SFRP2 Monoclonal Antibody Targets Tumor Associated Macrophages and Suppresses Metastatic Growth

MUSC Medical University of South Carolina | Hollings Cancer Center, An NCI-Designated Cancer Center

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BACKGROUND

SFRP2

- Secreted frizzled related protein 2 (SFRP2) is overexpressed in several tumor types
- SFRP2 has been shown to induce angiogenesis and inhibit tumor apoptosis
- Expression of SFRP2 in triple negative breast cancer (TNBC) is unknown

Tumor Associate Macrophages (TAMs)

- TAMs facilitate malignant progression
- TAMs are broadly classified as M1 and M2.
- M1 macrophages exhibit pro-inflammatory and anti-tumor activity, while M2 macrophages display pro-tumor activity
- A higher M1/M2 ratio indicates a dominance of pro-inflammatory M1-like TAMs, which boost T cell immune responses to tumors and improve immunotherapy effectiveness.

METHODS

- **Multiplex IHC with SFRP2, CD68, CD3, and CytoKeratin to assess SFRP2 and CD38 in the tumor microenvironment.** IHC on 4 human TNBC tumors was completed with primary antibodies to the markers mentioned. After staining, percent positivity of each marker was analyzed with via PhenoptrReports Open Source R Package.
- **Isolation of TAMs from mice**. E0771 cells ($1x10^6$) were injected into the mammary fat pad of C57/BL6 mice and grown to a diameter of 1 cm. TAMs were isolated using a tumor cell isolation kit. Macrophage isolation was confirmed with flow cytometry.
- **Western blot and qrt-PCR with hSFRP2 mAb and IgG1 control treated TAMs.** TAMs were plated and treated for 1 hour with hSFRP2 mAb (10 uM) and IgG1 control (10uM). Cell lysates were collected and underwent Western blot probing for IFN-ɣ or qrt-PCR.
- **Analysis of RNAseq data from TCGA comparing IFNγ and SFRP2 in breast cancer.** RNAseq data from 1075 breast cancer samples was collected from TCGA. Individual sample levels of SFRP2 protein and IFNγ were plotted and then analyzed with linear regression.
- **Treatment of TNBC lung mets with hSFRP2mAb in vivo**. Thirty female C57BL/6 mice received tail vein injections of E0771 cells, with hSFRP2 mAb treatment commencing 48 hours post-injection. The treatment regimen spanned 28 days, with hSFRP2 mAb administered at 8 mg/kg every three days or an IgG1 control . On the 29th day, the mice were humanely euthanized, and lung dissections were conducted.
- **M1/M2 Polarization**. FFPE serial lung slides containing EO771 metastases from mice treated with hSFRP2 mAb and IgG1 control were stained with IHC with antibodies to CD163 (M2 marker), CD86 (M1 Marker), and F4/80 (macrophage marker) and analyzed by spatial analysis software.
- **Detection of IFN-γ protein in the Serum of hSFRP2 mAb treated TNBC mice**. Serum from mice with metastatic EO771.LMB or PY8119 TNBC treated with IgG1 or hSFRP2 mAb was collected from mice and serum INF-γ was measured by ELISA.

RESULTS

SFRP2 Localizes to TAMs, TILS, and Tumor in TNBC Immune Microenvironment

A.

SFRP2	Mean±SEM
% Tumor Cells SFRP2 +	87.57±8.11
% TAMs SFRP2 +	90.34±5.59
% TILs SFRP2 +	96.38±2.21

