Inhibition of STAT3 in Gastric Cancer;

Role of SHP-1 Induction

Moon Kyung Joo M.D., Ph.D.

Division of Gastroenterology, Department of Internal Medicine
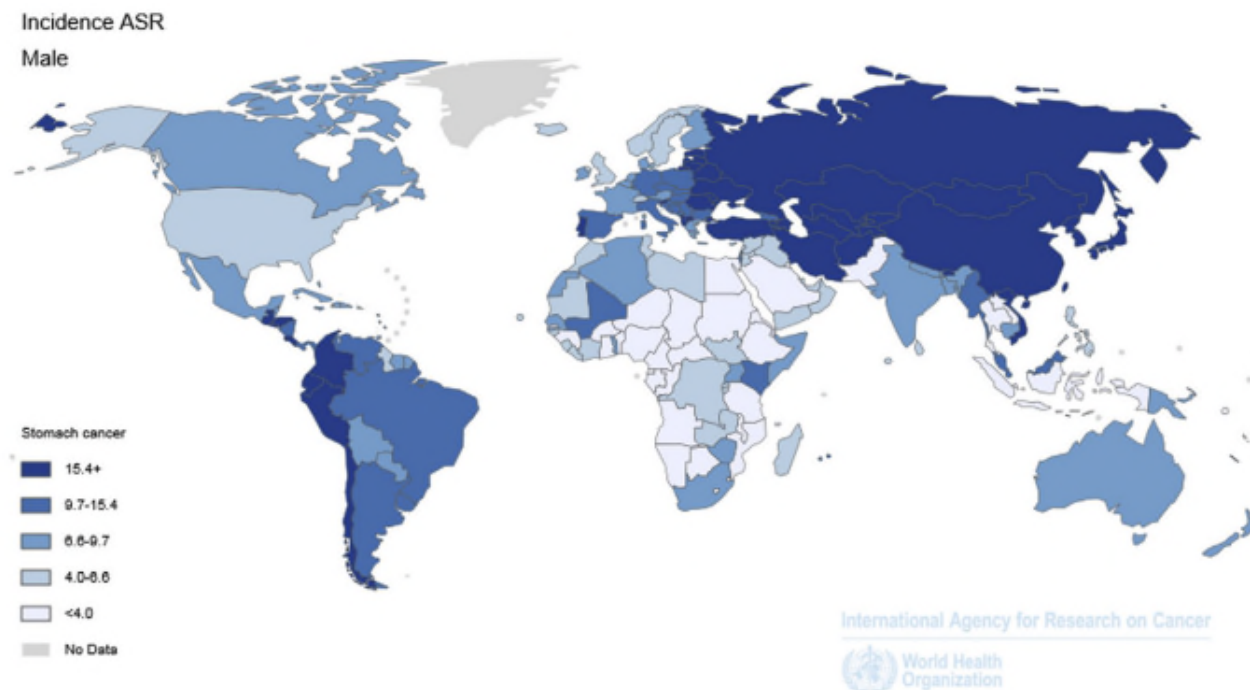
Korea University College of Medicine Guro Hospital

KU MC **KOREA** UNIVERSITY MEDICAL CENTER

KU MC 고려대학교구로병원 KOREA UNIVERSITY GURO HOSPITAL

Contents

Introduction

✓ Gastric cancer

- 3rd leading cancer-related cause of death
- 720,000 deaths per year worldwide

Incidence ASR
Male

Stomach cancer
- 15.4+
- 9.7-15.4
- 6.6-9.7
- 4.0-6.6
- <4.0
- No Data

International Agency for Research on Cancer

World Health Organization

Source: GLOBOCAN 2012 (IARC)

Herrero, R. et al. Best Pract Res Clin Gastroenterol 2014;28 (6):1107-14

Introduction

- ✓ Advanced gastric cancer
    - Chance for cure is getting lower dramatically
    - OS of non-curable stage IV gastric cancer ≤ 18 months
- ➢ A long way till the conquest of gastric cancer

Fujitani, K. et al. Lancet Oncol 2016;17 (3):309-18
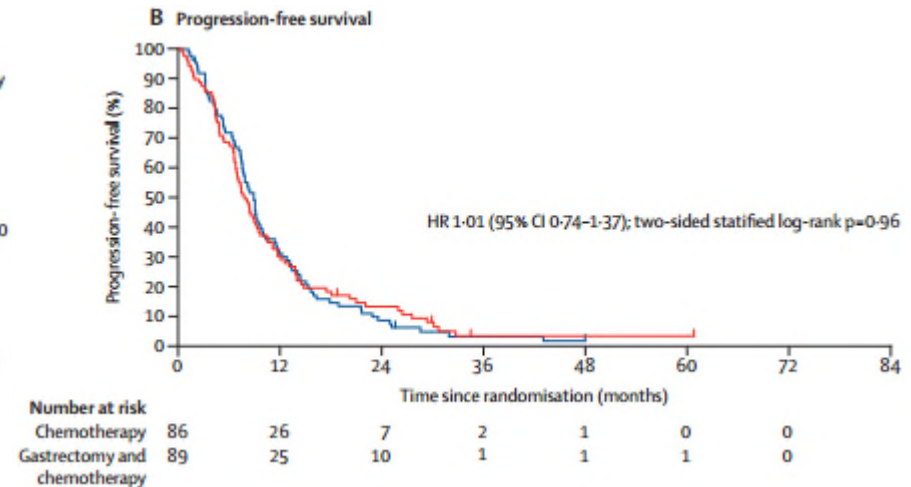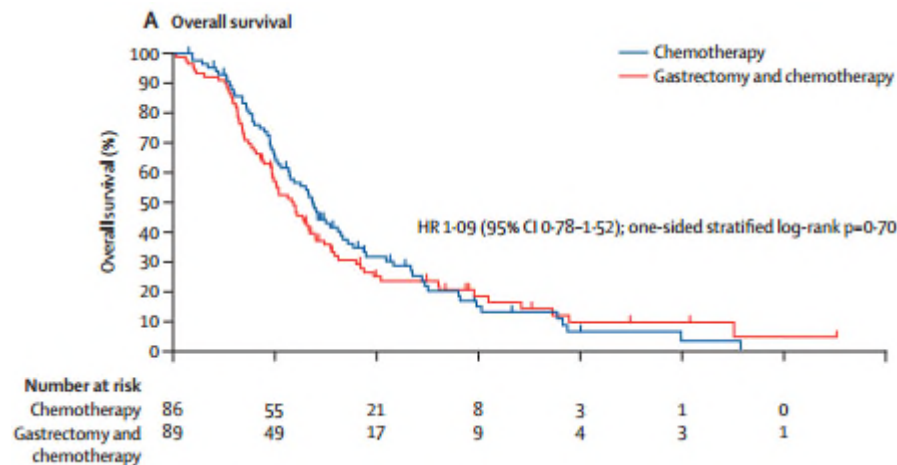
STAT3 Signaling in Gastric Cancer

- ✓ JAK2/STAT3 signaling pathway

  - Inflammatory process by chronic *H. pylori* infection

  - Interaction between gastric epithelium and microenvironmental stromal cells

  - Migration or invasion of cancer cells

- ➤ <u>Inhibition of JAK2/STAT3 signaling</u>: a reasonable option for control of multiple steps in gastric carcinogenesis and invasion

