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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Development of a Novel Humanized Monoclonal Antibody to Secreted Frizzled-Related Protein-2 That Inhibits Triple-Negative Breast Cancer and Angiosarcoma Growth In Vivo

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ABSTRACT

Background. We previously reported that secreted frizzled-related protein-2 (SFRP2) is expressed in a variety of tumors, including sarcoma and breast carcinoma, and stimulates angiogenesis and inhibits tumor apoptosis. Therefore, we hypothesized that a humanized SFRP2 monoclonal antibody (hSFRP2 mAb) would inhibit tumor growth.

Methods. The lead hSFRP2 antibody was tested against a cohort of 22 healthy donors using a time course T-cell assay to determine the relative risk of immunogenicity. To determine hSFRP2 mAb efficacy, nude mice were subcutaneously injected with SVR angiosarcoma cells and treated with hSFRP2 mAb 4 mg/kg intravenously every 3 days for 3 weeks. We then injected Hs578T triple-negative breast cells into the mammary fat pad of nude mice and treated for 40 days. Control mice received an immunoglobulin (Ig) G1 control. The SVR and Hs578T tumors were then stained using a TUNEL assay to detect apoptosis.

Results. Immunogenicity testing of hSFRP2 mAb did not induce proliferative responses using a simulation index (SI) ≥ 2.0 ($p < 0.05$) threshold in any of the healthy donors. SVR angiosarcoma tumor growth was inhibited in vivo, evidenced by significant tumor volume reduction in the hSFRP2 mAb-treated group, compared with controls ($n = 10$, $p < 0.001$). Likewise, Hs578T triple-negative breast tumors were smaller in the hSFRP2 mAb-treated group compared with controls ($n = 10$, $p < 0.001$). The hSFRP2 mAb treatment correlated with an increase in tumor cell apoptosis ($n = 11$, $p < 0.05$). Importantly, hSFRP2 mAb treatment was not associated with any weight loss or lethargy.

Conclusion. We present a novel hSFRP2 mAb with therapeutic potential in breast cancer and sarcoma that has no effect on immunogenicity.

Wnt ligands are secreted glycoproteins that activate downstream effectors through binding to cell surface frizzled G-protein-coupled transmembrane receptors. Activation of Wnt signaling regulates normal embryonic development, but dysregulation of this pathway has been implicated in tumor progression for various cancers.[1,2] Secreted frizzled-related proteins (SFRPs) were previously

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regarded as inhibitors of the canonical Wnt/β-catenin pathway,[1] suggesting a tumor suppressor function for secreted frizzled-related protein-2 (SFRP2). However, several recent studies have shown that SFRP2 can act as a β-catenin agonist rather than an antagonist,[3–7] supporting a role in tumor promotion.

In endothelial cells, SFRP2 activates the non-canonical Wnt/Ca^2 pathway, rather than the canonical Wnt/β-catenin pathway, to stimulate angiogenesis.[8,9] The Wnt/Ca^{2+} pathway is mediated through activated G-proteins and phospholipases. This leads to transient increases in cytoplasmic free calcium and activation of the phosphatase and calcineurin that dephosphorylates the nuclear factor of activated T-cells (NFAT), after which NFAT translocates from the cytoplasm to the nucleus. SFRP2 stimulates endothelial tube formation and migration, and is anti-apoptotic.[10,11] Antagonizing SFRP2 also directly inactivates NFAT in tumor cells and prevents tumor cell migration.[12]

Substantial evidence now strongly supports the contribution of SFRP2 in promoting tumor growth in breast cancer,[5,10,13] angiosarcoma,[10,13] osteosarcoma,[14] rhabdomyosarcoma,[15] alveolar soft part sarcoma,[16] malignant glioma,[17] multiple myeloma,[18] renal cell carcinoma,[2] prostate cancer,[19] lung cancer,[20] and melanoma.[21] Additionally, in vivo SFRP2 molecular imaging shows that SFRP2 expression increases proportionally with tumor size.[22] We then demonstrated that a murine SFRP2 monoclonal antibody inhibits angiosarcoma and breast cancer growth in vivo.[12] Moving toward clinical translation, we developed a humanized monoclonal antibody to SFRP2 (hSFRP2 mAb) that inhibits angiogenesis and tumor growth, while promoting tumor apoptosis.

METHODS

Humanization of hSFRP2 Monoclonal Antibody (mAb)

V region genes encoding the murine SFRP2 monoclonal antibody 80.8.613 were cloned and used to construct chimeric antibodies, and then tested for purity and binding to SFRP2 (electronic supplementary Methods).

Immunogenicity Testing

The lead fully-humanized anti-SFRP2 antibody and the reference chimeric anti-SFRP2 antibody were assessed for immunogenic potential (electronic supplementary Methods).