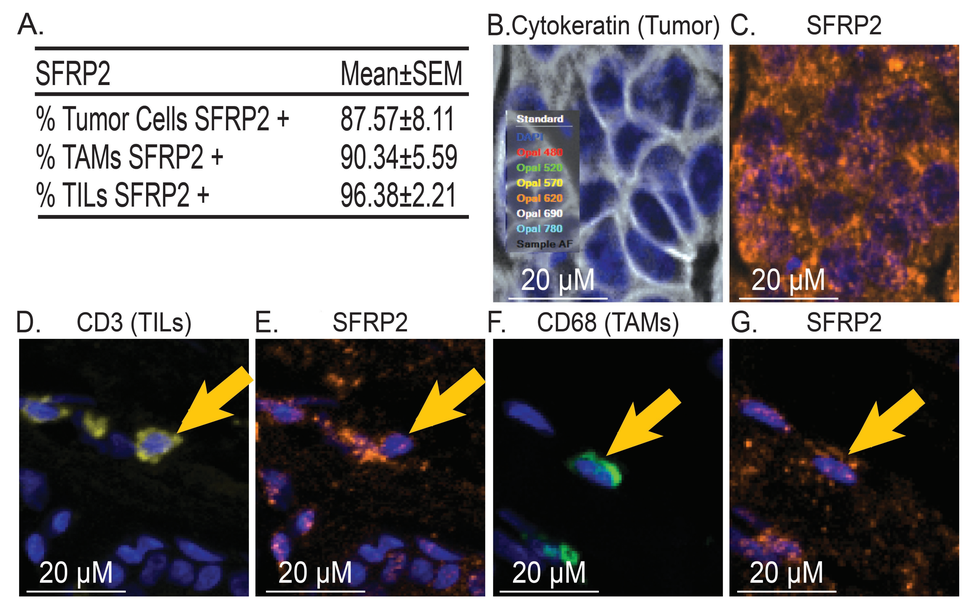

Figure 1. Four human TNBC tumors underwent multiplex IHC staining with antibodies to SFRP2, CD3 (TILs) CD68 (TAMs), and Cytokeratin (Tumor). **(A)** InForm spatial analysis showed a high degree of cells in the tumor, TAMs, and TILs, and staining positive for SFRP2. **(B)**. A human TNBC tumor stains positive for CytoKeratin, shown in white **(C)**, SFRP2, in orange **(D)**. The same tumor also stains positive for CD3, shown in yellow **(E)**, SFRP2, in orange **(F)**. Lastly, the tumor stains positive for CD68 are shown in green **(G)**, and SFRP2,.

