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SFRP1 and SFRP2 jointly regulate normal and malignant hematopoietic progenitors by influencing mesenchymal cells in the hematopoietic niche

Hikaru Tsutsumi, Fuki Kumagai, Kazuki Tsutsui, Akio Maekawa, Aya Ohno, Hikari Okamoto, Ayana Ueno, Ayano Kuga, Natsumi Hasegawa, Mitsuhiro Ito^{*}

Laboratory of Hematology, Division of Medical Biophysics, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe, 654-0142, Japan

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ABSTRACT

Secreted frizzled-related proteins (SFRPs) are secreted proteins identified as antagonists of the Wnt signaling pathway. *Sfrp1* or *Sfrp2* deletion in mice attenuates the hematopoietic stem/progenitor cell (HSPC) pool; however, the mechanisms by which SFRP1/2 support hematopoietic cells remain unclear. Here, we showed that SFRP1/2 functions as a niche factor for both normal and malignant HSPCs by acting on mesenchymal stromal cells (MSCs). Addition of SFRP1/2-blocking antibodies to a co-culture of OP-9 MSCs and normal mouse bone marrow hematopoietic cells reduced the long-term culture-initiating cell number. Addition of these antibodies to the co-culture of MSCs and MB-1 myeloblastoma cells reduced the cobblestone area, which is a characteristic feature of leukemic stem cells. During co-culture with MB-1 cells, these antibodies upregulated the target genes of the Wnt and bone morphogenetic protein pathways in OP-9 MSCs, but not in MB-1 cells. Additionally, addition of blocking antibodies to OP-9 MSC monoculture attenuated cell growth. Overall, our findings suggest that SFRP1 and SFRP2 maintain normal and malignant HSPC functions in the hematopoietic niche by modulating the Wnt and bone morphogenetic protein pathways in MSCs.

1. Introduction

Hematopoietic niche, a specialized microenvironment within the bone marrow (BM), plays a pivotal role in regulating the self-renewal and differentiation of hematopoietic stem/progenitor cells (HSPCs) to maintain homeostasis. Within this niche, mesenchymal stromal cells (MSCs) support HSPCs through adhesive interactions and biochemical cues, including chemokines and extracellular matrix components [1,2]. This concept is fundamental to understand the pathophysiology of hematological disorders as genetic alterations in MSCs initiate and promote hematopoietic malignancy [3]. Therefore, understanding these complicated interactions will provide valuable insights into HSPC regulation dynamics and hematological disease pathogenesis.

Mediator, a subcomplex of RNA polymerase II holoenzyme, serves as a convergence point for various intracellular signaling pathways. This Mediator plays an essential role in initiating transcription by recruiting general transcription factors and facilitating the assembly of a functional preinitiation complex [4–6]. Mediator subunit MED1, specific co-activator of various activators including nuclear receptors, is crucial for the niche functions of mesenchymal cells [7]. Using *Med1*-deficient

mouse embryonic fibroblasts (MEFs), we previously identified osteopontin, fibroblast growth factor 7, periostin, and matrix Gla protein as niche factors produced under MED1 regulation [7–10]. These findings underscore the essential roles of MED1 in hematopoietic niche functions.

Wnt signaling, initiated by the binding of cysteine-rich protein Wnt ligands to frizzled (FZD) receptors, regulates the proliferation and polarity of HSPCs [11,12]. Dysregulation of Wnt signaling is frequently associated with tumorigenesis, with elevated levels of β -catenin, a downstream effector of the canonical Wnt pathway, observed in the bone lineage cells of patients with myelodysplastic syndrome and acute myeloid leukemia [3,13].

Secreted frizzled-related protein (SFRP) family consists of five members, each containing approximately 300 amino acids, with a netrin-like domain and cysteine-rich domain homologous to that of FZD receptors; it inhibits Wnt signaling by binding directly to the Wnt ligands or FZD receptors [14]. Specifically, SFRP1 and SFRP2 interact with Wnt3a and play distinct roles in HSPC self-renewal [15]. Studies on *Sfrp1* and *Sfrp2* knockout mice have demonstrated that these genes are critical for maintaining the HSPC pool [16,17]; however, the specific

^{*} Corresponding author.

E-mail address: itomi@med.kobe-u.ac.jp (M. Ito).

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mechanisms remain unclear.

In this study, we conducted *in vitro* experiments using normal mouse BM hematopoietic cells and MB-1 myeloblastoma cells, which were established from a patient with chronic myeloid leukemia blast crisis [18,19], to investigate the roles and action mechanisms of SFRP1/2 in normal and malignant niches.