Determination of hSFRP2 mAb Binding Affinity, Half Maximal Effective Concentration and Equilibrium Dissociation Constant

A microplate solid-phase protein binding (ELISA) assay was used to determine the half maximal effective concentration (EC_{50}) for hSFRP2 mAb and its binding affinity for SFRP2, using the Cheng–Prusoff equation[23] (electronic supplementary Methods).

Cell Culture

The origin and methods of culture for 2H11 murine endothelial, Hs578T human triple-negative breast carcinoma, MDA-MB-231 human triple-negative breast carcinoma, and SVR murine angiosarcoma cell lines[24] are described in the electronic supplementary Methods. The SVR angiosarcoma tumor model was derived from the transfection of Ras into MS1 endothelial cells. MS1 cells were previously generated by immortalizing murine endothelial cells by expressing the temperature-sensitive large T antigen.[24] Upon implantation into mice, these cells form dormant hemangiomas.[24] MS1 cells were then transfected with Ras (SVR), and this cell line forms angiosarcomas when injected into nude mice.[24]

Endothelial Tube Formation Assay

2H11 endothelial cells were analyzed for effects on tube formation in vitro (electronic supplementary Methods).

Proliferation Assay

Proliferation of Hs578T, MDA-MB-231, and SVR cells was assessed after treatment with the hSFRP2 mAb (electronic supplementary Methods).

Apoptosis/Necrosis

The effects of hSFRP2 mAb on apoptosis and necrosis were analyzed in Hs578T, MDA-MB-231, and SVR cells (electronic supplementary Methods).

Microplate Solid-Phase Protein Binding (ELISA) Assay for the Pharmacokinetics of hSFRP2 mAb

ELISA was performed and pharmacokinetic estimates were generated using non-compartmental analysis (NCA)[25] (see the electronic supplementary Methods for further details).

In Vivo Studies

Animal experiment protocols were consistent with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and approved by the Animal Care and Use Committee. The methods for pharmacokinetic, maximum tolerated dose (MTD), angiosarcoma allografts, and Hs578T triple-negative breast xenograft studies are described in the electronic supplementary Methods.

Immunohistochemistry

Formalin-fixed paraffin-embedded SVR and Hs578T sections were analyzed for proliferation by immunohistochemistry staining with Ki67 antibody (electronic supplementary Methods).

TUNEL Assay

Formalin-fixed paraffin-embedded sections from resected Hs578T and SVR tumors were stained for apoptotic cells following the manufacturer's protocol for the Apoptag® Peroxidase In Situ Apoptosis Detection Kit (#S7100).

Statistics

For in vitro assays, statistical differences between IgG1 and hSFRP2 mAb treatments were calculated using a two-tailed Student's t test, with a p value ≤ 0.05 considered significant. For in vivo tumor studies in angiosarcoma, a two-tailed Student's t-test was used, and for Hs578T, a two-sample t test for each time point was used and compared the tumor volume between treated and control animals (electronic supplementary Methods).

RESULTS

Humanization of SFRP2 mAb

The chimeric antibodies and combinations of composite heavy and light chains (16 antibodies in total) were tested for binding to SFRP2 in a competition ELISA assay. This demonstrated that the binding efficiency of all composite antibodies to SFRP2 were broadly comparable with that of the chimeric antibodies, with all variants showing improvement when compared with the murine antibody (data not shown). The chimeric antibodies and composite variants of anti-SFRP2 were purified from cell culture supernatants on a Protein A Sepharose column, buffer exchanged into PBS pH 7.4, and quantified by $OD_{280\ nm}$, using an extinction coefficient (E_c [0.1%] = 1.76) based on the predicted amino acid sequence. Endotoxin testing of the lead hSFRP2 mAb showed endotoxin < 0.5 EU/m. Western blot analyses of the lead hSFRP2 mAb indicated two bands corresponding to heavy and light chains (Fig. 1).

Immunogenicity Testing of hSFRP2 mAb

The lead, fully humanized, and chimeric anti-SFRP2 antibodies were tested against a cohort of 22 healthy donors using an EpiScreen™ time course T-cell assay, in order to determine the relative risk of immunogenicity. Fully humanized anti-SFRP2 antibody induced no positive responses using a simulation index (SI) ≥ 2.0 ($p < 0.05$) threshold in any of the donors in the proliferation assay, whereas the chimeric anti-SFRP2 antibody induced positive T-cell proliferation responses in 23% of the donors. Results with the control antigen KLH show that there was a good correlation (< 10% interassay variability) between the positive and negative results in repeat studies, indicating high reproducibility in the assay (Fig. 2).

