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The SDF-1α/CXCR4 axis induces the expression of fatty acid synthase via sterol regulatory element-binding protein-1 activation in cancer cells

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Fatty acid synthase (FASN), a key enzyme that synthesizes long-chain fatty acids, is involved in both normal lipid synthesis and cancer development. Overexpression and increased activity of FASN represents one of the most frequent phenotypic alterations in cancer cells. Multiple growth factors and growth factor receptors have emerged as major contributors to FASN overexpression. However, the ultimate mechanisms responsible for tumor-associated FASN overexpression are not completely understood. Here, we show that the stromal cell-derived factor-1 alpha (SDF-1α)/CXCR4 axis can induce the FASN expression via the nuclear translocation of sterol regulatory element-binding protein-1, a major modulator of FASN transcription. We also identified that recombinant SDF-1α-induced phosphatidylinositol-3′-kinase/protein kinase B (Akt) phosphorylation was involved in the expression or activities of FASN. Finally, we demonstrated that FASN inhibition significantly reduced the SDF-1α-mediated G_1 cyclin expression and cell viability. Taken together, our findings manifest that the SDF-1α/CXCR4 axis is a novel upstream pathway of FASN expression and is associated with mediating its prosurvival effect.

Introduction

Most normal human tissues preferentially use dietary (exogenous) lipid for synthesis of new structural lipids, whereas *de novo* (endogenous) fatty acid (FA) synthesis is usually suppressed and fatty acid synthase (FASN) expression is maintained at low levels (1). In contrast, in cancer cells, *de novo* FA synthesis is elevated and the supply of cellular FA is highly dependent on the *de novo* synthesis. FASN is the key metabolic multienzyme that is responsible for the terminal catalytic step in FA synthesis (condensation of acetyl-CoA and malonyl-CoA to produce the saturated palmitate). Overexpression and increased activity of FASN represents one of the most frequent phenotypic alterations in cancer cells (2). Moreover, several reports have shown that FASN and related lipogenic enzymes play important roles in tumor cell survival at multiple levels (2,3). Tumor-associated FASN overexpression preferentially occurs through the modulation of the expression and/or maturation status of the sterol regulatory element-binding protein (SREBP)-1 that stimulates FASN transcription when interacting with an SREBP-binding site at the endogenous FASN promoter (4,5). SREBP-1s are synthesized as precursors (∼125 kDa) bound to the endoplasmic reticulum and nuclear envelope (6,7). Upon activation, SREBP-1s are released from the membrane into the nucleus as a mature protein (∼68 kDa) by a sequential two-step cleavage process (8).

The chemokine stromal cell-derived factor-1 (CXCL12/SDF-1) regulates many essential biological processes, including stem cell motility, cardiac and neuronal development, neovascularization and tumorigenesis (9). It binds to the expressed cell surface receptor CXCR4 and to the recently identified receptor CXCR7/RDC1 (10,11). The stromal cell-derived factor-1 alpha (SDF-1α)/CXCR4 axis is functional in evolutionarily distant organisms, such as zebra fish and mice, in which CXCR4 expression is a prerequisite for germ cell migration to SDF-1α-expressing gonads during development (12). The SDF-1 interaction with CXCR4 plays a prominent role in tumorigenesis and preferentially spreads to tissues, including lung, liver, bone marrow and lymph nodes. Adhesion to stromal cells supports the growth of neoplastic cells through the high-level expression of SDF-1α (13) and the SDF-1α/CXCR4 signaling is central to the survival and growth of invasive or micrometastatic tumor cells, reflecting downstream signaling through the adhesion-dependent tyrosine kinase focal adhesion kinase, the phosphatidylinositol-3′-kinase (PI3K)/Akt and etc. (14–17).

Growth factors and growth factor receptors have emerged as major contributors to FASN overexpression in tumor cells and are involved in activation and/or cross talk between multiple signal transcription pathways (18–20). In this study, we explored the functional relevance of FASN regulation by the SDF-1α/CXCR4 axis. The mechanisms by which SDF-1α/CXCR4 axis causes the enhancements of FASN expression were suggested following the findings that this axis induced the nuclear localization of SREBP-1. We have identified that the prosurvival activity of this signaling pathway through the concomitant induction of cyclins D1 and E1 can be significantly increased by FASN. This may show the novel upstream signaling pathway regulating overexpression and increased activity of FASN in cancer cells.

Materials and methods

Plasmids, reagents and antibodies

pcDNA3.1/Flag/SREBP-1a (human, amino acid 1–490) (21), pGL2B/rFAS-Luc (−1594/+65) (22) and pSynSRE-Luc were generously donated by Dr T.F.Osborne. pcDNA3/HA/SREBP-1c and pcDNA3/RFP/SREBP-1c were described previously (23).

The transfection reagents PolyFect and JetPEI were purchased from (QIAGEN Hilden, Germany) and (Polyplus-transfection, Illkirch, France), respectively. The recombinant SDF-1α (rSDF-1α) and cerulenin were purchased from (R&D Systems, Minneapolis, MN) and (Cayman, Ann Arbor, MI). All other reagents were purchased from (Sigma, St Louis, MO). The antibodies against SREBP-1 (sc-13551), cyclin D1 (sc-6281), cyclin E1 (sc-481) and Akt1/2 (sc-8312) were purchased from Santa Cruz Biotechnology, and actin (A2066), FASN (610962) and phospho-Akt (Ser473) (#9271) antibody were obtained from Sigma, (BD Biosciences, San Jose, CA) and (Cell Signaling, Danvers, MA), respectively.

Cell culture and transient transfection

Chang liver, Huh7, HepG2 and SKBr3 cell lines were maintained in Dulbecco's modified Eagle's medium–10% fetal bovine serum (Abclone, Victoria, Australia). Transient transfections of HepG2 and Chang liver cells were conducted using PolyFect or JetPEI reagents of cell cultures in 24-well culture plates with the indicated reporter plasmids and then cotransfected with mammalian expression vectors. Expression vectors were maintained at constant total amounts via the addition of empty vectors. Relative luciferase activities were assessed by luciferin (BD Biosciences).

