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FGF7 supports hematopoietic stem and progenitor cells and niche-dependent myeloblastoma cells via autocrine action on bone marrow stromal cells in vitro

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ABSTRACT

FGF1 and FGF2 support hematopoietic stem and progenitor cells (HSPCs) under stress conditions. In this study, we show that fibroblast growth factor (FGF7) may be a novel niche factor for HSPC support and leukemic growth. FGF7 expression was attenuated in mouse embryonic fibroblasts (MEFs) deficient for the MED1 subunit of the Mediator transcriptional coregulator complex. When normal mouse bone marrow (BM) cells were cocultured with *Med1*$^{+/+}$ MEFs or BM stromal cells in the presence of anti-FGF7 antibody, the growth of BM cells and the number of long-time culture-initiating cells (LTC-ICs) decreased significantly. Anti-FGF7 antibody also attenuated the proliferation and cobblestone formation of MB1 stromal cell-dependent myeloblastoma cells. The addition of recombinant FGF7 to the coculture of BM cells and *Med1*$^{-/-}$ MEFs increased BM cells and LTC-ICs. FGF7 and its cognate receptor, FGFR2IIIb, were undetectable in BM cells, but MEFs and BM stromal cells expressed both. FGF7 activated downstream targets of FGFR2IIIb in *Med1*$^{+/+}$ and *Med1*$^{-/-}$ MEFs and BM stromal cells. Taken together, we propose that FGF7 supports HSPCs and leukemia-initiating cells indirectly via FGFR2IIIb expressed on stromal cells.

1. Introduction

Postnatal mammalian hematopoietic stem and progenitor cells (HSPCs) are strictly regulated by specific microenvironmental niches in the bone marrow (BM). BM mesenchymal stem cells (MSCs) and their derived or related cells constitute important cellular components of the hematopoietic niche [reviewed in 1–3]. The endosteal region of the BM was first identified as the resting HSPC reservoir, where osteoblasts of mesenchymal origin primarily play a niche function. Meanwhile, recent studies have highlighted the BM sinusoidal vasculature and perivascular region, which are composed of MSCs or related stromal cells (Nestin^{+} MSCs, CXC chemokine ligand 12 (CXCL12)-abundant reticular cells, pericytes, and endothelial cells), as a major niche for HSPCs [4–6, reviewed in 1–3]. Molecular components of the HSPC niche include angiopoietin-1 (Ang-1), CXCL12, Wnt signaling, and N-cadherin, which in concert control HSPC behavior [reviewed in 1–3]. We have also reported osteopontin (OPN), produced by stromal cells, as an important niche factor [7].

Fibroblast growth factors (FGFs) comprise a large family of heparin-binding growth factors that regulate cell migration, growth, and differentiation/fate. A family of four receptor tyrosine kinases, designated FGF receptor (FGFR) 1 through FGFR4, acts as the specific receptors for FGFs to initiate intracellular signaling reviewed in [8,9]. FGFR1 and FGF2 appear to be dispensable for homeostatic hematopoiesis [10,11]. However, in response to stimulatory stresses to HSPCs, FGFR1 expressed on HSPCs reportedly promotes HSPC expansion through FGF1 (and possibly FGF4) produced by megakaryocytes [10], while FGF2 produced by megakaryocytes indirectly promotes HSPC expansion via the proliferation of stromal cells, which in turn change the levels of niche signals [11]. In this manner, some FGF signaling appears to be a key regulator of HSPC proliferation and recovery in stressed environments.

FGF7 (also known as keratinocyte growth factor), produced by mesenchymal cells, binds to and activates the specific receptor tyrosine kinase FGFR2IIIb, which in turn rapidly activates

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Ras-mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase-Akt signaling pathways. FGFR2IIIb, expressed mainly on epithelial cells, functions in epithelial cell growth, wound healing, and hair development [reviewed in 8,9]. Upon hepatic injury, Thy1^{+} mesenchymal cells produce FGF7 in close proximity to liver progenitor cells, which subsequently proliferate and contribute to liver regeneration. Thus, FGF7 appears to be a functional niche signal for the stimulation of liver progenitor cells [12]. However, the role of FGF7 in HSPC maintenance is not known.

The Mediator transcriptional coregulator complex, a subcomplex of RNA polymerase II holoenzyme, acts as the end-point integrator of a variety of activators and conveys intracellular signals to the general transcription machinery. Among circa 30 subunits, MED1 is crucial for various biological events through its specific interaction with distinct activators, such as nuclear receptors [reviewed in 13]. In this study, we show that FGF7 expression was profoundly attenuated in *Med1*$^{-/-}$ mouse embryonic fibroblasts (MEFs), that FGF7 produced by MEFs and BM stromal cells is a crucial niche factor for HSPC support and leukemic growth in vitro, and that FGF7 acts indirectly by stimulating stromal cells in an autocrine manner.