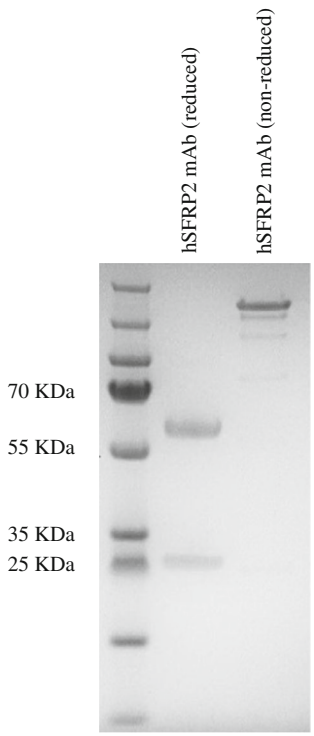

FIG. 1 Characterization of the purified candidate SFRP2 mAb by SDS-Page. One microgram of purified lead hSFRP2 mAb was loaded on a 4–12% NuPAGE SDS gel. PageRuler Plus pre-stained ladder was loaded to allow sizing of bands. Lane 1 was reduced with β-mercaptoethanol; two bands were present for the sample corresponding to the heavy and light chains. Lane 2 was non-reduced. *SFRP2* secreted frizzled-related protein-2, *mAb* monoclonal antibody, *hSFRP2* humanized SFRP2