RNA isolation and reverse transcription–polymerase chain reaction

Total RNA from HepG2 and Chang liver cells was prepared using TRIzol (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's recommendations. The complementary DNA was synthesized from 2 μg of total RNA with Moloney murine leukemia virus reverse transcriptase (Elpis Biotech, Daejeon, Korea) with a random hexamer (Cosmo, Seoul, Korea) at 37°C for 1 h. A 1/25th aliquot of the complementary DNA was subjected to polymerase chain reaction (PCR) amplification using gene-specific primers. The PCR primers were as follows: for SREBP-1 gene amplification: 5′-ACG GCA GCC CCT GTA ACG ACC ACT GTG A-3′ (sense) and 5′- TGC CAA GAT GGT TCC GCC ACT CAC CAG G-3′ (antisense); for FASN gene

Abbreviations: FA, fatty acid; FASN, fatty acid synthase; mRNA, messenger RNA; 25-OHC, 25-hydroxycholesterol; PCR, polymerase chain reaction; PI3K, phosphatidylinositol-3′-kinase; rSDF-1α, recombinant SDF-1α; SDF-1α, stromal cell-derived factor-1 alpha; siRNA, small interfering RNA; SREBP, sterol regulatory element-binding protein.

amplification: 5′-GAA ACT GCA GGA GCT GTC-3′ (sense) and 5′-CAC GGA GTT GAG GCG CAT-3′ (antisense); for cyclin D1 gene amplification: 5′-CGT CCA TGC GGA AGA TCG TC-3′ (sense) and 5′-GAA ATC GTG CGG GGT CAT TG-3′ (antisense); for cyclin E1 gene amplification: 5′-GCT GGG CAA ATA GAG AGG AAG-3′ (sense) and 5′-CTG GTG CAA CTT TGG AGG ATA G-3′; and for β-actin gene amplification: 5′-GAC TAC CTC ATG AAG ATC-3′ (sense) and 5′-GAT CCA CAT CTG CTG GAA-3′ (antisense). The complementary DNAs were amplified by PCR under the following conditions: 28 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 30 s and extension at 72°C for 30 s in a thermal cycler. The PCR products were examined by electrophoresis on 1.5% agarose gels.

Fluorescence microscopy in living cells

Fluorescence microscopy was conducted on Chang liver or Huh7 cells transfected with the pcDNA3/RFP/SREBP-1c or pcDNA3/GFP/SREBP-1c construct. Following transfection, the cells were incubated for 48 h. Prior to imaging, the cells were counterstained with Hoechst dye for 10 min at 37°C to stain the nuclei and were visualized with a Zeiss Axiovert 200M fluorescence microscope.

MTT assay

For cell viability assay, cells were seeded in a 24-well tissue culture plate and incubated for 24 h. Cells were transfected with the indicated expression vectors and then etoposide was treated in the indicated concentration. After 24 h, MTT 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (M5655; Sigma) solution (0.5 mg/ml) was added to each well. After incubation for 2 h at 37°C, formazan crystals in viable cells were solubilized in 200 μl of dimethyl sulfoxide. The soluble formazan product was spectrophotometrically quantified using an enzyme-linked immunosorbent assay leader at 570 nm.

RNA interference and transfection

For the small interfering RNA (siRNA)-mediated downregulation of FASN, FASN-specific siRNA and negative control siRNA were purchased from Bioneer (Daejeon, Korea). Chang liver cells were transfected with either the siRNA molecule specific for FASN or with a negative control siRNA sequence using HiPerFect transfection reagent (QIAGEN).

Statistical analysis

Statistical analyses were conducted via unpaired or paired *t*-tests as appropriate. All data were expressed as mean ± SD. *P* values of <0.05 were considered to be significant.

Results

The SDF-1α/CXCR4 axis induces the expression of FASN

To study the effects of SDF-1α on the expression of FASN in several cancer cell types, we assessed the expression of FASN protein in treatment of rSDF-1α using immunoblotting and determined that FASN was significantly increased in Chang liver, Huh7 and SKBr3 cells (Figure 1A). However, the level of FASN expression was not changed in HepG2 cells that are unresponsive to SDF-1α stimulation because of defects of receptor-mediated activation of the signaling cascade (24).

Recent reports demonstrated that CXCR7 also was identified as the cell surface receptor for SDF-1α (10,11). Therefore, in order to assess whether SDF-1α-induced FASN expression is mediated via CXCR4, we tested the effect of bicyclam AMD3100, which is a selective CXCR4 antagonist (25), on the SDF-1α-induced FASN expression. We observed that the treatments of AMD3100 dramatically reduced the FASN expression in the presence or absence of rSDF-1α treatment (Figure 1B). These results support the notion that SDF-1α can upregulate FASN proteins via a cell surface receptor of SDF-1α, CXCR4.

The SDF-1α/CXCR4 axis increases the activity of SREBP-1

We next assessed the potential mechanisms of the increased FASN expression in SDF-1α-treated cells. First, we explored whether the SDF-1α-induced FASN expression is regulated on the messenger RNA (mRNA) or protein level. As shown in Figure 2A, increased expression of FASN mRNA was observed in SDF-1α-treated cells consistent with protein levels indicated by the results of Figure 1A. However, SREBP-1, the expression of one of the major transcription factors for inducing FASN expression, was not led by the SDF-1α treatment on the mRNA levels. We then explored whether SDF-1α promotes the nuclear accumulation of SREBP-1 protein in hepatic cell lines. We examined that mature SREBP-1 (68 kDa) proteins were significantly increased by rSDF-1α treatments, in contrast to decreases of the premature SREBP-1 (125 kDa) in dose- or time-dependent manners (Figure 2B).