2. Materials and methods

2.1. Cell culture

Stable lines of *Med1*$^{+/+}$*p53*$^{-/-}$ and *Med1*$^{-/-}$*p53*$^{-/-}$ MEFs established from embryonic day 10.0 (E10.0) embryos derived from a single crossing of *Med1*$^{+/-}$*p53*$^{-/-}$ mice on a C57BL6 background were described previously [7]. Two lines of these MEFs were analyzed in all experiments. The MEFs were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C. The OP-9 BM stromal cells, distributed by RIKEN BRC through the National Bio-Resource of the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), MC3T3-E1 cells, and MS-5 cells [14] were maintained in α-modified Eagle's medium (αMEM) supplemented with 20% FBS, 10% FBS, and 20% horse serum, respectively. MB-1 stromal cell-dependent myeloblastic leukemia cells were maintained by coculture with mitomycin C-treated OP-9 cells in αMEM supplemented with 10% FBS, 1 × non-essential amino acids, and 100 μM β-mercaptoethanol [15,16].

2.2. BM culture and colony-forming cell assay

MEFs and MS-5 and OP-9 cells (2×10^5), treated with 100 μg/mL of mitomycin C to arrest the cell cycle, were plated on 0.1% gelatin-coated 12-well plates. The next day, 1×10^6 BM cells, harvested from the femurs of congenic wild-type mice, were added to each well and cultured in MyeloCult M5300 (Stem Cell Technologies, Canada) and 10 U/mL heparin in the absence or presence of various amounts of recombinant human (rh) FGF7 (R&D Systems), 0.2 μg/mL anti-mouse (m) FGF7 rabbit polyclonal IgG (H-73: sc-7882; Santa Cruz), or normal rabbit IgG (Sigma) at 33 °C.

For long-term culture, half of the medium was replaced with fresh medium each week. After an 8-week culture period, trypsinized cells containing HSPCs (adherent and nonadherent) were collected and cultured in complete methylcellulose medium (MethoCult M3434; Stem Cell Technologies) for all types of colonies at 37 °C for 14 days, and the colonies were counted.

2.3. DNA synthesis

Incorporation of bromodeoxyuridine (BrdU) into cells cultured in 24-well plates, after purging for 6 h, was measured by using a cell proliferation enzyme-linked immunosorbent assay (ELISA) for BrdU (chemiluminescence) (Roche).

2.4. Quantitation of mRNA

For quantitative PCR (qPCR), total RNA (0.5 μg) was used to prepare cDNA with the ReverTra Ace qPCR RT Master Mix with a gDNA Remover kit (Toyobo). The expression of various mouse genes was identified by qPCR (StepOnePlus Real-Time PCR system; Life Technologies). Mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference marker. The sequences of the primers used and the PCR conditions for amplification are available upon request.

2.5. Western blot analysis

For Western blot analysis, total cell lysates were separated by SDS–PAGE, blotted onto a nitrocellulose membrane, and probed with polyclonal antibodies against mFGF7 (above), β-actin (BioLegend), FGFR substrate 2 (FRS2) (H-91: sc-8318; Santa Cruz), Phospho-FRS2-α (Tyr196) (Cell Signaling), mitogen-activated protein (MAP) kinase ERK1/ERK2 (Millipore), and phospho-MAP kinase ERK1/ERK2 (Tyr202/204) (Cell Signaling) and with monoclonal antibody against mFGFR2(IIIb) (133730; R&D Systems). Chemiluminescence was detected by an ImageQuant LAS 4000mini (GE Healthcare).

2.6. Statistical analysis

All assays were performed in triplicate or quadruplicate in at least three separate experiments. The significance of the differences between independent means was assessed by Student's *t*-test. We considered a *P* value of <0.05 statistically significant.

3. Results

3.1. FGF7 expression is attenuated in *Med1*$^{-/-}$ MEFs

MEFs, primitive mesenchymal cells resembling MSCs, support HSPCs and thus mimic the hematopoietic niche [7]. We employed MEFs as an in vitro model to analyze the role of MED1 in the BM niche and previously reported that the ability of *Med1*$^{-/-}$ MEFs to promote BM cell proliferation and support HSPCs is reduced relative to that of *Med1*$^{+/+}$ MEFs [7]. Therefore, MED1 in MEFs has a crucial role in activating the transcription of genes encoding molecules with potency for HSPC support. Microarray analysis comparing *Med1*$^{+/+}$*p53*$^{-/-}$ and *Med1*$^{-/-}$*p53*$^{-/-}$ MEFs revealed reduced expression of *Fgf7* and *Opn* in *Med1*$^{-/-}$*p53*$^{-/-}$ MEFs (GEO accession number GSE22471) [7].

