Dong *et al. BMC Cancer* (2024) 24:1165
https://doi.org/10.1186/s12885-024-12907-1

BMC Cancer

RESEARCH Open Access

SFRP1 mediates cancer-associated fibroblasts to suppress cancer cell proliferation and migration in head and neck squamous cell carcinoma

Lei Dong[1,2], Yumei Li[1,2], Xiaoyu Song[2,3], Caiyu Sun[1,2*†] and Xicheng Song[1,2*†]

Abstract

Background Cancer-associated fibroblasts (CAFs), as key cell populations in the tumor microenvironment (TME), play a crucial role in tumor regulation. Previous studies on a prognostic signature of 8 CAF-related genes in head and neck squamous cell carcinoma (HNSCC) revealed that Secreted frizzled-related protein 1 (SFRP1) is one of the hub genes closely related to CAFs. SFRP1 is deficiently expressed in numerous types of cancer and is classified as a tumor suppressor gene. However, the role of SFRP1 in TME regulation in HNSCC remains unclear. This study aimed to explore the role of SFRP1 in the proliferation and migration of HNSCC cells by mediating CAFs and their regulatory mechanisms.

Methods The expression differences, prognosis, and immune infiltration of SFRP1 in HNSCC were analyzed using the TIMER and GEPIA2 databases. The expression of SFRP1 in HNSCC tumor tissues, as well as the expression and secretion of SFRP1 in CAFs and tumor cells, were examined. An indirect co-culture system was constructed to detect the proliferation, migration, and apoptosis of HNSCC cells, and to clarify the effect of SFRP1 on tumor cells by mediating CAFs. Furthermore, the expression and secretion of 10 cytokines derived from CAFs that act on immune cells were verified.

Results SFRP1 was differently expressed in HNSCC tumor tissues and highly expressed in CAFs. SFRP1 inhibited the proliferation and migration of tumor cells and promoted apoptosis by mediating CAFs. The detection of CAFs-derived factors suggested that the mechanism of action of SFRP1 was associated with the regulation of immune cells.

Conclusion SFRP1 inhibits the proliferation and migration of HNSCC cells by mediating CAFs, and the mechanism of action is related to the regulation of immune cells, which may provide new research directions and therapeutic targets for HNSCC.

[†]Caiyu Sun and Xicheng Song contributed equally to this work.

*Correspondence:
Caiyu Sun
876468133@qq.com
Xicheng Song
drxchsong@163.com

Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Keywords SFRP1, Cancer-associated fibroblasts, Head and neck squamous cell carcinoma, The tumor microenvironment

Background

Head and neck squamous cell carcinoma (HNSCC), the seventh most common type of cancer, poses an enormous threat and burden to human health [1]. The tumor microenvironment (TME) consists of not only cancer cells but also epithelial cells, stromal and immune cells, along with their surrounding matrix [2]. Cancer-associated fibroblasts (CAFs), which are fibroblasts with altered phenotypes, are the dominant cells in TME and facilitate the promotion of tumor proliferation, invasion, angiogenesis, immune escape, drug resistance, and metastasis [3, 4].

CAFs interact with adjacent cells through various mechanisms, such as autocrine, paracrine, and direct interactions. CAFs interact with tumor-infiltrating immune cells and other immune components within the TME by secreting various effector molecules, such as cytokines, growth factors, and chemokines, thereby producing an immunosuppressive TME that allows cancer cells to evade immune surveillance [5]. Further research on the role of CAFs in TME, especially the regulatory mechanism between CAFs and immune cells, is expected to provide novel strategies for subsequent targeted immunotherapies [6]. In a preliminary study, a prognostic signature for HNSCC, containing 8 genes associated with CAFs, was constructed and validated. Among 8 genes, Secreted frizzled-related protein 1 (SFRP1) was downregulated as a hub gene in HNSCC compared to the control [7].

SFRP1 is an important protein-coding gene that belongs to the SFRP family of secreted glycoproteins. As a regulator of the Wnt pathway, the loss or down-regulation of SFRP1 expression has been observed in various solid tumors [8]. However, the current understanding of the roles and potential mechanisms of SFRP1 in the TME remains unclear, especially its effects on CAFs and immune cells in the TME of HNSCC. Our study found that SFRP1 plays a pivotal role in the TME, is closely related to CAFs, which are key cells in the TME and exerts tumor-regulating effects by mediating CAFs, the mechanism of which may be related to the modulation of the biological properties of immune cells in the TME. These findings may provide new research directions for mechanistic studies of SFRP1 and are expected to reveal new tumor markers and therapeutic targets, provide new perspectives for understanding the complex interactions in the TME of HNSCC, and offer new strategies for HNSCC immunotherapy.

Methods

Gene expression analysis

The expression levels of SFRP1 in different human tumors and matched paracancer tissues were compared using the Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/). The expression levels of SFRP1 in HNSCC and paired normal tissues were measured using the Gene Expression Profiling Interactive Analysis 2 database (GEPIA2, http://gepia2.cancer-pku.cn/).

Survival and immune infiltration analyses

Survival analysis was determined using the GEPIA2 database. Patients with HNSCC were included in the overall survival analysis based on SFRP1. The relationship between SFRP1 and immune cells was explored using the TIMER database.

Patients and tissues

All surgical samples were obtained from patients with HNSCC treated at the Yantai Yuhuanding Hospital. The study protocol was approved by the Ethics Committee of Yantai Yuhuangding Hospital (ID: 2022−319), and informed consent was obtained from the patients. Cancer tissue and paracancer tissue (5 mm away from the tumor) were obtained by surgery, then rapidly frozen in liquid nitrogen and stored at -80 ° C.

Tumor-bearing mice and injection of SFRP1

The xenograft tumor model was established by subcutaneously injecting 0.1 mL of 10^6 SCC7 cells (resuspended in PBS) into the flanks of 6-week-old male C57BL/6J mice (Jinan Pengyue Laboratory Animal Breeding Co.). When visible tumors appeared, mice were divided into two groups based on the treatment. BSA or recombinant SFRP1 protein was subcutaneously injected at a 2 μg/mL (50 μL/mice) dose. After administration once a day for nine consecutive days, the mice were sacrificed. The peripheral blood, xenograft tumors, liver, kidney, and spleen were collected from mice for further statistical analysis. All mice were bred at the Experimental Animal Center of Yantai Yuhuangding Hospital under conditions without specific pathogens. All mouse experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee and were approved by the Medical Ethics Committee of Yantai Yuhuangding Hospital (ID: 2022−319).

Cell culture

To isolate primary CAFs, human HNSCC cancer tissues were cut into 1 mm^3 pieces, suspended in DMEM/F12 medium (Biological Industries, Israel) containing 0.1% type I collagenase (Coolaber, China), incubated for 2 h on a shaker at 37 °C, filtered through a 70 μm filter, and centrifuged to precipitate the cells (1500 rpm, 5 min). The cell precipitates were resuspended in the DMEM/F12 medium (Gibco, USA) containing 10% fetal bovine serum (SenBeiJia Biological Technology, China) and 1% penicillin and streptomycin (Gibco, USA).

Human laryngeal squamous cell carcinoma cell line AMC-HN-8 and mouse squamous cell carcinoma cell line SCC7 were purchased from ATCC (Virginia, USA) and cultured in RPMI 1640 medium (Gibco, USA) containing 10% fetal bovine serum. All the cells were cultured at 37 °C in a 5% CO_2 incubator.

Immunohistochemistry

Paraffin sections of HNSCC clinical samples were deparaffinized and heat-induced epitope retrieval was conducted in a microwave oven in sodium citrate antigen retrieval buffer (EE0005, Sparkjade, China). Primary anti-SFRP1 antibody (1:25, D160294, Sango Biotech, China) was applied to the tissue sections at 4 °C overnight. HRP-labeled secondary antibodies were applied to the tissue sections after three washes with PBST (0.2% Tween in PBS). A DAB detection kit (EE0017, Sparkjade, China) was used to visualize SFRP1. The nuclei were stained with hematoxylin (EE0012, Sparkjade, China). The sections were dehydrated and blocked with neutral balsam (abs9177, Absin, China).