2. Methods

2.1. Cell culture

Med1^{+/+}*p53*^{-/-} and *Med1*^{-/-}*p53*^{-/-} MEFs [7] were cultured in the Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with 10 % fetal bovine serum (FBS) (MP Biomedicals). Mouse BM MSC lines, OP-9 (RIKEN BioResource Center) and MS-5 cells [20], were maintained in the α -modified Eagle's medium (α MEM) (Thermo Fisher Scientific) supplemented with 20 % horse serum (Sigma-Aldrich) and 20 % FBS, respectively. MC3T3 osteoblastic cells were maintained in α MEM supplemented with 10 % FBS. MB-1 cells, stromal cell-dependent myeloblastic leukemia cells [18], were co-cultured with OP-9 or MS-5 cells growth-arrested by treating with 3 μ g/mL mitomycin C (Nacalai Tesque). Co-culture was performed in α MEM supplemented with 10 % FBS, 1 % non-essential amino acids (Wako), 100 μ M β -mercaptoethanol, and 1 % penicillin/streptomycin (Sigma-Aldrich). For OP-9 cell monoculture, 2×10^4 cells were plated in a 24-well plate and cultured at 37 °C. α -SFRP1 polyclonal (sc-13939 [Santa Cruz Biotechnology] or 26460-AP [Proteintech]) and α -SFRP2 (polyclonal [sc-13940; Santa Cruz Biotechnology] or monoclonal [SC-36524; Santa Cruz Biotechnology]) antibodies (0.5 μ g/mL) or an equivalent amount of normal IgG (A16104; Invitrogen) as a control were used in the tests.

2.2. Colony-forming assay and cobblestone area

BM hematopoietic cells (1×10^6) isolated from C57BL6J male mouse femora and tibiae were co-cultured with growth-arrested OP-9 or MS-5 cells in the MyeloCult M5300 medium (Stem Cell Technologies) in the presence of 0.5 μ g/mL α -SFRP1 or α -SFRP2 antibody (above) or normal IgG as a control. The culture medium was replaced weekly. After four weeks, all cells were harvested using trypsin and cultured in the methylcellulose medium (Methocult M3434; Stem Cell Technologies) at 37 °C for seven days, and number of colonies (long-term culture-initiating cells [LTC-ICs]) was recorded. Animal experiments were performed according to the institutional guidelines after obtaining ethical approval from the Kobe University Animal Experiments Committee.

A cobblestone area (CA) is defined as a group of four or more adjacent MB-1 cells exhibiting characteristic cobblestone morphology [18]. Here, CAs were observed in three randomly selected fields.

2.3. Immunofluorescence staining

OP-9 cells were cultured in a 12-well plate and fixed with 4 % paraformaldehyde (Wako) at 25 °C for 10 min. Permeabilization was performed using 20 % Triton X-100 (Wako). The cells were blocked with 10 % goat serum (Jackson Immuno Research) and incubated overnight at 4 °C with the α -SFRP1 polyclonal (26460-AP) or α -SFRP2 monoclonal (SC-36524) primary antibodies. The following day, the cells were incubated with the following secondary antibodies under light-protected conditions at 37 °C for 1 h: Alexa Fluor 488 goat anti-mouse IgG (2306579; Life Technologies) for α -SFRP1 and Alexa Fluor 555 goat anti-rabbit IgG (2298171; Life Technologies) for α -SFRP2. Finally, the nuclei were stained with 4',6-diamidino-2-phenylindole (Wako), and images were captured by using Axio Vert.A1 (Zeiss).

2.4. DNA synthesis

DNA synthesis was assessed via 5-bromo-2'-deoxyuridine (BrdU)

incorporation. The cells were treated with 100 μ M BrdU for 20 h. BrdU incorporation into DNA was subsequently measured using the Cell Proliferation ELISA for BrdU (chemiluminescence) kit (Roche).

2.5. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA, extracted from the cells using Isogen (Toyobo), was converted to cDNA using the ReverTra Ace qPCR RT Master Mix with a gDNA Remover kit (Toyobo). RNA expression levels of various mouse and human genes were quantified using the KOD SYBR qPCR Mix (Toyobo) and StepOnePlus Real-Time PCR system (Thermo Fisher Scientific). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. Primer sequences are listed (Supplementary Table 1).

2.6. Western blotting

Cell lysates were fractionated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and protein expression was examined using the α -SFRP1 rabbit polyclonal (ab4193; Abcam), α -SFRP2 rabbit polyclonal (12189-1-AP; Proteintech), and anti- β -actin (MAB1501; Merck Millipore) antibodies.

2.7. Statistical analyses

All experimental results are expressed as the mean $\pm$ standard deviation. The measurements were obtained from three independent samples for each experimental condition. Statistical analyses were conducted via Student's *t*-test or one-way analysis of variance, followed by Tukey's post-hoc test, as appropriate. Statistical significance was set at $p < 0.05$ and indicated as follows: * $p < 0.05$ and ** $p < 0.01$.

3. Results

3.1. Expression levels and distribution of SFRP1/2 in hematopoietic niche-associated cells and MEFs

We previously identified MED1 as a transcription factor critical for the regulation of the hematopoietic niche. Microarray analysis has demonstrated significantly reduced *Sfrp1* and *Sfrp2* expression levels in *Med1*^{-/-} MEFs (7). Therefore, protein expression levels of mouse SFRP1 and SFRP2 were examined in various mesenchymal cells, including OP-9 and MS-5 MSCs, MC3T3 osteoblastic cells, MEFs, and BM hematopoietic cells (Fig. 1A). Western blotting revealed that both SFRP1 and SFRP2 were highly expressed in the wild-type MEFs; however, their expression levels were profoundly reduced in *Med1*^{-/-} MEFs. Minimal SFRP1 and SFRP2 expression was observed in BM hematopoietic cells. Mesenchymal cells, MS-5, OP9, and MC3T3 strongly expressed SFRP1 but only marginally expressed SFRP2. These findings highlight the cell type-specific differences in the expression levels of SFRP1 and SFRP2, suggesting their potential roles in the hematopoietic niche.