RT-qPCR (Fig. 1A) and Western blot (Fig. 1B) analyses confirmed the reduced level of FGF7 in *Med1*$^{-/-}$ MEFs. However, *Fgf7* expression was not restored after MED1 was reintroduced into *Med1*$^{-/-}$ MEFs (Rev-*Med1*$^{-/-}$ MEFs) (Fig. 1A), indicating that the reduction in *Fgf7* expression was an indirect effect of *Med1* ablation.

3.2. FGF7 is expressed in BM mesenchymal cells but not in BM hematopoietic cells

RT-qPCR (Fig. 1A) and Western blot (Fig. 1B) analyses showed that FGF7 was expressed in BM mesenchymal cells, including stromal cells (MS-5 and OP-9) and osteoblasts (MC3T3-E1), as in *Med1*$^{+/+}$ MEFs, but *Fgf7* mRNA was undetectable in hematopoietic cells (Fig. 1A). This implies that the quantity of FGF7 secreted by stromal cells reflected FGF7 levels in the coculture niche model.

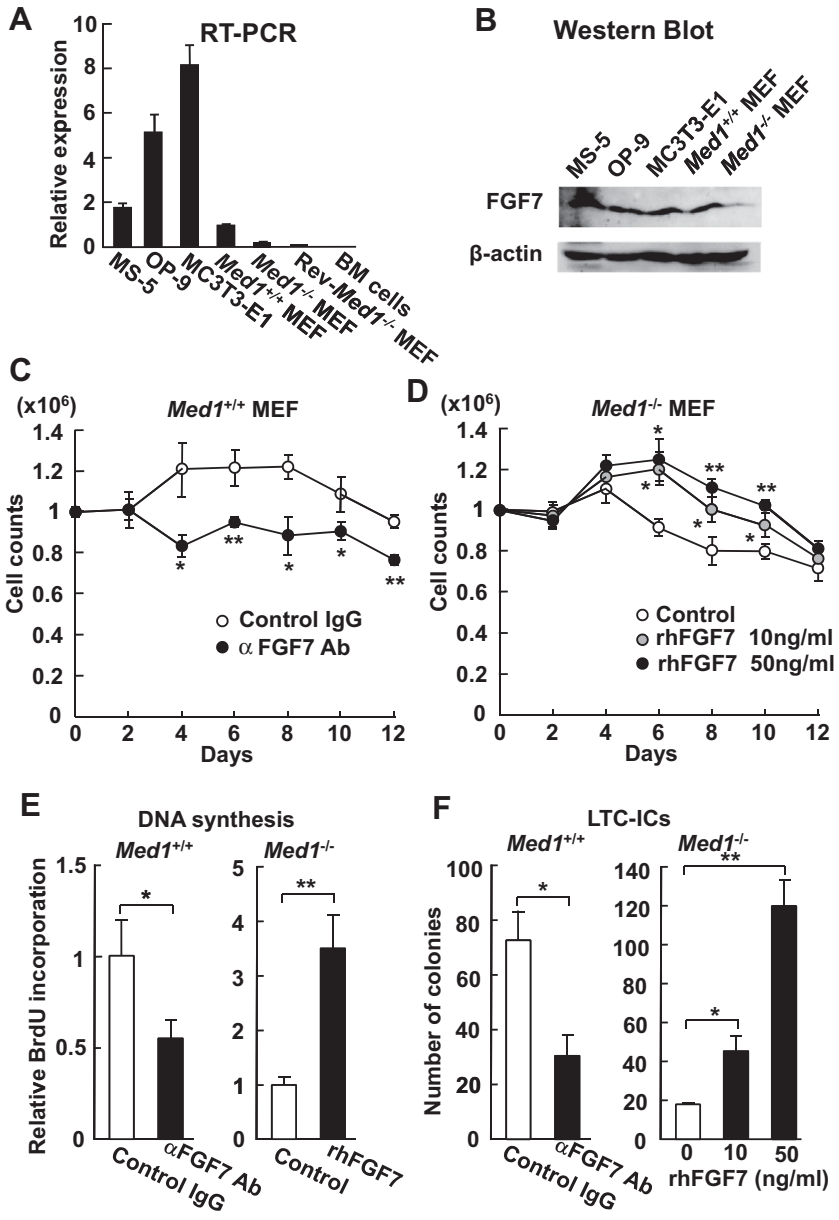

Fig. 1. MEF-produced FGF7 promotes BM cell growth and supports LTC-ICs on MEFs. (A and B) Expression of FGF7. Quantitative PCR (A) and Western blot analysis (B). FGF7 was expressed in *Med1*$^{+/+}$ MEFs, BM stromal cells (MS-5 and OP-9), and osteoblasts (MC3T3-E1), and its expression was reduced in *Med1*$^{-/-}$ MEFs (A and B). *Fgf7* mRNA expression was not restored in Rev-*Med1*$^{-/-}$ MEFs and was not detected in BM hematopoietic cells (A). (C) The number of BM cells, when cocultured on *Med1*$^{+/+}$ MEFs, decreased in the presence of anti-FGF7 antibody (Ab). α, anti. (D) The number of BM cells, when cocultured on *Med1*$^{-/-}$ MEFs, increased in the presence of rhFGF7. (E) BM cell DNA synthesis on *Med1*$^{+/+}$ MEFs, measured by BrdU incorporation, decreased in the presence of anti-FGF7 Ab. BM cell DNA synthesis on *Med1*$^{-/-}$ MEFs increased in the presence of rhFGF7. (F) The number of LTC-ICs