Buchert, M. et al. Oncogene 2016;35 (8):939-51
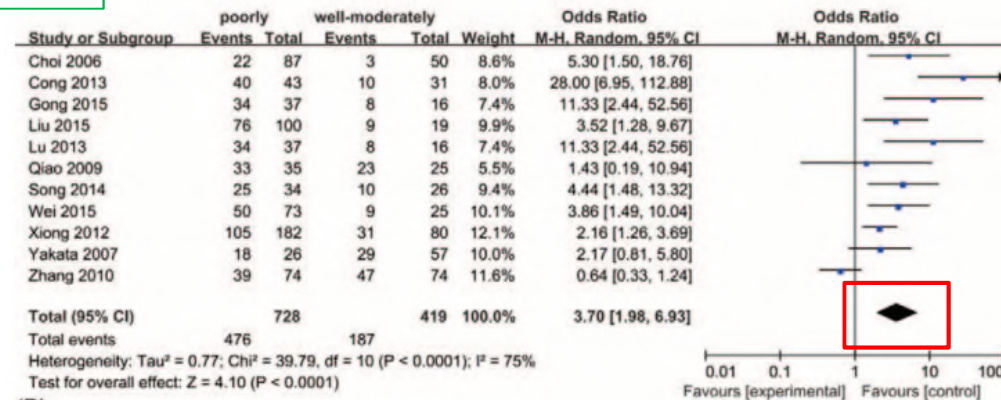
Pathways Activating JAK–STAT3 Signaling in Cancer
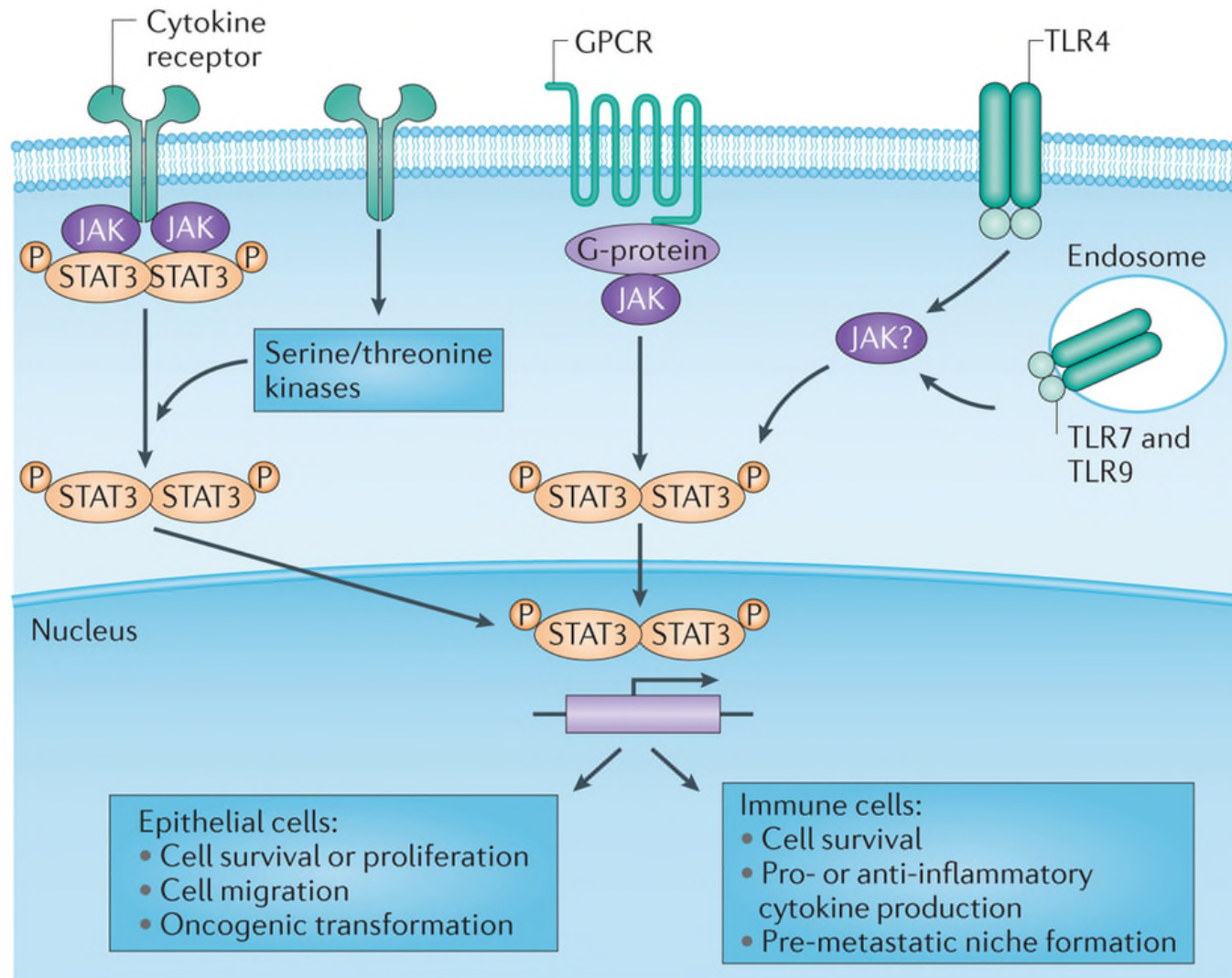
Constitutive Activation of STAT3 in Gastric Cancer

A. Lymph node metastasis
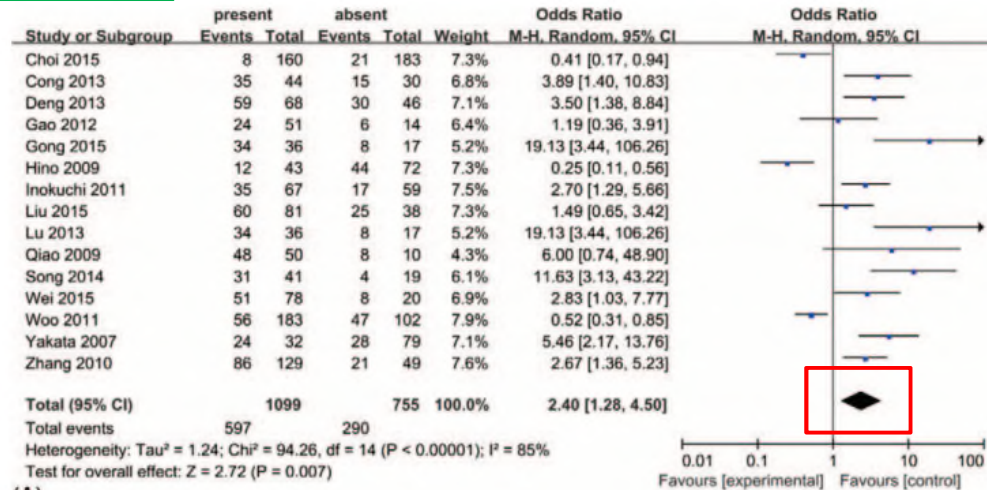
(A)

B. Tumor differentiation

(B)

Ji, K. et al. Medicine (Baltimore) 2016;95(5):e2641

STAT3 inhibition in gastric cancer

✓ Most of STAT3 inhibitors have been evaluated in experimental studies.

✓ Strategies to inhibit STAT3 activity

- Direct inhibition of STAT3 targeting

    - Src homology 2 (SH2) domain

    - DNA binding domain

    - N-terminal domain

    - Oligonucleotide-based inhibition (i.e. siRNA, decoy oligonucleotide technology)

- Indirect inhibition of upstream intracellular kinases (i.e. JAK2, Src kinase)

Schematic structural domains of the STAT3 proteins

Experimental STAT3 Inhibitors in Gastric Cancer

Inhibitor	Study design	Functional effects	Suggested mechanism of STAT3 inhibition	Reference
Flucoxanthin	*In vitro*	Cell cycle arrest ↑ Apoptosis	Not presented	(Yu et al. 2011)
DDP	*In vitro*	↓ Chemoresistance ↑ Apoptosis	Not presented	(Huang et al. 2012)
Honokiol	*In vitro/in vivo*	↓ Angiogenesis and peritoneal Dissemination	Increase of SHP-1	(Liu et al. 2012)
OPB-31121	*In vitro/in vivo*	↑ Apoptosis Synergism with 5-FU and cisplatin ↑ Apoptosis	Downregulation of JAK2 SH2 domain of STAT3	(Kim et al. 2013)
Pantoprazole	*In vitro*	↓ Secretion of pro-inflammatory cytokine (IL-6)	Not presented	(Huang et al. 2013)
AZD 1480	*In vivo*	↑ Apoptosis ↓ IL-11	Inhibition of JAK1/2 phosphorylation	(Stuart et al. 2014)
WP1066	*In vitro/in vivo*	↓ Pro-inflammatory cytokines (IL-11, IL-6, IL-1β)	Inhibition of JAK2 phosphorylation	(Judd et al. 2014)
Plumbagin	*In vitro*	↓ Migration/invasion ↑ Apoptosis	Induction of SHP-1	(Joo et al. 2015)
Salinomycin	*In vitro/in vivo*	↓ Angiogenesis ↓ VEGFR2 phosphorylation	Not presented	(Li et al. 2016)
JSI-124	*In vitro/in vivo*	↓ Angiogenesis ↓ VEGF	Not presented	(Wu et al. 2016)

STAT3 Inhibitors in Gastric Cancer
- Limitations -

- ✓ Only evaluated in pre-clinical studies.

- ✓ Clinical outcomes including sufficient number of gastric cancer patients are especially lacking.

- ✓ Technical difficulties to develop more suitable and effective agent directly targeting STAT3.

- ➢ New strategy for inhibition of STAT3 activity is suggested.