SFRPs translocate to the nucleus in malignant cells, where they directly bind to β -catenin and modulate its pathway [21]. To investigate SFRP1/2 distribution, immunofluorescence staining was performed using OP-9 cells (Fig. 1B). SFRP2 was localized on the cell membrane, whereas SFRP1 was distributed not only on the cell membrane but also within the cytoplasm, but it was not detected in the nucleus. As SFRP1/2 are minimally expressed in hematopoietic cells and do not localize to the nuclei in MSCs, these results suggest that SFRP1/2 are secretory proteins that have minimal or no role in directly binding to β -catenin and suppressing its activity in the nucleus under normal hematopoietic micro-environmental conditions.

3.2. Oscillated expression levels of genes encoding SFRP1/2

Stromal cell-dependent MB-1 cells were maintained via co-culture

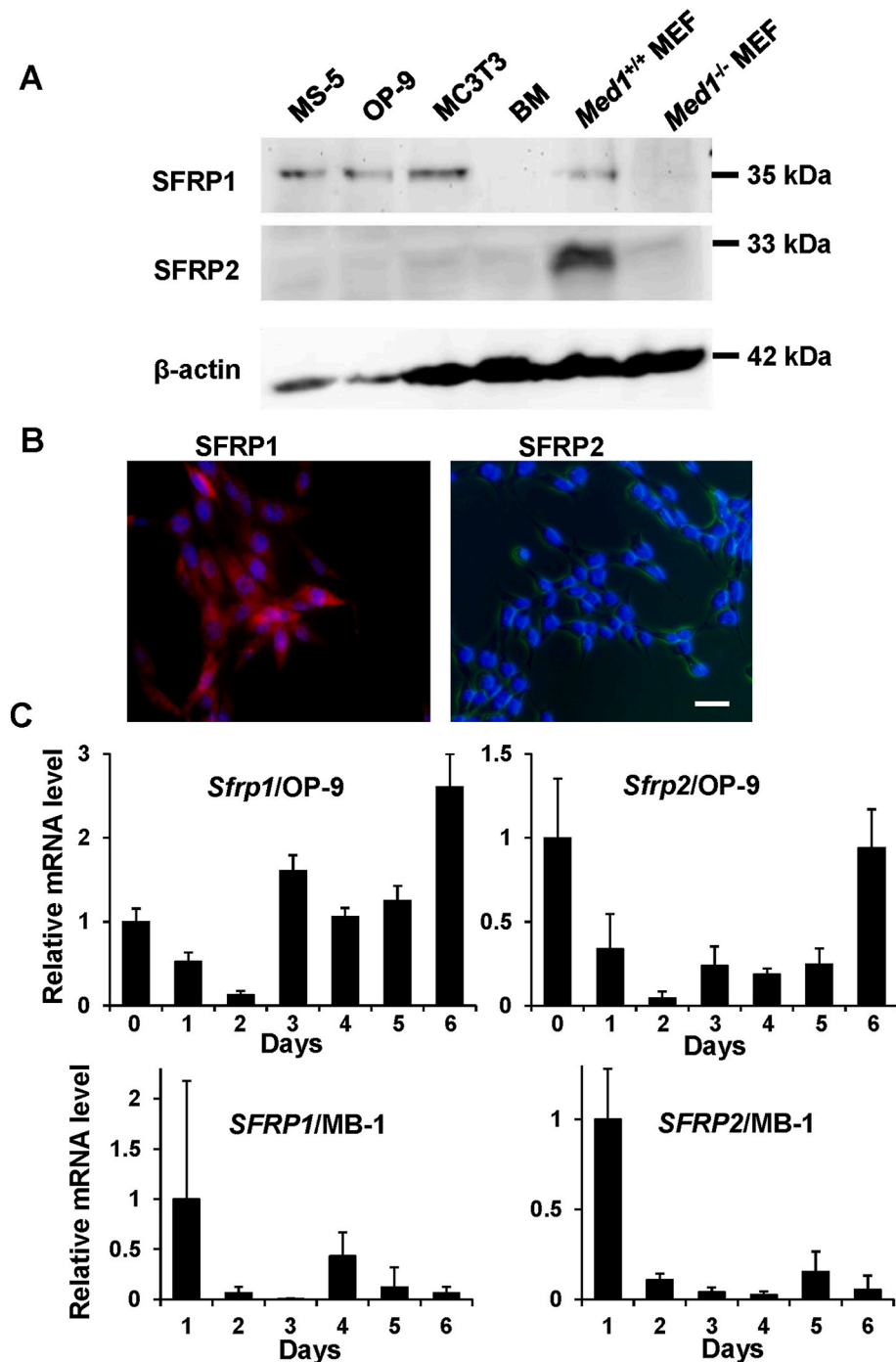


Fig. 1. Expression levels of secreted frizzled-related protein (SFRP)-1/2 in hematopoietic niche-associated cells. (A) Western blot showing the SFRP1/2 expression levels in mesenchymal stromal cells (MSCs; MS-5 and OP-9), osteoblastic cells (MC3T3), bone marrow (BM) hematopoietic cells, and mouse embryonic fibroblasts (MEFs). (B) Immunofluorescence staining of OP-9 cells. SFRP1 was localized in the cytoplasm, whereas SFRP2 was localized in the cell membrane. Red, SFRP1; green, SFRP2; blue, 4',6-diamidino-2-phenylindole (DAPI). Bar, 100 μ m. (C) Daily expression levels of *Sfrp1*/2 in OP-9 and *SFRP1*/2 in MB-1 cells during co-culture were quantified via reverse transcription-polymerase chain reaction (RT-PCR). *Sfrp1*/2 and *SFRP1*/2 were reciprocally expressed during the six days of co-culture. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

with OP-9 MSCs. MB-1 cells have the characteristic ability to proliferate by migrating beneath the stromal cells and forming CAs, a property thought to accurately replicate the stochastic model of leukemic stem cells (LSCs) [18,19].

To elucidate the mechanisms by which the SFRP1/2 levels were temporally regulated in the co-culture, we analyzed the mRNA levels of *Sfrp1*/2 and *SFRP1*/2 in OP-9 and MB-1 cells, respectively, via RT-qPCR using species-specific primer pairs (Fig. 1C). In OP-9 cells, *Sfrp1* and

