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# STAT3 and SHP-1: Toward Effective Management of Gastric Cancer

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The importance of signal transducer and activator of transcription 3 (STAT3) signaling in gastric carcinogenesis was firmly evaluated in the previous studies. Fully activated STAT3 induces various target genes involving tumor invasion and epithelial-mesenchymal transition (EMT), and mediates interaction between cancer cells and microenvironmental immune cells. Thus, suppression of STAT3 activity is an important issue for inhibition of gastric carcinogenesis and invasion. Unfortunately, data from clinical studies of direct inhibitor targeting STAT3 have been disappointing. SH2-containing protein tyrosine phosphatase 1 (SHP-1) effectively dephosphorylates and inhibits STAT3 activity, which has not been extensively studied gastric cancer research field. However, by summarizing recent data, it is evident that protein and gene expression of SHP-1 are minimal in gastric cancer cells, and induction of SHP-1 effectively downregulates phosphorylated STAT3 and inhibits cellular invasion in gastric cancer cells. Several SHP-1 inducers have been investigated in the experimental studies, including proton pump inhibitor, arsenic trioxide, and other natural compounds. Taken together, we suggest that modulation of SHP-1/STAT3 signaling axis may present a new way for treatment of gastric cancer, and development of effective SHP-1 inducer may be an important task in the future search field of gastric cancer.

**Key Words**: Gastric cancer, SH2-containing protein tyrosine phosphatase 1, Signal transducer and activator of transcription 3

## INTRODUCTION

Gastric cancer is one of the most frequently diagnosed cancers and the 3rd leading cancer-related cause of death with more than 720,000 deaths per year worldwide. The global incidence rate of gastric cancer, which shows the highest incidence in East Asia including China, Japan and Korea.[1] If gastric cancer is diagnosed in advanced stage with distant metastasis, chance for cure is getting lower dramatically. Recent pivotal study showed that if there is single metastasis of gastric cancer outside stomach (i.e. liver), there was no significant difference of overall and progression-free survival between chemotherapy alone and chemotherapy plus gastrectomy group, and overall survival was less than 18 months in both groups.[2] Thus, much remains to be done to achieve complete conquest of stomach cancer, and the development of methods to effectively inhibit stomach cancer progression remains one of the most important challenges.

In this article, we briefly review the importance of signal transducer and activator of transcription 3 (STAT3) signaling and inhibition of STAT3 activity in gastric cancer, and the role of SH2-containing protein tyrosine phosphatase 1 (SHP-1) in inactivating STAT3, and recent researches to induce SHP-1 activity by pharmacologic interventions.

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## STAT3 Signaling and Inhibition in Gastric Cancer

Janus kinase 2 (JAK2)/STAT3 signaling pathway mediates multiple biologic reactions associated with gastric cancer, such as chronic inflammation by *Helicobacter pylori* (*H. pylori*) infection or interaction between gastric epithelium and microenvironmental stromal cells to promote stemness of cancer cells.[3,4] Thus, suppression of JAK2/STAT3 activity is considered as an optimal strategy for inhibition of multiple steps in gastric carcinogenesis and invasion.[5] Pathway activating JAK/STAT3 signaling in epithelial cancer cells and surrounding immune cells is summarized as follows; various sti-

 

**Table 1.** Experimental Inhibitors of STAT3 for Gastric Cancer Treatment

| Author | Inhibitor | Study design | Functional effects | Suggested mechanism |
|---|---|---|---|---|
| Kim et al.[8] | OPB-31121 | *In vitro/in vivo* | ↑ Apoptosis<br>Synergism with 5-FU and cisplatin | Downregulation of JAK2 SH2 domain of STAT3 |
| Stuart et al.[9,10] | AZD 1480 | *In vivo* | ↑ Apoptosis<br>↓ IL-11 | Inhibition of JAK1/2 phosphorylation |
| Judd et al.[11] | WP1066 | *In vitro/in vivo* | ↓ Pro-inflammatory cytokines (IL-11, IL-6, IL-1$\beta$) | Inhibition of JAK2 phosphorylation |
| Huang et al.[12] | Proton pump inhibitor | *In vitro* | ↑ Apoptosis<br>↓ Secretion of pro-inflammatory cytokine (IL-6) | Not presented |