In order to examine whether SDF-1α-induced SREBP-1 maturation is mediated via CXCR4, we tested the effect of AMD3100 on the SDF-1α-induced SREBP-1 maturation. The treatment of AMD3100 significantly diminished the expression of nuclear form of SREBP-1 in the presence or absence of rSDF-1α (Figure 2C). We next assessed whether SDF-1α is able to affect the transcriptional activities of SREBP-1 consistent with the increased maturation of SREBP-1. Transient transfection of the pSynSRE-Luc plasmid, a reporter gene with SREBP-response element, or the plasmid for FASN promoter, with or without rSDF-1α, was used to test transcriptional activation of SREBP-1 by SDF-1α treatment. As shown in Figure 2D, the rSDF-1α treatment reinforced the transcriptional activity of SREBP-1a or SREBP-1c in a dose-dependent manner. These findings indicate that SREBP-1-dependent FASN induction can be positively modulated through CXCR4 activation by SDF-1α.

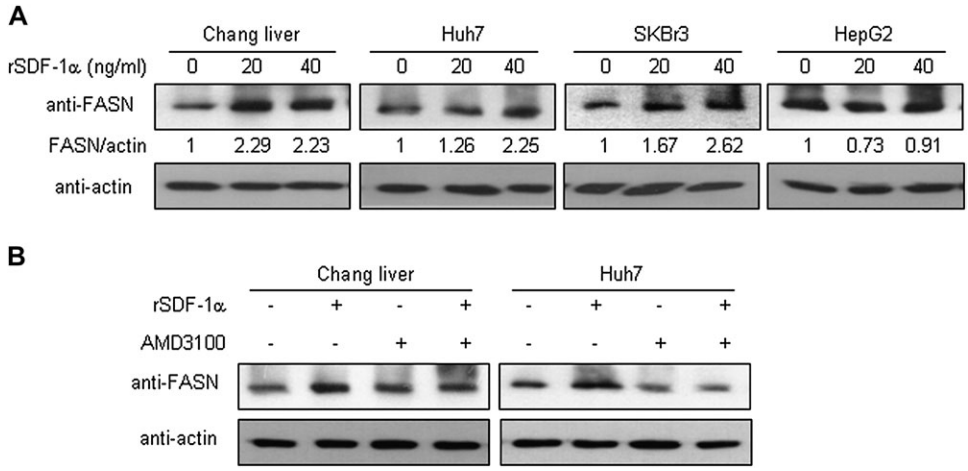

Fig. 1. The SDF-1α/CXCR4 axis induces the expression of FASN. (**A**) Effects of CXCL12 on the expression of FASN in the various cell lines. Chang liver, Huh7, SKBr3 and HepG2 cell lines were incubated with the indicated concentrations of rSDF-1α for 48 h. Data from western blot were quantified and normalized relative to the actin protein using ImageJ version 1.42 (NIH Image). (**B**) Effects of the CXCR4 antagonist on the CXCL12-induced FASN expression. Chang liver or Huh7 cells were pretreated with or without a CXCR4 antagonist, AMD3100 (20 μM), for 4 h and were further incubated in the absence or presence of 40 ng/ml rSDF-1α. Whole-cell lysates were separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and detected using specific antibodies for FASN or actin.