Cellular immunofluorescence

CAFs were fixed with 4% paraformaldehyde and then permeabilized with 0.25% Triton X-100, followed by blocking in PBS containing 4% BSA. The slides were washed with PBS three times and then incubated with anti-Vimentin (1:100, abs149750, Absin, China), anti-α-SMA (1:100, abs130621, Absin, China), and anti-FAP antibodies (1:100, abs131443, Absin, China) at 4 °C overnight. After washing with PBS three times, a fluorescently conjugated secondary antibody was added and incubated at room temperature in the dark for 2 h, and the DNA-binding dye 4′,6-diamidino-2-phenylindole dihydrochloride was added to stain the nuclei for 10 min. Images were captured using a fluorescence microscope.

In vitro cell transfection

10 nM FAM-siRNAs (Jikai Gene Biological Inc., China) were transfected into CAFs using Lipofectamine 3000 (Invitrogen, USA) to knock down SFRP1 in CAFs. After 6 h of transfection, the complete DMEM/F12 medium was replaced to continue the culture. After transfection, the expression of fluorescent protein expression was observed through fluorescence microscopy at 48 h. CAFs were collected on the 4th and 7th day to quantify SFRP1 expression by qPCR. Culture supernatants were collected on the 4th day and stored at -80 °C for subsequent assays.

Cell co-culture

CAFs (2×10^5/well) were plated in 6-well plates, and AMC-HN-8 cells (3×10^5/well) were plated in 6-well Transwell® polyester permeable supports (Corning, USA) with 0.4 μm pore size. After 72 h of co-culture, the AMC-HN-8 cells were collected separately for subsequent cell proliferation, migration, qPCR, ELISA, and apoptosis assays.

Cell proliferation assay

The co-cultured AMC-HN-8 cells were seeded in a 96-well culture plate with 5000 cells/well, and cell viability was detected using the CCK-8 assay kit (Sparkjade, China) at 0, 24, 48, and 72 h. The optical density (OD) at 450 nm was recorded after 2 h of incubation for measurement.

Wound healing assay

The co-cultured AMC-HN-8 cells were seeded in a 6-well culture plate and cultured into a monolayer, and then wounds were scraped with a sterile 1 mL tip. The cells were photographed under a microscope at 0, 12, 24, and 48 h. The wound healing areas were calculated using the ImageJ software.

Transwell assay

The migratory capacity of the cells was assessed using 24-well plates. In the upper chamber, co-cultured AMC-HN-8 cells were seeded in a serum-free medium. Full culture medium was supplied to the lower chamber, and the chambers were incubated for 24 h. The number of migrating cells was counted under a microscope.

qPCR

Total RNA from tissue homogenates and cells was extracted using RNAiso Plus (TaKaRa, Japan) according to the manufacturer's instructions. cDNA was synthesized using a PrimeScript RT Reagent Kit and gDNA Eraser Kit (TaKaRa, Japan). Quantitative PCR was performed using a TB Green Premix Ex Taq kit (TaKaRa, Japan) on an FTC-3000 A fluorescence quantitative PCR instrument (Funglyn Biotech, Canada). Gene expression levels were quantified using the $2^{-\Delta\Delta CT}$ method based on Ct values and normalized to a reference gene, GAPDH. All samples were made in triplicate, and the mean values were used for comparative analyses (the primers are listed in Supplementary Table 1 (Sango Biotech, China)).

ELISA analysis
The secretion of SFRP1, interleukin-6 (IL-6), and C-X-C motif chemokine 12 (CXCL12) in the culture supernatants of CAFs were measured using the corresponding ELISA kits (ABclone, USA) according to the manufacturer's instructions.

Apoptosis assay
The apoptosis of co-cultured AMC-HN-8 cells was measured using an apoptosis detection kit (Sparkjade, China). The cells were resuspended in 100 μL 1×buffer, 5 μL Annexin V-FITC staining solution, and 5 μL PI, and incubated at room temperature in the dark for 10 min. Then the cells were detected and analyzed by flow cytometry within 1 h of staining.

Statistical analysis
All experiments were made in duplicate and repeated three times. All data were analyzed using GraphPad Prism software, and the results were expressed as the mean value ± standard deviation. Intergroup differences were evaluated using an unpaired t-test, and statistical significance was set at $P < 0.05$.

Results
SFRP1 is downregulated in HNSCC tissues
While analyzing the expression levels of SFRP1 in pan-cancer and normal tissues using the TIMER database, we found that SFRP1 expression levels were significantly lower in most solid tumors than in paired normal tissues (Fig. 1A). Comparison of the gene expression levels of SFRP1 in HNSCC and paired normal tissues using the GEPIA2 database showed that the expression level of SFRP1 was significantly downregulated in HNSCC tumor samples compared with control samples. And the gene expression profile interaction analysis demonstrated a higher overall survival rate of HNSCC in the SFRP1 high-expression group compared with that of HNSCC in the low-expression group (Fig. 1B, C). Furthermore, we performed immunohistochemical analysis and qPCR assay of clinical samples from patients with three types of HNSCC, including laryngeal squamous cell carcinoma (LSCC), hypopharyngeal squamous cell carcinoma (HSCC), and nasopharyngeal squamous cell carcinoma (NPSCC). The results showed that SFRP1 was expressed at low levels in tumor tissues and at high levels in the corresponding paracancer tissues (Fig. 1D, E). Thus, SFRP1 was confirmed to be downregulated in HNSCC in both database and clinical samples.

SFRP1 is highly expressed in CAFs
To investigate whether SFRP1 mediated CAFs, it was necessary to first confirm the expression of SFRP1 in CAFs, and then knockdown of highly expressed SFRP1 in CAFs provided a basis for subsequent experiments. First, the CAFs in HNSCC were isolated and identified markers (vimentin, α-SMA, and FAP) (Fig. 2A, B). The qPCR results showed that SFRP1 mRNA was highly expressed in CAFs and very low in AMC-HN-8 cells. Similarly, ELISA results showed that the secretion of SFRP1 was significantly higher in CAFs than in AMC-HN-8 cells (Fig. 2C, D). Following FAM-siRNA transfection, the expression of SFRP1 in CAFs was verified by qPCR on the 4th and 7th day after transfection, suggesting the high efficiency of FAM-siRNA transfection (Fig. 2E, F). SFRP1 was highly expressed in CAFs compared with that in AMC-HN-8 cells, and the high expression of SFRP1 in CAFs was knocked down by in vitro cell transfection.

SFRP1 inhibited the proliferation and migration of HNSCC cells
An indirect co-culture system of CAFs and AMC-HN-8 cells was constructed to determine whether SFRP1 regulated the biological functions of AMC-HN-8 cells by mediating CAFs. CAFs or CAFs after knockdown of SFRP1 were co-cultured with AMC-HN-8 cells and subsequent functional analyses were performed after 72 h (Fig. 3A). The OD450 values of the co-cultured AMC-HN-8 cells were measured using CCK-8 assay to investigate the effect of SFRP1 on the proliferation of AMC-HN-8 cells. The results showed that at 24, 48, and 72 h, the OD450 values of the co-cultured AMC-HN-8 cells with SFRP1-siRNA CAFs were significantly higher than those of the co-cultured AMC-HN-8 cells with CAFs and higher than those of the control group (Fig. 3B), indicating that SFRP1 inhibited the proliferation of AMC-HN-8 cells by mediating CAFs.

To confirm the function of SFRP1 in AMC-HN-8 cell migration, wound healing and transwell assays were performed. The areas of healing in the co-cultured AMC-HN-8 cells with SFRP1-siRNA CAFs were smaller than those in the co-cultured AMC-HN-8 cells with CAFs and smaller than those in the control group at 24 and 48 h (Fig. 3C, D). Consistent results were also observed in the transwell assay, where the number of migrating cells was significantly higher in the group co-cultured with SFRP1-siRNA CAFs than in the group co-cultured with CAFs and higher than in the control group at 24 h (Fig. 3E, F). These results suggested that SFRP1 inhibited AMC-HN-8 cell migration by mediating CAFs.

