 (Garcia Fortanet et al., 2016).

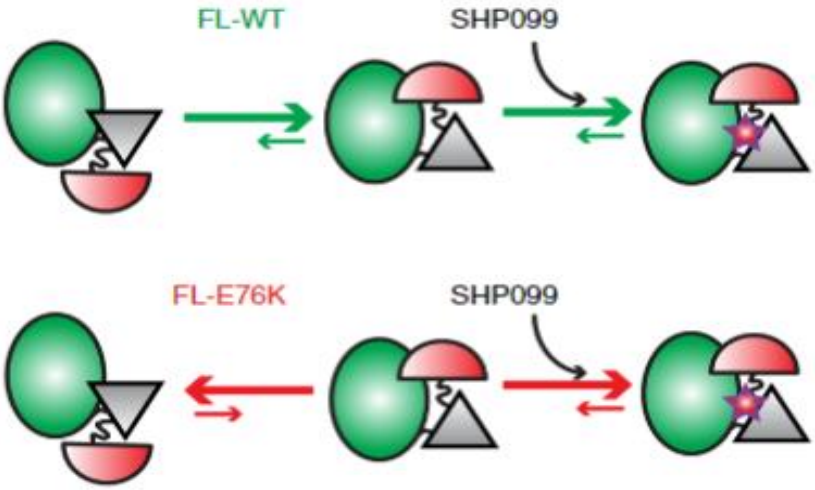

Figure 13: Conformational changes in presence of SHP099 (Pádua et al., 2018).

SHP394 was identified as an orally efficient inhibitor showing improved potency and increased lipophilic efficiency. It has been studied that many kinds of SHP2 inhibitors after discovering clinical trials are currently ongoing for further information (Sarver et al., 2019).

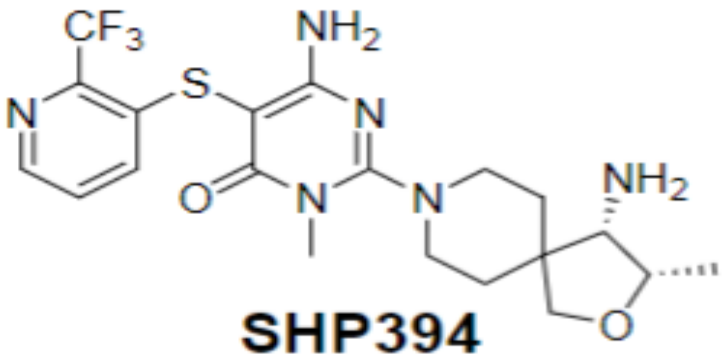

Figure 14: Structure of SHP394 (Sarver et al., 2019).

Studies have been conducted to optimize the structures of several candidates to form a new HH-3 compound. Initial molecular docking research revealed that the novel compounds affinity strongly for the SHP2 catalytic region. This is clear because they outperformed the reference catalytic inhibitor in affinity. The presence of a 5-aminosalicylate moiety was critical

for catalytic affinity. Salicylate (-COOH and–OH) groups were able to form a network of significant hydrogen bond connections with catalytic site residues. Moreover, carboxylic acid works as an alternative for sulfonic acid (Vazhappilly et al., 2018).

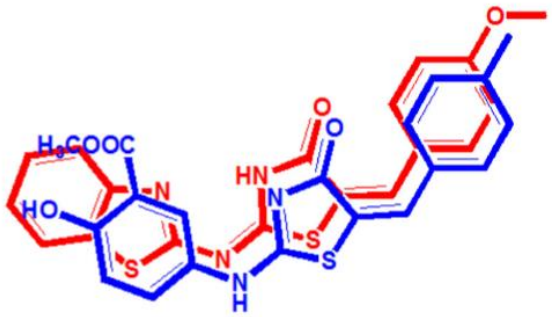

Figure 15: Structure of HH-3 compound (Vazhappilly et al., 2018).

