Growth Factors, Cytokines, Cell Cycle Molecules

Oval Cell Response in 2-Acetylaminofluorene/Partial Hepatectomy Rat Is Attenuated by Short Interfering RNA Targeted to Stromal Cell-Derived Factor-1

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Stromal cell-derived factor-1 (SDF-1) is known to play an essential role in the regulation of stem/progenitor cell trafficking. During hepatic stem, or oval, cell activation, SDF-1 has been reported to be up-regulated within the liver, implying a possible role in oval cell-aided liver regeneration. In the present study, SDF-1 expression was knocked down in the liver of 2-acetylaminofluorene/partial hepatectomy-treated rats using short interfering RNA delivered by recombinant adenovirus. The oval cell response was compromised in these animals, as evidenced by a decreased number of OV6-positive oval cells. In addition, knockdown of SDF-1 expression caused a dramatic decrease in α -fetoprotein expression, implying impaired oval cell activation in these animals. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling assay showed no significant apoptosis related to SDF-1 suppression. Instead, as revealed by Ki67 immunohistochemistry, the suppression of SDF-1 resulted in decrease of hepatic cell proliferation, implying the repair process had been inhibited in these animals. These results indicate that SDF-1 is an essential molecule needed in oval cell activation. (*Am J Pathol* 2006, 169:2066–2074; DOI: 10.2353/ajpath.2006.060211)

In general, the liver relies on two types of responses to regenerate after major tissue loss: proliferation of existing hepatocytes and, to a lesser extent, the activation of the stem/progenitor cell compartment. Mature hepatocytes have a remarkable replication capability and are very efficient in restoring hepatic parenchyma after liver injury caused by a variety of methods [ie, partial hepatectomy

(PHx), hepatic toxins, hepatatrophic virus infection, and so on] and thus are enlisted as the first line of regeneration. However, in some situations where hepatocyte replication is suppressed, such as after treatment with 2-acetylaminofluorene (2AAF), oval cells will proliferate and differentiate to replenish the hepatic mass. Oval cells in this case have been regarded as facultative liver stem cells capable of differentiating into both hepatocytes and bile duct epithelial cells.^{1,2}

The question of the origin of oval cells remains open. It has been suggested that oval cells or their precursors reside within or adjacent to the canal of Hering and expand into the liver parenchyma after activation.³ Several reports suggest that bone marrow-derived stem cells might be an alternative source of the liver progenitor cells,^{4,5} migrating to and engrafting in the liver, giving rise to oval cells and then hepatocytes. There are also reports suggesting otherwise.⁶ Regardless of the origin, oval cells or their precursors must depend on proper signal(s) to mediate activation, migration, and differentiation. The molecular signaling microenvironment at the site of liver injury consists of a complex array of growth factors, cytokines, chemokines, extracellular matrix (ECM), and cell-cell contacts. Factors that have been associated with the oval cell response include, but are not limited to, hepatocyte growth factor,^{7–10} transforming growth factor- α ,^{7,11} acidic fibroblast growth factor,^{7,12} tumor necrosis factor,^{13,14} leukemia inhibitory factor,¹⁵ stem cell factor (SCF),^{16,17} γ -interferon,¹⁸ and the plasminogen activator/plasmin system,¹⁹ but the precise roles of these proteins are still unclear.

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Stromal cell-derived factor-1 (SDF-1) is a member of the CXC chemokine family first identified from bone marrow stromal cells and later found in most major solid organs in the body including liver. One of the functions of SDF-1 is to direct cell migration along a SDF-1 gradient, from low concentration to high concentration. This is triggered by binding of SDF-1 to the G-protein-coupled receptor CXCR4 on the surface of responding cells. The SDF-1/CXCR4 axis plays an essential role in hematopoiesis presumably through directing hematopoietic stem cells to their final niches.^{20,21} The SDF-1/CXCR4 interaction may have a more general role during embryogenesis and postnatal tissue regeneration involving various tissue-committed stem cells. For example, some neural precursors,²² endothelial progenitors,²³ and primordial germ cells²⁴ also express functional CXCR4 on their surfaces, and the importance of SDF-1/CXCR4 interaction on these cells have been illustrated by the defects of brain,²⁵ large vessel,²⁶ and germ cells²⁴ found in the embryos of CXCR4^{-/-} mice. Previous findings from this laboratory have reported that SDF-1 was up-regulated during oval cell activation but not during normal liver regeneration. In the 2-acetylaminofluorene/partial hepatectomy (2AAF/PHx) oval cell activation model, SDF-1 was expressed by hepatocytes,²⁷ whereas its receptor CXCR4 was expressed on the oval cell surface.^{27,28} *In vitro* migration assays demonstrated that oval cells migrate to a gradient of higher SDF-1 concentration.²⁷ These observations suggest the possibility that the SDF-1/CXCR4 axis may play a role in oval cell activation, although the significance of this interaction on the oval cell response is yet to be determined.