on *Med1*$^{+/+}$ MEFs was reduced in the presence of anti-FGF7 Ab. The number of LTC-ICs on *Med1*$^{-/-}$ MEFs increased in the presence of rhFGF7. The values are the mean ± SD (A, C–E) or mean ± SE (F) of a representative experiment performed in triplicate (A) or quadruplicate (C–F) ($^{*}P < 0.05$; $^{**}P < 0.01$).

3.3. MEF FGF7 mediates mitogenic signal(s) to BM cells and the support of long-term culture-initiating cells

We asked whether FGF7 is responsible for the phenotypes of *Med1*$^{-/-}$ MEFs. To answer this question, we first analyzed the effect of FGF7 on the mitogenicity of cocultured BM cells. When BM cells on *Med1*$^{+/+}$ MEFs were cocultured for 2 weeks in the presence of anti-FGF7 IgG or control rabbit whole IgG, the number of BM cells in cultures with anti-FGF7 IgG was lower than in the control cultures (Fig. 1C, Supplementary Fig. 1A). This result, which could be due to a specific blocking effect of the antibody, prompted us to culture BM cells on *Med1*$^{-/-}$ MEFs in the presence or absence of rhFGF7. As expected, the number of BM cells increased in an FGF7 dose-dependent manner (Fig. 1D, Supplementary Fig. 1B).

To assess if changes in the cell number reflect altered mitogenicity, DNA synthesis was measured. After 1 week of coculture, the incorporation of BrdU into BM cells on *Med1*$^{+/+}$ MEFs was lower in the presence of anti-FGF7 antibody than in the control, and the incorporation into BM cells on *Med1*$^{-/-}$ MEFs was higher in the presence of rhFGF7 (Fig. 1E). Together, these data strongly suggest that FGF7 produced by MEFs had mitogenic activity on the cocultured BM cells.

We then conducted a long-term BM culture to assess whether FGF7 affected HSPC support. We first cultured BM cells on *Med1*$^{+/+}$