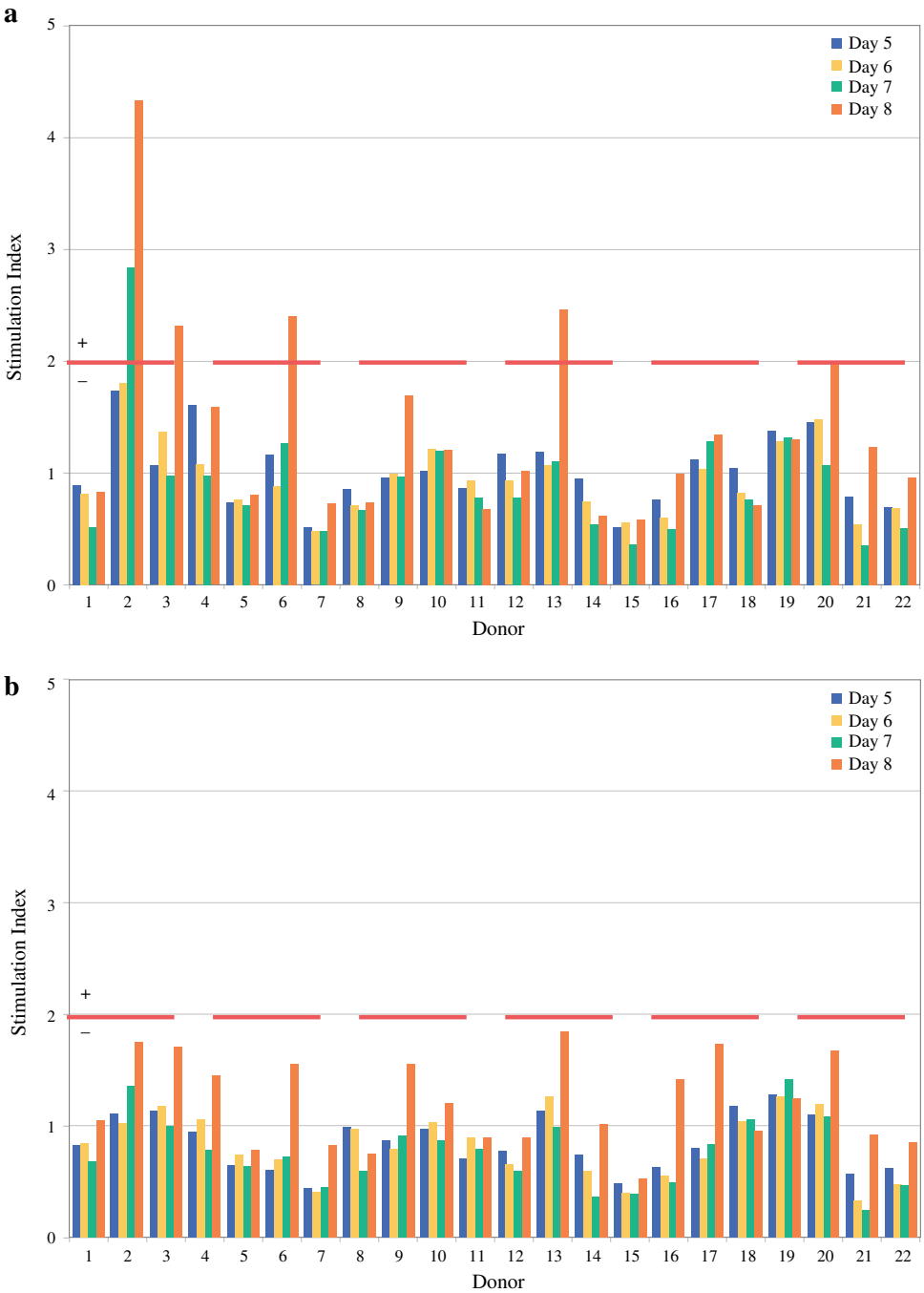

FIG. 2 Healthy donor T-cell proliferation responses to test antibodies. PBMCs from bulk cultures were sampled and assessed for proliferation on days 5, 6, 7, and 8 after incubation with the three test samples. Proliferation responses with an SI $\geq$ 2.0 ($p < 0.05$), indicated by the red dotted line, that were significant ($p < 0.05$) using an unpaired, two-sample Student's *t* test, were considered positive. None of the test antibodies tested positive. **a** Chimeric antibody; **b** fully humanized antibody. *PBMCs* peripheral blood mononuclear cells, *SI* simulation index

hSFRP2 mAb Binds SFRP2 with High Affinity

To determine binding affinity of the lead hSFRP2 mAb to SFRP2, SFRP2 (1 μM) was incubated with increasing concentrations of hSFRP2 mAb in a microplate solid-phase protein-binding ELISA assay. The hSFRP2 mAb bound SFRP2 with an EC_{50} of 8.7 nM and an equilibrium dissociation constant of 74.1 pM (Fig. 3a).

hSFRP2 mAb Inhibits Endothelial Tube Formation, Tumor Cell Proliferation, and Promotes Tumor Apoptosis

Consistent with previous reports,[8] SFRP2 induced an increase in the number of endothelial branch points in an endothelial tube formation assay compared with control cells ($n = 4$, $p \leq 0.05$) (Fig. 3b). Conversely, increasing concentrations of hSFRP2 mAb significantly counteracted SFRP2 effects on tube formation ($n = 4$, $p \leq 0.05$). The