In the present study, RNA interference was used to knock down the SDF-1 signal in the livers of 2AAF/PHx-treated rats, providing a more clear view of the role of the SDF-1/CXCR4 axis during oval cell activation. The oval cell response was assessed by histology and Western blot analyses. These results indicate that the oval cell response was compromised when SDF-1 expression was suppressed within the regenerating liver, suggesting an important role of SDF-1 in oval cell activation.

Materials and Methods

Animal Experiment

Two-month-old female F-344 rats were used for all experiments. A 2AAF pellet (70 mg, released over a period of 28 days) was implanted into the peritoneal cavity. A 70% partial hepatectomy was performed 7 days after 2AAF implantation, and 6×10^{10} pfu of recombinant adenovirus was infused through the tail vein immediately after PHx. Animals were sacrificed at day 9 or 13 after PHx, and liver tissues were collected for further studies. The control group ($n = 3$ for each time point) received adenovirus expressing scramble RNA; the SDF-1-knock-down group ($n = 3$ for each time point) received adenovirus expressing short interfering RNA (siRNA) targeted to SDF-1. All animal studies were conducted according to the National Institutes of Health guidelines for animal use and institutionally approved protocols.

Recombinant Adenovirus

siRNA Expression Cassette

Invert repeat DNA fragments based on rat SDF-1 coding sequence were inserted at the +1 position of mouse U6 promoter. The transcribed RNA was therefore predicted to form a small hairpin, which will be further processed into siRNA within the target cell. Control vector was constructed in a similar way except that a scrambled sequence was used.

Mouse U6 promoter (-315 to +1) was amplified from mouse genomic DNA by PCR using primer pairs 5'-AC-TAGTGATCCGACGCCGCCATCTCTAGGC-3' and 5'-GGCCCAACAAGGCTTTTCTCCAAGGGATATTTA-3'. Oligonucleotide pairs 5'-TGTGCATTGACCCGAAATTC-AAGAGAATTTTCGGGTCAATGCACACTTTTGGTAC-3' and 5'-CAAAAAGTGTGCATTGACCCGAAATTCCTTG-AAATTTTCGGGTCAAGTCACA-3' were annealed to form invert repeat DNA template for siRNA against SDF-1 (siSDF). Oligonucleotide pairs for scrambled siRNA templates are 5'-GCATATGTGCGTACCTAGCATTCAA-GAGATGCTAGGTACGCACATATGCCTTTTGGTAC-3' and 5'-CAAAAAGGCATATGTGCGTACCTAGCATCTC-TTGAATGCTAGGTACGCACATATGC-3'.

Generation of Recombinant Adenovirus

Adeno-X expression system (BD Biosciences Clontech, Palo Alto, CA) was used to make adenoviral vectors containing the siSDF expression cassette and control adenoviral vector containing the scrambled siRNA cassette. U6 promoter and invert repeat DNA were inserted into pShuttle vector, and the expression cassettes were further transferred to BD Adeno-X vector. The adenoviral vectors were then used to transfect AD-293 cells (Stratagene, La Jolla, CA) to produce adenovirus. Recombinant adenoviruses were enriched and purified using ViraBind Adenovirus purification kit (Cell Biolabs Inc., San Diego, CA) and titrated using Adeno-X rapid titer kit (BD Biosciences Clontech). All procedures were performed following the manufacturer's instructions. The adenovirus containing the siSDF cassette was designated as Ad-siSDF, and the control virus, Ad-scramble.

Detection of Viral DNA

DNA was isolated from rat liver using genomic DNA purification kit (Promega, Madison, WI) following the manufacturer's instruction. Polymerase chain reaction (PCR) was performed to detect recombinant adenovirus DNA component using primer set 5'-AAGCCTTGCCCTGTTGTAGC-3' and 5'-TAATGAGGGGGTGGAGTTTG-3'. The size of the positive PCR product is expected to be about 500 bp.