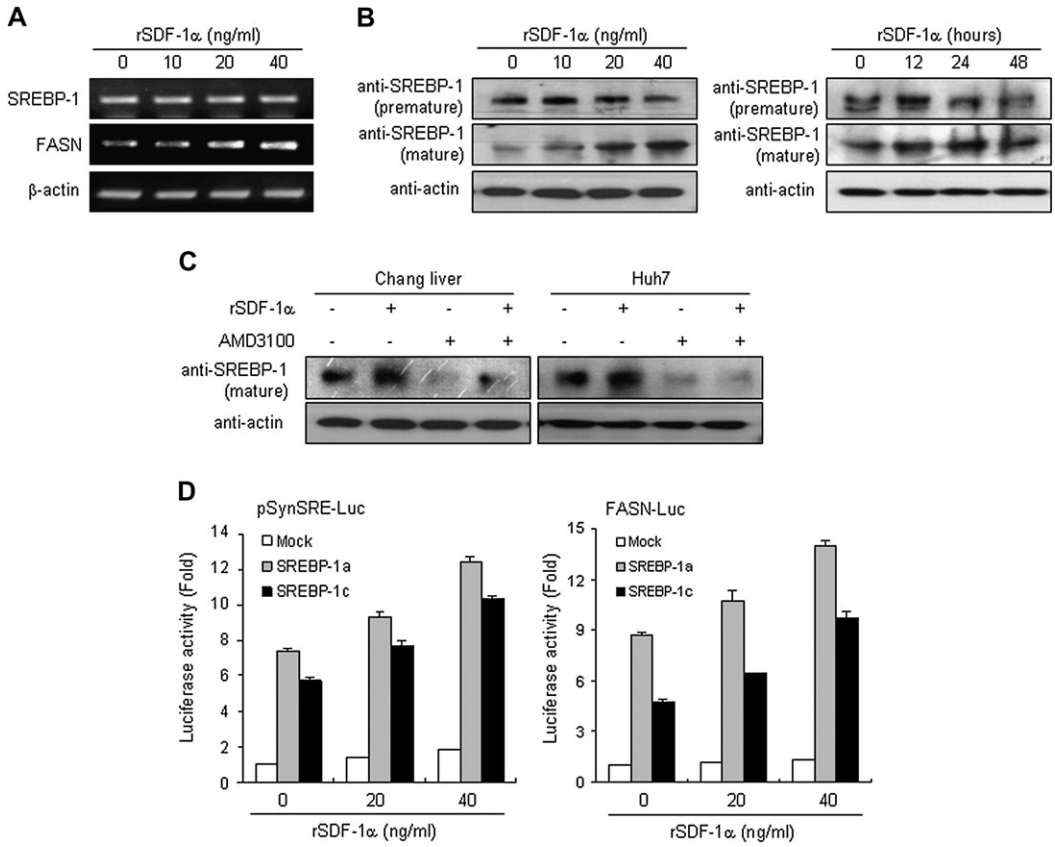

Fig. 2. The SDF-1α/CXCR4 axis increases the activity of SREBP-1. (**A**) Effects of the expression of SREBP-1 in the treatment of rSDF-1α. Chang liver cells were treated with the indicated concentrations of rSDF-1α for 48 h. The SREBP-1 or FASN mRNA level was detected using RT–PCR analysis, and β-actin was used as an mRNA-loading control. (**B**) Chang liver cells were treated with the increasing concentration of rSDF-1α for 48 h (left) or 40 ng/ml rSDF-1α for the indicated amount of time (right). (**C**) Effects of the CXCR4 antagonist on the CXCL12-induced SREBP-1 expression. Chang liver or Huh7 cells were pretreated with or without a CXCR4 antagonist, AMD3100 (20 μM), for 4 h and were further incubated in the absence or presence of 40 ng/ml rSDF-1α. Whole-cell lysates were separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and detected using specific antibodies for SREBP-1 or actin. (**D**) The transactivities of SREBP-1 in the presence of rSDF-1α. pSynSRE-Luc (100 ng) or FASN promoter (100 ng) was cotransfected into Chang liver cells with the eukaryotic expression vectors for the constructs for SREBP-1a or SREBP-1c. The transfected cells were incubated for 24 h in the presence or absence of the indicated concentrations of rSDF-1α, and luciferase activity was measured. The values are expressed as mean ± SD for at least three independent experiments. RT–PCR, reverse transcription–polymerase chain reaction.

The SDF-1α/CXCR4 axis induces the nuclear translocation of SREBP-1

Based on evidence that SDF-1α-induced SREBP-1 maturation plays an important role in regulating FASN expression (Figure 1), it was assumed that abrogation of SREBP-1 nuclear translocation would invariably inactivate this protein. Chang liver cells were pretreated for 6 h in the presence of 25-hydroxycholesterol (25-OHC) to suppress the nuclear translocation of SREBP-1. After this preincubation, cells were stimulated with rSDF-1α for 48 h. We examined that the treatments of 25-OHC significantly inhibited SDF-1α-induced maturation of SREBP-1 and FASN protein expression (Figure 3A). Also, we identified that the mRNA level of FASN was not increased by SDF-1α treatment in the presence of 25-OHC (Figure 3B).

We next assessed the subcellular localization of SREBP-1c in the presence or absence of SDF-1α via fluorescence microscopy in living cells using red fluorescence protein-fusion proteins of SREBP-1c. It was examined that vehicle-treated SREBP-1c was presented in both cytoplasm and nucleus. In rSDF-1α-treated cells, most proteins of RFP/SREBP-1c were located in nucleus. However, in the presence of 25-OHC, SREBP-1c was not localized at nucleus, even though treatments of rSDF-1α. This is consistent with the idea that SDF-1α-induced SREBP-1 activation leads to the expression of FASN via the nuclear localization of SREBP-1 (Figure 3C). Together, these results suggest that the increased expression of FASN by SDF-1α might result from efficient mobilization of SREBP-1 from cytoplasm to nucleus, followed by transcriptional activation on FASN promoter.

The PI3K/Akt pathways are associated with SDF-1α-mediated FASN expression

To understand the molecular mechanism by which SDF-1α increases the activation of SREBP-1, we next analyzed the signaling pathways involved in the SDF-1α-induced SREBP-1 activation. Consistent with previous observations (17,26), we found that the phosphorylation of Akt in the presence of rSDF-1α was much stronger compared with vehicle-treated cells. Furthermore, as shown in Figure 4A, LY294002, the specific inhibitor for the PI3K/Akt pathway, attenuated the increase of SDF-1α-induced SREBP-1 maturation and FASN expression in mRNA and protein levels.

In addition, we determined whether the PI3K/Akt pathway is involved in the enhancement of SDF-1α-mediated SREBP-1 activity. The reporter assay showed that increased activity of SREBP-1 by rSDF-1α treatments on the sterol-responsive element or FASN promoter was attenuated by treatment of LY294002. Therefore, these results indicate that the activation of PI3K/Akt pathway is associated with the SDF-1α-mediated SREBP-1 activation and FASN expression (Figure 4B).

The SDF-1α/CXCR4 axis or FASN expression enhances the G_1 cyclin-mediated cell proliferation

Prior to studying whether the increased FASN expression could have physiological relevance to the SDF-1α/CXCR4 or not, we tested