Following the development of SHP099, revolution medicine has been described named RMC-4550 and their derivatives showing high inhibitory activity for SHP2. Here, the observation shows an IC_{50} value of less than 10 μM. If taken orally with a dose of 30 mg/kg, it can reduce tumor growth. Yet, RMC-4550 could not avoid the viability and p-ERK level in the CT26 cell line. Also, it was not effective if the mice lacked B and T lymphocytes. In addition, it showed the depletion of CD4+ and CD8+ T cells and immune competency (Yuan et al., 2020).

TNO155 is a potent discovery in nature, orally bio-available, and highly effective with dose-dependent pathway inhibition and anticancer efficacy. Unfavorable chemical-based toxicity of the drug can also be avoided to ensure optimal potency in the allosteric pocket (Lamarche et al., 2020). TNO155 was the first to be welcomed in the clinic to prevail over the RAS's activation mediated by RTK in five combinations. The combinations include KRAS mutant G12, BRAF, EGFR, an antibody of anti-PD-1, and CDK4/6 (Liu et al., 2020).

4.2.3 PROTAC strategy

A PROTAC molecule is a bi-functional molecule containing two ligands: one attached to the target protein and another that initiates an E3 ligase system. A chemical linker is used to

connect the ligands. Arvinas scientists have developed two PROTAC compounds that target the androgen and estrogen receptors. For instance, SHP2-D26 shows fast and effective inhibition of SHP2 (>95%). In cancer cell lines, this substance is more active in inhibiting ERK activation (Wang et al., 2020).

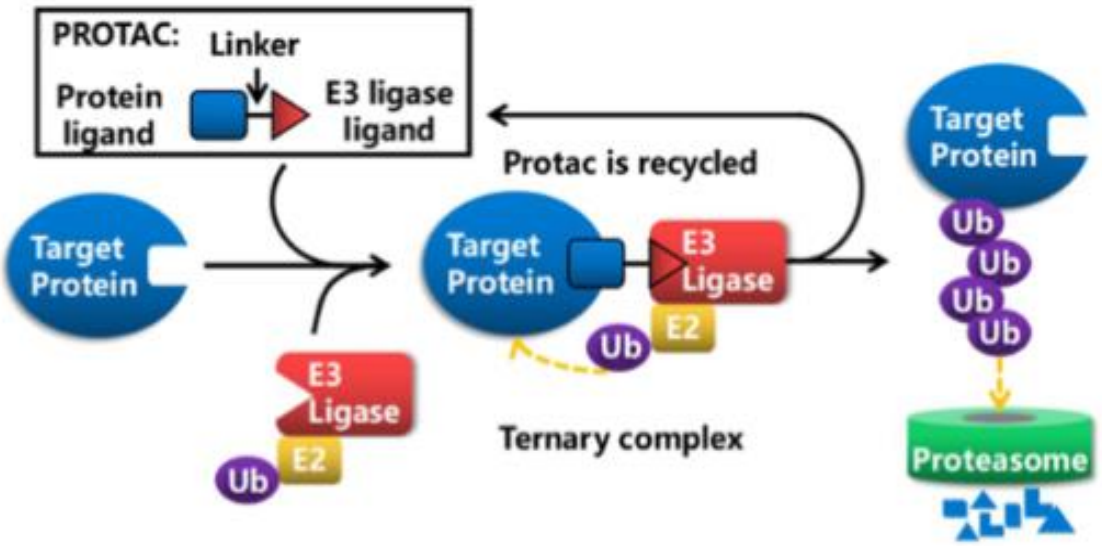

Figure 16: Schematic representation of PROTACs (Zheng et al., 2021).