Immunohistology

Immunofluorescent Staining for SDF-1

Formalin-fixed liver tissues were embedded in paraffin and cut into 5- μ m sections. After deparaffinization and

Figure 1. Immunofluorescent staining of SDF-1 in normal rat liver and 2AAF/PHx rat liver. Normal liver (**A**) and 2AAF/PHx (**B**) rat, day 9. **C:** Primary antibody replaced by normal goat IgG (negative control) 2AAF/PHx rat, day 9. **Arrows** point to SDF-1-positive hepatocytes. SDF-1 (green) expression can be seen in most of the hepatocytes within the pericentral region on the liver of 2AAF/PHx rat. **C:** Notice that in this model, most cells within portal triads are SDF-1-negative. **A:** SDF-1 expression is not detected in normal liver. Autofluorescence is displayed as orange color under the dual-path fluorescein isothiocyanate-Texas Red filter used here. PT, portal triad; CV, central vein. Original magnification, $\times 20$.

hydration, sections were microwaved for 7 minutes in 0.01 mol/L citrate buffer, pH 6.0. The sections were then washed with Tris-buffered saline, blocked with normal rabbit serum, and incubated with goat polyclonal anti-SDF1 (diluted 1:50; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour at room temperature. Fluorescein isothiocyanate-conjugated rabbit anti-goat IgG (diluted 1:200; Vector Laboratories, Burlingame, CA) was used as the secondary antibody.

Immunostaining for OV6

Cryostat sections (5 μ m) were fixed in acetone (-20°C) for 10 minutes. After serum blocking and avidin/biotin blocking, sections were incubated with mouse anti-OV6 antibody (diluted 1:150; a gift from Dr. Stewart Sell, Ordway Research Institute and Wadsworth Center, Albany, NY) for 1 hour at room temperature and later with biotinylated anti-mouse IgG (Vector Laboratories) for 30 minutes at room temperature. The staining reaction was developed using Vectastain elite ABC kit (Vector Laboratories) and diaminobenzidine tetrahydrochloride substrate (Vector Laboratories).

Immunostaining for Ki67

Five-micrometer paraffin sections were microwaved for 7 minutes in 0.01 mol/L citrate buffer (pH 6.0) after deparaffinization and hydration. The sections were then washed with Tris-buffered saline. After normal serum blocking and avidin/biotin blocking, sections were incubated with mouse anti-Ki67 antibody (diluted 1:100; PharMingen, San Diego, CA) for 1 hour at room temperature and later with biotinylated anti-mouse IgG (Vector Laboratories) for 30 minutes at room temperature. The staining reaction was developed using Vectastain elite ABC kit (Vector Laboratories) and diaminobenzidine tetrahydrochloride substrate (Vector Laboratories).

Western Blotting

Liver lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to

Immun-Blot PVDF membrane (Bio-Rad, Hercules, CA) using standard technique. For α -fetoprotein (AFP) detection, goat polyclonal anti-AFP IgG (Santa Cruz Biotechnology) and horseradish peroxidase (HRP)-conjugated anti-goat IgG (Santa Cruz Biotechnology) were used as primary and secondary antibody, respectively. ECL plus Western blotting detection kit (Amersham Biosciences, Piscataway, NJ) was used for development of the membrane. Membranes were stripped and blocked before detection of different proteins. For SDF-1 detection, goat polyclonal anti-SDF1 (Santa Cruz Biotechnology) was used as primary antibody, and HRP-conjugated anti-goat IgG (Santa Cruz Biotechnology) was used as secondary antibody. For β -actin detection, mouse anti-actin IgG (Abcam, Cambridge, MA) was used as primary antibody, and HRP-conjugated anti-mouse IgG (Amersham Life Sciences, Arlington Heights, IL) was used as secondary antibody.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick-End-Labeling (TUNEL) Analysis

TUNEL assay was performed on paraffin-embedded liver sections to detect apoptotic cells using the ApoAlert DNA fragmentation assay kit (BD Biosciences Clontech) following the manufacturer's instruction.