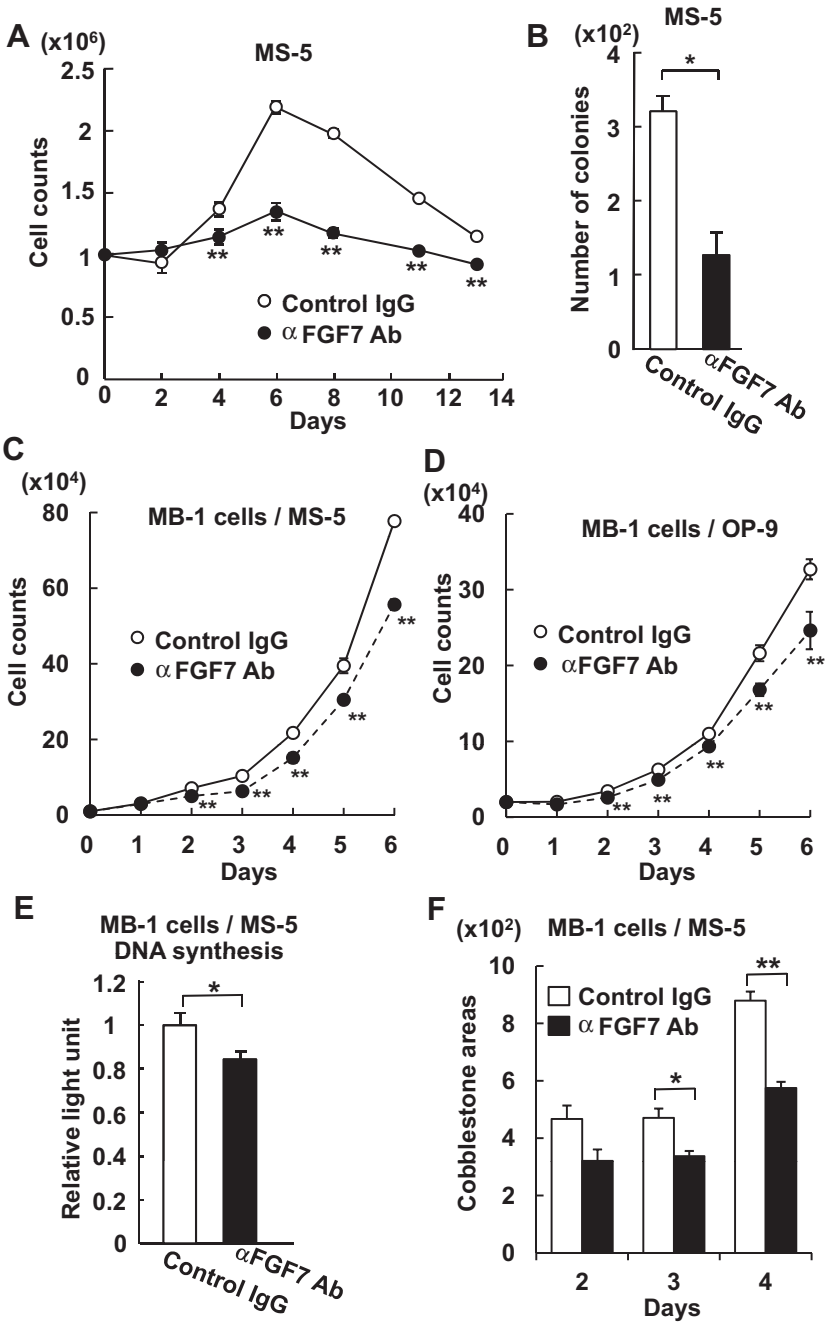

Fig. 2. FGF7 depletion attenuates the growth of BM cells and MB-1 niche-dependent myeloblastoma cells and reduces the number of LTC-ICs cocultured on BM stromal cells. (A and B) BM cells (A) and LTC-ICs (B) on MS-5 BM stromal cells were counted in the presence of anti-FGF7 Ab. The numbers of BM cells and LTC-ICs on stromal cells decreased in the presence of anti-FGF7 Ab. (C and D) The number of MB-1 cells on MS-5 (C) or OP-9 (D) BM stromal cells decreased in the presence of anti-FGF7 Ab. (E) BrdU incorporation into MB-1 cells cocultured with MS-5 cells was impaired in the presence of anti-FGF7 Ab. The values of BrdU incorporation are plotted as the fold increase versus the value with control IgG. (F) The number of cobblestone areas per visual field formed by MB-1 cells cocultured with MS-5 cells was counted. The number decreased in the presence of anti-FGF7 Ab. The values are the mean ± SD (A, C, D) or SE (B, E, F) of a representative experiment performed in quadruplicate ($^{*}P < 0.05$; $^{**}P < 0.01$).

MEFs in the presence of anti-FGF7 or control IgG for 8 weeks and counted the resulting long-term culture-initiating cells (LTC-ICs) by colony formation assay. The number of LTC-ICs was reduced in the presence of anti-FGF7 antibody, probably through its blocking effect on FGF7 secreted by MEFs (Fig. 1F). BM coculture on *Med1*$^{-/-}$ MEFs in the presence of rhFGF7 enhanced the number of LTC-ICs in an FGF7 dose-dependent manner (Fig. 1F). These data suggested a role for FGF7 in HSPC support in a MEF-based long-term BM culture.

3.4. BM stromal cell FGF7-mediated growth of BM cells and LTC-IC support

Two independently established mouse BM stromal cell lines, MS-5 and OP-9, represent a BM niche and provide long-term HSPC support in vitro [14,17]. Therefore, we performed similar experiments using these cells. As expected, when BM cells were cocultured with MS-5 or OP-9 cells for 2 weeks, the addition of