IL, interleukin; JAK2, Janus kinase 2; STAT3, signal transducer and activator 3

mulant are binding to their receptors, they serve the interacting site for the phosphorylation of internal tyrosine kinase JAK1 and JAK2, which, in turn, phosphorylate STAT3. Fully phosphorylated STAT3 then forms homodimer to translocate into the nucleus and finally activate different target genes involved in cellular proliferation, survival, migration and invasion in epithelial cancer cells as well as surrounding immune cells.[6]

Thus, effective suppression of STAT3 activity is an important topic for effective treatment of gastric cancer. Various drugs or materials which are designed to suppress STAT3 activity have been developed and evaluated in the experimental and clinical studies. There are two strategies for inhibition of STAT3 activity. First one is direct inhibition of STAT3 targeting Src homology 2 (SH2) domain, DNA binding domain, or N-terminal domain of STAT3, and oligonucleotide-based inhibition of STAT3 such as siRNA or decoy oligonucleotide technology. Second one is indirect inhibition of STAT3 targeting upstream intracellular kinases such as JAK2 or Src kinase.[7] STAT3 inhibitors which have been evaluated for the management of gastric cancer are as follows; direct inhibitor (OPB-31121),[8] inhibition of JAK1/2 phosphorylation (AZD1480, WP1066)[9,10] or unknown mechanism[11] (Table 1). However, there are several limitations of the STAT3-inhibitors. Most of them have been only evaluated in pre-clinical studies, and significant clinical outcomes are lacking. Furthermore, there are some technical difficulties to develop more suitable and effective agent directly targeting STAT3.[7] Thus, new strategy for inhibition of STAT3 activity might be necessary.

## Role of SHP-1: A Phosphatase to Inactivate STAT3

SHP-1 is a non-receptor protein tyrosine phosphatase, which in abundantly expressed in hematopoietic cell lineage and dephosphorylates STAT3 as well as JAK2, and in turn downregulates STAT3 activity and inhibits various STAT3 mediated target genes such as cell proliferation and survival, angiogenesis, cellular migration or invasion.[12] However, only few studies have evaluated the aberrant expression of SHP-1 in epithelial cancer cells, such as estrogen receptor-negative breast cancer cell lines or several prostate or pancreas cancer cell lines.[13,14] In epithelial cells in GI tract, a previous study showed that CpG promoter hypermethylation of SHP-1 was frequently observed in colorectal cancer cells, and demethylating agents (i.e. DNA methyltransferase inhibitor or 5-Aza-2'-deoxycytidine) induced SHP-1 expression to downregulate JAK2/STAT3 signaling.[15]

However, data about SHP-1 expression in gastric cancer are very limited, and several previous studies only briefly reported methylation rate of SHP-1 in gastric cancer tissue as 40 to 70%.[16] We previously investigated the promoter hypermethylation and gene expression of SHP-1 in various gastric cancer cells as well as the anti-invasive effect of SHP-1 by suppression of JAK2/STAT3 activity in SHP-1 negative gastric cancer cells. We found that protein and mRNA expression of SHP-1 were reduced or absent in most of gastric cancer cells by aberrant CpG island promoter hypermethylation. Furthermore, reinforced SHP-1 expression in SHP-1 negative gastric cancer cells downregulated p-JAK2/p-STAT3 as well as their target genes including cyclin D1, matrix metallopeptidase 9 (MMP-9), vascular endothelial growth factor 1 (VEGF1) and survivin. which, in turn, significantly suppressed cell proliferation, migration and invasion in SHP-1 transfected gastric cancer cells.[17] However, underlying mechanism of aberrant SHP-1 expression in gastric cancer is rarely reported. Recently, Prof. Fang and their colleagues demonstrated that the transmembrane protein with epidermal growth factor and two follistatin motifs 2 (TMEFF2) is a