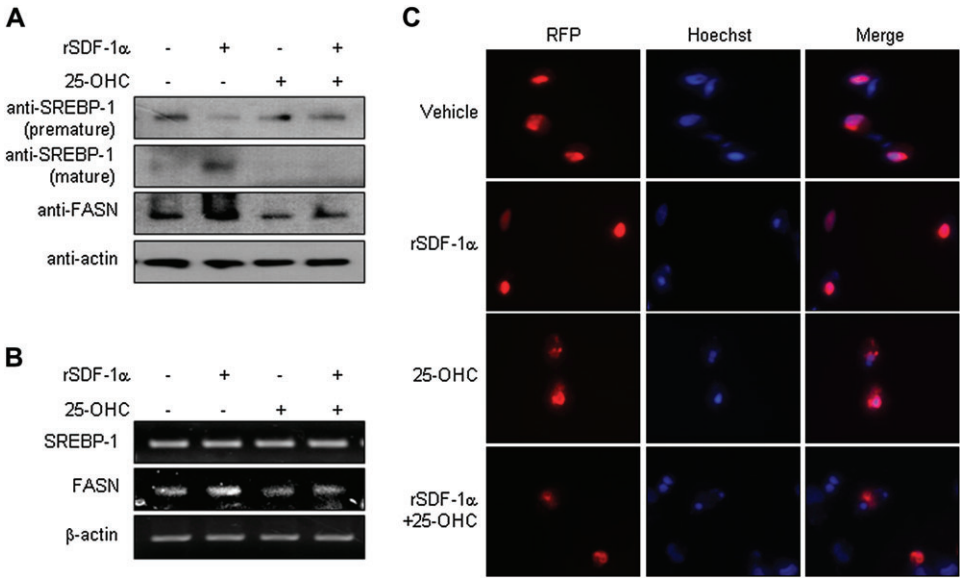

Fig. 3. The SDF-1α/CXCR4 axis induces the translocation of SREBP-1. (**A**) Immunoblot analysis of SREBP-1 and FASN in the presence of rSDF-1α and/or 25-OHC. Chang liver cells were preincubated with or without 25-OHC (20 μM) for 4 h and were further incubated in the absence or presence of 40 ng/ml rSDF-1α. Whole-cell lysates were separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and detected using specific antibodies for SREBP-1, FASN or actin. (**B**) RT–PCR analysis of SREBP-1 and FASN in the treatment of rSDF-1α and/or 25-OHC. (**C**) Subcellular localization of REP-tagged SREBP-1c with or without rSDF-1α and/or 25-OHC. Chang liver cells transiently expressed REP-fusion proteins of SREBP-1c were incubated in presence or absence of rSDF-1α and/or 25-OHC for 48 h prior to Hoechst staining. REP-fusion proteins were visualized via fluorescence microscopy in living cells. RT–PCR, reverse transcription–polymerase chain reaction.

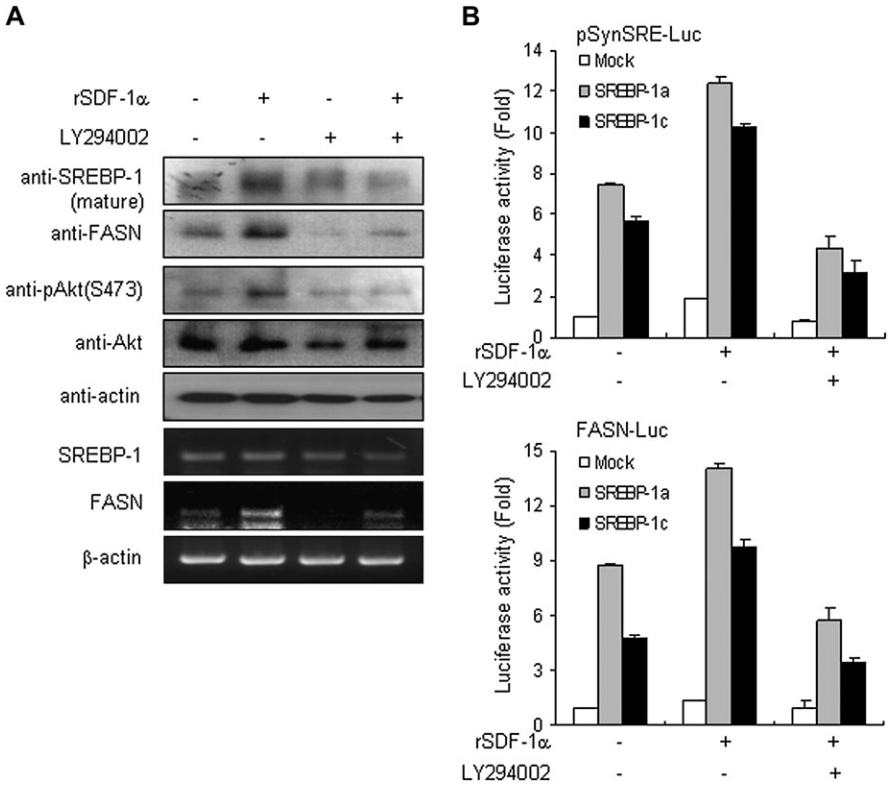

Fig. 4. The PI3K/Akt pathway associates with SDF-1α-mediated FASN expression. (**A**) Effect of the PI3K/Akt inhibitor on rSDF-1α-induced FASN expression. Chang liver cells were pretreated with or without LY294002 (25 μM) for 4 h and were further incubated in the absence or presence of 40 ng/ml rSDF-1α. Whole-cell lysates were separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and detected using specific antibodies for SREBP-1, FASN, total Akt, phospho-Akt (Ser473) or actin. The SREBP-1 or FASN mRNA level was detected using RT–PCR analysis, and β-actin was used as an mRNA-loading control. (**B**) The transactivities of SREBP-1 by the PI3K/Akt inhibitor on rSDF-1α-induced cells. pSynSRE-Luc (100 ng) or FASN promoter (100 ng) was cotransfected into Chang liver cells with the eukaryotic expression vectors for the constructs for SREBP-1a or SREBP-1c. The transfected cells were preincubated for 4 h in the presence or absence of LY294002 (25 μM) and were further incubated in the absence or presence of rSDF-1α (40 ng/ml), and luciferase activity was measured. The values are expressed as mean ± SD for at least three independent experiments. RT–PCR, reverse transcription–polymerase chain reaction.

whether each pathway regulates the common targets for cell proliferation. In view of the established roles of cyclins D1 and E1 in G_1/S transition, we identified whether treatment of rSDF-1α or expression of FASN regulates their expression. As shown in Figure 5A, compared with vehicle-treated cells, mRNA and protein levels of both cyclins were increased after incubation of rSDF-1α. In addition, an increase of cell viability in MTT assay was observed in rSDF-1α-treated cells in a manner of dose dependency (Figure 5B).