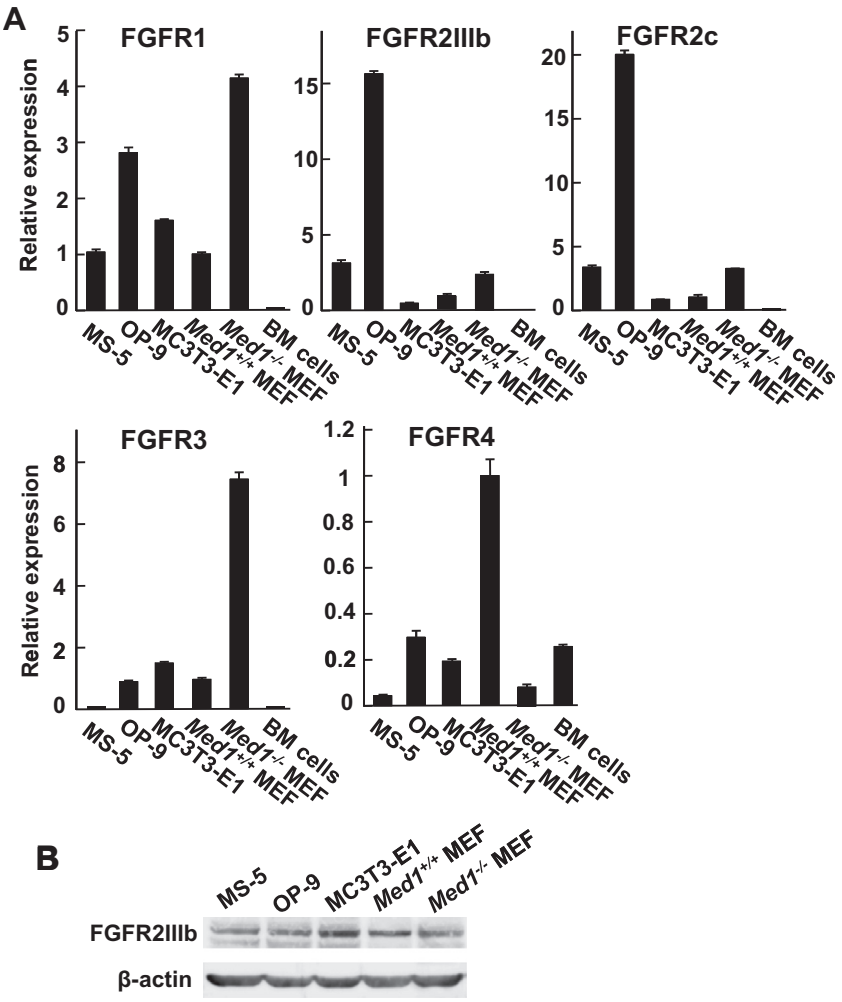

Fig. 3. *Fgfr2IIIb* is expressed in MEFs and BM stromal cells, but not in BM hematopoietic cells. (A) Quantitative PCR. *Fgfr1*, *2IIIb*, *2c*, *3*, and *4* mRNAs were expressed in BM stromal cells (MS-5 and OP-9), osteoblasts (MC3T3-E1), and MEFs. BM hematopoietic cells expressed *Fgfr1*, *2c*, *3*, and *4* mRNAs but not *Fgfr2IIIb* mRNA. The values (mean ± SD of a representative experiment performed in triplicate) are plotted as the fold increase versus the value in *Med1*$^{+/+}$ MEFs. (B) Western blot. FGF7 receptor FGFR2IIIb was expressed comparably in BM stromal cells, osteoblasts, and MEFs.

anti-FGF7 antibody attenuated the growth of the cocultured BM cells (Fig. 2A and Supplementary Fig. 2A). Similarly, when BM cells were cocultured for 8 weeks, the number of LTC-ICs was reduced by the addition of anti-FGF7 antibody (Fig. 2B and Supplementary Fig. 2B). These data further support the MEF-based conclusion that FGF7 has a role in BM cell growth and HSPC support in vitro.

3.5. BM stromal cell FGF7-mediated growth of niche-dependent myeloblastic leukemia cells

MB-1 niche-dependent myeloblastoma cells, originally derived from a patient with myeloid blast crisis chronic myeloid leukemia, require BM stromal cells such as OP-9 and MS-5 cells for survival and optimal growth. During coculture, typically with OP-9 and MS-5 BM stromal cells, MB-1 cells form cobblestone areas, which most probably reconstitute a stochastic model of leukemic stem or initiating cells in vitro [15,16]. We next asked if FGF7 produced by MS-5 or OP-9 BM stromal cells has a role in cocultured MB-1 cell growth. When anti-FGF7 antibody was added to MB-1 cells cocultured with MS-5 or OP-9 cells, the proliferation of MB-1 cells was significantly attenuated, although the cells were alive, as judged by trypan blue exclusion (Fig. 2C and D). Anti-FGF7 antibody also attenuated DNA synthesis in MB-1 cells cocultured with MS-5 or OP-9 cells (Fig. 2E, Supplementary Fig. 3A). The number of cobblestone areas was also reduced significantly when anti-FGF7 antibody was added to the coculture, indicating reduced stem cell-like features of MB-1 cells (Fig. 2F, Supplementary Fig. 3B). These results clearly indicate that BM stromal cell FGF7 is important for the optimal growth and support of stromal cell-dependent myeloblastic leukemia cells; it may constitute a leukemic stem cell niche.