Types of inhibition	**Study type**	**Result**	**Mechanism of action**	**Reference**
Catalytic site inhibitors	*In vitro*	NSC-87877 (IC_{50} value of 0.32 μM for SHP2 and 0.36 μM for SHP1.)	Inhibition of the catalytic pocket of PTP.	(Guo et al., 2021; Yuan et al., 2020)
		PHPS4: PHPS1+ carboxylic ethyl ester group (IC_{50} values for		

	In vitro and *in vivo*	SHP1= 1.8 μM, PTP1B = 2.1 μM SHP2E76K HEK293 cells).		
		GS-493 as an improved inhibitor assayed in HPAF II pancreatic cancer cells (IC_{50} value of 71 nM for SHP2).		
		Cinnamic acid derivatives for several cell lines of cancer: AML. (IC_{50} value of around 2.1 μM while the other showed an IC_{50} value of approximately 0.45 μM.)		
		Cryptotanshinone as a dual inhibitor (for SHP1, the value of IC_{50} was 39.5 μM, and for SHP2, the value of IC_{50} was found 22.5 μM).		
		NSC-117199 (SHP2 with the IC_{50} value of 46.8 μM)		

		II-B08 (IC_{50} value of 5.5 μM)		
		Cefsulodin (IC_{50} value of 16.8 μM for SHP2)		
		Fumisorinone (IC_{50} value of 6.3 μM for SHP2).		
		5FU (IC_{50} value of 6.31 μM).		
Allosteric site inhibitors	*In vivo*	• SHP836 (IC_{50} value of >100 μM.) • SHP099 in MDA-MB-468 and KYSE520. (IC_{50} value of 0.07 μM) • RMC-4550 in PC9 cell line (IC_{50} value of <10 μM.)	Inhibition of SHP2 by binding outside the catalytic pocket of PTP.	(Guo et al., 2021; Lamarche et al., 2020; Lu et al., 2019; Mostinski et al., 2020; Sarver et al., 2019; Vazhappilly et al., 2018; Yuan et al., 2020)
	In vivo	Dose-dependent combination therapy with trametinib. 50/25 mpk/every other day of		

		SHP099 with 0.3 mpk/day dose of trametinib.		
	In vitro	HH-3 compound was discovered. (IC_{50}= 0.48±0.02 in HeLa, IC_{50}= 0.30±0.045 MCF-7 in MDA-MB-231 cells)		
	In vitro	TNO155 (IC_{50} value of 0.011 μM for SHP2)		
PROTAC strategy	*In vivo*	In KYSE520 cells and MV4;11 cells, SHP2-D26 shows the value of 6 nm and 2.6 nm respectively for DC50	E3 ligase system.	(Wang et al., 2020)

Table 1: Types of inhibitors summary with experimental results and mechanism of action.

Chapter 5: SHP2 inhibition and its role on different types of cancer

5.1 Leukemia

Acute myeloid leukemia (AML) is a malignancy that targets the myeloid hematopoietic stem and has a poor 5-year prognosis. TET2 and DNMT3A are genes that regulate DNA methylation, mutate, and react with activating mutations in FMS-like tyrosine kinase-3 (FLT3). Patients have an unfavorable prognosis since they do not adapt well to conventional treatments (Song et al., 2022). SHP2 activated mutations have been reported in 35% of the incidence of juvenile myelomonocytic leukemia (JMML), 10% of incidences of myelodysplastic syndrome, 7% of B-cell acute lymphoblastic leukemia, and 4% of acute cases of myeloid leukemia (AML) (Wang et al., 2020). SHP099, an SHP2 allosteric inhibitor, is required to stop cytokine receptor signaling. Its inhibitory impact can slow tumor growth and induce leukemia cell differentiation. Furthermore, it was discovered that inhibiting both Syk kinase and SHP2 phosphatase simultaneously lowers STAT5 over-activation and proliferation in acute myeloid leukemia. The core problem with chronic myeloid leukemia is acquired tyrosine kinase inhibitor resistance (CML) (Song et al., 2022). LY6 can also be an effective inhibitor of SHP2 for leukemia, where the IC_{50} value for the compound was observed to be very high to be around 9.8 μM for SHP2. The result showed that the compound was 7 fold more selective for SHP2 than SHP1 (Wu et al., 2018).

