SRMS as a Novel Therapeutic Target in Gastric Cancer Peritoneal Metastases

Jaqueline Quintanilla[1,2], Ailing W Scott[1], Jiankang Jin[1], Yibo Fan[1], Lang Ma[1], Jaffer A Ajani[1], Shumei Song[1]

[1] Department of GI Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

[2] Department of Health & Biomedical Sciences, The University of Texas Rio Grande Valley, Brownsville, TX 78520, USA

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Introduction

Affecting over 1 million people in the past year, gastric adenocarcinoma (GAC) is the 5th most common cancer in the population and the 4th most common cause of cancer fatalities.[1] GAC with peritoneal carcinomatosis (PC) is common affecting ~45% of patients during the course of GAC and leads to poor survival, but the molecular events leading to PC are not clear.[2] Our recent RNAseq of PC specimen revealed that SRMS (Src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristylation sites), a nonreceptor tyrosine kinase, was amplified in PC samples and highly upregulated in the diffuse type and signet-ring cell (SRC) subtypes of GAC with especially poor prognosis. We aim to elucidate its function and whether it could be a therapeutic target in GAC with PC.

Methods

Western blot and real-time polymerase chain reaction (qPCR) were used to determine the expression of SRMS in GAC cell lines and tissues. Transfection of SRMS cDNA in AGS cells utilized the jetPRIME® protocol to study the function and signaling activated by SRMS. GA0518 patient-derived PC cells, AGS GAC cells and Flo-1 radiation resistant XTR cells were used to study the functions of SRMS including proliferation, invasion, and tumor sphere forming capacity *in vitro.* Dasatinib, an inhibitor of SRC, being reported to suppress SRMS and a novel inhibitor HJC0378 were tested for their suppression of SRMS and other oncogenic signaling as well as on inhibition of tumor cell malignant phenotypes.

Results

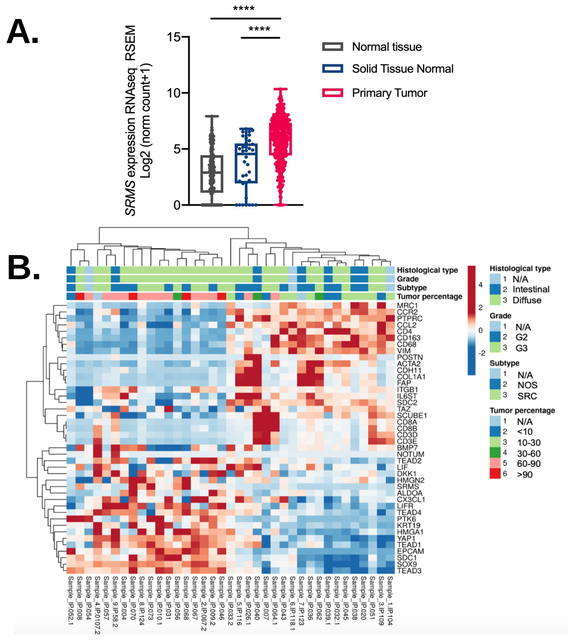


Fig. 1 SRMS was highly expressed in GAC tumor tissues compared to normal and adjacent normal **(A: TCGAA)** and highly expressed in metastatic PC samples by RNAseq **(B)**

SRMS Expression in GAC

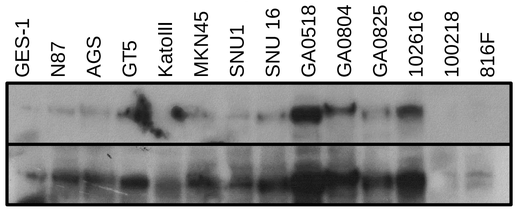
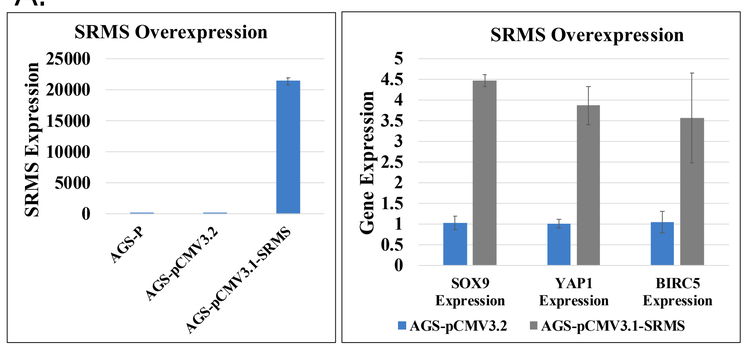


Fig. 2 Overexpression of SRMS in patient-derived metastatic PC cells is much higher than normal and other cells. Western blot identifying expression of SRMS in various cell lines (GES-1: Gastric Epithelial Cells; N87-SNU16: Primary Gastric Cancer Cells; GA0518-GA0825: Patient-derived PC cells; 102616-0816F: Cancer-Associated Fibroblasts)

SRMS Overexpression

A.