3.6. FGF7 receptor FGFR2IIIb is expressed on BM mesenchymal cells but not on BM hematopoietic cells

We next investigated the mechanism of FGF7 in BM cell growth and HSPC support. First, we performed RT-qPCR analysis to measure the expression of FGF receptors on BM hematopoietic cells and found that some FGFRs were variously expressed. However, FGFR2IIIb, the sole and specific receptor for FGF7, was completely undetectable (Fig. 3A).

Therefore, FGF7 may act on stromal cells in an autocrine manner. Various mesenchymal cells were checked for the expression of each FGFR. BM mesenchymal stromal cells (MS-5 and OP-9), osteoblastic cells (MC3T3-E1), and MEFs (*Med1*$^{+/+}$ and *Med1*$^{-/-}$) expressed all of the FGFRs at various levels (Fig. 3A). The expression of FGFR2IIIb was also examined at the protein level by Western

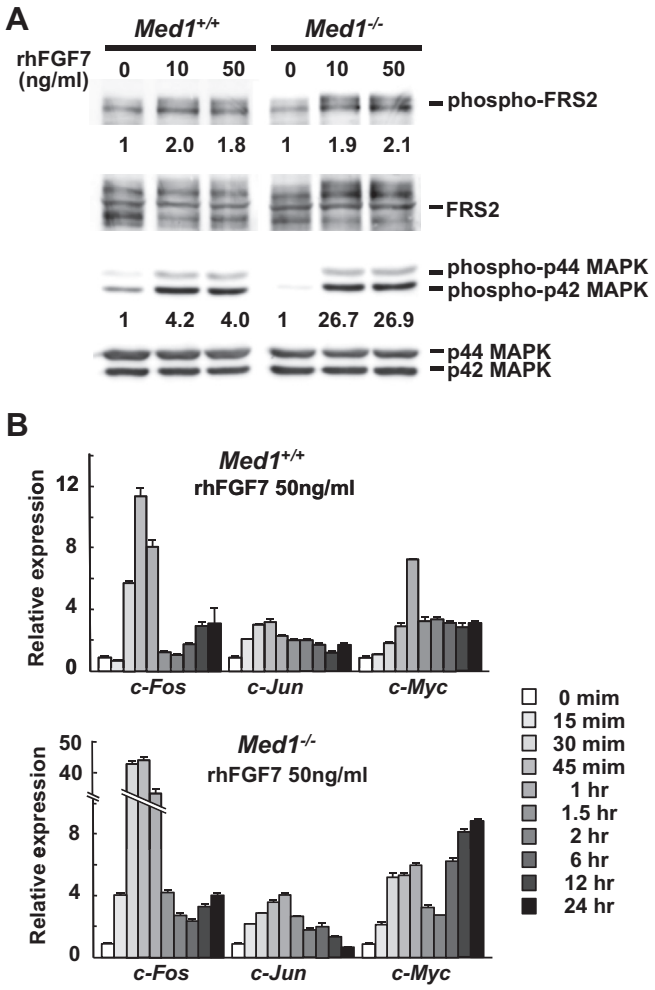

Fig. 4. FGF7 initiates FGFR2IIIb-mediated intracellular signaling in both *Med1*$^{+/+}$ and *Med1*$^{-/-}$ MEFs. (A) The phosphorylation of FRS2 and MAP kinases was induced 10 min after rhFGF7 addition in MEFs. (B) The expression of immediate early genes, c-*Fos*, c-*Jun*, and c-*Myc*, was induced after rhFGF7 addition in MEFs.

blot and was comparable in all of these cells (Fig. 3B). The data suggest that FGF7 might act on BM stromal cells in an autocrine (or paracrine) manner to elicit niche function indirectly by these cells.

3.7. FGF7 initiates FGFR-mediated intracellular signaling in MEFs and BM stromal cells

Because FGF7 appeared to act on stromal cells in the niche model system, we asked if treating these cells with FGF7 elicited intracellular signaling. To this end, we assessed the phosphorylation of FRS2 (the immediate target of FGFR tyrosine kinase), the phosphorylation of MAP kinase (the intermediate hub of various intracellular signals), and the mRNA levels of immediate early response genes [8,9].

In both *Med1*$^{+/+}$ and *Med1*$^{-/-}$ MEFs, the phosphorylation of FRS2 and MAP kinases was upregulated 10 min after the addition of 10 or 50 ng/mL FGF7 (Fig. 4A), indicating that FGF7 effectively elicited FGFR2IIIb tyrosine kinase activity with subsequent activation of the downstream signaling cascade of FGFR2IIIb. We next analyzed the response of immediate early gene expression. In both *Med1*$^{+/+}$ and *Med1*$^{-/-}$ MEFs, c-*Fos*, c-*Jun*, and c-*Myc* were robustly transcribed after the addition of 10 ng/mL (Supplementary Fig. 4) or 50 ng/mL (Fig. 4B) of FGF7, with a peak within 1 h, followed by attenuation and a second wave of transcription (Fig. 4B, Supplementary Fig. 4).