5.2 Lung cancer

SHP2 plays varying effects in different tumors. Hence, in non-small cell lung cancer (also known as NSCLC), the expression and progression of SHP2 are not clear yet. Western blot approaches aimed at protein expression in twenty NSCLC tumors and similar normal lung tissues. It was discovered that expression of Hook1 in lung tumor samples was also much lower than that in surrounding tissue using densitometry analyses. Fifty-nine of the 101 lung tumor

tissues were immune reactive with the SHP2 antibody, while 46 tumor tissues had no evidence of Hook1 protein expression. However, Forty-one of the fifty-nine individuals who had positive results for SHP2 expression also had good results for Hook1. In lung tumor tissue, SHP2 expression was highly linked with Hook1 expression (He et al., 2019).

SHP2 may play a vital role in tumor stemness regulation. SHP2 inhibition has been shown to restore the sensitivity in NSCLC cells in recent research. In addition, inhibiting SHP2 reduces adaptive susceptibility to MEK inhibitors. SHP2 suppression reduced the stemness of KRAS-mut NSCLC cells with TKI therapy but had no effect under normal cell culture conditions. It implies that SHP2 has particular micro environmental effects. As a result, it can be noted that inhibiting SHP2 via inactivating MEK signaling reduced the stemness of KRAS-mut NSCLC cells (Jiang et al., 2019).

Recently, small-molecule inhibitors of protein tyrosine kinases have progressed well, with excellent specificity, selectivity, and safety. TKIs are commonly utilized to treat epidermal growth factor receptor (EGFR) mutant NSCLC. The deletion of PTPN11 significantly reduces the risk of lung cancer. As a result, treating lung cancer with a KRAS mutation with MEK and SHP2 inhibition is a viable option. SHP2 is also a crucial component in the activation of ALK inhibition, according to a recent study. So, combining the SHP2 inhibitor SHP099 with the ceritinib (ALK inhibitor) could improve ceritinib's efficacy. Also, the combination can avoid drug resistance (Song et al., 2022).

Furthermore, inhibition of SHP2 affects the therapeutic response to tepotinib in tumors. Tepotinib is an orally administered MET inhibitor approved to treat metastatic non-small cell lung cancer (NSCLC). In treatment-naive and tepotinib resistant, an SHP2 inhibitor detained the development of tepotinib resistance. Alternative signaling pathways may decrease the efficacy of tepotinib monotherapy, and combining tepotinib with an SHP2 inhibitor allows tumor progression to be inhibited in cells with MET alteration genetically. By bypassing

signaling, it leads to meditating the resistance. In a treatment-naive lung cancer cell line with MET amplification, adding an SHP2 inhibitor to tepotinib can delay the emergence of tepotinib resistance. However, combining tepotinib and an SHP2 inhibitor stopped the proliferation of cells. Furthermore, there are some limitations of the combination where the dose of the SHP2 inhibitors may vary depending on the patients to conduct a successful treatment (Pudelko et al., 2020).

5.3 Gastroesophageal cancer

KRAS has been acknowledged as the most commonly mutated oncogene in cancer. Besides, the majority of RAS-driven cancer research focuses on RAS coding mutations. However, another method has been explored where the KRAS gene is absent from coding abnormalities. Then, the somatic copy number change (SCNA) in gastric, esophageal, and colorectal cancer was evaluated, and it was discovered that KRAS was the most amplification-prone gene. The expansion of wild-type KRAS is linked to the elevated expression pattern of KRAS as well as the high mortality rate in gastric cancer patients. In KRAS-amplified gastric cancer, the combination of SHP099 with MEK inhibitor displays remarkable efficacy *in vitro* and *in vivo* (Song et al., 2022).