Sfrp2 levels decreased on day 1 of co-culture, followed by a gradual return to baseline levels over subsequent days. Conversely, in MB-1 cells, *SFRP1* and *SFRP2* levels were upregulated on day 1 of co-culture and subsequently decreased. These findings indicate the dynamic expression patterns, in which SFRP1 and SFRP2 predominantly originate from MB-1 cells at the early stage of co-culture but are gradually replaced by the OP-9 cell-derived ones over time.

3.3. SFRP1/2 support the maintenance/proliferation of hematopoietic cells in normal and malignant niches

To elucidate the functions of SFRP1/2 in the hematopoietic micro-environment, we conducted experiments using a simplified *in vitro* hematopoietic niche model. This model involves the co-culture of mitomycin C-treated OP-9 cells with BM hematopoietic cells to assay the number of LTC-ICs, reflecting the survival of HSPCs. During the initial two weeks of co-culture, addition of α -SFRP1, α -SFRP2, or both antibodies significantly reduced the hematopoietic cell number among OP-9 cells by day 4 compared to that in the controls. On day 8, groups treated with both antibodies showed a more pronounced reduction in cell

number than those treated with either antibody alone (Fig. 2A). Antibody-effected reduction in hematopoietic cell number was reproduced when BM hematopoietic cells were co-cultured with MS-5 cells using the other pair of α -SFRP1/2 antibodies (Supplementary Fig. 1A). Although not statistically significant, DNA synthesis revealed a similar trend of reduction in each antibody-treated group compared to that in the control group, with the greatest decrease observed in the group treated with both antibodies (Supplementary Fig. 1B).

After four weeks of co-culture, LTC-IC number was measured. OP-9 cell-supported LTC-IC number was profoundly reduced in the presence of either antibody (Fig. 2B). Notably, myeloid and erythroid colonies were equally reduced, indicating that the α -SFRP1/2 antibody-mediated