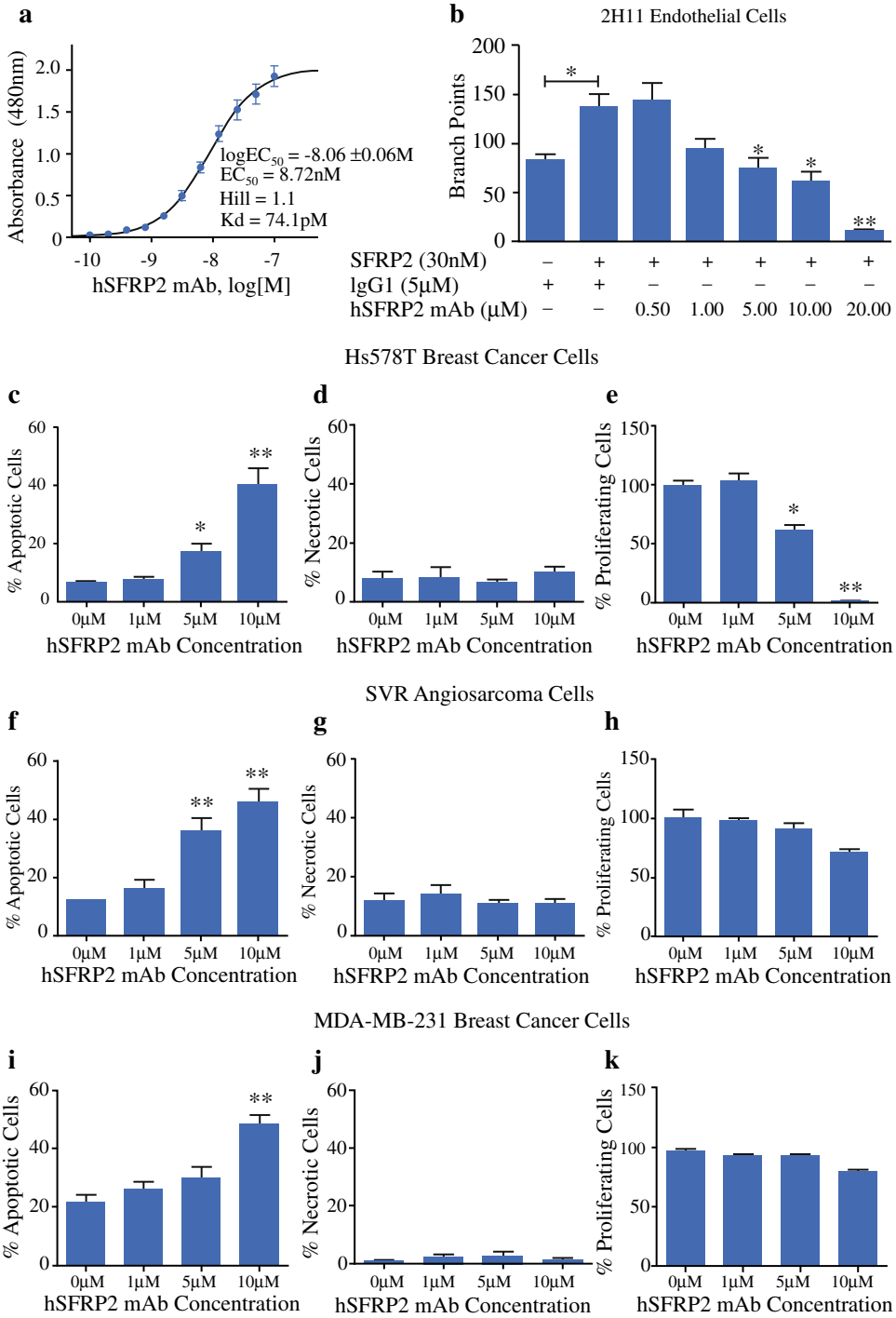

FIG. 3 hSFRP2 mAb in vitro activity. **a** Humanized SFRP2 mAb binds recombinant hSFRP2 protein with high affinity. Concentration–response curve showing the 480 nm absorbance measured after binding increasing concentrations of hSFRP2 mAb to a preset concentration of 1 μM SFRP2 in an ELISA assay ($n = 16$). **b** Effects of SFRP2 and hSFRP2 mAb on 2H11 endothelial tube formation. 2H11 cells were incubated and either treated with IgG1 control only (5 μM), IgG1 (5 μM) + SFRP2 protein (30 nM), or a combination of SFRP2 (30 nM) and hSFRP2 mAb (from 0.5 to 10 μM) [$n = 4$; *$p \leq 0.05$, **$p \leq 0.001$]. **c–h** Effects of increasing concentrations of hSFRP2 mAb (0–10 μM) on apoptosis (**c**, **f**, **i**) and necrosis (**d**, **g**, **j**) proliferation (**e**, **h**, **k**), in Hs578T breast cancer cells (**c–e**), SVR angiosarcoma cells (**f–h**), and MDA-MB-231 cells (**i–k**) [*$p \leq 0.05$, **$p \leq 0.001$]. Proliferation was measured using Cyquant®, while apoptosis and necrosis were measured using Annexin V and propidium iodide. Results are a compilation of three independent experiments containing four wells each ($n = 12$) for Hs578T and SVR, and two experiments containing four repeats for MDA-MD-231 ($n = 8$). *SFRP2* secreted frizzled-related protein-2, *hSFRP2* humanized SFRP2, *mAb* monoclonal antibody, EC_{50} half maximal effective concentration, *Kd* equilibrium dissociation constant, *Hill* Hill coefficient, *ELISA* enzyme-linked immunosorbent assay, *IgG1* immunoglobulin G1

half maximal inhibitory concentration (IC_{50}) for hSFRP2 mAb inhibition of SFRP2-stimulated tube formation was calculated after Cyquant proliferation assay, and was found to be 4.9 ± 2 μM.

SFRP2 antagonism with murine SFRP2 mAb has been shown to increase apoptosis without affecting proliferation.[12] We therefore evaluated whether the hSFRP2 mAb affects tumor cell proliferation, apoptosis and necrosis in Hs578T triple-negative breast carcinoma, MDA-MB-231 breast carcinoma, and SVR angiosarcoma cells, in vitro. Treatment with hSFRP2 mAb for 2 h significantly increased tumor apoptosis in Hs578T cells (*$p \leq 0.05$ for 5 μM, **$p \leq 0.001$ for 10 μM hSFRP2 mAb compared with control; $n = 12$) (Fig. 3c); SVR angiosarcoma cells (**$p \leq 0.001$ for 5 μM and 10 μM hSFRP2 mAb compared with control; $n = 12$) (Fig. 3f); and MDA-MB-231

cells (**$p \leq 0.001$ for 10 μM compared with control; $n = 8$) (Fig. 3i). There was no change in necrosis with hSFRP2 mAb treatment in any of the three cell lines (Fig. 3d, g, j). Treatment with hSFRP2 mAb had no effect on SVR or MDA-MB-231 proliferation at 72 h (Fig. 3h, k), but significantly reduced tumor cell proliferation of Hs578T cells compared with control (5 μM: *$p \leq 0.05$; 10 μM: **$p \leq 0.001$; $n = 12$) (Fig. 3e).