SH2-containing Protein Tyrosine Phosphatase 1 (SHP-1)
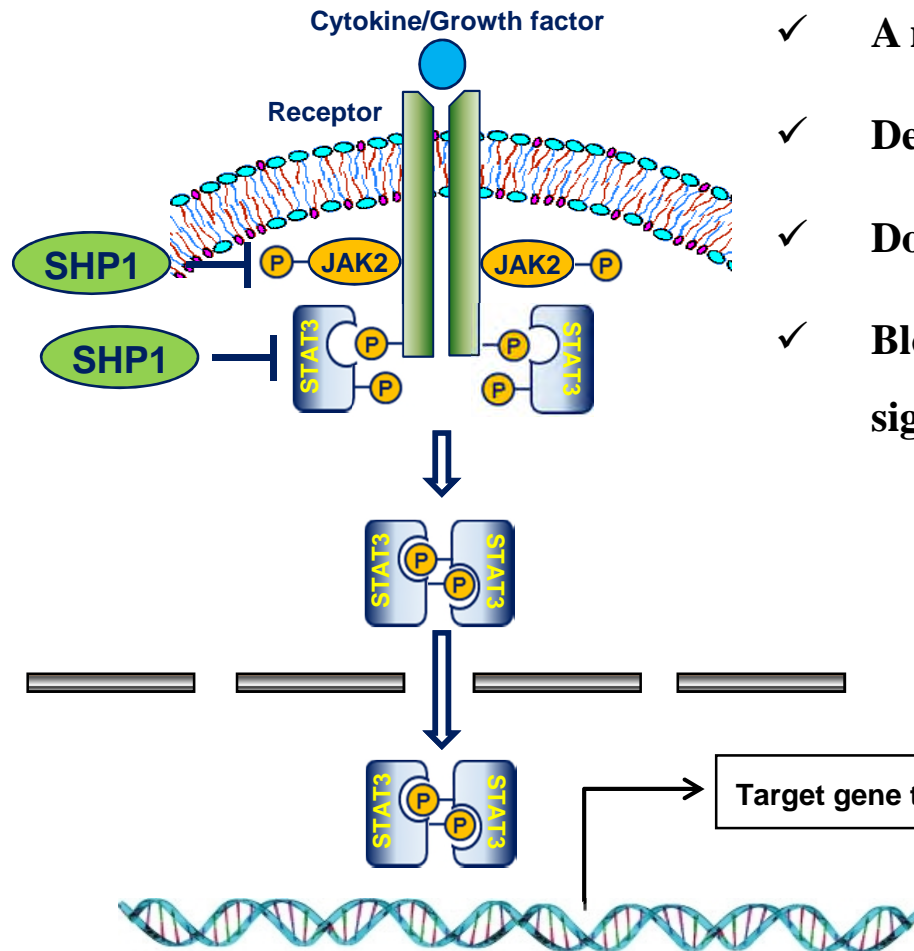
- ✓ A non-receptor type protein Tyr phosphatase

- ✓ Dephosphorylation of JAK2 and STAT3

- ✓ Downregulation STAT-3 activity

- ✓ Blockage of the STAT3-mediated cellular signaling pathways

SHP-1 Expression in Epithelial Cancer Cells

✓ Lack of data

- Estrogen receptor-negative breast cancer cell lines

- Prostate or pancreas cancer cell lines

✓ Epithelial cells in gastrointestinal (GI) tract

- CpG promoter hypermethylation of SHP-1 in colorectal cancer cells

- Demethylating agents (DNA methyltransferase inhibitor, 5-Aza-2'-deoxycytidine) restored SHP-1 expression to dephosphorylate JAK2/STAT3

Yip, S. et al. Int J Cancer 2000;88 (3):363-8

Zapata, P. D. et al. J Clin Endocrinol Metab 2002;87 (2):915-26

SHP-1 Expression in Gastric Cancer

- ✓ <u>Limited data</u>

  - Methylation rate: 40~70% in gastric carcinoma tissues

- ➢ Our previous study

  - Promoter hypermethylation and gene/protein expression of SHP-1 in various gastric cancer cell lines.

  - Functional effects of <u>SHP-1</u> on the cell proliferation and migration through the <u>modulation of JAK2/STAT3 pathway</u>.

SHP-1 Expression in Gastric Cancer Cells

A. Gene and protein expression
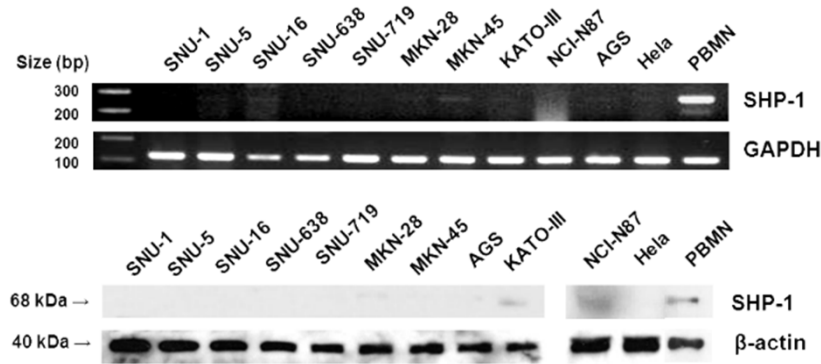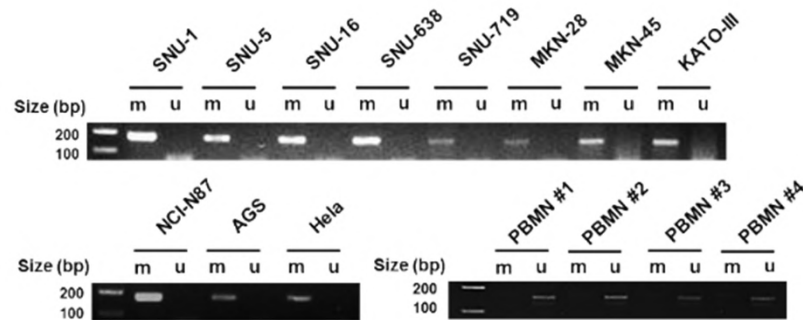
C. Bisulfite pyrosequencing

B. Methylation-specific PCR

Joo, M.K. et al. Tumour Biol 2016;37 (4):4603-12
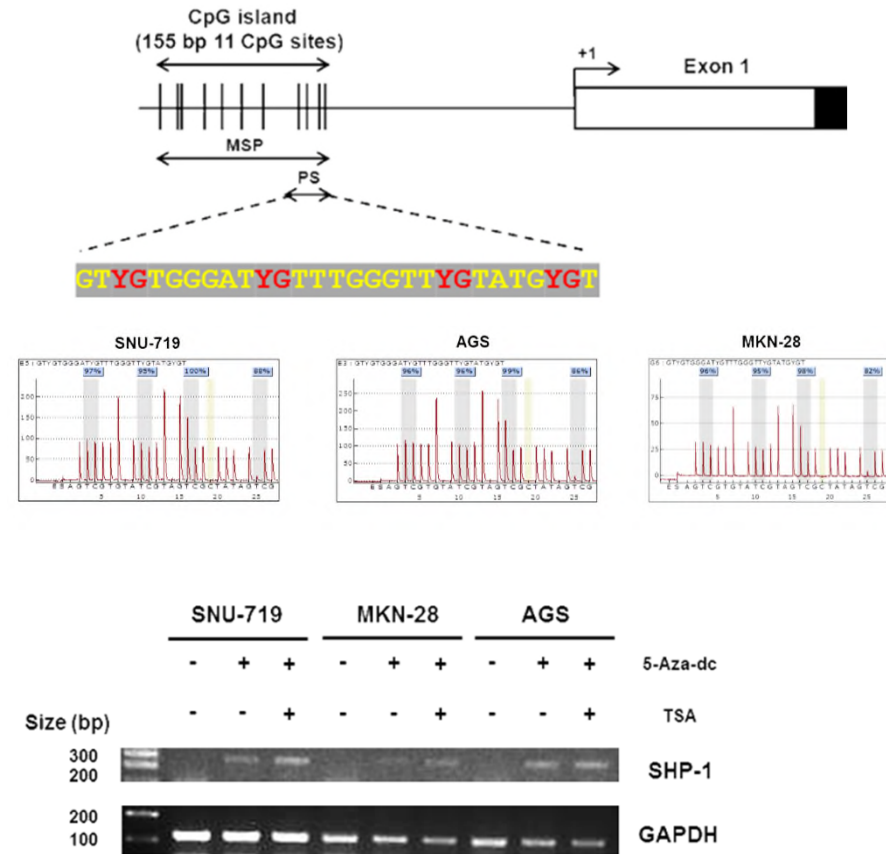
Reinforced SHP-1 Expression in SHP-1 (-) Gastric Cancer Cells