Furthermore, mRNA and protein levels of both cyclins were significantly increased in transfected cells with the FASN expression plasmid. These effects could be reversed by RNA interference. Downmodulation of FASN expression by an RNA interference construct targeting FASN silenced FASN expression and reduced cyclins D1 and E1 expression (Figure 5C). Consistent with these results, an increase or decrease of cell viability was remarked in cells with overexpressed or downregulated FASN (Figure 5D).

The inhibition of FASN reduces the SDF-1α-mediated cell proliferation

To test that the FASN induction could be physiologically associated with SDF-1α-induced cell viability, we investigated the effect of SDF-1α when the activity or expression of FASN is prevented. First, we used one of the FASN inhibitors, cerulenin ((2S,3R)-2,3-epoxy-4-oxo-7,10-dodecadienoylamide), preventing the chemical reaction between the elongating FA chain and successive acetyl or malonyl residues (27). Previous reports have shown that cerulenin is a cytotoxic agent for several tumor cells through induction of apoptosis (28,29). Therefore, we tested the cellular viability by treatment of cerulenin to determine the appropriate dose that have little an effect on cellular cytotoxicity. As shown in Figure 6A, MTT assay was achieved in Chang liver or Huh7 cells and cells were derived to cell death in high concentration than 1 μg/ml of cerulenin. Therefore, we used cerulenin in the concentration of 0.5 μg/ml for diminishing the effect of cellular cytotoxicity. Interestingly, the cell viability was significantly reduced in cerulenin-treated cells, even though the treatment of rSDF-1α. In accordance with these data, mRNA and protein levels of both cyclins D1 and E1 were diminished in the cerulenin-treated cells, even though the treatment of rSDF-1α (Figure 6B).

We also assessed whether inhibition of FASN expression is involved with SDF-1α-induced cell viability. Knockdown of FASN with the specific siRNA decreased the cell viability in rSDF-1α-treated cells (Figure 6C). In addition, we observed a similar decrease in cyclins D1 and E1 expression in both mRNA and protein levels after FASN knockdown in rSDF-1α-induced cells (Figure 6D). Taken together, these findings support that FASN plays an important role in the SDF-1α/CXCR4-mediated cell viability.

Discussion

Overexpression and increased activity of FASN represents one of the most frequent phenotypic alterations in cancer cells. Immunohistochemical studies have suggested high levels of FASN in many human epithelial cancers and their preneoplastic lesions, including breast, prostate, lung and hepatocellular carcinomas (2). The mechanisms responsible for tumor-associated FASN overexpression were demonstrated in some reports. Growth factors and growth factor receptors are important contributors at the transcriptional level to FASN overexpression in cancer cells (30). Also, post-translation regulation induces FASN expression in tumor cells (31).

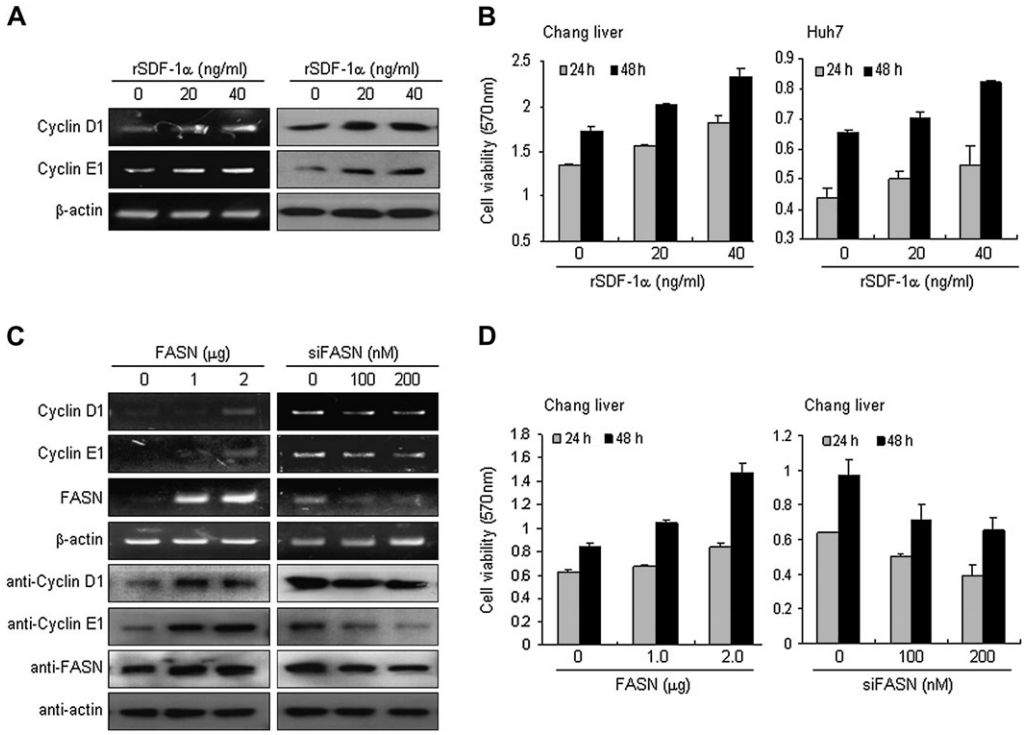

Fig. 5. The SDF-1α/CXCR4 axis or expression of FASN enhances the G_1 cyclin-mediated cell proliferation. (**A**) Effect of cyclin D1 or E1 expression on the rSDF-1α treatments. Chang liver cells were treated with indicated concentration of rSDF-1α for 48 h. Cyclin D1 or E1 mRNA level was detected using RT–PCR analysis and β-actin was used as an mRNA-loading control. Cell lysates were assessed using western blotting with antibodies for cyclin D1, E1 or actin. (**B**) Quantification of cell viability measured by MTT assay in Chang liver or Huh7 cells with treatment with rSDF-1α. Chang liver or Huh7 cells stimulated with rSDF-1α for 24 and 48 h showed the increased cell viability relative to untreated controls. (**C**) Cyclin D1 or E1 expression in the FASN-overexpressed or knockdowned cells. Chang liver cells were transfected with empty vector of FASN expression vector. After 48 h of incubation, total RNA or protein extracts were subjected to RT–PCR or western blot analysis. For the siRNA-mediated downregulation of FASN, negative control siRNA or FASN-specific siRNA was transfected into Chang liver cells. The transfected cells were analyzed by RT–PCR or western blotting. (**D**) Quantification of cell viability assessed by MTT assay by the FASN expression. Chang liver cells transfected with FASN expression vector or siRNA for FASN for 24 and 48 h were assessed by measuring the cell viability. RT–PCR, reverse transcription–polymerase chain reaction.