key co-factor of SHP-1, and both cooperatively work to suppress STAT3 activity in gastric cancer cells. The immunofluorescence stain showed the co-expression of TMEFF2 and SHP-1 in the cytoplasm of gastric cancer cells. Furthermore, silencing of SHP-1 significantly attenuated the TMEFF2-induced anti-proliferative and pro-apoptotic effects. Thus, authors concluded that TMEFF2 acts as a tumor suppressor in gastric cancer through direct interaction with SHP-1 via its intercellular domain, and the SH2 1/2 domains of SHP-1 are important for its interaction with TMEFF2 and the tumor suppressive function of TMEFF2.[18] They also showed that mRNA expression of SHP-1 is the highest in normal gastric tissues, followed by intestinal metaplasia, dysplasia and the lowest in gastric cancer tissues. Aberrantly reduced expression of SHP-1 in precancerous lesions and gastric cancers is governed by epigenetic mechanism, and methylation rate of SHP-1 promoter was gradually increased in intestinal metaplasia, dysplasia and cancer compared with normal stomach tissues. Furthermore, tumors with lower expression of both TMEFF2 and SHP-1 significantly showed the worst prognosis among gastric cancer patients, which suggested that both SHP-1 and TMEFF2 are significantly associated with prognosis of gastric cancer.

## Induction of SHP-1 to Inhibit STAT3 in Gastric Cancer Cells

The molecular structure of SHP-1 is that two SH2 domains in N-terminus, phosphatase domain, and C-terminus, which enables autoinhibition of ligand-free SHP-1. That is, SH2 domains of SHP-1 are located at N-terminus, and inserted into the catalytic cleft, thereby repressing the activity of SHP-1. By binding of a phosphotyrosil peptide to SH2 domains or phosphorylation of tyrosines in C-terminus lead to disruption of the interaction between SH2 domains and N-terminus, and in turn, activate phosphatase of SHP-1.[19] A few compounds have been reported to effectively suppress constitutive STAT3 activity by enhancing SHP-1 expression in cancer cells. Among them, Prof. Chen and his colleagues have nicely showed that several chemotherapeutic agents have anti-STAT3 effect via induction of SHP-1 in hepatocellular carcinoma (HCC).[20] They include oral multiple kinase inhibitors such as sorafenib,[21] dovitinib[22] and regorafenib,[23] and their analogues.[24] They showed that SHP-1 tyrosine phosphatase activity is enhanced by its potential to dock to the inhibitory N-SH2 domain and the catalytic phosphatase domain of SHP-1 or directly relieve the inhibitory N-SH2 domain of SHP-1.

Gene and protein expression of SHP-1 are very lacking in most of gastric cancer tissues or cell lines. Thus, upregulation of SHP-1 appears to be a reasonable option for effective inhibition of constitutive STAT3 activity in gastric cancer cells. We recently focused on the role of pantoprazole, which is a well-known proton pump inhibitor (PPI), and is also shows some unexpected effects such as anti-proliferation and enhancing chemosensitivity. We tried to investigate the anti-invasive effect of pantoprazole, and the role of SHP-1/JAK2/STAT3 signaling axis under the effect (under submission). Treatment of pantoprazole upregulated SHP-1, downregulated the p-JAK2/p-STAT3, upregulated epithelial marker (E-cadherin) and downregulated mesenchymal marker (Snail1, vimentin). Pantoprazole also showed same effect in IL-6 stimulated STAT3 activated MKN-28 cells. Immunofluorescence stain also showed that pantoprazole upregulated SHP-1 and downregulated STAT-3 in AGS cells. Pantoprazole also showed significantly increased wound closure distance, decreased number of invasive cells and spindle-like cellular projection, which indicate anti-invasion/migration effect. However, modulation of SHP-1/p-JAK2/p-STAT3 expression and anti-invasion/migration effect by pantoprazole were attenuated by the pharmacologic inhibitor or knockdown of SHP-1, which suggested that SHP-1 might be an important mediator for downregulation of STAT3 activity by pantoprazole. In xenograft tumor model, intraperitoneal injection of pantoprazole significantly reduced the tumor size and volume, which was attenuated by concomitant injection of pharmacologic inhibitor. Taken together, we suggest that pantoprazole downregulates JAK2/STAT3 signaling through induction of SHP1 expression to modulate the expression of EMT markers, and inhibit cellular migration and invasion in gastric cancer cell.