5.4 Breast cancer

Triple-negative breast cancer has the most significant mortality rate among all breast cancer subtypes. It has no effective treatment at the moment. The lack of targeted medicines and the diversity of molecular illnesses are the primary causes of poor clinical prognosis. SHP2 promotes breast cancer growth by increasing essential transcription factors (c-Myc and ZEB1). SHP2 was detected to be active in most breast cancers with a poor prognosis, emphasizing the importance of SHP2 in malignant breast tissue. By upregulating the signaling pathways of numerous RTKs, suppression of SHP2 examined in BTBC cells could limit tumor incidence

and metastasis. At the same time, promoting the transformation and invasion of BTBC cells can occur. It was recently shown that the SHP2 is required for tumorigenesis initiated by ERBB2. In the breast cancer model mice, the knockout of the PTPN11 gene eliminates the formation of cancers (Song et al., 2022). SHP2 is upregulated in cells responsible for breast cancer, and several SHP2 regulation mechanisms have been discovered. SHP2 signature genes, for example, are concurrently enabled in a vast segment of tumor tissues. The outcome has given insight into influencing tumor-initiating cells. SHP2 contacts GFR bound protein 2/Grb2 that associates binding protein 1 (Grb2/Gab1) to regulate tumor growth. It works as a signal transducer and activates transcription 1 (also known as Stat1) regulation, thus promoting the signaling pathway of breast cancer markers (Zhang et al., 2015).

An extra nuclear estrogen receptor (ER) pathway is essential for estrogen's signal, which initiates cytoplasmic kinase cascades. The ER route is associated with cell proliferation, migration, secretions, and apoptosis. Understanding these has recently improved the views on breast carcinogenesis despite the vague mechanisms. In the mice model, SHP2 enhances the expansion of human breast epithelial MCF10A cells. SHP2 elimination has been shown to inhibit the development and penetration of MCF10A cells. Though the process is not transparent yet, a hypothesis shows that SHP2 may be known to contribute to an estrogen signal and exhibit a role in estrogen-related breast cancer. SHP2 expression in breast tumors is connected with estrogen receptors (ERs), and E2 promotes the production of Gab2 (an SHP2 pattern protein) in breast cancer cells. A recent study showed that SHP2 undergoes overexpression in the breast tumor based on the Fischer test though it has no accurately known clinical relationship with the ER pathway. Also, due to the suppression of SHP2, tumor progression was blocked in the mammary glands of tested rodents undergoing DMBA treatments. Again, there has been proved of another hypothesis is that by acquainting with the estrogen membrane receptor complex, SHP2 may mediate the estrogen signal (Li et al., 2014).

In addition, inhibiting SHP2 prevents breast cancer cells from reforming, as revealed by the formation of epithelial morphology, absence of anchorage-independent proliferation, and differentiating in 3D LRBM matrigel (Zhou & Agazie, 2008).

Also, according to earlier research, SHP2 is capable of selectively dephosphorylating target phosphotyrosine substrates. Additional acidic residues are essential for selective binding and dephosphorylation. In addition, PTPase activity *in vitro* and SHP2-mediated signaling in cells can both be inhibited by a tyrosine-phosphorylated peptide. Based on this knowledge, the SHP2 inhibitor with the structure was designed and chemically manufactured. This compound's chemical name is CNBDA, and its formula weight is 512. The 4′-carboxylate group represents phosphate, butanoic acids. They imitate carboxylic side chains in natural SHP2 substrates. Additionally, they are connected to the biphenyl ring, which serves as the compound's core. Moreover, the aliphatic group was added to increase cellular permeability. CNBDA inhibits SHP2 with an IC_{50} of 5M *in vitro* PTPase tests, but the IC_{50} for SHP1 inhibition was 125M. Even though the effect of CNBDA on other PTPs was not analyzed in-depth, the 25-fold selectivity for SHP2 compared to SHP1. CNBDA is expected to be more specific to SHP2 because of the extensive interactions with the active site of SHP2. It also mediates specialized interactivity. Differences in CNBDA effectiveness *in vitro* and in cells were another critical finding. Even though the IC_{50} for SHP2 in PTPase assays was 5M, the IC_{50} for cell viability assays was 300-400 nM. The chemical likely binds to full-length SHP2 overexpressed in cancer cells more effectively than the separated PTP domain employed in enzyme experiments. One of SHP2's well-known biological functions is to regulate RTK signaling (Hartman et al., 2020).