A. Western blot

C. Migration and Matrigel invasion assay

B. RT-PCR

Joo, M.K. et al. Tumour Biol 2016;37 (4):4603-12
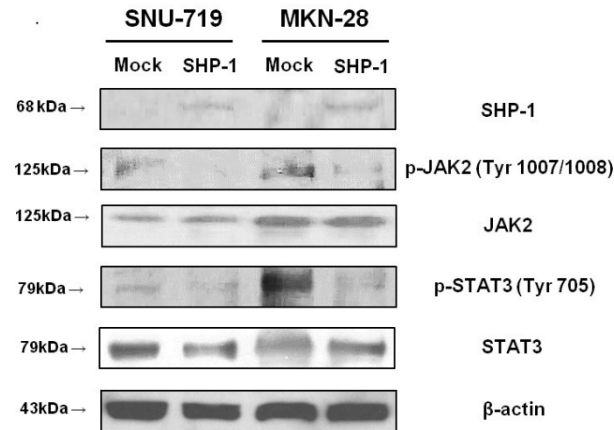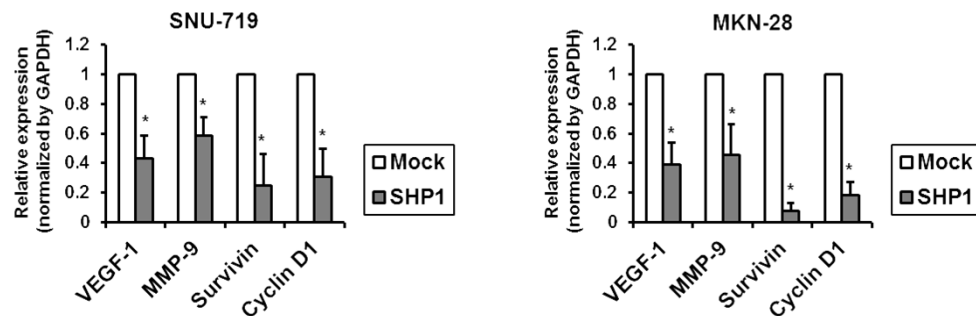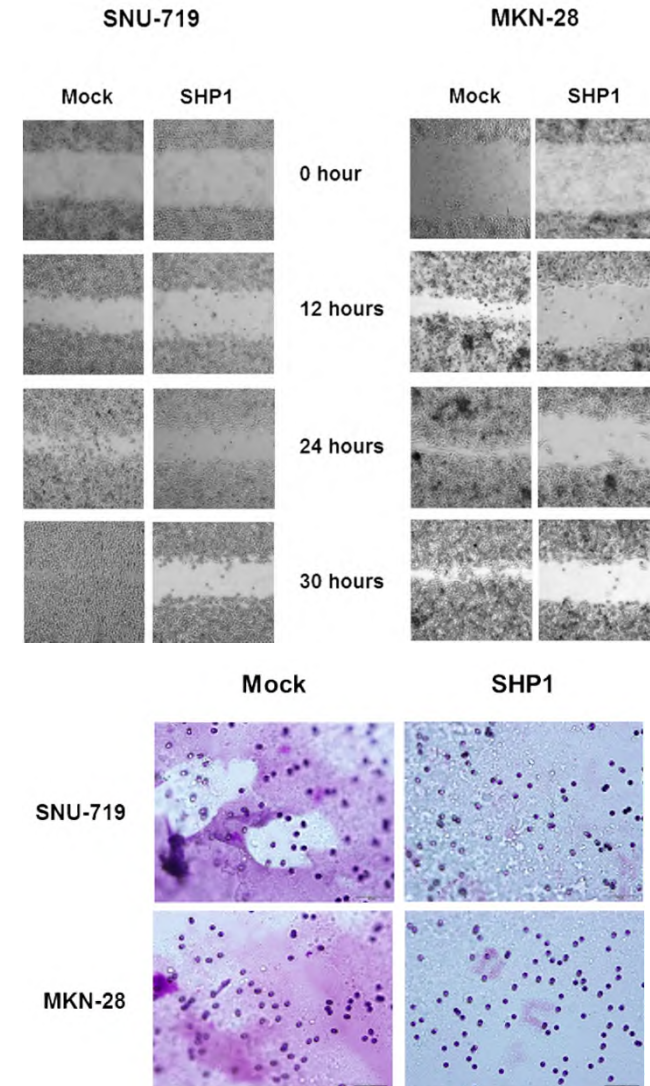
SHP-1 in Gastric Cancer Cells

✓ Protein and mRNA expression of SHP-1 was reduced or absent in most of gastric cancer cells by aberrant CpG island promoter hypermethylation.

✓ Reinforced SHP-1 expression in SHP-1 (-) gastric cancer cells inhibited JAK2/STAT3 activity and their target genes including cyclin D1, MMP-9, VEGF1 and survivin.

✓ This, in turn, suppressed cell proliferation, migration and invasion in SHP-1 transfected gastric cancer cells.

Joo, M.K. et al. Tumour Biol 2016;37 (4):4603-12

Mechanism of Aberrant SHP-1 Expression in Gastric Cancer
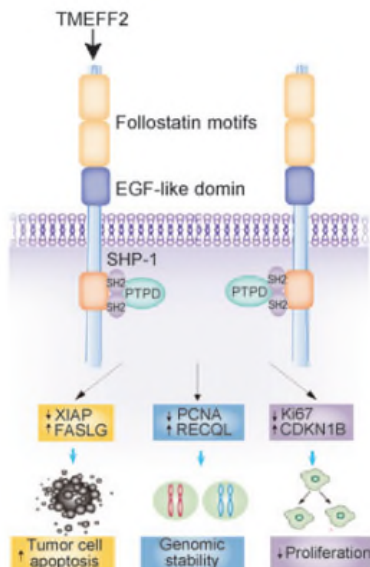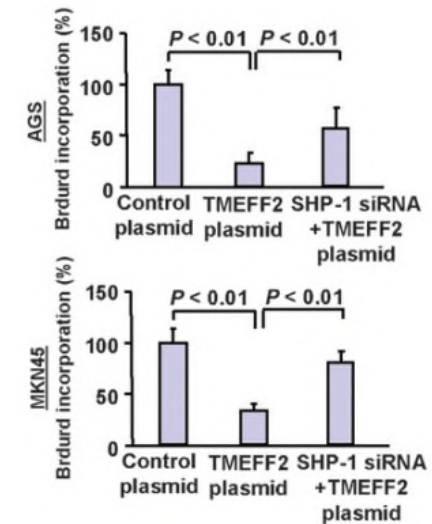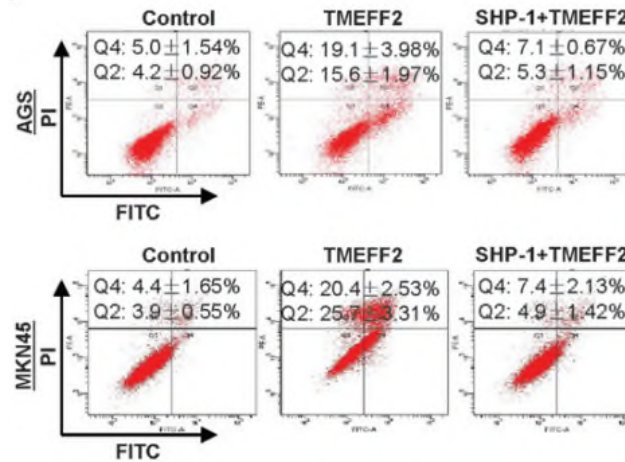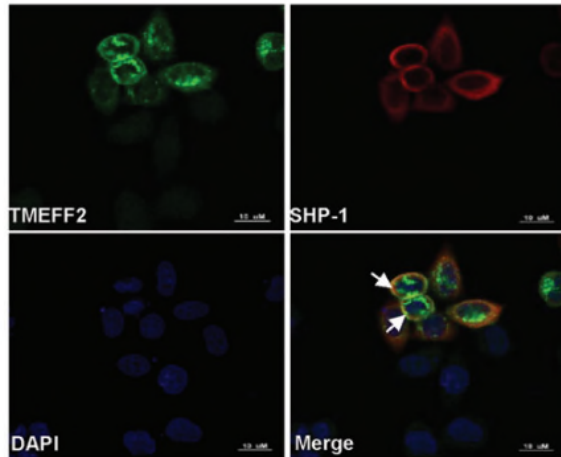
- ✓ TMEFF2 acts as a tumor suppressor in gastric cancer through direct interaction with SHP-1 via its intercellular domain.

- ✓ The SH2 1/2 domains of SHP-1 are important for its interaction with TMEFF2 and the tumor suppressive function of TMEFF2.