SFRP1 inhibited tumor growth in mice
We constructed a mouse tumor-bearing model by subcutaneous injection of SCC7 cells (Fig. 4A). Through subcutaneous injection of mouse SFRP1 recombinant protein, we found that it can significantly inhibit tumor growth in mice (Fig. 4B-D). However, there were no significant changes in the weight and liver and kidney toxicity of

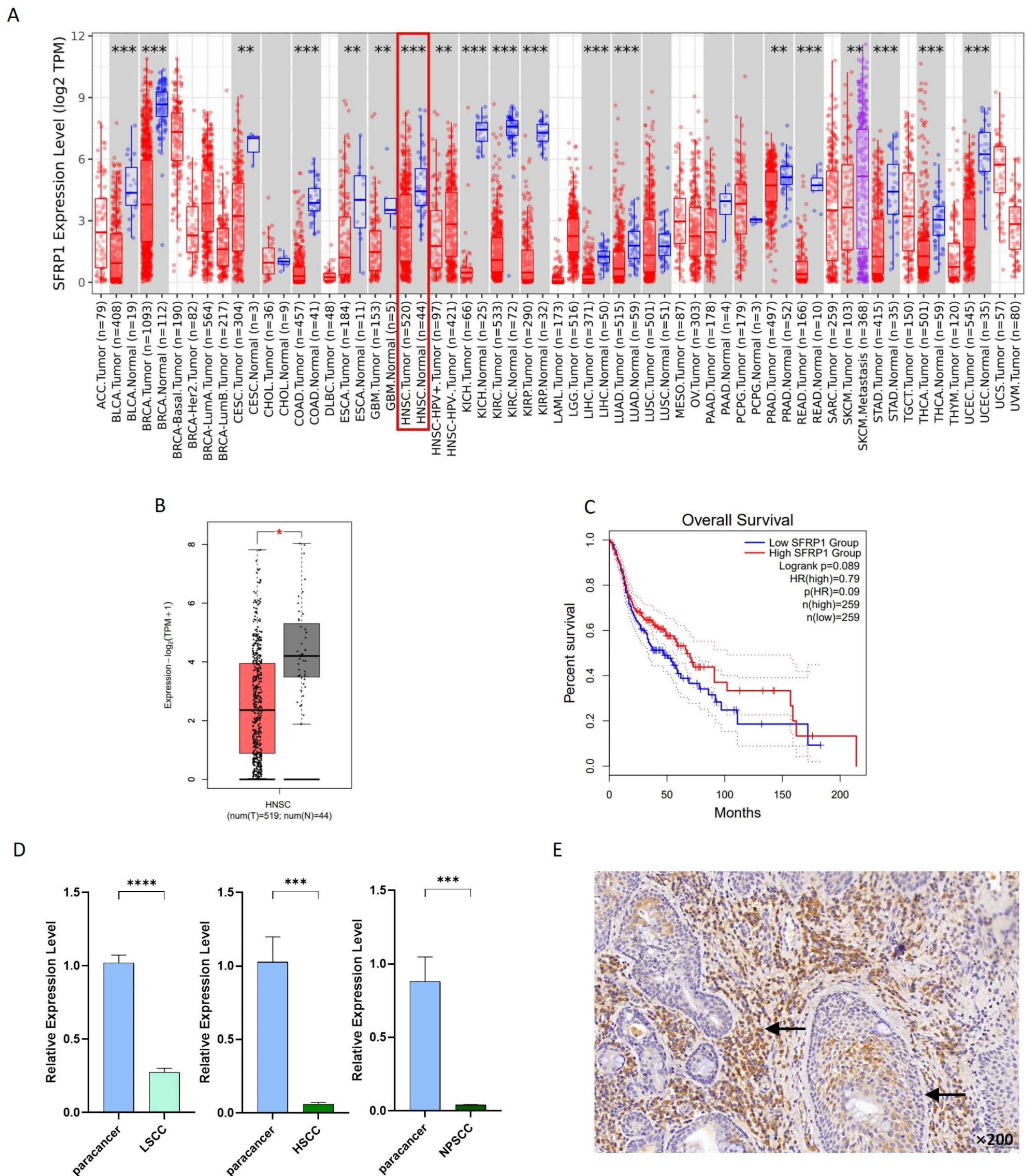

Fig. 1 SFRP1 expression in HNSCC and associated with HNSCC prognosis. (**A**) SFRP1 expression levels in different cancer types from the TCGA database analyzed by the TIMER database. (**B**) SFRP1 in HNSCC and paired normal tissue in the GEPIA2 database. (**C**) The overall survival of HNSCC in high and low SFRP1 expression groups were analyzed in the GEPIA2 database. (**D**) qPCR validation of the expression of SFRP1 mRNA in NHSCC tissue and paracancer tissue. (**E**) Representative images of IHC in NHSCC