5.5 Pancreatic cancer

Pancreatic cancer arises from the ductal epithelium. The epithelial presence of SHP2 was discovered in patients with pancreatic ductal adenocarcinoma (PDAC). The deletion of the

PTPN11 gene, which encodes the SHP2 protein, prevents KRASG12D-driven pancreatitis. Furthermore, researchers discovered that the absence of SHP2 slows tumor development and makes tumor cells more susceptible to MEK inhibition. Novartis researchers found that SHP099 of SHP2 can inhibit cancer cell growth (Song et al., 2022). Also, GSP-493 significantly reversed the transition of the HGF/SF-induced endothelial mesenchymal in the HPAF II pancreatic cancer cells in an analysis based on scatter assay (Yuan et al., 2020). SHP2 is a signaling node that triggers different RAS pathways. The survival and expansion of cancer cells are dependent on RAS activation. As a result, an SHP2 inhibitor with acceptable properties could be developed into a broad-spectrum anticancer medication. Furthermore, as the PTK and SHP2 signaling pathways overlap, a combination of SHP2 inhibitors kinase inhibitors can be employed to inhibit interrelated signaling pathways simultaneously. This combination therapy is more successful than monotherapy in preventing drug-derived resistance (Song et al., 2022).

5.6 Neuroblastoma

Neuroblastoma is a peripheral nerve system tumor prevalent in extra cranial solid tumors. It is clinically and physiologically heterogeneous. Most patients suffering from low to intermediate neuroblastoma can be treated with surgery or chemotherapy in low doses. However, in the case of high-risk diagnosis, patients' mortality rate is very high. Reoccurring somatic mutations are relatively uncommon in NB at diagnosis. The most common ones include 20% MYCN amplification, 23% TERT rearrangements, 6% NF1-loss, 9% ALK/ 3.5% PTPN11 mutations. The RAS-MAPK pathway was predicted to be activated by 78 % of mutations that have been found in relapse samples. Reduced sensitivity to SHP2 inhibitors, e.g., NSC-87877, RMC-4550, II-B08, and SHP099, is related to RAS mutations in NB. Also, NRASQ61K mutation provides resistance to inhibition of SHP2. In NB cells, inhibition of SHP2 and RAS effectors RAF, MEK, or ERK exerts synergistic effects, and combining SHP099 with MEK inhibitor

trametinib enhances survival. These findings imply that combinations of drugs targeting the RAS-MAPK signaling pathway could be appropriate therapies. As direct targeting of RAS has failed in the past, additional molecular-based techniques to inactivate RAS and the downstream MAPK pathway need to focus. This is where targeting of SHP2 can be effective, which results in the silencing of the RAS-MAPK pathway. Preclinical indications indicate SHP2 inhibitors as a promising treatment for RAS-related cancers, currently undergoing numerous phase I trials (Valencia-Sama et al., 2020).

5.7 Liver cancer

SHP2 has the ability to inhibit the onset of hepatocellular cancer (HCC), a significant liver cancer type. SHP2 expression was elevated in 65.9% (394 out of 598) of individual HCCs. SHP2 overexpression is linked to the malignant clinicopathological features of HCC. It predicted a bad outcome for patients. Inhibition of SHP2 expression decreased the maturation of HCC xenografts *in vivo* and suppressed the proliferation of hepatoma cells *in vitro*. In mice, SHP2 downregulation decreased hepatoma cell adhesion and movement, as well as metastasized HCC genesis. Furthermore, lowering SHP2 expression increased the sensitivity of hepatoma cells to sorafenib. Also, patients with low SHP2 expression had a better response to sorafenib. Thus, it indicated that SHP2 could be used as a patient prognostic biomarker (Han et al., 2015).