Sun, T. et al. Clin Cancer Res 2014;20 (17):4689-704

Aberrant Expression of SHP-1 and TMEFF2 in Gastric Cancer Tissues

A. Gene expression of SHP-1
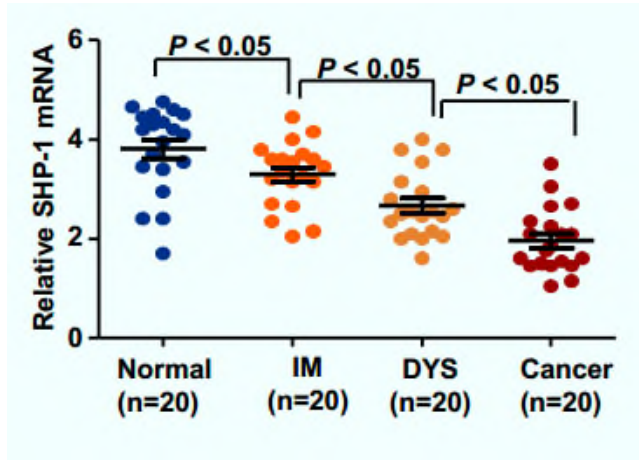
B. Methylation-specific PCR
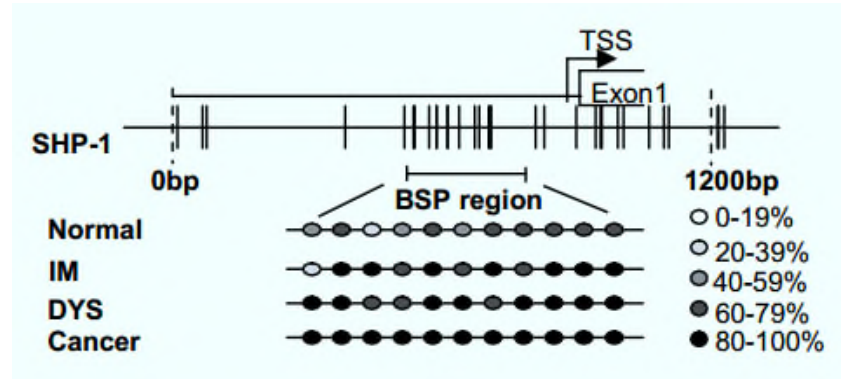
C. Overall survival according to the TMEFF2 and SHP-1 expression
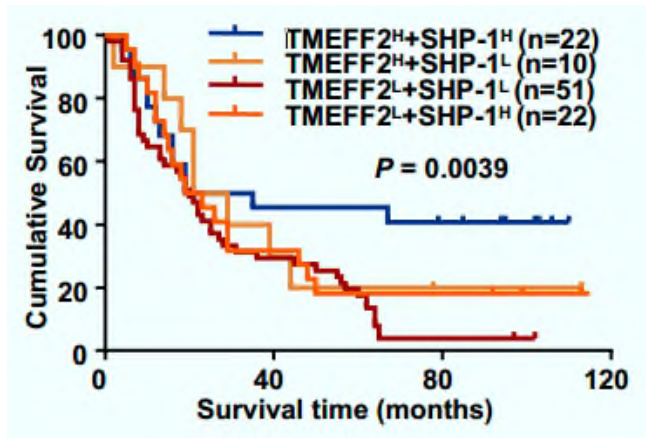
Induction of SHP-1 to Inhibit p-STAT3 in Gastric Cancer

✓ <u>Expression of SHP-1 is minimal</u> in most of gastric cancer tissues or cell lines.

✓ <u>SHP-1-induction strategy</u> appears to be reasonable to effectively inhibit constitutive STAT3 activity and alternative therapeutic option in gastric cancer.

Induction of SHP-1 to Antagonize p-STAT3 in Gastric Cancer Cells: Pantoprazole

- ✓ Pantoprazole (PPZ)

  - An effective proton pump inhibitor

  - Unexpected effects; anti-proliferation, enhancing chemosensitivity in cancer cell

  - Suggested underlying mechanism: inhibition of p-STAT3

- ➤ To investigate the <u>anti-invasive effect of PPZ</u> in gastric cancer cell

- ➤ To investigate <u>the role of SHP-1-JAK2-STAT3 signaling axis</u> associated with anti-invasive effect of PPZ in gastric cancer cell

Materials and Methods

Human Gastric Cancer Cell Lines
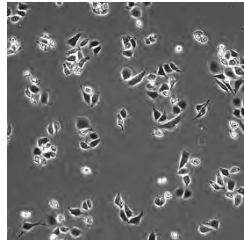
(AGS cell)

⬇

Minimal SHP-1 expression

Constitutive p-JAK2/p-STAT3 activity

Mesenchymal phenotype

⬇

Pantoprazole treatment

✓ **Functional studies**

  - 3-D spheroid culture assay

  - Wound closure assay

  - Matrigel invasion assay

✓ **Western blot / immunofluorescence**

  - p-JAK2/p-STAT3, SHP-1

  - Mesenchymal marker (Snail1, vimentin)

  - Epithelial marker (E-cadherin)

✓ **Validation of SHP-1-mediated PPZ effect**

  - Pre-treatment with PTPase inhibitor / transfection siRNA

✓ **Xenograft tumor model by using male nude mouse**

  - Intraperitoneal injection of

   ; PPZ (180mg/kg, 2 times weekly)

   ; PV co-IP injection (18mg/kg, 2 times weekly)