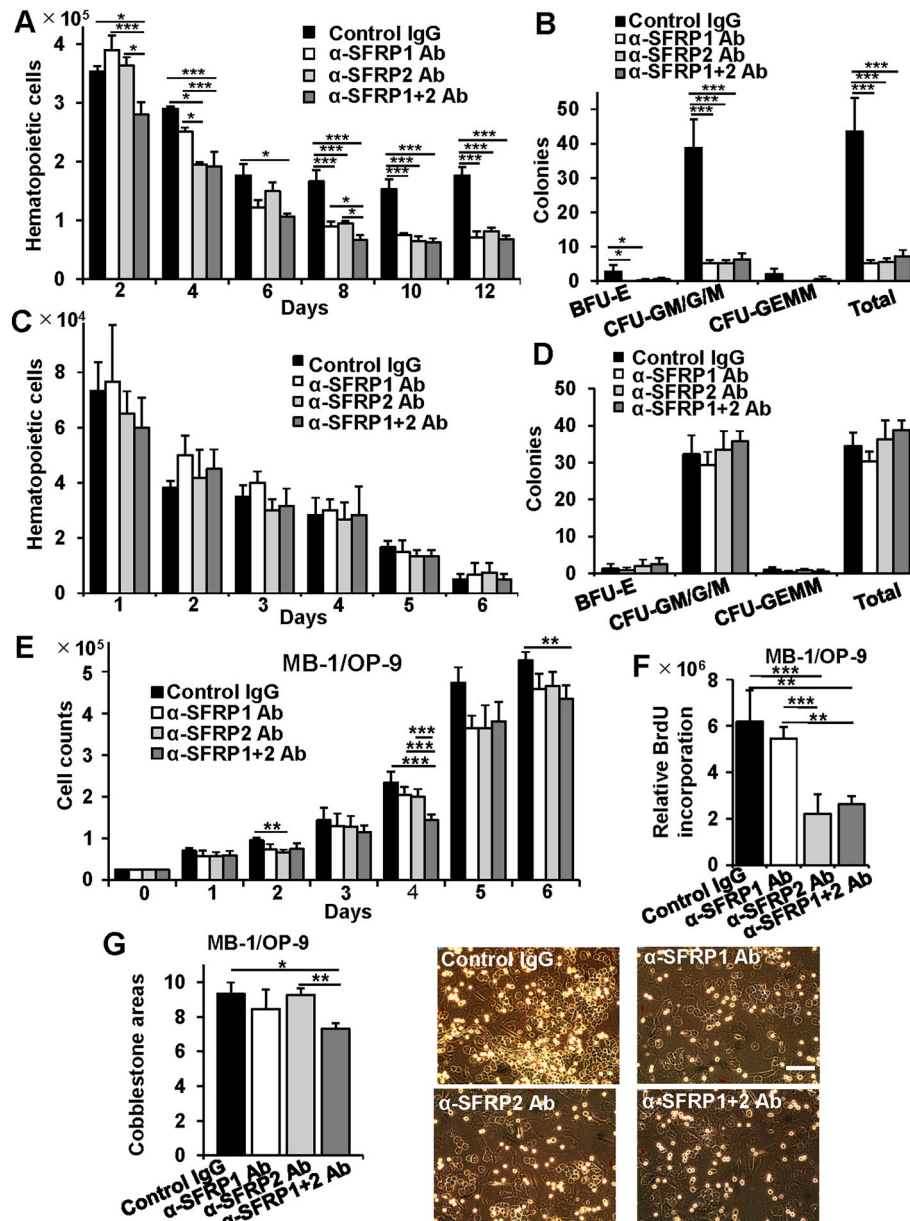


Fig. 2. SFRP1/2 contribute to the maintenance of hematopoietic stem/progenitor cells (HSPCs) and MB-1 cells during co-cultures with OP-9 cells. (A and B) Co-culture with BM hematopoietic cells. Addition of α -SFRP1/2 antibodies resulted in the additive reduction in BM hematopoietic cell numbers during first two weeks (A). Long-term culture-initiating cells (LTC-ICs) assessed after four weeks revealed profound reduction by the antibodies. Erythroid and myeloid colonies were equally depressed (B). (C and D) Culture of BM hematopoietic cells without OP-9 cells. Addition of α -SFRP1/2 antibodies had no effect during a week on cell numbers (C) or LTC-ICs (D). (E–G) Co-culture with MB-1 myeloblastoma cells. Addition of α -SFRP1/2 antibodies resulted in the additive reduction in cell numbers (E). DNA synthesis on day 4 was significantly decreased in the groups treated with α -SFRP2 alone and both antibodies (F). Cobblestone areas (CAs) on day 3 were significantly reduced in the group treated with both antibodies (G). Bar, 100 μ m.

reduction of the hematopoietic cell number during co-culture (Fig. 2A) was not biased but directed to both myeloid and erythroid/megakaryocytic lineages (above). Co-culture with MS-5 cells in the presence of the other pair of SFRP1/2-blocking antibodies reproduced the results, but the LTC-IC number was further profoundly reduced when both antibodies were added (Supplementary Fig. 1C). These findings suggest that SFRP1/2 jointly function as niche factors essential for the maintenance of normal HSPCs.

To elucidate whether the α -SFRP1/2 antibody-mediated reductions of the hematopoietic cell and LTC-IC numbers originate in the direct action on hematopoietic cells, we treated BM hematopoietic cells (1×10^5) alone with α -SFRP1/2 antibodies without mesenchymal cells. The number of hematopoietic cells decreased rapidly without MSCs, being reduced to less than one tenths on day 6, and the antibodies had no effect (Fig. 2C). These antibodies did not alter the colony-forming units (CFUs), either (Fig. 2D). Hence, the effect of α -SFRP1/2 antibodies on

hematopoietic cells appears to be indirect and caused by the action on the hematopoietic niche.

To further investigate whether SFRP1 and SFRP2 also play a role in maintaining LSCs, we conducted co-culture experiments using MB-1 and OP-9 cells. A significant reduction in MB-1 cell number was observed from day 4 in the antibody-treated groups, and MB-1 cells treated with both antibodies showed a more pronounced decrease than the groups treated with either antibody alone (Fig. 2E). DNA synthesis, assessed on day 4, also exhibited a greater reduction in the group treated with the α -SFRP2 antibody (Fig. 2F). Furthermore, CA, an indicator of LSCs, decreased by approximately 70 % in the group treated with both antibodies compared to that in the control group (Fig. 2G). Similar results were obtained with the other set of α -SFRP1 and α -SFRP2 antibodies (Supplementary Fig. 2A and B). These experiments indicate SFRP1/2 as niche factors influencing the maintenance and growth of normal and malignant HSPCs in both the normal and malignant hematopoietic