Results

SDF-1 Expression in Rat Liver after 2AAF/PHx

SDF-1 expression was not detected by immunostaining on quiescent liver cells before oval cell induction (Figure 1A). By day 9 after 2AAF/PHx, most of the hepatocytes located within the pericentral region were positive for SDF-1, whereas most of the cells located within portal regions were SDF-1-negative (Figure 1C). This staining pattern was further confirmed using two other antibodies targeted to different epitopes of SDF-1 molecule (supplemental material at <http://ajp.amjpathol.org>). This finding is consistent with our previous finding.²⁷

Figure 2. Ad-siSDF knocks down SDF-1 expression in 2AAF/PHx rat livers and inhibits the oval cell reaction in these animals. **A:** PCR detection of viral DNA in the livers day 9 after 2AAF/PHx and virus infusion. DNA from day-9 2AAF/PHx-treated liver was used as negative control. **B** and **C:** Liver sections from 2AAF/PHx rat treated with Ad-scramble, day 9. **D** and **E:** Liver sections from 2AAF/PHx rat treated with Ad-siSDF, day 9. **B** and **D:** Immunofluorescence of SDF-1 (green) counterstained with DAPI (blue) for nuclear. SDF-1 was knocked down in Ad-siSDF-treated animal liver (compare **B** with **D**). **C** and **E:** H&E staining of liver sections showing suppressed oval cell response in Ad-siSDF-treated animals (**E**) compared with control animal (**C**). **Arrows** in **B** and **D** point to SDF-1-positive hepatocytes. **Arrows** in **C** and **E** point to oval cells. Original magnification, $\times 20$.

Suppression of SDF-1 Expression by siSDF in Livers of 2AAF/PHx Rats

Recombinant adenovirus has been used for hepatic gene transfer to express therapeutic gene products in mice, rats, rabbits, and dogs. From these studies, intravenously administered adenovirus has proven to be highly efficient in transducing nondividing hepatocytes.²⁹ In the present study, adenovirus was used to deliver the siRNA expression cassette into rat liver after 2AAF/PHx. The recombi-

nant adenovirus successfully infected the liver cells, as demonstrated by PCR detection of the viral DNA in the liver (Figure 2A). Nine days after virus infusion, SDF-1 expressions in Ad-siSDF-transduced livers were remarkably knocked down in most hepatocytes (Figure 2D), whereas the Ad-scramble treatment did not inhibit up-regulation of SDF-1 (Figure 2B). Western blot analyses of Ad-siSDF-treated livers further confirmed decreased SDF-1 expression level (Figure 3). It is unlikely that suppression of SDF-1 production was due to nonspecific

Figure 3. Knockdown of SDF-1 and decreased AFP expression after siSDF treatment. SDF-1 and AFP protein level in rat liver was detected by Western blot. Protein sample (10 μ g) from the liver of each animal was loaded into each lane. Relative quantity of each band is determined by normalizing its total density to that of loading control in the same lane. Ad-siSDF-induced RNA interference results in considerable decrease in SDF-1 expression, accompanied by a decrease of AFP expression from these rat livers ($P < 0.05$, Student's *t*-test).

inhibition of protein synthesis by foreign RNA or adenovirus infection because similar levels of SDF-1 protein were seen between Ad-scramble-treated 2AAF/PHx liver and 2AAF/PHx liver (Figure 3).

Inhibition of Oval Cell Response by Knocking Down SDF-1

On day 9 after 2AAF/PHx and Ad-scramble infusion, numerous oval cells appeared at the portal regions (Figure

2C). Oval cells featured an ovoid-shaped nucleus and high nucleus-to-cytoplasm ratio when stained with hematoxylin and eosin. A remarkable decrease in oval cell proliferation was seen in the liver of 2AAF/PHx rats treated with Ad-siSDF (Figure 2E). Consistent with the morphological findings, the number of OV6⁺ oval cells was dramatically decreased in Ad-siSDF-treated 2AAF/PHx rats compared with that of Ad-scramble-treated rats (Figure 4). Western blot analyses on AFP showed that this oval cell marker protein was dramatically reduced at the

Figure 4. OV6 immunostaining of rat liver sections. **A:** 2AAF/PHx rat treated with Ad-scramble, day 9. **B:** High magnification of the bracketed area in **A**. **C:** 2AAF/PHx rat treated with Ad-siSDF, day 9. **D:** High magnification of bracketed area in **C**. Less OV6-positive (brown) oval cells are seen in Ad-siSDF-treated animals (**C** and **D**) than in control animals (**A** and **B**). **E:** Comparison of the number of OV6-positive cells in different group of animals. OV6-positive oval cells were counted from 25 randomly selected fields ($\times 40$) in sections from each group. The number of oval cells per field was 88.9 ± 29.8 in control rat and 24.2 ± 17.4 in Ad-siSDF-treated rat (mean $\pm$ 2 SD, $P < 0.01$, Student's *t*-test). **Arrows** point to oval cells. Original magnification: $\times 10$ (**A** and **C**); $\times 40$ (**B** and **D**).

livers of Ad-siSDF-treated 2AAF/PHx rat (Figure 3), which further confirmed that the oval cell activation was compromised in these animals.