Pharmacokinetics

To study the pharmacokinetic properties of the antibody, a single dose of hSFRP2 mAb of 4 mg/kg was injected via the tail vein into nude mice, and blood samples were collected at different time points (Fig. 4a). The half-life of the antibody in the serum of the animals was 4.1 ± 0.5 days, with a maximum serum concentration (C_{max}) of 7.8 ± 1.0 mg/L and a clearance of 13.0 ± 0.6 mL/h.

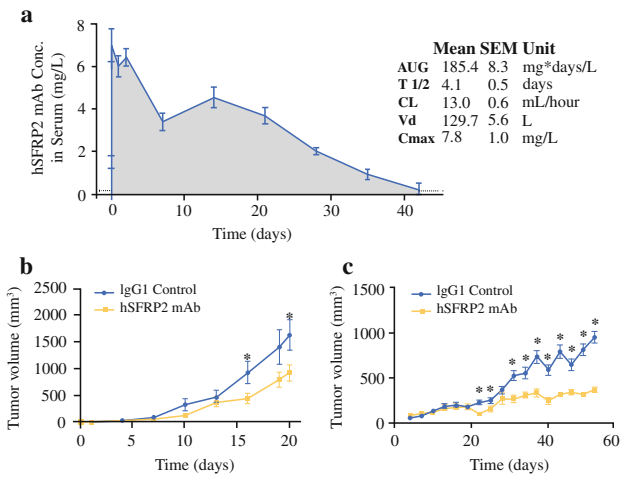

FIG. 4 hSFRP2 mAb inhibits tumor growth in angiosarcoma and breast cancer. **a** Pharmacokinetic plot showing the decrease in concentration of hSFRP2 mAb in the serum of mice over time after a single intravenous injection of 4 mg/kg. Each data point represents the mean ± SEM of the measurements of at least three independent samples ($n = 3$ per time point). **b** Nude mice with SVR angiosarcoma allografts were treated with hSFRP2 mAb or IgG1 control. There was a 43% reduction in tumor growth in the hSFRP2 mAb- treated mice ($n = 10$, *$p < 0.05$). **c** Effects of IgG1 treatment versus hSFRP2 mAb on Hs578T tumor volume in vivo over time. Nude mice with Hs578T xenografts were treated with hSFRP2 mAb or IgG1 control. Day is counted from the day of the first treatment, which was 30 days from tumor inoculation). There was a 61% reduction in tumor volume in the hSFRP2 mAb-treated mice ($n = 11$; *$p < 0.05$). *hSFRP2* humanized secreted frizzled-related protein-2, *mAb* monoclonal antibody, *AUC* area under the curve; $t_{½}$ half-life, *CL* clearance, *Vd* volume of distribution, *Cmax* maximum serum concentration, *SEM* standard error of the mean, *IgG1* immunoglobulin G1, *Conc.* concentration

Determination of Toxicity of hSFRP2 mAb In Vivo

Mice injected with SVR angiosarcoma cells were treated with hSFRP2 mAb at dosages of 2, 4, 10 and 20 mg/kg intravenously every 3 days, or IgG1 control for 21 days. There was no weight loss or lethargy in any of the antibody-treated mice. Hematoxylin and eosin sections of kidney and liver from all mice, at a 20 mg/kg dose, were reviewed by a board-certified pathologist (LS), and there were no pathologic changes in the liver or kidney between control and hSFRP2 mAb-treated mice (electronic supplementary Fig. 1). At the end of the experiment, body weights remained similar among the groups (32.2 ± 1.4 g for controls; 31.3 ± 1.1 g for 2 mg/kg; 32.1 ± 0.5 g for 4 mg/kg; 31.8 ± 0.9 g for 10 mg/kg; and 32.7 ± 1.0 g for 20 mg/kg). The dose with the maximum effect was 4 mg/kg, where there was a 69% reduction in tumor volume ($n = 5$ per group, $p = 0.05$).

Determination of Efficacy of hSFRP2 mAb In Vivo

To confirm the efficacy of the 4 mg/kg dose identified in the MTD experiment, we repeated the experiment with the SVR angiosarcoma tumors on a larger number of animals ($n = 10$ animals/group), treating them with 4 mg/kg hSFRP2 mAb. As described in Fig. 4b, after 3 weeks, tumors treated with hSFRP2 mAb were 43% smaller than tumors treated with the IgG1 control (1631.3 ± 283 mm^3 for control, 928.5 ± 148 mm^3 for hSFRP2 mAb; $p \leq 0.05$).

Next, we asked whether hSFRP2 mAb could affect the growth of other tumor types. Mice with Hs578T breast xenografts were treated with hSFRP2 mAb or IGg1 control.