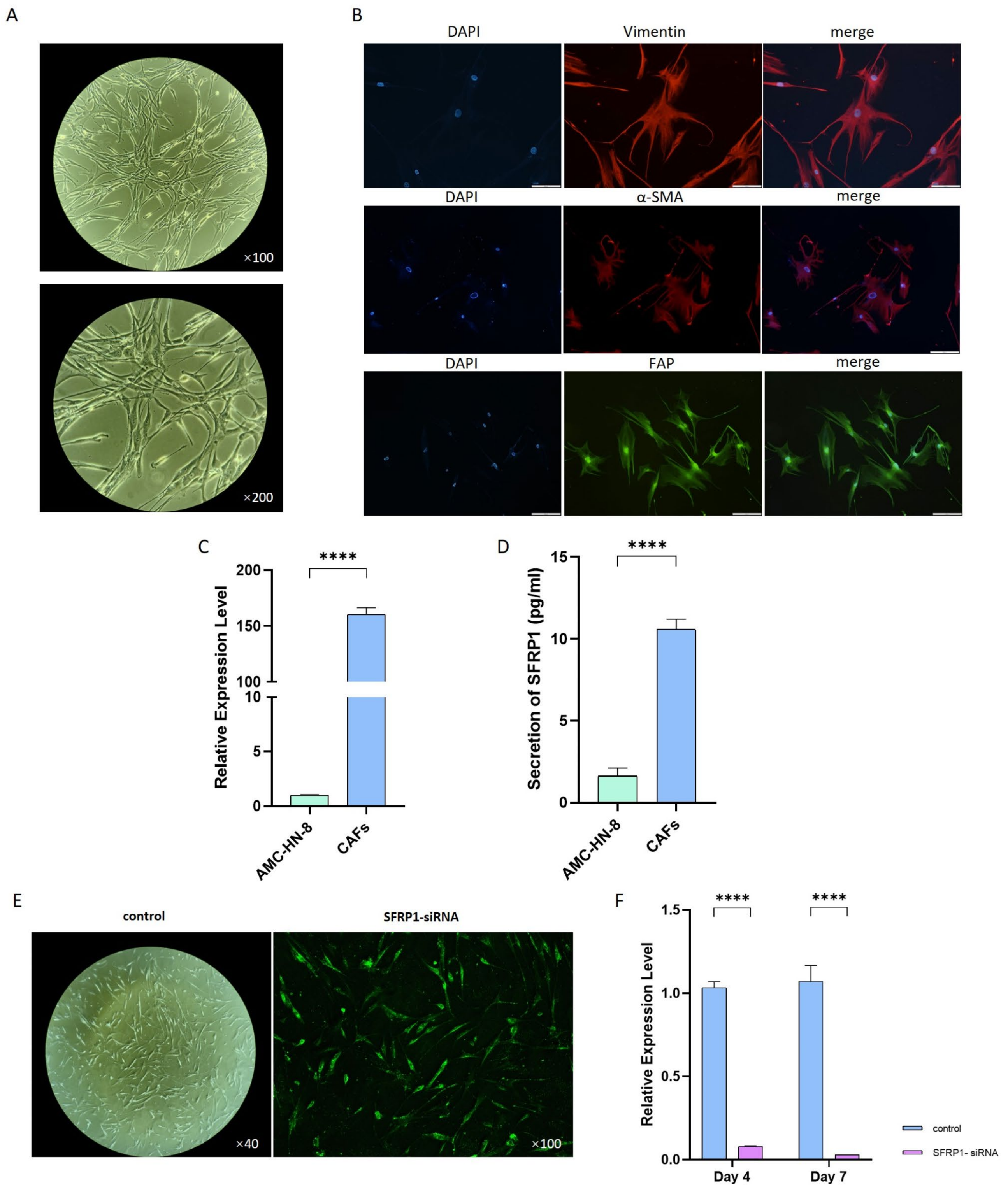

Fig. 2 SFRP1 expression in CAFs (**A**) In vitro primary culture of the CAFs. (**B**) Identification of the CAFs. (**C**) The mRNA levels of SFRP1 in AMC-HN-8 cells and CAFs were tested by qPCR. (**D**) The secretion of SFRP1 in AMC-HN-8 cells and CAFs was detected by ELISA. (**E**) SFRP1 was knocked down in CAFs by FAM-siRNA transfection. (**F**) The mRNA levels of SFRP1 in CAFs were tested by qPCR on the 4th and 7th day after transfection

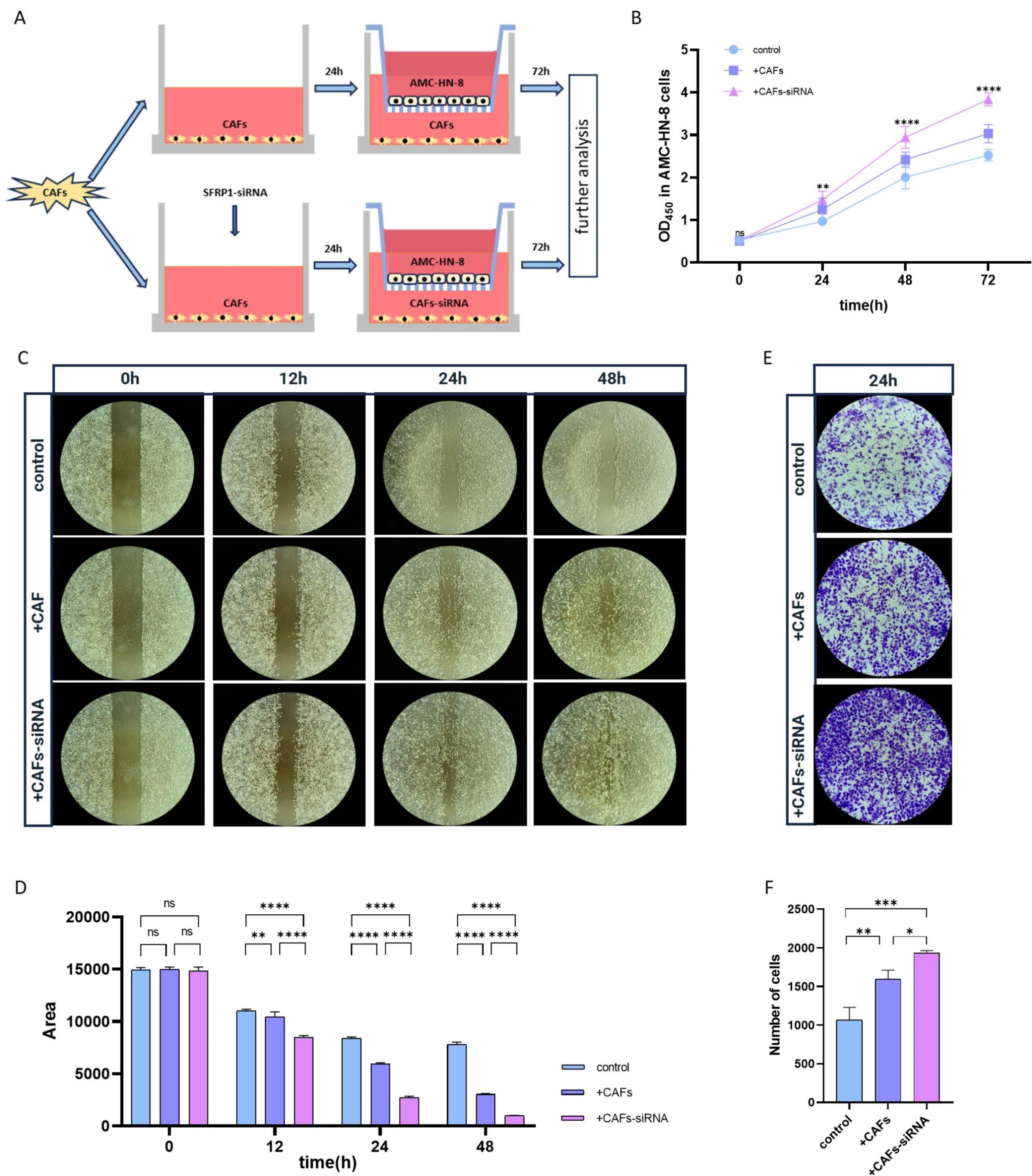

Fig. 3 Effects of SFRP1 on the proliferation and migration of HNSCC cells. (**A**) Schematic diagram of the CAFs and AMC-HN-8 cells co-culture system. (**B**) The OD450 of AMC-HN-8 cells in the control group, the co-culture group with CAFs, and the co-culture group with SFRP1-siRNA CAFs were examined at 0, 24, 48, and 72 h. (**C**) Wound healing pictures of AMC-HN-8 cells in the control group, the co-culture group with CAFs, and the co-culture group with SFRP1-siRNA CAFs were shown at 0, 12, 24, and 48 h. (**D**) The areas of wound healing of AMC-HN-8 cells in the control group, the co-culture group with CAFs, and the co-culture group with SFRP1-siRNA CAFs were calculated by Image J. (**E**) Migration pictures of AMC-HN-8 cells in the control group, the co-culture group with CAFs, and the co-culture group with SFRP1-siRNA CAFs were shown at 24 h. (**F**) The number of cell migrations of AMC-HN-8 cells in the control group, the co-culture group with CAFs, and the co-culture group with SFRP1-siRNA CAFs quantified by were calculated by Image J