Decrease of Oval Cell Number Was Not Related to Oval Cell Apoptosis

Because SDF-1 has been shown, in some studies, to be capable of protecting CD34⁺ progenitor cells from apoptosis,³⁰ it was considered that the decreased number of oval cells seen in Ad-siSDF-treated animal was the result of apoptosis of those cells. To address this issue, TUNEL staining of the liver sections was used to assess apoptosis in the livers of all rats used in these studies. Apoptotic cells were rarely detected in all three groups of rats

(Figure 5). Hence, suppression of SDF-1 did not increase oval cell apoptosis evidently. The decrease in oval cell numbers seen in Ad-siSDF-treated animals likely results from suppressed proliferation of oval cells. Consistent with this notion, Ki67 staining revealed that the number of cells entering active cell cycle was far lower in Ad-siSDF-treated rat than in control rat in days 9 and 13 after the treatment. (Figure 6) The majority of the proliferating cells that reside outside of the sinusoid were believed to be oval cells and their progenies—new hepatocytes and bile duct cells. This result indicates that knockdown of SDF-1 expression in the damaged liver inhibits the proliferation of hepatic cells and thus hinders oval cell-aided liver regeneration.

Discussion

A role for the SDF-1/CXCR4 axis in stem cell-aided liver regeneration has been suggested by several studies, all of which demonstrated a correlation between SDF-1 expression and stem cell accumulation in the liver.^{27,28,31} In the present study, the well-characterized 2AAF/PHx model was used to determine the role of SDF-1 in oval cell activation. In 2AAF/PHx model, oval cell number increases markedly by day 5 after PHx and peaks at approximately day 9.³² Consistent with the previous findings, SDF-1 was expressed mostly in the pericentral region of the liver during oval cell activation (Figure 1). This finding is in contradiction with that of Mavrier et al,²⁸ who demonstrated in a similar model that SDF-1 was strongly expressed by oval cells, although only rarely detected in hepatocytes. To further confirm the expression pattern of SDF-1 in this rat model, two polyclonal antibodies targeted to full-length and N-terminal SDF-1, respectively, were used to detect SDF-1 expression by immunofluorescence. The SDF-1 signal was present mainly in the hepatocytes within the pericentral region, although the sensitivities of the antibodies may vary (supplemental material; <http://ajp.amjpathol.org>).

According to our present findings, production of SDF-1 was polarized across the liver lobule with high levels of expression near the central vein and virtually no expression around the portal triad. SDF-1 is known to bind heparin sulfate-associated proteoglycan on cell mem-

Figure 5. No significant apoptosis detected by TUNEL staining in 2AAF/PHx rat livers. **A:** 2AAF/PHx rat, day 9. **B:** 2AAF/PHx/Ad-scramble-treated rat, day 9. **C:** 2AAF/PHx/Ad-siSDF-treated rat, day 9. **Arrows** point to apoptotic cells (green), which are sporadic on the liver sections from all animals. The extent of apoptosis in the liver is similar between all three groups at this time point. Original magnification, $\times 10$.

Figure 6. Knockdown of SDF-1 hinders hepatic cell proliferation. **A:** 2AAF/PHx/Ad-scramble-treated rat, day 9. **B:** 2AAF/PHx/Ad-siSDF-treated rat, day 9. **C:** 2AAF/PHx/Ad-scramble-treated rat, day 13. **D:** 2AAF/PHx/Ad-siSDF-treated rat, day 13. **Arrows** point to proliferating oval cells and **arrowheads** point to proliferating hepatocytes. Original magnification, $\times 20$. **E:** Comparison of the number of proliferating cells in different group of animals. Ki67-positive cells (brown nucleus) were counted as proliferating cells from 25 randomly selected fields in sections of each group. The cells residing within sinusoids were intentionally ignored because they were not related to oval cells. The number of proliferating cells per field (under $\times 20$ magnification) was 388.7 ± 49.1 in control rat versus 106.4 ± 40.6 in Ad-siSDF-treated rat at day 9 and 465.8 ± 53.7 in control rat versus 157.5 ± 20.2 in Ad-siSDF-treated rat at day 13 (mean $\pm$ 2 SD, $P < 0.01$, Student's *t*-test).