Inhibition of STAT3 via Induction of SHP-1 by PPZ in Gastric Cancer Cells

Joo M.K. et al. EHMSG 2017
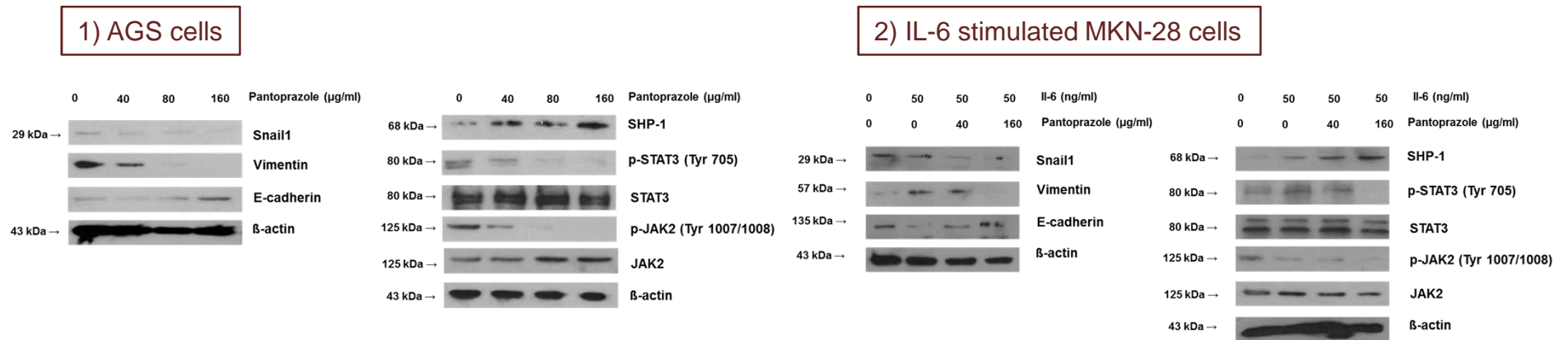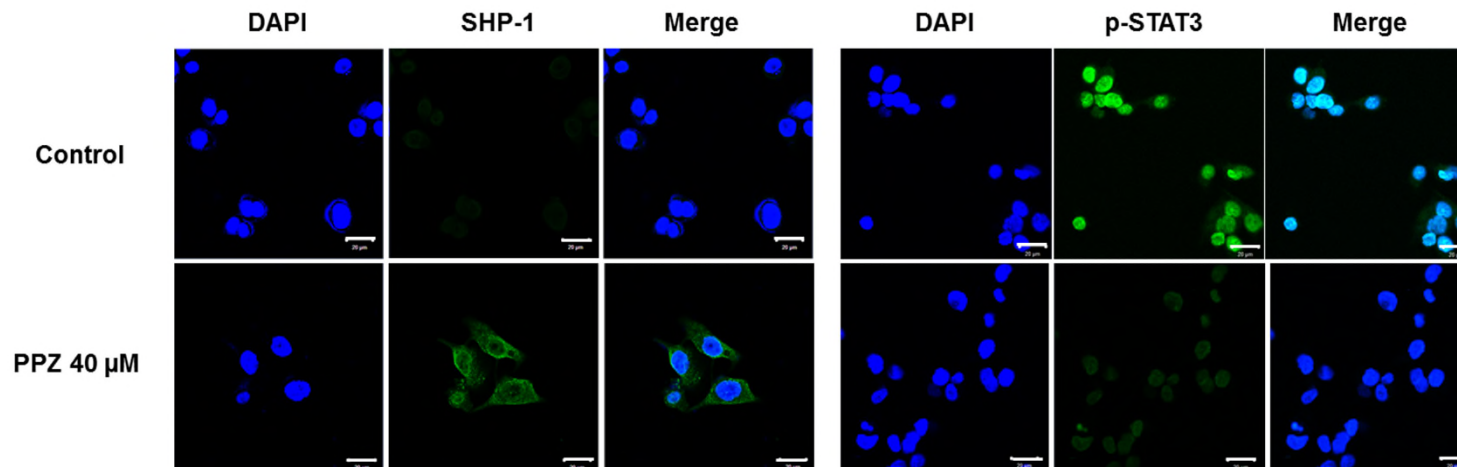
Anti-invasive Effect of PPZ via Induction of SHP-1 in Gastric Cancer Cells
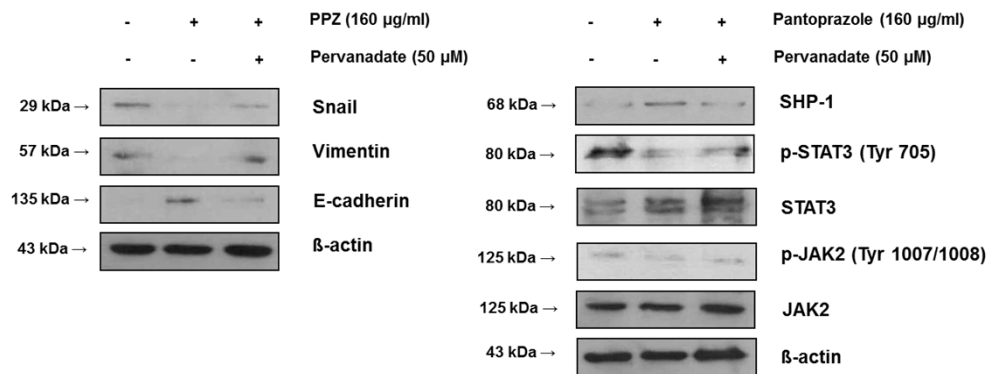
A. Migration and Matrigel invasion assay
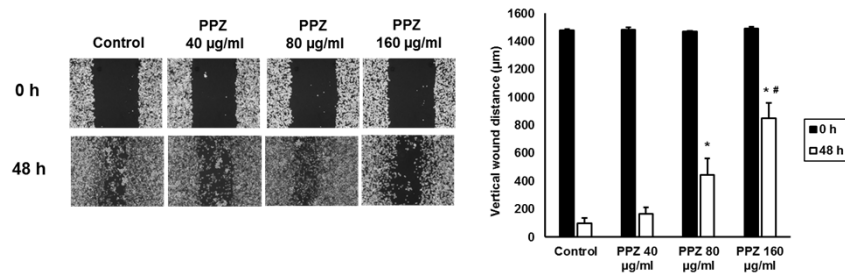
B. 3-D spheroid culture invasion assay
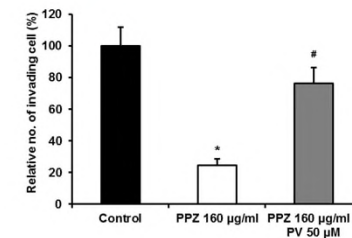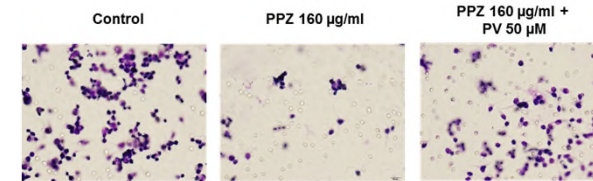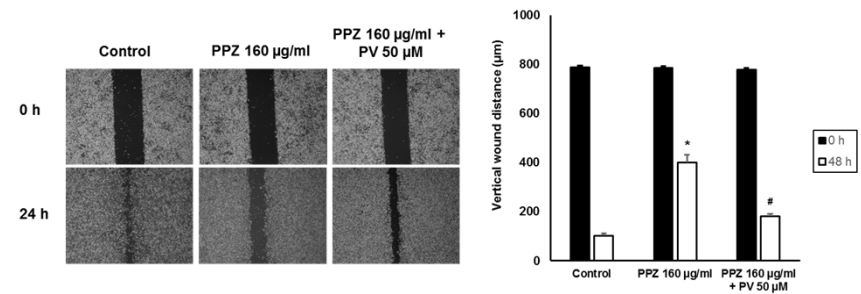

Joo M.K. et al. EHMSG 2017
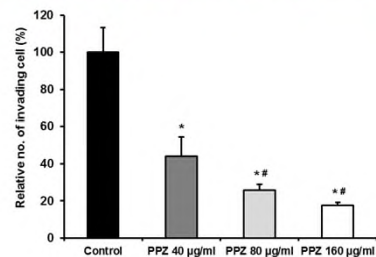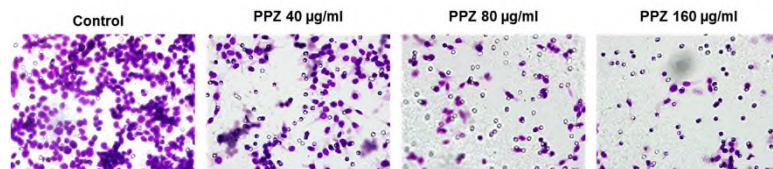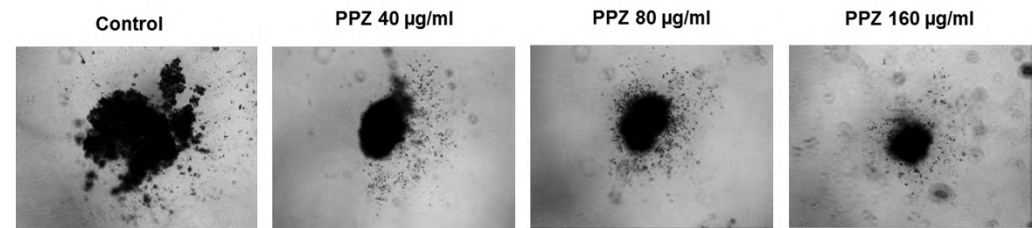
Anti-invasive Effect of PPZ in Gastric Cancer Cell is Attenuated by PTPase Inhibitor

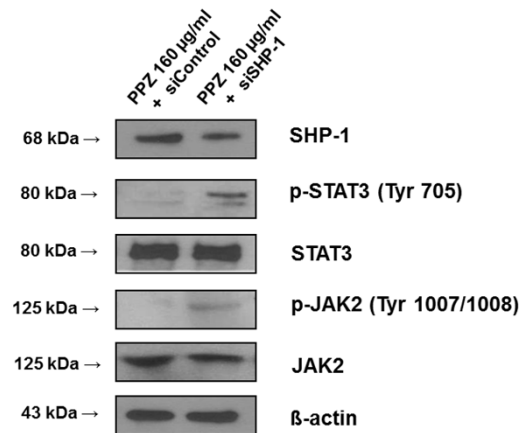**A. Western blot**

B. Migration and Matrigel invasion assay

C. 3-D spheroid culture invasion assay
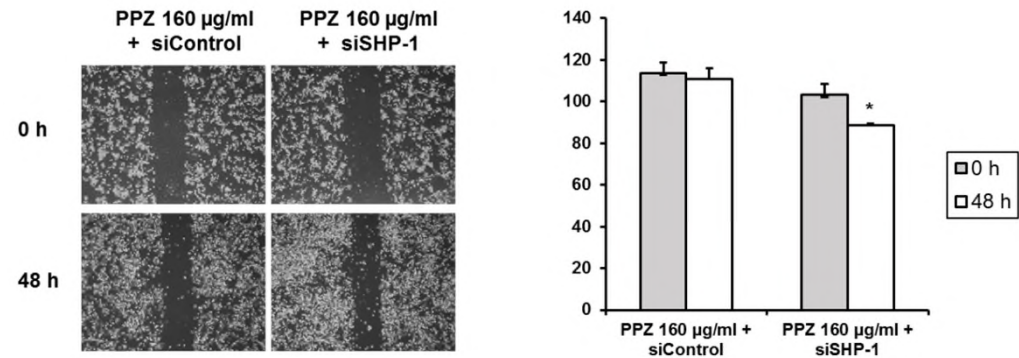

Joo M.K. et al. EHMSG 2017
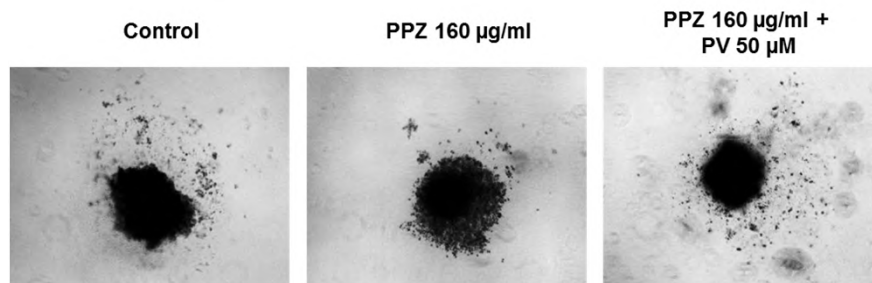
Anti-invasive Effect of PPZ in Gastric Cancer Cell is Attenuated by SHP-1 siRNA