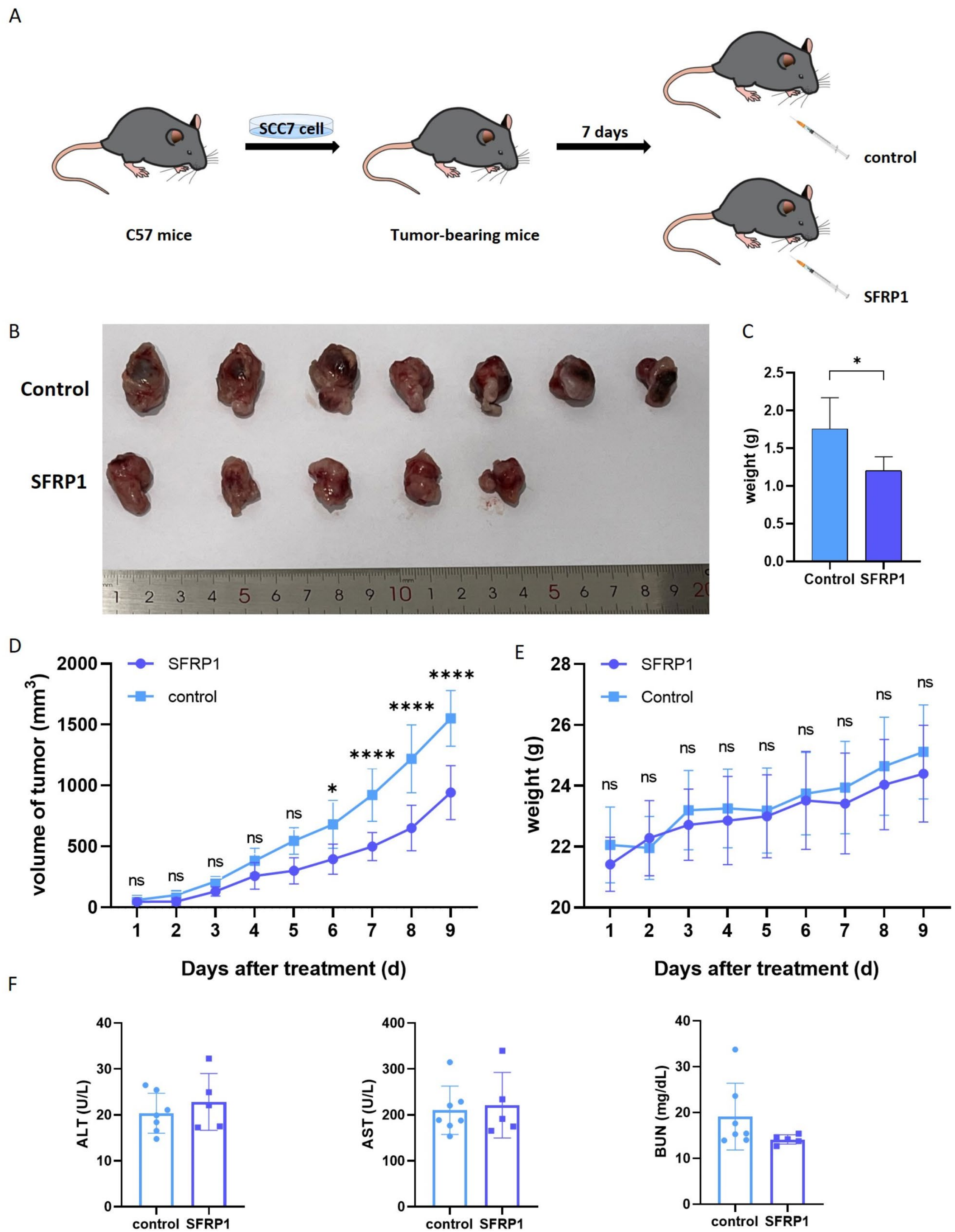

Fig. 4 Subcutaneous injection of SFRP1 recombinant protein for the treatment of subcutaneous tumor-bearing mice. (**A**) Schematic of experimental timeline and procedures. (**B**) Representative images of subcutaneous tumors with indicated treatment. (**C**) The weight of tumors with indicated treatment. (**D**) Relative tumor growth curves of subcutaneous tumors with indicated treatment. (**E**) The body weights of mice were analyzed and compared. (**F**) Liver and kidney function of the mouse were compared

the mice (Fig. 4E). Therefore, the SFRP1 protein has the potential for clinical treatment of tumors.

Knocking down SFRP1 inhibited apoptosis of HNSCC cells

To further explore the mechanism, apoptosis assays were performed. The percentage of apoptotic AMC-HN-8 cells in the group co-cultured with SFRP1-siRNA CAFs was lower than that in the group co-cultured with CAFs and the control group (Fig. 5A, B). These results suggested that SFRP1 promoted apoptosis of AMC-HN-8 cells by mediating CAFs.

On the vector of an indirect co-culture system, CAFs or CAFs with SFRP1 knockdown were co-cultured with AMC-HN-8 cells to observe changes in the cell function of AMC-HN-8 cells. SFRP1 inhibited the proliferation and migration of AMC-HN-8 cells by mediating CAFs, and the mechanism of action of SFRP1 may be related to apoptosis.

Effect of SFRP1 on CAFs-derived factors associated with immune cells

To better understand the characteristics of SFRP1 in the TME and its relationship with immune cells, the correlation between the abundance of SFRP1 and immune cells was analyzed using the TIMER database. The results showed that SFRP1 expression correlated with CD4+T cells, CD8+T cells, macrophages, neutrophils, and dendritic cells in the HNSCC microenvironment (Fig. 6A).

After consulting several studies, 10 CAFs-derived factors that may theoretically act on immune cells were

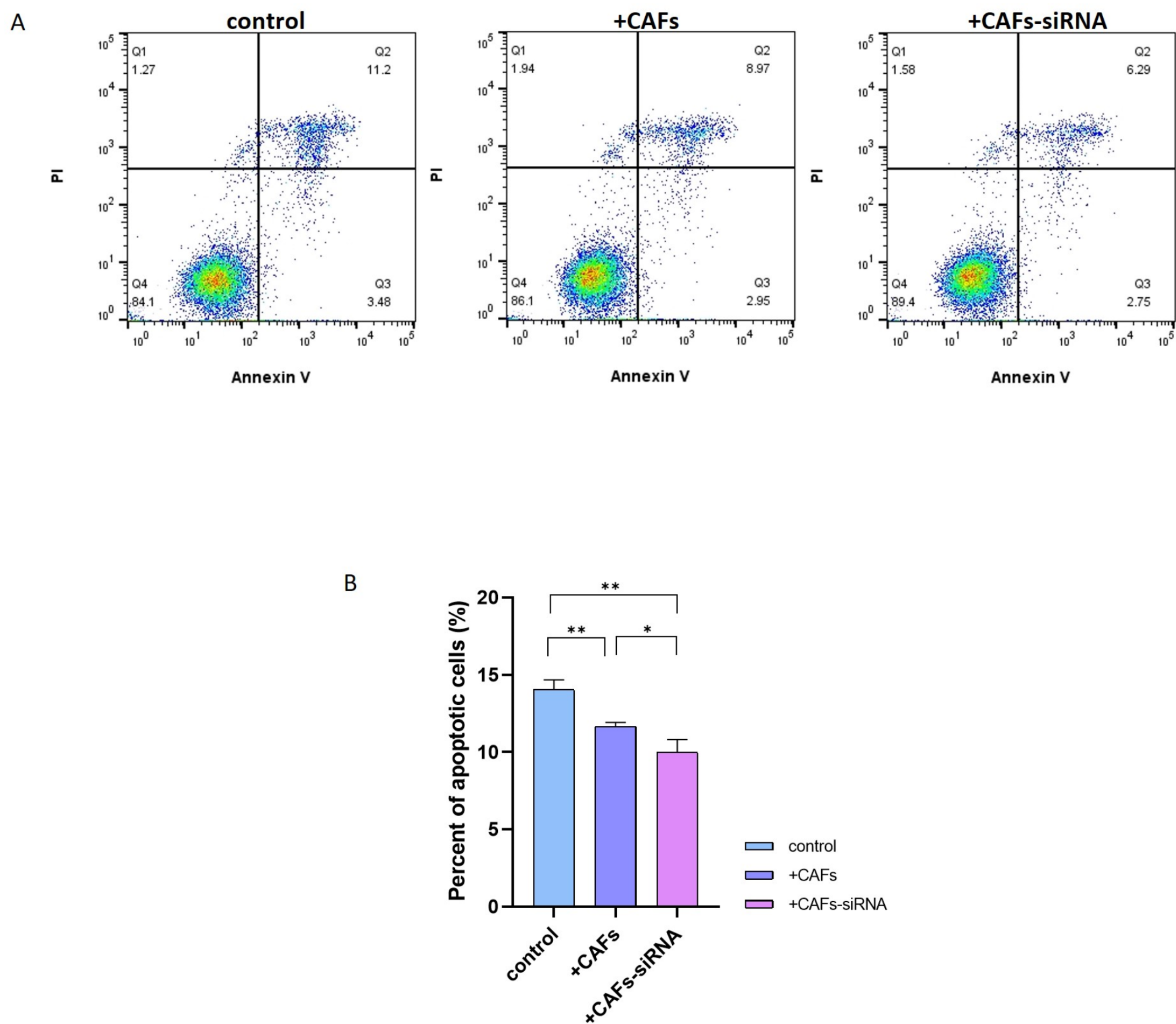

Fig. 5 SFRP1 mediates the mechanism of CAFs on tumor cells. (**A**) Apoptosis of AMC-HN-8 cells in the control group, the co-cultured group with CAFs, and the co-cultured group with SFRP1-siRNA CAFs. (**B**) Analysis of apoptosis of AMC-HN-8 cells in the control group, the co-culture group with CAFs, and the co-culture group with SFRP1-siRNA CAFs by flow cytometry after Annexin-V/PI staining