Experiments using MS-5 BM stromal cells yielded similar results, with prompt activation of FRS2 and MAP kinases and subsequent transcription of c-*Fos* and c-*Myc* (Supplementary Fig. 5A and B). The phosphorylation of FRS2 and MAP kinases was also evident after the addition of FGF7 to OP-9 cells (Supplementary Fig. 5A). Taken together, these results clearly show that FGF7 effectively elicits FGFR2IIIb activation and subsequent intracellular signaling in MEFs and BM stromal cells and that MED1 is dispensable in these systems.

4. Discussion

We determined the role of FGF7 in a hematopoietic niche model. Our findings suggest that FGF7 and its cognate receptor FGFR2IIIb are specifically expressed in mesenchymal stromal cells, that FGF7 supports HSPC maintenance, and that this activity may be elicited indirectly by FGF7-activated stromal cells in an autocrine or paracrine manner. BM stromal cell FGF7 also constitutes a niche for myeloblastic leukemia cells.

FGF systems have been highlighted in recent years as important molecular components of microenvironmental signals both in hematopoietic (FGF1 and FGF2) and liver (FGF7) systems in stress conditions. In the liver niche, FGF7 produced by fibroblastic stroma-like cells acts on liver progenitor cells, thus constituting a genuine functional niche signal [12]. The FGF7-FGFR2IIIb system in the hematopoietic niche model and liver niche contrasts with the FGF1- and FGF2-elicited HSPC expansion in that, whereas FGF1 and FGF2 are supplied primarily by megakaryocytes, FGF7 is produced by stromal cells both in the hematopoietic niche (this study) and liver niche [12]. In addition, FGF7 acts on FGFR2IIIb on mesenchymal stromal cells in an autocrine or paracrine manner, whereas FGF1 and FGF2 act on cells distinct from their origin.

The mechanism of niche function by FGF7 appears to be essentially indirect: it acts not on HSPCs (nor, most likely, on leukemic stem cells) but on stromal cells themselves, which subsequently act on normal and malignant HSPCs by an as yet unknown mechanism(s). A precedent is the indirect action of FGF7 on thymic mesenchymal cells to mediate thymocyte proliferation and T cell maturation [18]. The thymic mesenchymal cells express FGFR2IIIb, and these cells reportedly produce bone morphogenic protein 2 (BMP2), BMP4, Wnt5b, and Wnt10b, probably through an indirect mechanism downstream of transcription factors such as p53 and NF-κB [18]. It will be interesting to see if these molecules are altered in BM mesenchymal cells as well and if they contribute to HSPC support and leukemic growth.

Although FGF7 is downregulated in *Med1*$^{-/-}$ MEFs, the inability to restore its expression by reintroducing MED1 in the cells indicates that the effect of MED1 in this process is again indirect; it might reflect subtle differences in the differentiation status of *Med1*$^{+/+}$ MEFs and *Med1*$^{-/-}$ MEFs, reflected in their different manner of myogenic differentiation when MyoD is added [19]. In *Med1*$^{-/-}$ MEFs, we previously showed that niche molecules, including angiopoietin-1, Jagged-1, N-cadherin, Wnt, and BMP4, remain unchanged [7]. However, the transcription of genes encoding the chemokines CXCL1, CXCL5, CXCL10, and CXCL15 is significantly reduced in *Med1*$^{-/-}$ MEFs (GEO accession number GSE22471) [7]. These chemokines, whose functions in the BM microenvironment are essentially unknown, might contribute to the niche function uniquely for HSPC support and leukemic cell growth. The action of these chemokines on HSPCs in the presence or absence of FGF7 must be carefully examined in the future.

The BM microenvironment is complex in terms of cell types, molecules, oxygen concentration, and gravity, etc., which contribute to HSPC maintenance, proliferation, and differentiation.

Therefore, the effect of niche molecules, especially those in families, such as FGFs, may not be detected because of redundancies within a living animal. Thus, a simplified niche model system, such as the one adopted in this study, is an ideal strategy for assaying molecules of interest. However, having established FGF7 as a potentially important (both normal and malignant) niche factor in vitro, the next step is to determine whether it has the same role in BM mesenchymal stromal cells in vivo. A series of mouse knockout models, with single or double ablation of genes including *Fgf7* or *Fgfr2IIIb*, as well as transgenic models, will help answer this question in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.09.044.

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