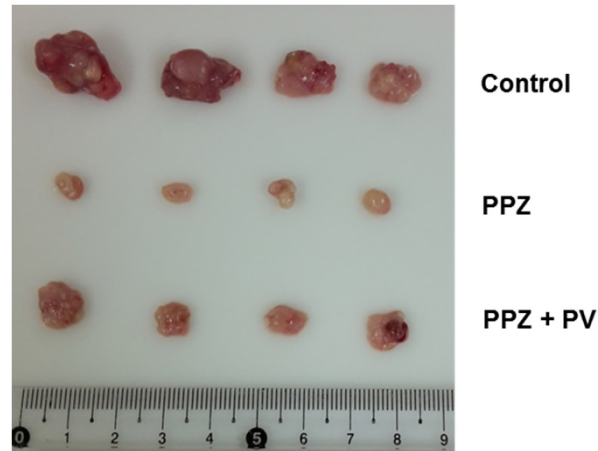**A. Western blot**

B. Migration and Matrigel invasion assay
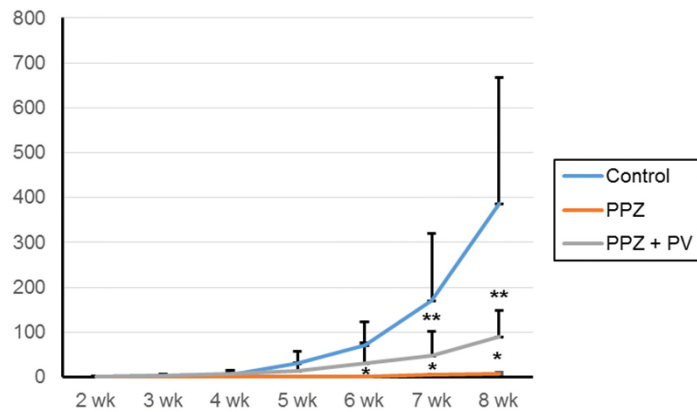

C. 3-D spheroid culture invasion assay

Joo M.K. et al. EHMSG 2017
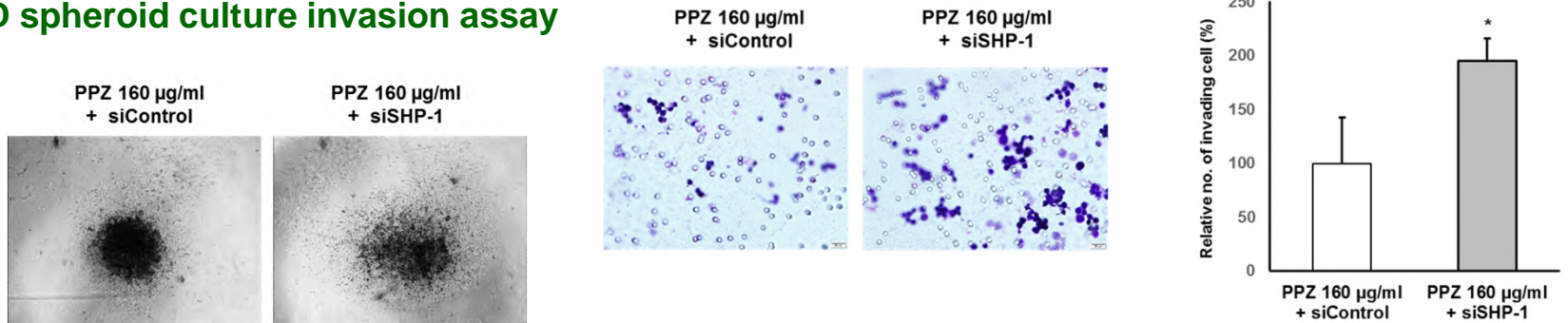
Anti-Tumor Effect of PPZ in Xenograft Tumor Model

A. Gross picture of xenograft tumors

B. Difference of tumor volume

C. Difference of body weigt

Joo M.K. et al. EHMSG 2017
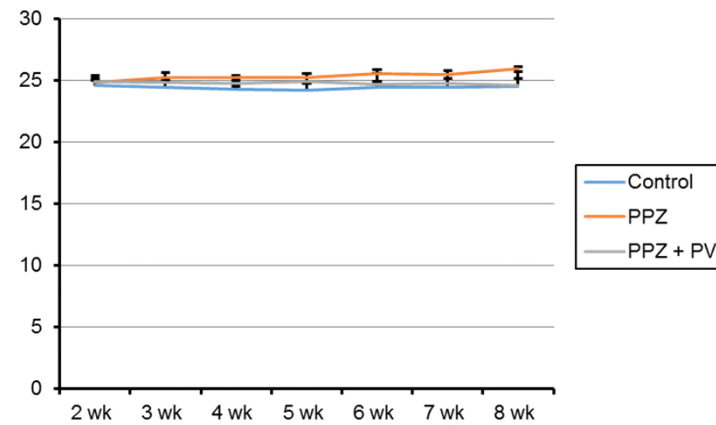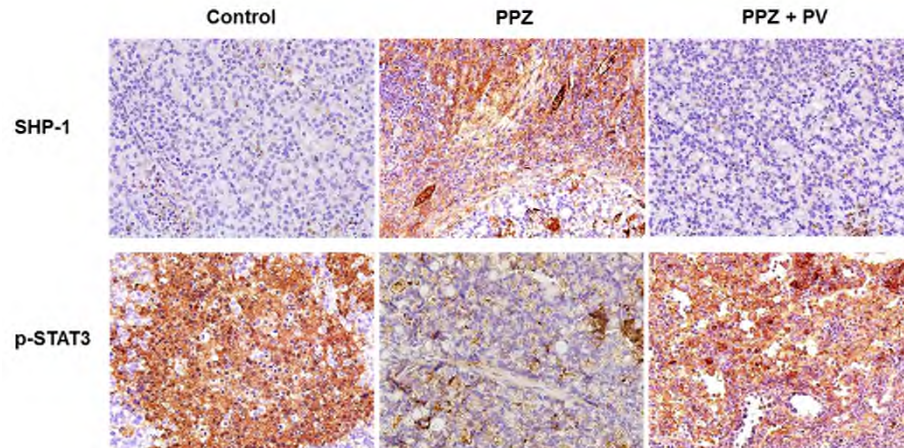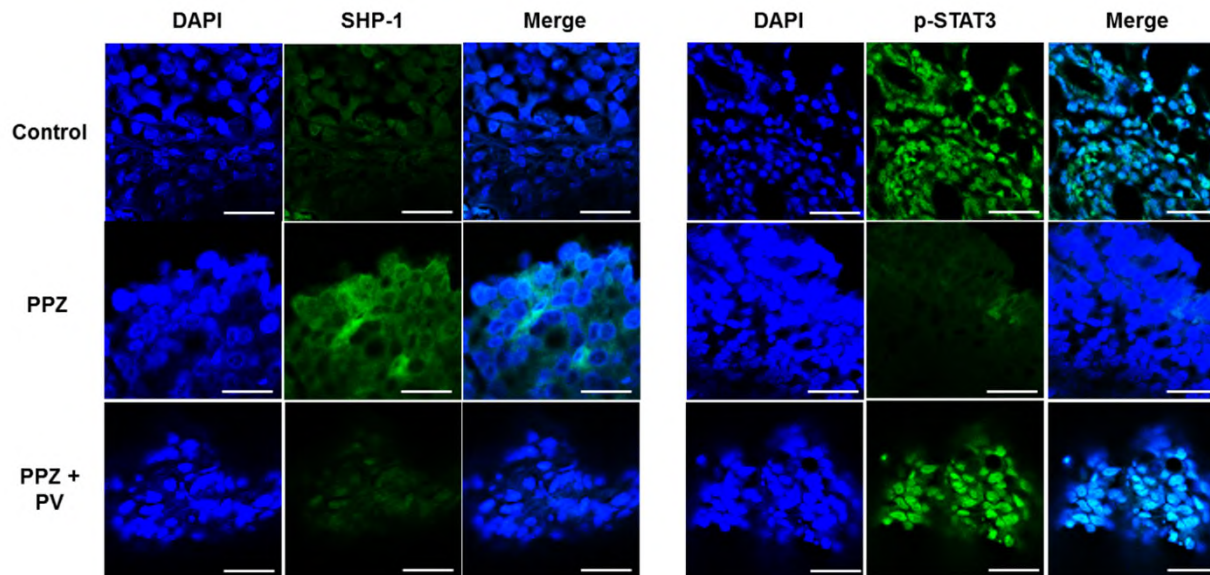
Anti-Tumor Effect of PPZ in Xenograft Tumor Model