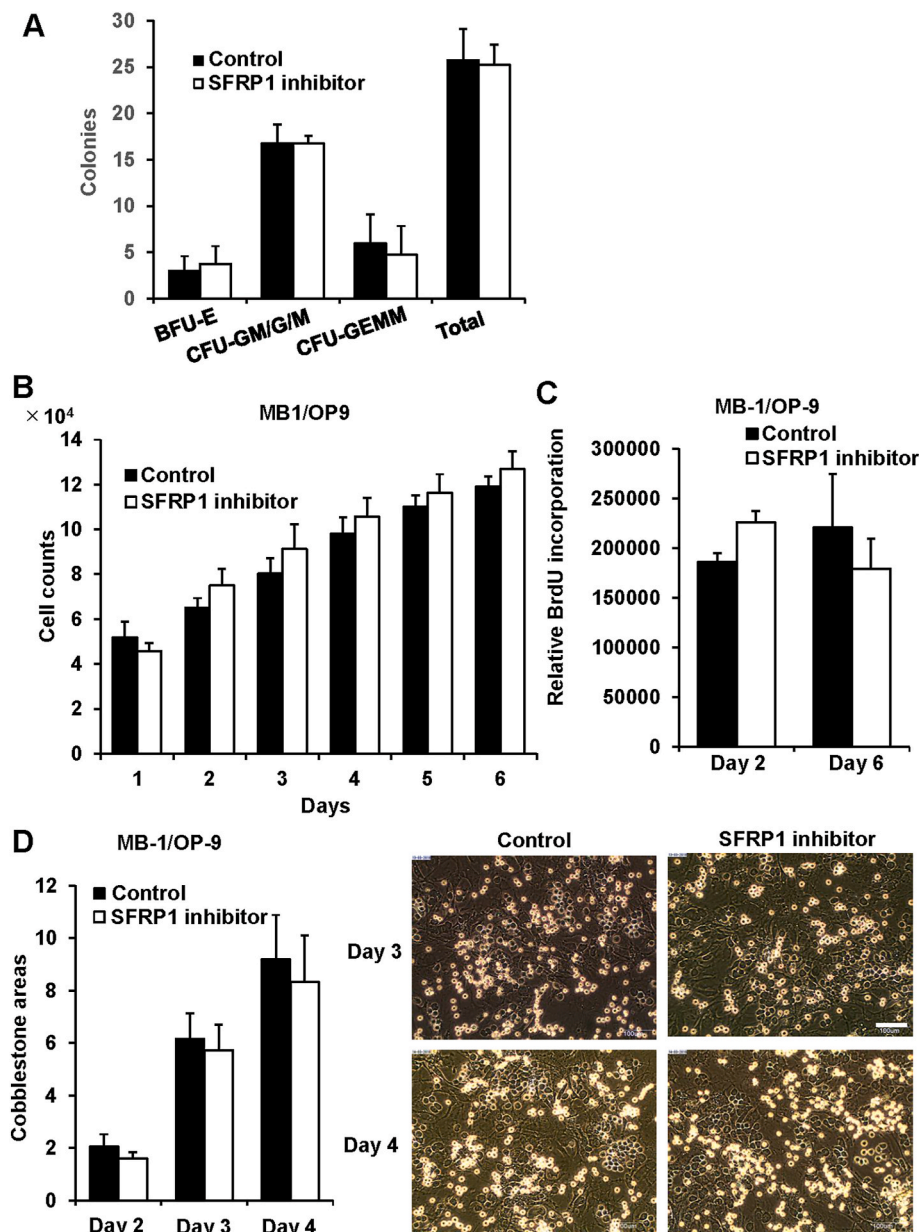


Fig. 3. SFRP1 inhibitor does not impact the niche functions.

(A) Co-culture of BM hematopoietic and OP-9 cells. LTC-ICs were unaffected by the SFRP1 inhibitor, WAY 316606 hydrochloride, which ablated the SFRP1–Wnt interaction.

(B–D) Co-culture of MB-1 and OP-9 cells. SFRP1 inhibitor did not affect the cell number (B), DNA synthesis (C), and CA (D). Bar, 100 μ m.

niches.

3.4. SFRP1 action on hematopoietic niche is not solely attributed to the Wnt pathway

SFRP1 primarily functions as an antagonist of the Wnt pathway that possibly contributes to the maintenance and proliferation of the hematopoietic niche via Wnt pathway inhibition [16]. To investigate the influence of SFRP1–Wnt binding on LTC-ICs and evaluate the characteristics of MB-1 cells, a potent SFRP1 inhibitor (WAY 316606 hydrochloride; Biotechne) was added to the co-cultures at 8 $\mu\text{mol/L}$.

When the SFRP1 inhibitor was added to the co-culture of BM hematopoietic and OP-9 cells, numbers of hematopoietic cells during the initial two weeks and LTC-ICs assessed after four weeks of co-culture were similar to those in the control group (Fig. 3A and B). Moreover,

addition of the SFRP1 inhibitor to the MB-1/OP-9 co-culture did not cause any difference in the cell number, DNA synthesis, and CA compared to those in the control group (Fig. 3C and D). Therefore, unlike the addition of the α -SFRP1 blocking antibody, SFRP1 inhibitor did not affect LTC-ICs or LSC features of MB-1 cells. These data suggest that the effect of SFRP1 on Wnt signaling is not sufficient for hematopoietic niche function.