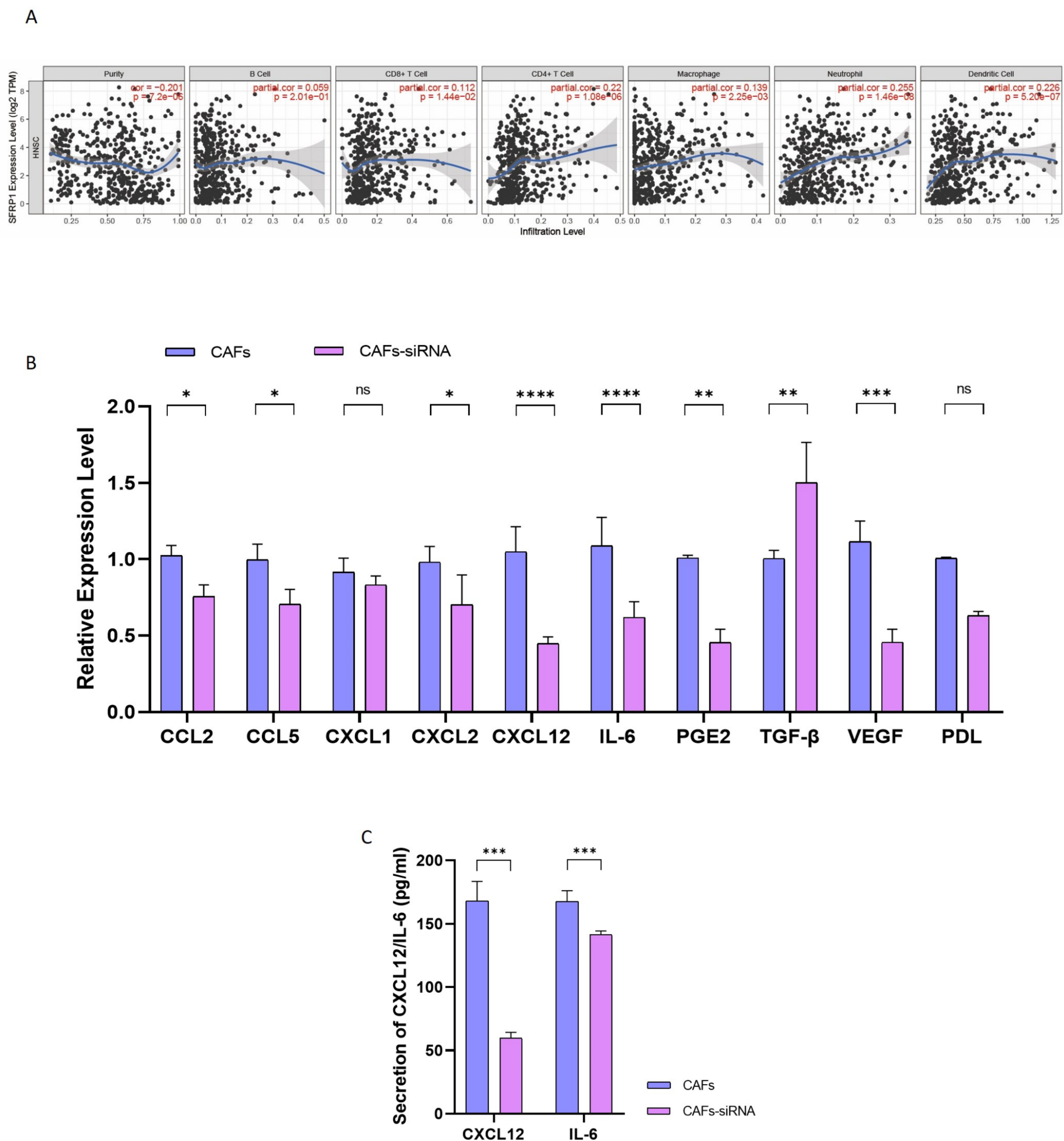

Fig. 6 The correlation between SFRP1 and immune cells in HNSCC. (**A**) Correlation analysis of SFRP1 and immune cell infiltration in HNSCC analyzed by the TIMER database. (**B**) 10 tumor cell and immune cell related mRNA expression in CAFs and CAFs-siRNA were tested by qPCR. (**C**) Secretion of CXCL12 and IL-6 from CAFs and CAFs-siRNA were detected by ELISA. $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$

selected for further exploration. The effects of SFRP1 on 10 cytokines in CAFs were detected using qPCR and ELISA. The qPCR results showed significant differences in the expression of CCL2, CCL5, CXCL2, CXCL12, IL-6, PGE2, TGF-β and VEGF in CAFs after SFRP1 knockdown. In contrast, there were no significant differences in CXCL1 and PDL (Fig. 6B). Among the differentially expressed cytokines, secretion in the supernatant of CAFs was detected by ELISA. The results showed that the secretion of CXCL12 and IL-6 differed markedly between the two groups of CAFs (Fig. 6C). These findings suggested that the mechanism of action of SFRP1 may involve altering the CAFs-derived factors, such as CXCL12 and IL-6, which are associated with immune

cell function in the TME. These results may provide more clues for exploring the mechanism of SFRP1 regulation in the TME by mediating CAFs.

Discussion

Despite significant improvements in the screening, diagnosis, and multidisciplinary treatment of HNSCC, the 5-year survival rate of patients remains less than 50% [2]. In recent years, immunotherapy has become a novel treatment for HNSCC, and several studies have been conducted to determine the mechanisms of tumor immunotherapy focused on the important role of the TME. CAFs are a major cell type in the TME and serve the role of regulating TME homeostasis [4]. CAFs play an immunomodulatory role through their interactions with immune cells in the TME, forming a tumor-supportive environment that plays a role in immune escape and immunotherapeutic resistance, thereby regulating tumorigenesis and development [9–12]. Not only CAFs themselves but also the importance of CAF-related genes on the prognosis and immune cell infiltration of a variety of solid tumors has been confirmed in several studies [13, 14].

SFRP1 is deficiently expressed in a wide range of cancers, including triple-negative breast [15], prostate [16], lung [17], colorectal [18], and liver cancers [19], and HNSCC [20–23], and is therefore classified as a tumor suppressor gene [24]. Previous studies have focused on the mechanism of action of SFRP1 on the Wnt pathway regulation and epigenetics. SFRP1 participates in the Wnt signaling pathway through multiple mechanisms and exerts antitumor activity. Some studies have reported that SFRP1 inhibits Wnt activity by directly binding to the Wnt protein-ligand via its netrin domain [25], SFRP1 bypasses the interaction with the Wnt ligand and binds to β-catenin in the cytoplasm directly to antagonize the Wnt signaling pathway [26], SFRP1 directly binds to Fz receptors through cysteine-rich domain motif, preventing the binding of the Wnt ligand to the receptor, thereby inhibiting the Wnt pathway [27]. Epigenetics is an important mechanism that regulates SFRP1 silencing. Endogenous SFRP1 expression increases in a dose-dependent manner after demethylation, indicating that DNA methylation is the main mechanism underlying SFRP1 silencing [24, 28]. Epigenetic silencing of SFRP1 has been demonstrated in many tumor types [29, 30]. The mechanism of action of SFRP1 has been increasingly revealed with further research. SFRP1 plays an important role in regulating the growth and differentiation of specific cell types (e.g., CAFs) [31–35], has both pro- and anti-angiogenic activities depending on the context [24], prevents Thrombospondin 1-mediated adhesion and migration of cancer cells [36]. However, the regulatory role and mechanism of SFRP1 in the TME concerning CAFs and immune cells remains unclear.