hSFRP2 mAb increases IFN-γ in TAMs

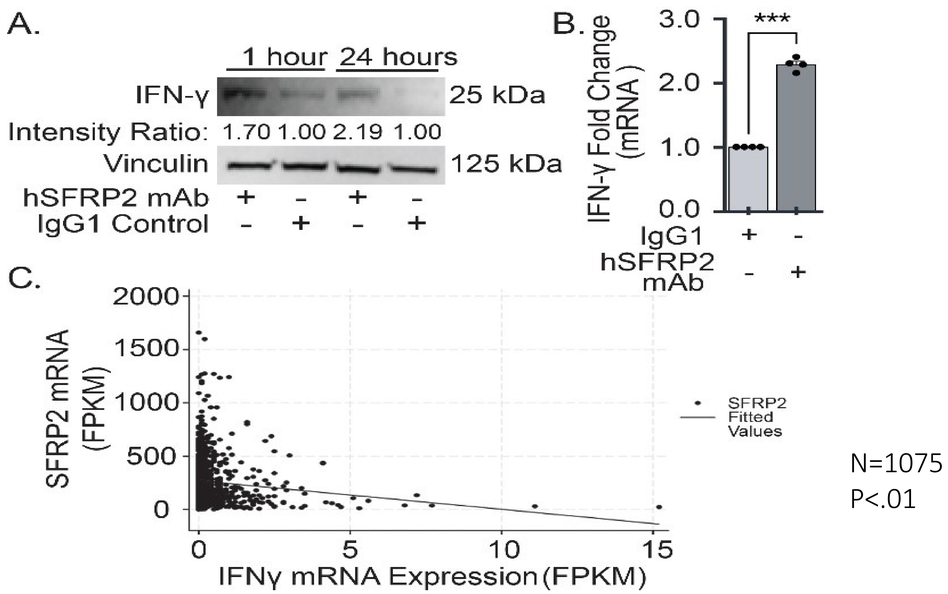

Fig 2. Murine TAMs were isolated from breast tumors and treated with hSFRP2 mAb 10 μM. Cell lysates were analyzed via Western blot, and analysis showed that hSFRP2 mAb treatment increased IFNγ when compared to IgG1 control A). RTqPCR was performed on the same cell lysate and showed a significant increase in IFNγ mRNA in hSFRP2 mAb treated TAMs. C. Analysis of INF-γ and SFRP2 expression TCGA data from 1075 entries showed a statistically significant inverse correlation between INF-γ and SFRP2

hSFRP2 mAb Inhibits Metastatic TNBC in vivo, Increases M1/M2 TAM Ratio, and Increases Serum IFN-ɣ

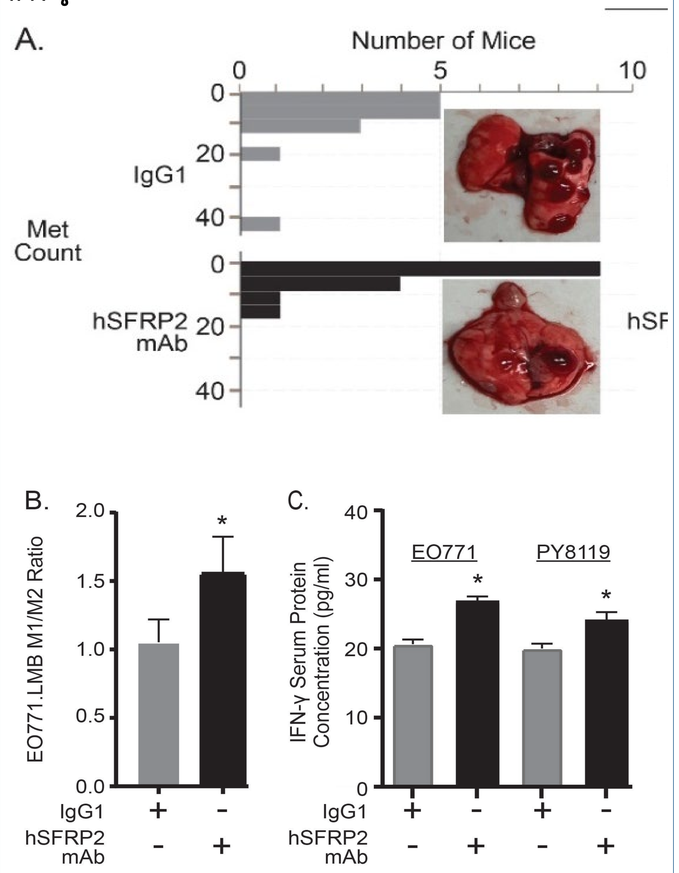

Figure 3. Lungs and serum from mice with metastatic TNBC from Fig. 5 treated with IgG1 control or hSFRP2 mAb were analyzed for tissue M1/M2 ratio and serum IFN-γ. (A) FFPE E0771 lung slices were stained with antibodies to CD86, CD163, and F4/80. M1/M2 ratios for hSFRP2 mAb treated mice were increased compared to IgG1 treated mice (*p=0.028, n=3). (B) ELISA revealed an increase of IFN-γ protein in the serum of EO771.LMB (p<0.0001 n=5) and PY8119 (p<0.0001 n=7) tumor-bearing mice following treatment with hSFRP2 mAb.

CONCLUSIONS

- SFRP2 localizes to macrophages, T cells, and tumor epithelial cells in the tumor microenvironment,
- hSFRP2 mAb increases IFN-ɣ protein and mRNA from TAMS
- hSFRP2 mAb inhibits the growth of metastatic TNBC, increases the M1/M2 TAM ratio, and increases serum IFN-ɣ

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