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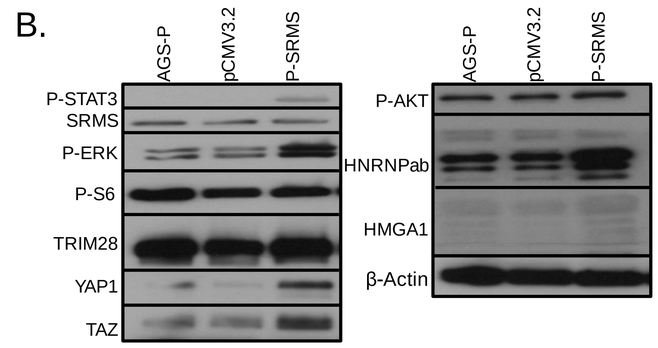


Fig. 3 A. Overexpression of SRMS in AGS cells by transfection of SRMS cDNA (pcDNA3.1SRMS) in AGS cells increased SRMS and SOX9, YAP1, BIRC5 oncogene expression and **B.** phospho-Stat3, phospho-S6 in AGS cells indicating SRMS activate these oncogenic signaling in GAC cells

Efficacy of Novel Inhibitor HJC0378 in Reducing SRMS Expression

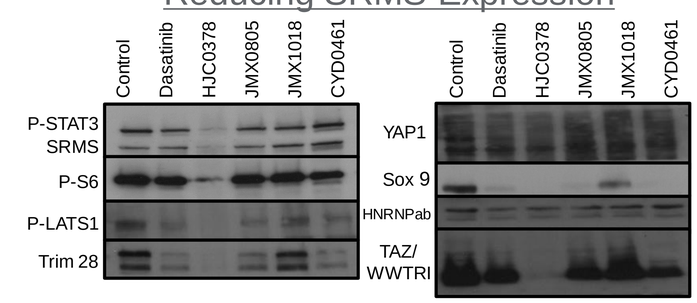


Fig. 4 HJC0378, a P-STAT3 inhibitor, demonstrates to be more effective than Dasatinib in inhibiting SRMS and other important proteins, such as Sox9 and Trim28

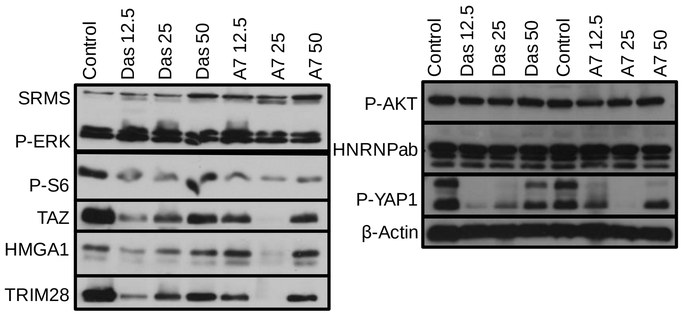
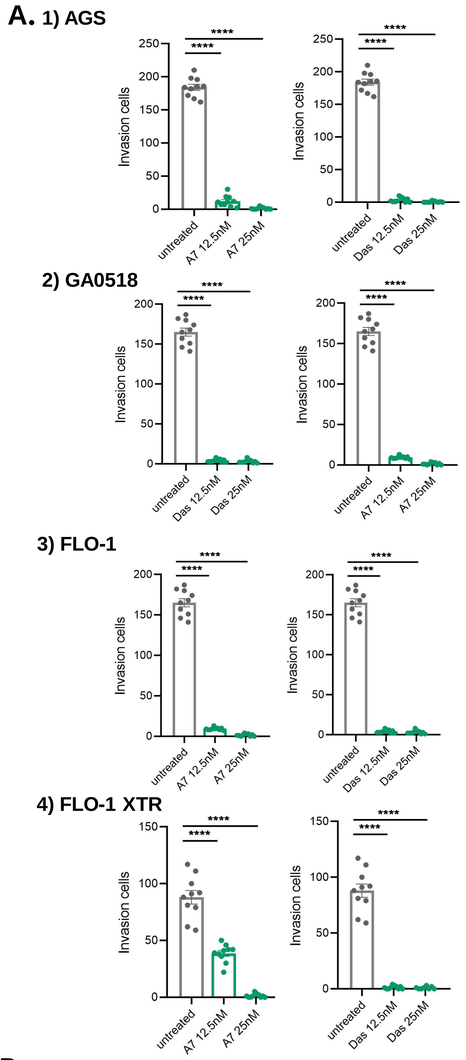