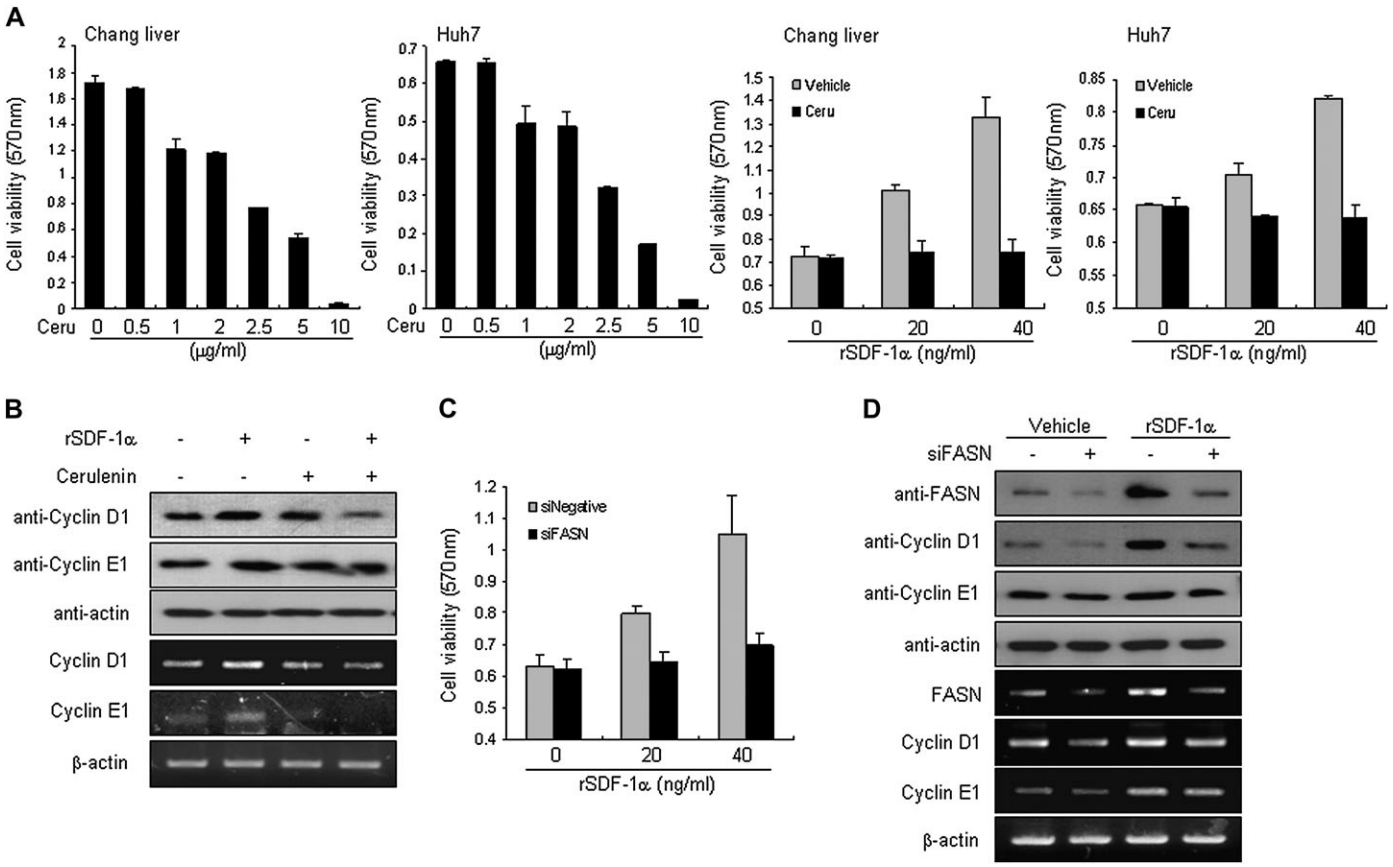

Fig. 6. The inhibition of FASN impedes the SDF-1α-mediated cell proliferation. (**A**) Left: Quantification of cell viability assessed by MTT assay by the FASN inhibitor. Chang liver or Huh7 cells stimulated with the indicated concentrations of cerulenin for 48 h showed the decreased cell viability relative to untreated controls. Right: Effect of the FASN inhibition on the rSDF-1α-mediated cell viability. Chang liver or Huh7 cells were pretreated with the 0.5 μg/ml cerulenin for 2 h and further incubated with or without the indicated concentration of rSDF-1α for 48 h. Cells were assessed by measuring the cell viability by MTT assay. (**B**) Effect of the expression of cyclin D1 or E1 expression on the FASN inhibitor and/or rSDF-1α treatments. Chang liver cells were pretreated with the 0.5 μg/ml cerulenin for 2 h and further incubated with or without the indicated concentration of rSDF-1α for 48 h. Cell lysates were assessed using western blotting with antibodies for cyclin D1, E1 or actin. Cyclin D1 or E1 mRNA level was detected using RT–PCR analysis and β-actin was used as an mRNA-loading control. (**C**) Quantification of cell viability by the FASN knockdown in the rSDF-1α treatments. Chang liver cells were transfected with siRNA for negative control or FASN for 24 h and further incubated with or without the indicated concentration of rSDF-1α. Cell viabilities were assessed by measuring MTT assay. (**D**) Effect of the expression of cyclin D1 or E1 expression on the FASN knockdown and/or rSDF-1α treatments. Chang liver cells were transfected with the siRNA for FASN for 24 h and further incubated with or without the indicated concentration of rSDF-1α for 48 h. Cell lysates were assessed using western blotting with antibodies for cyclin D1, E1 or actin. Cyclin D1 or E1 mRNA level was detected using RT–PCR analysis and β-actin was used as an mRNA-loading control. RT–PCR, reverse transcription–polymerase chain reaction.