3.5. SFRP1/2 regulate the hematopoietic niche by inhibiting the Wnt and bone morphogenetic protein (BMP) pathways

As ablation of the SFRP1–Wnt interaction had no effect on the maintenance of normal and malignant HSPC functions, we investigated whether SFRP1/2 is involved in pathways other than Wnt signaling. Based on a report that SFRP2 inhibits the BMP pathway in MSCs [22],

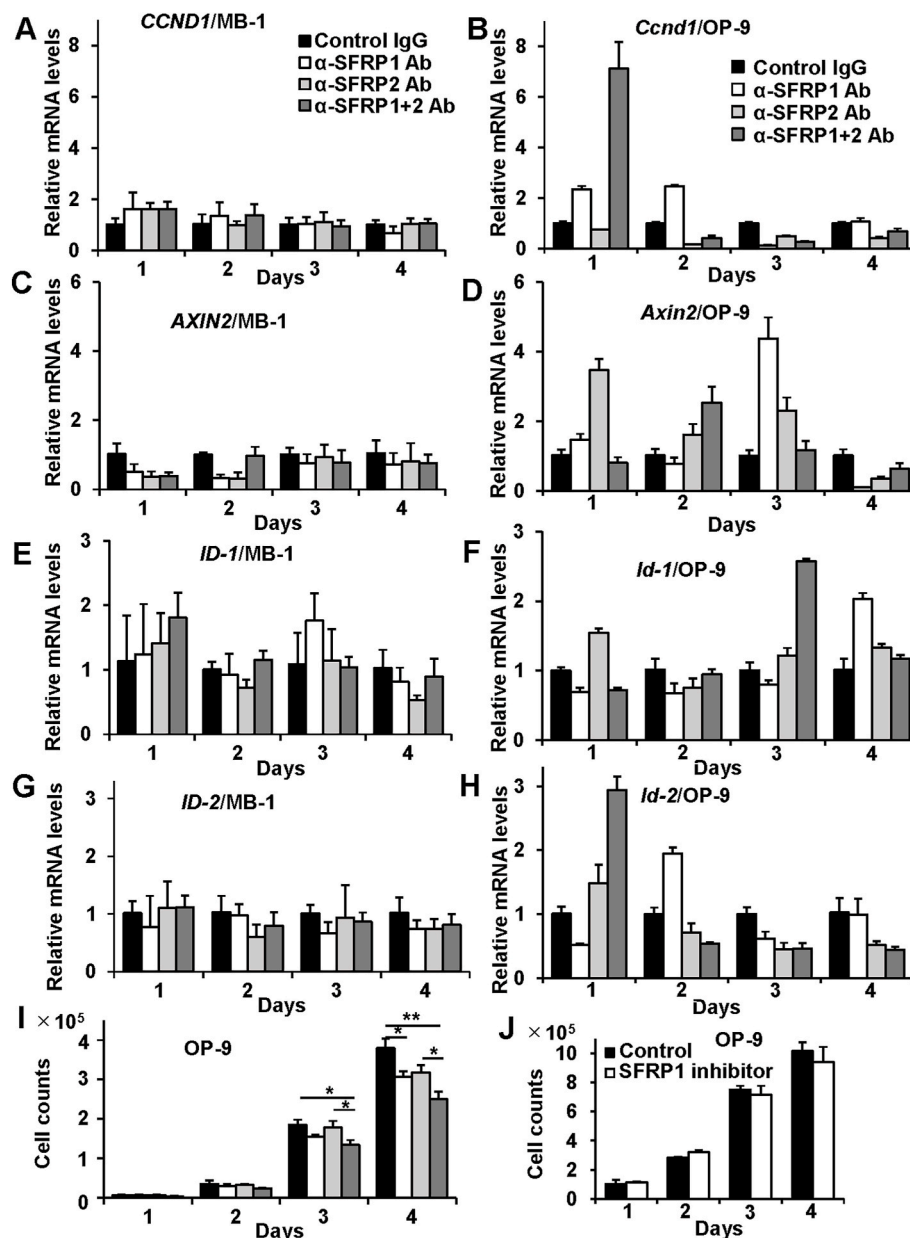


Fig. 4. Anti-SFRP1/2 antibody-induced upregulation of Wnt- and bone morphogenetic protein (BMP)-targeted gene expression levels in OP-9 cells. (A–H) Quantitative RT-PCR for MB-1 and OP-9 cell co-culture. Anti-SFRP1/2 antibodies did not upregulate the levels of Wnt-targeted genes encoding cyclin D1 and AXIN, not BMP-targeted genes encoding inhibitor of DNA-binding (ID)-1 and ID2 in MB-1 cells (A, C, E, and G), but upregulated them in OP-9 cells in a spiked manner (B, D, F, and H). (I and J) OP-9 cell monoculture. Cell number was decreased by the α -SFRP1/2 antibodies in an additive manner (I), but not by the SFRP1 inhibitor (J).

we analyzed the expression levels of Wnt- and BMP-targeted genes in culture systems treated with the α -SFRP1 and/or α -SFRP2 antibodies using species-specific primer pairs (Fig. 4A–H).