We also found that arsenic trioxide, a chemotherapeutic agent that is widely used in the treatment of hematopoietic malignancies, showed same effect in gastric cancer cells, which suggested that demethylation of SHP-1 promoter may be one of molecular link for induction of SHP-1 and downregulation STAT3 activity in gastric cancer cells.[25] A previous study also showed that treatment of honokiol, a small-molecular weight natural product, downregulated p-STAT-3 expression, which was reversed by pervanadate or transfection of siSHP-1 in AGS cells. They also showed that intraperitoneal injection of honokiol significantly upregulated SHP-1 expression in xenograft tumors. Thus they suggested that honokiol upregulates SHP-1 expression through increase of calpain-II, a calcium-activated nonlysosomal cysteine protease, and in turn, dephosphorylate STAT-3 in gastric cancer cells.[26]

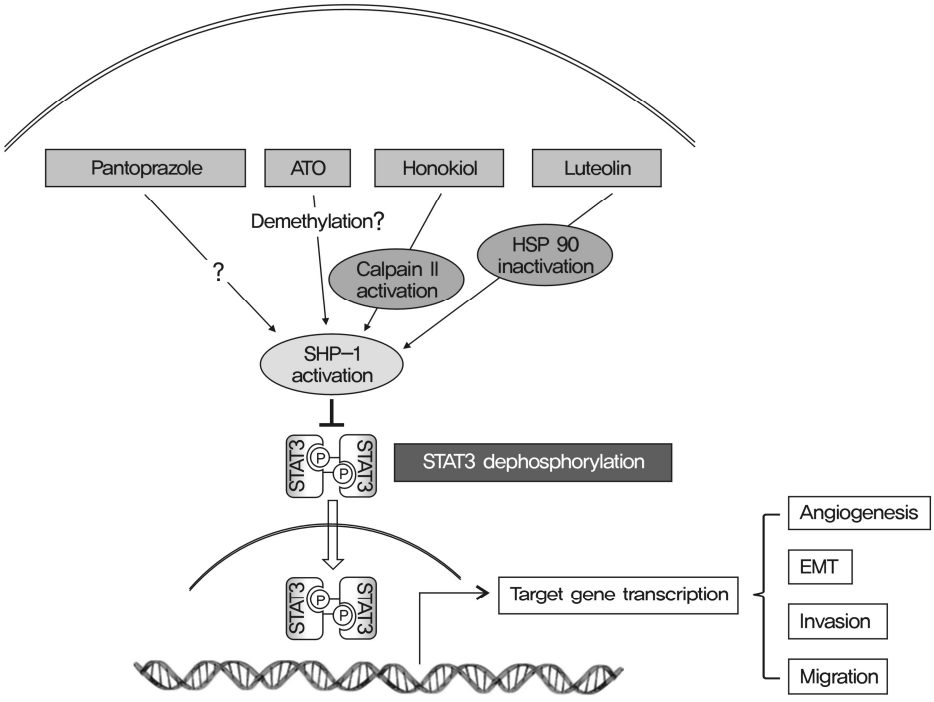


**Fig. 1.** Signaling pathway of SHP-1 activation by pharmacologic intervention in gastric cancer cells. Demethylation by ATO, activation of calpain-II by honokiol and inhibition of binding of HSP-90 to STAT3 may mediate the activation of SHP-1 and dephosphorylation of STAT3. ATO, arsenic trioxide; HSP-90, heat shock protein 90; SHP-1, SH2-containing protein tyrosine phosphatase 1; STAT3, signal transducer and activator.

Recently, Dr. Gao and their colleagues showed that luteolin, a plant flavonoid, inhibited STAT3 activation through disrupting the binding of HSP-90 to STAT3, which promoted its interaction to SHP-1, resulted in the dephosphorylation of STAT3 (Fig. 1).[27]

## CONCLUSION

In summary, the biologic impact of STAT3 in gastric carcinogenesis and progression has been widely accepted in the previous studies. A lot of efforts have been made to effectively inhibit the STAT3 activity including direct STAT3 inhibitors, which unfortunately showed no significant impacts in the clinical studies. SHP-1 as an effective phosphatase for inactivation of JAK2/STAT3 activity may be applied in gastric cancer. The exploration of more effective enhancer of SHP-1and its underlying mechanisms in gastric cancer need to be investigated.

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### Competing Interests

All authors have no potential competing interests (financial, professional, or personal) that are relevant to this publication.

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