The functional consequence of CXCR4 expression on cancer cells would be varied based on the numerous roles of the SDF-1α/CXCR4 signaling axis. For example, the combination of CXCR4 expression and interaction with stromal or nurse-like cells in chronic lymphocytic leukemia (32) and multiple myeloma (33) may account for resistance to spontaneous/drug-induced apoptosis and cell adhesion-mediated drug resistance, essentially providing a protective niche (34). Recently, CXCR4 expression on hepatocellular carcinoma was suggested to correlate with local tumor progression, lymphatic and distant metastasis, as well as a negative impact of the 3 year survival rate of the patients (35,36).

In this study, we found that the SDF-1α/CXCR4 axis enforces the expression of FASN. However, the SDF-1α treatment did not induce the expression of SREBP-1, one of the major transcription factors for inducing the FASN expression, on the mRNA levels. We examined the effect of 25-OHC to suppress the nuclear translocation of SREBP-1 on the SDF-1α-induced SREBP-1 translocalization. It was noted that oxygenated sterols such as 25-OHC were >50-fold more potent than cholesterol in reducing the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (37–39). When 25-OHC is treated with cells or when cholesterol is delivered in low-density lipoprotein, SREBP cleavage-activating protein becomes trapped in the endoplasmic reticulum. The bound SREBP is no longer carried to the Golgi apparatus, and the N-terminal domain cannot enter the nucleus (40). We identified that the mRNA level of FASN was not increased by SDF-1α treatment in the presence of 25-OHC (Figure 3).

In order to examine whether SDF-1α-induced SREBP-1 maturation is mediated via CXCR4, we tested the effect of AMD3100 on the SDF-1α-induced SREBP-1 maturation. The specific CXCR4 inhibitor, AMD3100, has been shown to specifically inhibit human immunodeficiency virus entry into cells via blocking CXCR4 interactions (25,41). The pharmacokinetics and safety have already been assessed in humans and AMD3100 was found to be well tolerated (42). Previous reports suggested that AMD3100 can be used as a therapeutic molecule for some cancers. The treatment of animals with AMD3100 resulted in decreased activation of the mitogen-activated protein kinase and Akt pathways in xenograft brain tumors, which are pathways downstream of CXCR4 that promote survival, proliferation and migration (43). Also, it was reported that AMD3100 had significant antitumor activity, which represents a novel strategy for targeting highly metastatic colorectal cancer cells (44). In contrast, it has been identified that PI3K/protein kinase B (Akt) signaling has been extensively implicated in cell survival and there is some evidence that Akt

might function in both upstream and downstream of FASN (19). Also, SDF-1α can activate the PI3K/Akt pathway, potentially leading to contribute to the survival and growth of tumor cells (17,26). Therefore, further studies are necessary on how the inhibition of CXCR4 by AMD3100 is associated with expression or activity of FASN *in vivo*.

The FA synthesis pathway involves two key enzymatic reactions. The first step is the carboxylation of acetyl-CoA in the cytosol to form malonyl-CoA, which is catalyzed by acetyl-CoA carboxylase α. The second step is sequentially mediated by FASN. Recently, it was identified that not only FASN but also acetyl-CoA carboxylase α is essential to breast cancer cell survival (45). Recent report demonstrates that SDF-1α induced HER2-neu tyrosine kinase transactivation in breast cancer cells and that this transactivation involved the activation of Src kinase (46) and it also was examined that the SDF-1α–CXCR4 interaction leads to HER2 activation in prostate cancer and that this transactivation occurs in lipid rafts (47). Also, molecular cross talk between the HER2 and FASN signaling pathways at the level of transcription, translation and biosynthetic activity was identified and FASN and acetyl-CoA carboxylase α are increased by HER2 overexpression (36). Additionally, it is possible that the SDF-1α/CXCR4 axis-induced FASN expression can be associated with the activation of HER2, especially in breast or prostate cancer. Furthermore, a recent publication has emphasized the link between glycolysis, lipogenesis and tumor cell proliferation (48). Downregulation of adenosine triphosphate citrate lyase, a key enzyme associating with glucose metabolism to lipid synthesis via the conversion of citrate to acetyl-CoA, affects glucose-dependent *de novo* FA synthesis in tumor cells (48). Accordingly, studies about the effect of the overall metabolic changes by the SDF-1α/CXCR4 should be attractive and studies can be interesting to explain a number of mechanisms, which can be associated with the SDF-1α/CXCR4 axis-mediated cancer cell survivals.

The D- and E-type cyclins induce progression through the G_1 phase of the cell cycle (49). Also, these cell cycle regulators are overexpressed or amplified at high frequencies in a variety of human cancers and in some cancers, such alterations are found in ∼50% of cases (50). We found that FASN plays an important role in the SDF-1α/CXCR4-mediated cell viability with regulation of G_1 phase of cyclins. Collectively, we demonstrated that the SDF-1α/CXCR4 axis induces Akt phosphorylation and activates SREBP-1 and enforces the expression of FASN. Taken together, in view of the coordinated regulation of FASN in cancer and the dysregulation of the SDF-1α/CXCR4 axis and the signaling pathway, our findings suggest that activation of the SREBP-1 by the SDF-1α/CXCR4 axis may contribute to the upregulation of FASN expression in a substantial subset of cancers.

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