A. Immunohistochemical stain

B. Immunofluorescence stain

Joo M.K. et al. EHMSG 2017

Anti-Invasive Effect of PPZ in Gastric Cancer Cells

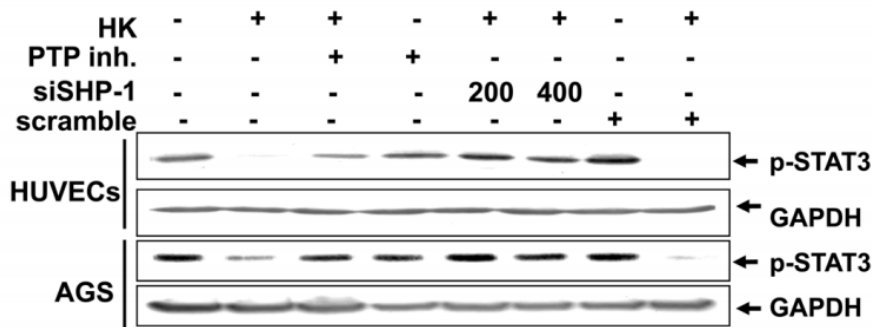**PPZ downregulates JAK2/STAT3 signaling through induction of SHP-1 expression to modulate expression of EMT markers and inhibit cellular migration/invasion in gastric cancer cell.**
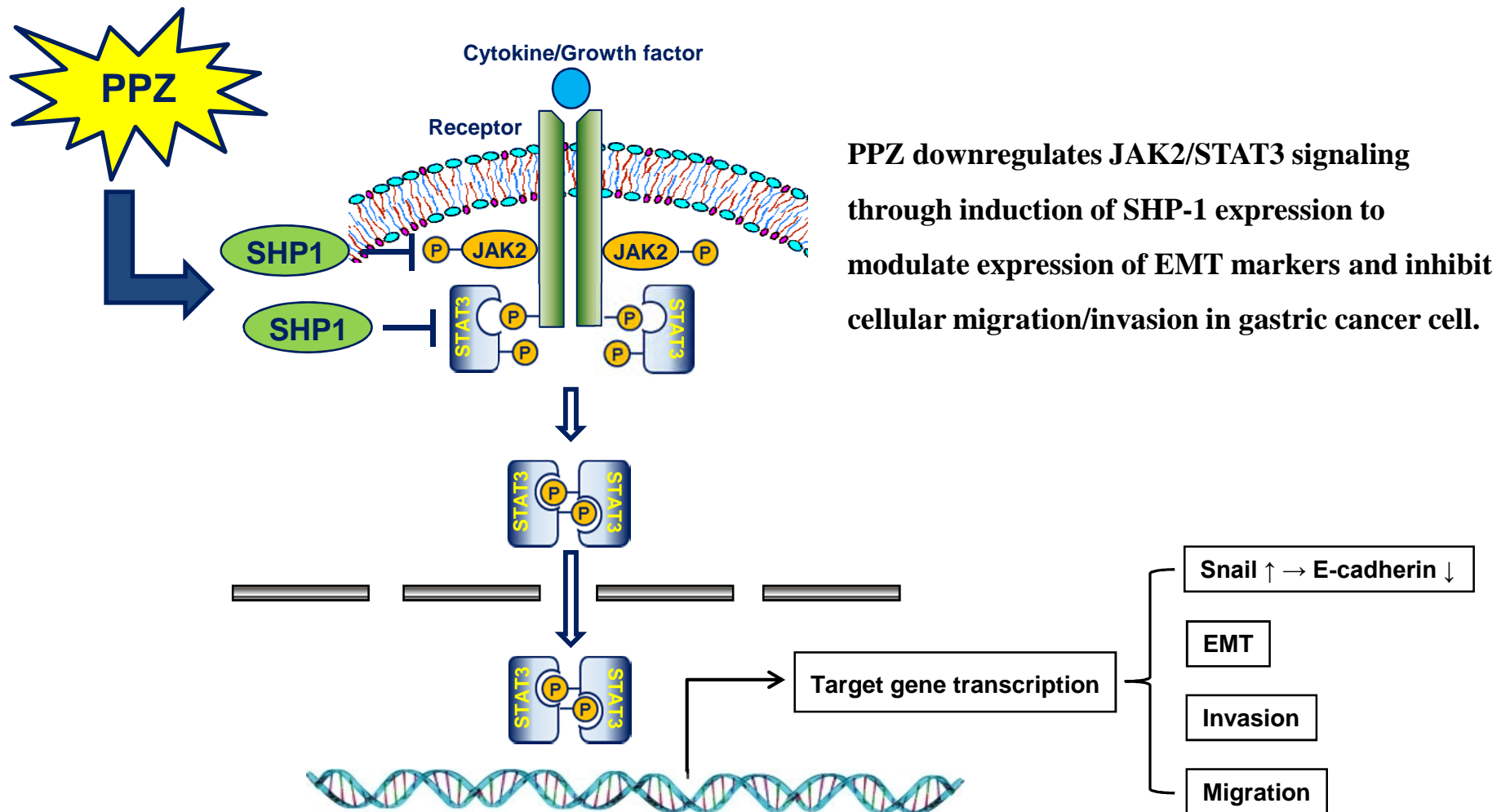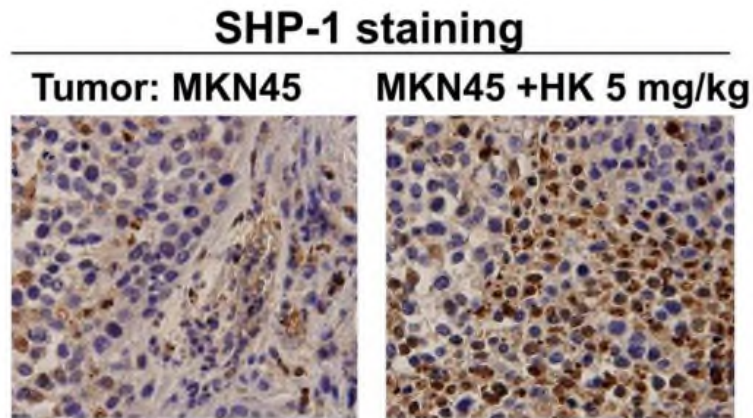
Effect of Honokiol through the Calpain II/SHP-1/STAT-3 Axis in Gastric Cancer

A. Western blot

B. Immunohistochemistry of metastatic nodule

C. A proposed mechanism for honokiol

Liu, S. H. et al. PLoS One 2012;(8):e43711
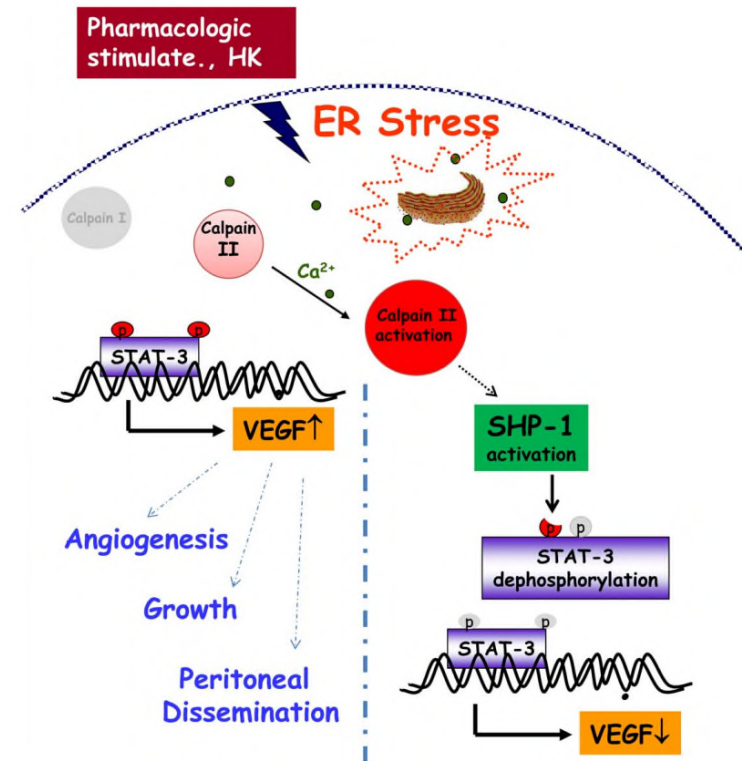
Summaries

➤ 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 less significant clinical impacts.

➤ SHP-1 as an effective phosphatase for inactivation of JAK2/STAT3 may be applied in gastric cancer.

➤ The exploration of more effective enhancer of SHP-1 and its underlying mechanisms in gastric cancer need to be investigated.

Thank you for your attention.