In our study, SFRP1 was significantly downregulated in clinical HNSCC samples obtained from public databases, which is consistent with its validation in LSCC, HSCC, and NPSCC. Based on survival analysis, patients with low SFRP1 expression had worse overall survival than those with high SFRP1 expression. These results suggested that SFRP1 is a tumor suppressor gene in HNSCC. Immune infiltration analysis showed that SFRP1 was positively correlated with immune cells, especially in terms of the abundance of CD4+T cells, CD8+T cells, macrophages, neutrophils, and dendritic cells, suggesting that it may be a relevant gene that affects the TME in HNSCC. Combined with our previous studies and further experimental validation, SFRP1 was more highly expressed in CAFs than in HNSCC cells. Considering the critical role of CAFs in the TME and SFRP1 as a characteristic hub gene of CAFs, it is reasonable to believe that SFRP1 participates in the regulation of immune cell function in the HNSCC TME by mediating CAFs. To further explore the role of SFRP1 in HNSCC by mediating CAFs, an indirect co-culture system of CAFs and HNSCC cells was constructed. Biological functional experiments have shown that knocking down SFRP1 in CAFs can enhance the proliferation activity of AMC-HN-8 cells, rendering AMC-HN-8 cells more motile. Correspondingly, SFRP1 knockdown in CAFs attenuated the apoptosis of AMC-HN-8 cells. Meanwhile, the tumor-suppressive potential of SFRP1 in HNSCC was verified by tumor-bearing mice.

Through bidirectional signaling with other cells mediated by CAFs-derived cytokines, chemokines, growth factors, and exosomes, CAFs exert their effects on tumor cells and immune cells within the TME [37–39]. Several studies including gene signature and mass spectrometry analysis have shown that the secretion profile of CAF, including CXCL1, CXCL2, CXCL5, CXCL12, CCL5, IL-1β, IL-6, IL-10, VEGF, and TGFβ, plays a major role in modulating the TME by regulating immune cell recruitment and functions within tumors [9, 40–47]. Based on the literature, 10 CAFs-derived factors that may be involved in the regulation of immune cells were selected for further exploration. The experimental results indicated that among the 10 CAFs-derived factors, knockdown of SFRP1 significantly reduces the expression and secretion levels of CXCL12 and IL-6 in CAFs. Notably, CAFs secretion of CXCL12 may contribute to an increase in CD8+T cell numbers in tumor tissues and is involved in regulating CD8+T cell depletion and exclusion from tumor nests [48, 49]. Recent studies have confirmed that CXCL12 is a key chemokine for CD8+T cell recruitment in HNSCC [50–52]. The importance of the CXCL12 signaling pathway in the regulation of tumor-infiltrating CD8+T cell migration induced by FAP+CAFs has been

confirmed in several reports [47, 53]. On the other hand, the release of IL-6 induced neutrophil activation in the TME. Research suggests that CAFs-derived IL-6 induces PDL1+neutrophils through the activation of the JAK-STAT3 pathway and impairs T cell function through PD1/PDL1 signaling, thereby creating favorable conditions for tumor progression [54]. CAFs remodel immunosuppressive CD8+and FoxP3+T cells in the TME by secreting high amounts of IL-6. Therapies that block IL-6 signaling or directly target CAFs may improve T cell trafficking, migration, and tumor immunosuppression, thereby improving the prognosis of patients with various cancers [55].

Therefore, it is reasonable to speculate that SFRP1 may affect the number and function of immune cells by altering the secretion factors of CAFs, thereby regulating the TME, which may be achieved by targeting immune cells such as CD8+T cells and/or neutrophils. The results of this study provide new clues and directions for future mechanistic studies on SFRP1 in the TME.

Conclusion

SFRP1 inhibits cancer cell proliferation and migration in HNSCC by mediating CAFs, and the mechanism of action is related to immune cell regulation, which may provide new research directions and therapeutic targets for HNSCC.

Abbreviations

CAFs	Cancer associated fibroblasts
CXCL12	C-X-C motif chemokine 12
HNSCC	Head and neck squamous cell carcinoma
HSCC	Hypopharyngeal squamous cell carcinoma
IL-6	Interleukin-6
LSCC	Laryngeal squamous cell carcinoma
NPSCC	Nasopharyngeal squamous cell carcinoma
OD	Optical density
SFRP1	Secreted frizzled-related protein 1
TME	The tumor microenvironment

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12907-1.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

Lei Dong and Yumei Li contributed to the conception of the study; Lei Dong performed the experiment; Lei Dong and Xiaoyu Song contributed significantly to analysis and manuscript preparation; Lei Dong and Caiyu Sun performed the data analyses and wrote the manuscript; Xicheng Song and Caiyu Sun helped perform the analysis with constructive discussions.

Funding

This work was supported by grants from the Taishan Scholar Project (No. ts20190991); the Key R&D Project of Shandong Province (No.2022CXPT023); the Natural Fund Program of Shandong Province (No. ZR202211250135); Yantai Science and Technology Program of Yantai City (No. 2022YD007); the China Postdoctoral Science Foundation under Grant Number 2023M741861; the National Natural Science Foundation of Shandong Province (No. ZR2023QH358).

Data availability

Data is provided within the manuscript or supplementary information files. All relevant data supporting this study's findings are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Yantai Yuhuangding Hospital ID: 2022 – 319, and the patients provided their informed consent for study participation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Otorhinolaryngology Head and Neck Surgery, Yantai Yuhuangding Hospital, Shandong University, No.20, Yuhuangding East Road, Zhifu District, Yantai 264000, Shandong, China
[2]Shandong Provincial Clinical Research Center for Otorhinolaryngologic Diseases, Yantai Yuhuangding Hospital, Yantai, China
[3]Qingdao University, Qingdao, China

Received: 18 June 2024 / Accepted: 5 September 2024
Published online: 19 September 2024

References

1. Alsahafi E, et al. Clinical update on head and neck cancer: molecular biology and ongoing challenges. Cell Death Dis. 2019;10(8):540.
2. Curry JM, et al. Tumor microenvironment in head and neck squamous cell carcinoma. Semin Oncol. 2014;41(2):217–34.
3. Cirri P, Chiarugi P. Cancer associated fibroblasts: the dark side of the coin. Am J Cancer Res. 2011;1(4):482–97.
4. Alcolea S, et al. Interaction between head and neck squamous cell carcinoma cells and fibroblasts in the biosynthesis of PGE2. J Lipid Res. 2012;53(4):630–42.
5. An Y, et al. Crosstalk between cancer-associated fibroblasts and immune cells in cancer. J Cell Mol Med. 2020;24(1):13–24.
6. Mao X, et al. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Mol Cancer. 2021;20(1):131.
7. Dong L, et al. Identification and verification of eight cancer-associated fibroblasts related genes as a prognostic signature for head and neck squamous cell carcinoma. Heliyon. 2023;9(3):e14003.
8. Liu JB, et al. Plasma DNA methylation of wnt antagonists predicts recurrence of esophageal squamous cell carcinoma. World J Gastroenterol. 2011;17(44):4917–21.
9. Costa A, et al. Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. Cancer Cell. 2018;33(3):463–e47910.
10. Buchsbaum RJ, Oh SY. Breast cancer-associated fibroblasts: where we are and where we need to go. Cancers (Basel). 2016;8(2).
11. Sun DY, et al. Cancer-associated fibroblast regulate proliferation and migration of prostate cancer cells through TGF-β signaling pathway. Life Sci. 2019;235:116791.
12. Ham IH, Lee D, Hur H. Role of cancer-associated fibroblast in gastric cancer progression and resistance to treatments. J Oncol. 2019;2019:p6270784.
13. Navab R, et al. Prognostic gene-expression signature of carcinoma-associated fibroblasts in non-small cell lung cancer. Proc Natl Acad Sci U S A. 2011;108(17):7160–5.
14. Ko YC et al. Index of cancer-associated fibroblasts is superior to the epithelial-mesenchymal transition score in prognosis prediction. Cancers (Basel). 2020;12(7).