Treatment with IgG1 or hSFRP2 mAb began at day 30 when the Hs578T mice had palpable tumors of approximately 100 mm^3. Comparison between control and each treated group at each time point shows that treatment days,[22,25] and all time points from day 31 until the last day of treatment were statistically significant (Fig. 4c, $p < 0.05$). At the end of the experiment, i.e. day 58 from tumor inoculation, after 28 days of treatment with hSFRP2 mAb, the Hs578T tumor volume was reduced by 61% (*$p < 0.05$) (Fig. 4c).

Humanized SFRP2 mAb Induces Apoptosis, with No Change in Proliferation in Tumors In Vivo

Since hSFRP2 mAb induces apoptosis in vitro and inhibits proliferation in breast cancer cells, we investigated if these phenotypes were retained in vivo. While the proportion of proliferative (Ki67-positive) cells was not affected by hSFRP2 mAb treatment compared with IgG1

control tumors (23 ± 1.6% vs. 29 ± 4.2% for SVR tumors; 18 ± 2.7% vs. 18 ± 2.8% for Hs578T tumors; p = non-significant), the proportion of apoptotic cells increased by 188% in SVR tumors (8.4 ± 0.9 in IgG1 control, and 24.2 ± 3.5 in hSFRP2 mAb-treated; $n = 10$, $p \leq 0.05$) and by 181% in Hs578T tumors (15.1 ± 4.9 in IgG1 control, and 42.4 ± 3.9 in hSFRP2 mAb tumors; $n = 10$, $p \leq 0.05$) (Fig. 5).

DISCUSSION

In this study, we report the development of an hSFRP2 mAb that is not immunogenic and binds to recombinant human SFRP2 protein with high affinity. Our in vitro and in vivo studies show a significant increase in apoptosis in both Hs578T and SVR angiosarcoma tumor cells, which correlate with our previous findings with the murine form of the SFRP2 mAb.[13] Hs578T and MDA-MB-231 cells were chosen because they are triple-negative breast cancer cell lines, for which there is a great need for novel therapies, and the SVR angiosarcoma cell line[24] was chosen because angiosarcomas have been shown to represent the signaling abnormalities of pathologic angiogenesis.[26] SVR cells produce SFRP2 protein,[8] and silencing SFRP2 inhibited angiosarcoma tube formation in a Matrigel tube formation assay.[8] 2H11 endothelial cells were chosen because they were demonstrated to be the best murine endothelial cell line to model tumor endothelium for studying the antiangiogenic activity of therapeutic compounds in vitro.[27]

Most importantly, the hSFRP2 mAb significantly reduced tumor growth in both Hs578T triple-negative breast and SVR angiosarcoma tumors in vivo, with no signs of toxicity. These antitumorigenic effects are particularly important in the setting of sarcoma and breast carcinosarcoma, which are aggressive malignancies with poor responses to known chemotherapeutic regimens.[28,29] Sarcomas are a heterogeneous group of malignancies that includes > 50 different subtypes, each with unique clinical and pathological characteristics. In general, there is a 50% mortality rate, and cures are only achieved with complete surgical resection. The efficacy of chemotherapeutic agents for unresectable or metastatic disease have been disappointing, with minimal long-term benefit, and a 5-year survival for patients with metastatic disease of only 15%.[28] Additionally, patients with late or metastatic disease rely on chemotherapy as the primary treatment. Unfortunately, doxorubicin has response rates of 10–25% in the clinic.[30]

Angiosarcoma is a vascular sarcoma arising from soft tissues in various anatomic locations that has a particularly poor prognosis, with an average 5-year survival of 35% and an aggressive course, with as many as 44% of patients presenting with metastatic disease. This malignancy has poor response to chemotherapeutic options, with the tendency to develop chemoresistance.[29,31] The SVR angiosarcoma line in this study is a transformed endothelial cell line that is a model of aggressive angiosarcoma in nude mice.[32] Thus, inhibition of tumor growth in this mouse model supports its potential efficacy in human angiosarcoma.