Fig. 5 GA0518's (highest expressing SRMS cell line) expression of some proteins, such as TAZ and TRIM28, were significantly reduced when treated with three concentrations (12.5, 25, and 50 nM) of SRMS inhibitors Dasatinib and HJC0378 (A7)

Ability of SRMS Inhibitors to Reduce Tumor Growth and Invasion in four GAC cell lines



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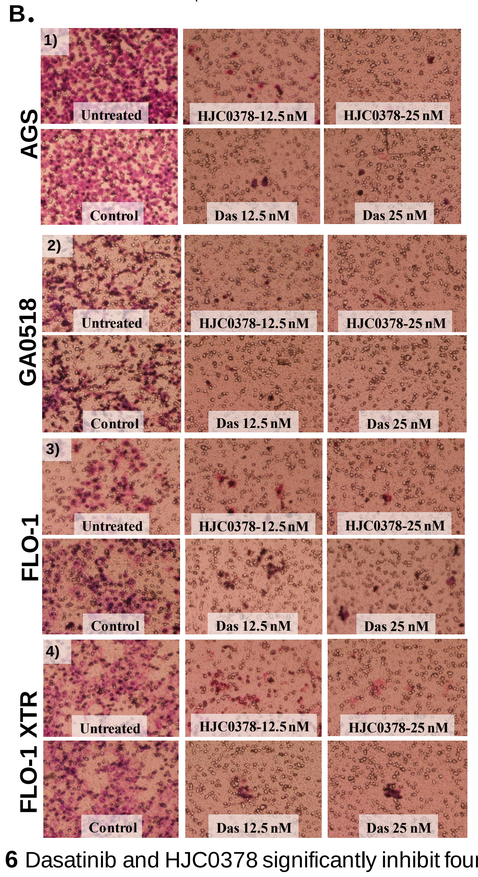

Fig. 6 Dasatinib and HJC0378 significantly inhibit four tumor cell invasion. **A.** Invasion of GAC cells was reduced when four GAC cells (1) AGS 2) GA0518 3) FLO-1 4) FLO-1 XTR) were treated with 12.5 and 25 nM of Dasatinib and HJC0378 respectively; **B.** representative images of invasion cells treated with or without inhibitors

Discussion

- TCGA data and western blot images show that patient-derived PC cells, such as GA0518 and GA0804, have higher expression of SRMS than primary tumor cells and normal gastric cells (Figure 1 and 2)
- Inhibitor HJC0378 ($C_{22}H_{24}ClN_3O_4S$) was able to suppress SRMS and other oncoproteins' expression in GA0518 cells (highest expressing SRMS cells) (Figure 4 and 5)
- Both HJC0378 and Dasatinib were able to significantly suppress tumor cell growth and invasive capacity (Figure 6)
- pCMV3.1-SRMS overexpression transfection in AGS cells (lowest expressing SRMS cells), increased the expression of several other genes including SOX9 and YAP1, which's protein were previously inhibited alongside SRMS by inhibitors HJC0378 and Dasatinib (Figure 3-5)
- There is a correlation between some oncoproteins' increase in expression, such as TRIM28, YAP1, phosphor-STAT3, and phosphor-S6, alongside SRMS' overexpression (Figure 3)

Conclusion

- SRMS is overexpressed in patient-derived metastatic PC cells compared to normal gastric cells or primary gastric cancer cells.
- Overexpression of SRMS in GAC cells increased YAP1/TAZ and SOX9 oncoproteins and also increased p-STAT3 and phospho-S6, a marker for mTOR activation suggesting the role of SRMS in tumor progression.
- SRC inhibitor Dasatinib can suppress SRMS and other oncoproteins and significantly suppress GAC tumor cell invasion.
- Most importantly, we discovered a novel inhibitor HJC0378 ($C_{22}H_{24}ClN_3O_4S$) that has proven to be a more effective SRMS inhibitor.
- Thus, SRMS is a potential novel target in GAC with PC.

References

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2) Wang R, Song S, Harada K, et al. Multiplex profiling of peritoneal metastases from gastric adenocarcinoma identified novel targets and molecular subtypes that predict treatment response. Gut. 2020;69(1):18-31.