15. Bernemann C, et al. Influence of secreted frizzled receptor protein 1 (SFRP1) on neoadjuvant chemotherapy in triple negative breast cancer does not rely on WNT signaling. Mol Cancer. 2014;13:174.
16. Li Q, et al. PHF21B overexpression promotes cancer stem cell-like traits in prostate cancer cells by activating the Wnt/β-catenin signaling pathway. J Exp Clin Cancer Res. 2017;36(1):85.
17. Cho SH, et al. Rab37 mediates exocytosis of secreted frizzled-related protein 1 to inhibit wnt signaling and thus suppress lung cancer stemness. Cell Death Dis. 2018;9(9):868.
18. Caldwell GM, et al. The wnt antagonist sFRP1 in colorectal tumorigenesis. Cancer Res. 2004;64(3):883–8.
19. Shih YL, et al. SFRP1 suppressed hepatoma cells growth through wnt canonical signaling pathway. Int J Cancer. 2007;121(5):1028–35.
20. Sunkara RR, et al. SFRP1 in skin tumor initiation and cancer stem cell regulation with potential implications in epithelial cancers. Stem Cell Rep. 2020;14(2):271–84.
21. Chen C et al. Expression of tumor suppressor SFRP1 predicts biological behaviors and prognosis: a potential target for oral squamous cell carcinoma. Biomolecules. 2022;12(8).
22. Paluszczak J, et al. Frequent hypermethylation of WNT pathway genes in laryngeal squamous cell carcinomas. J Oral Pathol Med. 2014;43(9):652–7.
23. Ren XY, et al. Low SFRP1 expression correlates with poor prognosis and promotes cell invasion by activating the Wnt/β-Catenin signaling pathway in NPC. Cancer Prev Res (Phila). 2015;8(10):968–77.
24. Baharudin R et al. Epigenetics of SFRP1: the dual roles in human cancers. Cancers (Basel). 2020;12(2).
25. Lopez-Rios J, et al. The netrin-related domain of Sfrp1 interacts with wnt ligands and antagonizes their activity in the anterior neural plate. Neural Dev. 2008;3:19.
26. Liang CJ, et al. SFRPs are biphasic modulators of wnt-signaling-elicited cancer stem cell properties beyond extracellular control. Cell Rep. 2019;28(6):1511–e15255.
27. Mao W, Wordinger RJ, Clark AF. Focus on molecules: SFRP1. Exp Eye Res. 2010;91(5):552–3.
28. Hattori N, et al. Novel prodrugs of decitabine with greater metabolic stability and less toxicity. Clin Epigenetics. 2019;11(1):111.
29. van Loon K, Huijbers EJM, Griffioen AW. Secreted frizzled-related protein 2: a key player in noncanonical wnt signaling and tumor angiogenesis. Cancer Metastasis Rev. 2021;40(1):191–203.
30. Müller D, Győrffy B. DNA methylation-based diagnostic, prognostic, and predictive biomarkers in colorectal cancer. Biochim Biophys Acta Rev Cancer. 2022;1877(3):188722.
31. Cruciat CM, Niehrs C. Secreted and transmembrane wnt inhibitors and activators. Cold Spring Harb Perspect Biol. 2013;5(3):a015081.
32. Mosa MH, et al. A Wnt-induced phenotypic switch in cancer-associated fibroblasts inhibits EMT in colorectal cancer. Cancer Res. 2020;80(24):5569–82.
33. Kato M, et al. Heterogeneous cancer-associated fibroblast population potentiates neuroendocrine differentiation and castrate resistance in a CD105-dependent manner. Oncogene. 2019;38(5):716–30.
34. Kasashima H, et al. Stromal SOX2 upregulation promotes tumorigenesis through the generation of a SFRP1/2-expressing cancer-associated fibroblast population. Dev Cell. 2021;56(1):95–e11010.
35. Warr N, et al. Sfrp1 and Sfrp2 are required for normal male sexual development in mice. Dev Biol. 2009;326(2):273–84.
36. Martin-Manso G, et al. sFRP-1 binds via its netrin-related motif to the N-module of thrombospondin-1 and blocks thrombospondin-1 stimulation of MDA-MB-231 breast carcinoma cell adhesion and migration. Arch Biochem Biophys. 2011;509(2):147–56.
37. Martinez-Outschoorn UE, Lisanti MP, Sotgia F. Catabolic cancer-associated fibroblasts transfer energy and biomass to anabolic cancer cells, fueling tumor growth. Semin Cancer Biol. 2014;25:47–60.
38. Kobayashi H, et al. Cancer-associated fibroblasts in gastrointestinal cancer. Nat Rev Gastroenterol Hepatol. 2019;16(5):282–95.
39. Farhood B, Najafi M, Mortezaee K. Cancer-associated fibroblasts: secretions, interactions, and therapy. J Cell Biochem. 2019;120(3):2791–800.
40. Givel AM, et al. miR200-regulated CXCL12β promotes fibroblast heterogeneity and immunosuppression in ovarian cancers. Nat Commun. 2018;9(1):1056.
41. Barrett R, Puré E. Cancer-associated fibroblasts: key determinants of tumor immunity and immunotherapy. Curr Opin Immunol. 2020;64:80–7.
42. Baker AT, et al. Cancer-Associated fibroblasts and T cells: from mechanisms to outcomes. J Immunol. 2021;206(2):310–20.
43. Chakravarthy A, et al. TGF-β-associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure. Nat Commun. 2018;9(1):4692.
44. Ziani L, Chouaib S, Thiery J. Alteration of the antitumor immune response by cancer-associated fibroblasts. Front Immunol. 2018;9:414.
45. Monteran L, Erez N. The dark side of fibroblasts: cancer-associated fibroblasts as mediators of immunosuppression in the tumor microenvironment. Front Immunol. 2019;10:1835.
46. Kieffer Y, et al. Single-cell analysis reveals fibroblast clusters linked to immunotherapy resistance in cancer. Cancer Discov. 2020;10(9):1330–51.
47. Yang X, et al. FAP promotes immunosuppression by cancer-associated fibroblasts in the tumor microenvironment via STAT3-CCL2 signaling. Cancer Res. 2016;76(14):4124–35.
48. Lavie D, et al. Cancer-associated fibroblasts in the single-cell era. Nat Cancer. 2022;3(7):793–807.
49. Grout JA, et al. Spatial positioning and Matrix Programs of Cancer-Associated fibroblasts promote T-cell exclusion in human lung tumors. Cancer Discov. 2022;12(11):2606–25.
50. Xu Z, et al. Anatomically distinct fibroblast subsets determine skin autoimmune patterns. Nature. 2022;601(7891):118–24.
51. Hornburg M, et al. Single-cell dissection of cellular components and interactions shaping the tumor immune phenotypes in ovarian cancer. Cancer Cell. 2021;39(7):928–e9446.
52. Li C, et al. Spatial and single-cell transcriptomics reveal a cancer-associated fibroblast subset in HNSCC that restricts infiltration and antitumor activity of CD8 + T cells. Cancer Res. 2024;84(2):258–75.
53. Kraman M, et al. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science. 2010;330(6005):827–30.
54. Cheng Y, et al. Cancer-associated fibroblasts induce PDL1 + neutrophils through the IL6-STAT3 pathway that foster immune suppression in hepatocellular carcinoma. Cell Death Dis. 2018;9(4):422.
55. Fang Y, et al. Cancer-associated fibroblast-like fibroblasts in vocal fold leukoplakia suppress CD8(+)T cell functions by inducing IL-6 autocrine loop and interacting with Th17 cells. Cancer Lett. 2022;546:215839.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.