In MB-1 cells, expression levels of Wnt signaling-targeted genes (*AXIN2*, and *CCND1* encoding cyclin D1 [*CCND1*]) and BMP signaling-targeted genes (*ID1* encoding inhibitor of DNA-binding 1 [*ID1*], and *ID2*) did not change after antibody treatment. In contrast, surged over-expression of both Wnt and BMP pathway-targeted genes by the α -SFRP1/2 antibodies was observed in OP-9 cells. Although a transient reduction was observed in some gene levels, all target genes showed transcription levels exceeding twice those observed in the control at specific time points, depending on the antibody type (Fig. 4A–H). These results suggest that SFRP1/2 coordinate with hematopoietic cells by modulating the Wnt and BMP pathway activity in mesenchymal cells, thereby maintaining the hematopoietic cell functions in the hematopoietic niche.

To further investigate the biological effects of SFRP1/2 on OP-9 cells, antibodies were added to the untreated OP-9 cells. Addition of these antibodies upregulated the Wnt- and BMP-targeted gene levels (Supplementary Fig. 3A–D), as observed in the co-culture. Moreover, either antibody alone reduced the cell number by 20 %, whereas their combination reduced the cell number by 40 % (Fig. 4I). Notably, SFRP1 inhibitor, which impeded the SFRP1–Wnt interaction, had no effect (Fig. 4J). Collectively, these findings suggest that SFRP1/2 maintain the hematopoietic microenvironment by acting on MSCs.

4. Discussion

To the best of our knowledge, this study is the first to report that SFRP1/2 jointly function as niche factors in both normal and malignant hematopoietic niches by acting on MSCs rather than on hematopoietic cells via the Wnt and BMP pathways. SFRP1/2 apparently maintain the stemness of MSCs in this manner [22]. It also provides novel insights into the roles of SFRP1/2 in supporting HSPCs and LSCs in the hematopoietic microenvironment. A precedent for this notion has been set in breast carcinoma. Although SFRP1 is often characterized as a tumor suppressor owing to its role as a Wnt signaling antagonist, suppressing excessive pathway activation, it also exhibits a dual nature, as evidenced by its high expression levels in basal-like and triple-negative breast cancers [23].

SFRP1 and SFRP2 from MSCs and leukemic cells alternately replace each other while participating in crosstalk. β -catenin activation in HSPCs inhibits their self-renewal and differentiation, leading to HSPC depletion in transgenic mice [24]. Similarly, in this study, SFRP1 and SFRP2 inhibition possibly disrupted the natural crosstalk between MSCs and HSPCs or LSCs. This disturbance possibly results in stem cell depletion *in vivo*, as observed in previous studies [16,17,25].

Inhibition of the interaction between SFRP1 and Wnt is insufficient to suppress the maintenance or proliferation of hematopoietic cells. This is possibly due to the inability of the inhibitor to block the BMP pathway, which is regulated by SFRP2 through unknown mechanisms [22], warranting further investigation into the interactions between SFRPs and BMP signaling. In this regard, surmising the mechanisms of action of the antibodies in this study might be helpful. The common domains of the immunogens for the antibodies are SFRP1(164–250) and SFRP2 (156–295), which are adjacent to the cysteine-rich domain and contain the netrin-like domain [14]. The antibodies bound to this region of SFRP1/2 must have hindered not only the SFRP–Wnt interaction but also the SFRP-mediated suppression of BMP signaling.

Involvement of bypass pathways mediated through regions of SFRP1 outside its inhibitor-binding sites has also been suggested. SFRPs bind to β -catenin via their cysteine-rich domain or netrin-like domain and translocate to the nucleus, where they either activate or inhibit β -catenin-induced transcription depending on the binding mode [21]. In this study, owing to the nature of the co-culture system, immunofluorescence staining was limited to MSCs. Future investigations are necessary

to examine possible nuclear translocation of SFRPs and associated β -catenin pathway activity in LSCs. Although SFRP1/2 are minimally expressed in normal BM hematopoietic cells, high mRNA levels of *SFRP1/2* were detected in MB-1 cells in this study. Considering the ectopic expression of SFRPs in leukemic cells, future studies should explore the nuclear translocation of SFRPs in malignant niches and their associated effects on β -catenin signaling activity.

SFRP expression patterns possibly differ in different leukemia cells, resulting in varying stem cell-supporting functions. Upon further confirmation of the stem cell-supporting functions of SFRPs in other leukemia cells and elucidation of the differences between the inhibitor and blocking antibody mechanisms, SFRPs can serve as promising therapeutic targets specifically acting on the niche environment of LSCs.

CRedit authorship contribution statement

Hikaru Tsutsumi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Fuki Kumagai:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation. **Kazuki Tsutsui:** Conceptualization, Data curation, Formal analysis, Investigation, Validation. **Akio Maekawa:** Data curation, Formal analysis, Investigation. **Aya Ohno:** Data curation, Investigation. **Hikari Okamoto:** Data curation, Investigation. **Ayana Ueno:** Data curation, Investigation, Validation. **Ayano Kuga:** Data curation, Investigation, Validation. **Natsumi Hasegawa:** Conceptualization, Data curation, Formal analysis, Project administration, Supervision. **Mitsuhiro Ito:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2025.152081>.

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