brane and ECM.³³ It is likely that ECM and cells within the central zone become decorated with SDF-1 during oval cell activation, establishing a concentration gradient that may aid in oval cell trafficking. SDF-1 is critical for cell trafficking in a number of important biological events including hematopoietic stem cell homing,^{20,21} cancer metastasis,³⁴ and primordial germ cell migration.^{24,35} During oval cell activation in the 2AAF/PHx rat model, oval cells proliferate and radiate from the periportal region (possibly from the canal of Hering) toward the pericentral region of the liver. Because oval cells express CXCR4 on their surface, it is possible that oval cells arising from the periportal region respond to the SDF-1

gradient across the liver lobule and migrate into the parenchyma, where the microenvironment is favorable for their proliferation or differentiation.

The effect of SDF-1 on cell survival/anti-apoptosis remains controversial. Recent studies found that activation of the SDF-1/CXCR4 axis prevents certain hematopoietic stem/progenitor cells or cell lines from apoptosis *in vitro*,^{30,36,37} whereas others did not.^{38,39} Apoptosis has been reported in later stages of oval cell-aided liver regeneration. Yano et al⁴⁰ observed apoptosis of hepatocytes surrounding oval cells at day 9 after 2AAF/PHx. In another study, oval cell apoptosis was seen to peak at day 10 after 2AAF/PHx and was believed to represent a

mechanism for modulating hepatic cell number and remodeling of liver parenchyma in the final stages of oval cell-aided liver regeneration.⁴¹ In our oval cell activation model, apoptosis was not evident at day 9 after 2AAF/PHx under either SDF-1 suppression or up-regulation. However, because survival of oval cells was not compromised by knocking down of SDF-1, the decrease in the number of oval cells observed in treated animals most likely reflects a decrease in proliferation of oval cells, suggesting a possible role of SDF-1 in oval cell proliferation.

SDF-1 was found to promote the proliferation of astrocytes⁴² and some tumor cell lines such as glioblastoma cells⁴³ and ovarian cancer cells.⁴⁴ This effect has been shown to correlate with the activation of extracellular signal-regulated kinase (ERK) 1/2 or phosphoinositide-3 kinase (PI3K)-AKT pathways within the target cells.^{42,44} However, another *in vitro* study has shown that SDF-1-induced activation of these pathways did not affect proliferation/survival of several hematopoietic cell lines.³⁹ This suggests that activation of other signaling pathways is required in the target cells to enhance their proliferation or survival. Moreover, there is evidence showing that SDF-1 acts synergistically with other cytokines such as granulocyte-macrophage colony-stimulating factor, SCF, and thrombopoietin, enhancing the survival of CD34⁺ progenitor cells.⁴⁵ The SDF-1/CXCR4 interaction also induces epidermal growth factor receptor phosphorylation to enhance proliferation of ovarian cancer cells.⁴⁴ All of these data support the notion that crosstalk between the SDF-1/CXCR4 axis and other cytokine signaling pathways plays an important role in regulating stem cell proliferation or survival. As shown by the present study, down-regulation of SDF-1 expression within the liver causes reduction in the number of proliferating oval cells and hepatocytes after 2AAF/PHx (Figure 6), implying an important role of SDF-1 in promoting cell proliferation during oval cell-aided liver regeneration.

Oval cell-aided liver regeneration is regulated by an array of cytokines and chemokines such as hepatocyte growth factor, transforming growth factor- α , acidic fibroblast growth factor, tumor necrosis factor- α , leukemia inhibitory factor, SCF, and γ -interferon.^{7–18} SDF-1/CXCR4 interaction may represent an essential component of this complex controlling network. Besides its direct action on oval cells, it is possible that SDF-1 also has an effect on other hepatic cells. Further studies aimed to elucidate the function of SDF-1 on various hepatic cells and to dissect the connections between SDF-1 and other cytokine signaling pathways in the context of oval cell-aided liver regeneration will be critical for a better understanding of the pathophysiology of liver regeneration.

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