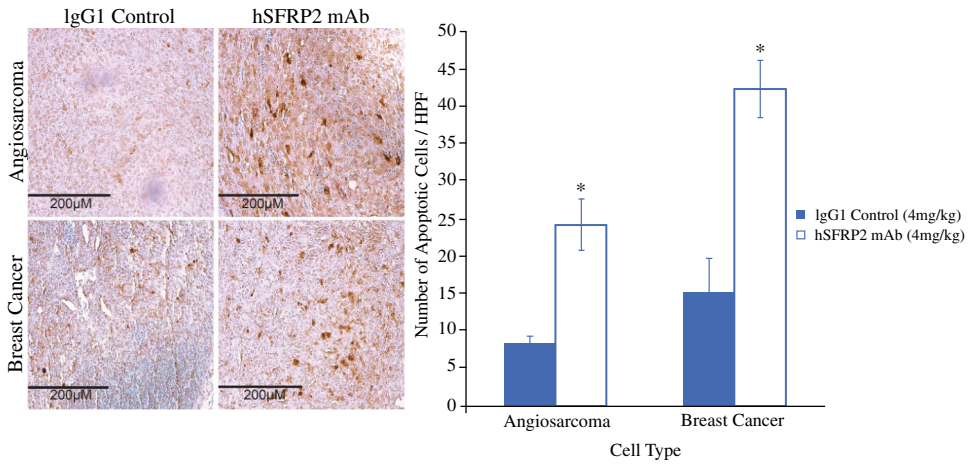

FIG. 5 hSFRP2 mAb treatment promotes apoptosis in tumors. Left: Paraffin-embedded Hs578T breast and SVR angiosarcoma tumors were sectioned and processed for TUNEL staining. For each tumor, a total of five fields were photographed, the number of apoptotic cells (brown) was counted in each field, and then averaged for each tumor. A total of 10 SVR tumors per treatment ($n = 10$) and 11 Hs578T tumors ($n = 11$) were used for the analysis. Right: Increase in the number of apoptotic cells in tumors treated with SFRP2 mAb (white bars) compared with IgG1 control-treated tumors (black bars) [$*p \leq 0.05$]. *hSFRP2* humanized secreted frizzled-related protein-2, *mAb* monoclonal antibody, *IgG1* immunoglobulin G1

Carcinosarcoma of the breast, sometimes referred to as metaplastic carcinoma, is a rare sarcoma-like cancer. It has an aggressive clinical course with poor prognosis compared with infiltrating ductal or lobular carcinomas. Breast carcinosarcomas are commonly triple-negative, meaning they do not express estrogen (ER) or progesterone receptors (PR), nor do they overexpress the HER2/neu oncogene. This genetic profile significantly limits a therapeutic approach due to the lack of response to estrogen-blocking treatments such as tamoxifen, or the HER2/Neu-targeting therapy trastuzumab.[33,34]

The Hs578T cell line is a triple-negative human cell line that is categorized as a basal cancer, meaning it is in the basal layer of the epithelium where progenitor cells reside.[35] It is further classified as basal B type, which has expression similar to stem cells, with overlapping features with the triple-negative tumor type.[36,37] This cell line has been found to express epidermal growth factor receptor and the hypermethylate E-cadherin gene, which is a mutation that has been implicated in change in response to chemotherapeutics.[38] A microRNA study of multiple breast cancer cell lines categorized Hs578T into a mesenchymal-like cell line with epithelial to mesenchymal transition features, and is similar to carcinosarcoma cell lines (metaplastic), which are frequently ER/PR- and HER2-negative.[33] Growth inhibition of this tumor in vivo is encouraging and could potentially lead to studies investigating utilization of hSFRP2 mAb on metaplastic breast cancers. Additionally, we previously showed that 85% of human triple-negative breast cancers have SFRP2 present,[8] and a murine SFRP2 mAb inhibits human MDA-MB-231 in mice.[13] We also confirmed that the hSFRP2 mAb induces apoptosis in vitro on MDA-MB-231. Therefore, the hSFRP2 mAb may also be a treatment, in general, for triple-negative breast cancer.

CONCLUSIONS

Targeted immunotherapies are a rapidly growing class of cancer treatments that have shown significant response and improvement in overall survival in various cancers. Recent investigation into combination therapies with other immunotherapies, or in conjunction with traditional chemotherapeutic agents, has also demonstrated promising results and is continuing to expand. In particular, several clinical trials investigating immunotherapy in sarcomas are ongoing, however only a few drugs are approved for use with metastatic disease. While there is some antitumor activity of targeted agents in sarcoma, improved therapeutic agents and novel combinations of therapeutics are essential to improve response and outcome. In this study, we present a novel humanized monoclonal antibody to SFRP2 with great potential applications in clinic as it inhibits tumor growth and increases apoptosis in both aggressive sarcoma and carcinosarcoma models in vivo.

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DISCLOSURE Novel Targets for Regulation of Angiogenesis, US Patent No. 8,734,789. Inventor: Nancy Klauber-DeMore, MD. Combination therapy of a humanized antibody to secreted frizzled-related 2 with PD-1 inhibition for the treatment of cancer. US Provisional App. No. 62/737,155. Inventor: Nancy Klauber-DeMore, MD. Dr. Klauber-DeMore is co-founder, shareholder, Chief Scientific Officer, and Board Member of Enci Therapeutics, Inc.

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