Interestingly, SHP2 has a dual role in liver cancer. The GOF T507K mutation is the only mutation discovered in this type of cancer. The activation of the RAF/ERK pathway is partly responsible for these effects. Acting as a tumor promoter in the disorders mentioned above, SHP2 can also act as a tumor inhibitor in hepatocellular carcinogenesis. Some patients with this cancer have been found to have SHP2 downregulation. SHP2 that is hyper-activated due to the GOF T507K mutation and SHP2 that is deficient due to low expression can both promote

cancer growth by separate signaling pathways. As a result, it is critical to determine the status of SHP2 and adopt the appropriate therapeutic strategy in this cancer (Shen et al., 2020). In addition, there has been observed a high expression for SHP2 patients' chemo-resistant hepatocellular carcinomas (HCCs) and recurring HCCs. SHP2 dephosphorylated CDC73 in the hepatoma cells. According to the researchers, the dephosphorylated CDC73 linked beta-catenin and promoted nuclear translocation. This caused hepatoma cells to become dedifferentiated. SHP2 elevated beta-catenin accumulation in liver cancer stem cells by suppressing GSK3-mediated beta-catenin degradation. Subsequently, it accelerates liver cancer stem cell self-renewal (Xiang et al., 2017).

Chapter 6: Conclusion and Future Prospects

6.1 Conclusion

SHP2 as a proto-oncogene initiates cancer through mutation, upregulation, and overexpression. Moreover, SHP2 is implicated in several signaling pathways, including RAS-ERK, RAS-MAPK, JAK-STAT, PI/3 K-AKT, etc. These pathways exhibit a distinctive role in regulating cell proliferation, transfer, division, and other functions where SHP2 is accountable for the gene activation (i.e., RAS). Also, overexpression of SHP2 can lead to the dephosphorylating and inhibition of STAT5 and STAT3 respectively. Furthermore, SHP2 shows an inhibitory role in the PI/3 K/Akt pathway and PD-L1/PD-L2 pathway. Hence, SHP2 has earned a particular focus on tumor pathology studies and anticancer medication development to develop targeted treatment for oncology. The recent drug design and their experimental outcome strongly indicate that SHP2 inhibitors could have a lot of medicinal potential. Through inhibition, SHP2 undergoes notable conformational modifications and causes a significant induction of senescence by canceling cancer initiation. A considerable investment has been dedicated to developing catalytic and allosteric SHP2 inhibitors. Though all of these are in the early stages of clinical investigations, specific barriers need to be avoided through more research in the future.

6.2 Future Prospects of SHP2 inhibitors

SHP2 has limited membrane permeability and poor inhibitor selectivity due to the conservative PTP domain. Hence, no active-site inhibitors have made it to the hospital as a treatment option. Consequently, allosteric inhibitors and many inhibitors have become the focus of attention in the laboratory (Guo et al., 2021). In preclinical investigations, combination therapy is also more efficient than monotherapy and an efficient strategy to mitigate drug resistance. Besides, SHP2 inhibitors combined with the other kinase inhibitors are much more successful

than single-agent therapy and have a lower risk of drug resistance. Developing multi-target inhibitors is also needed to be investigated in the future. Furthermore, immune chemotherapy can limit tumor cell proliferation and increase the immunity of T cells to tumor cells, which is an exciting study direction. The current experimentation delivers an excellent theoretical basis for using SHP2 inhibitors with medicines that specifically attack the immune system in clinical studies (Liu et al., 2021).

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