Biochemical and Biophysical Research Communications 394 (2010) 503–508

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Sp7/Osterix up-regulates the mouse pro-α3(V) collagen gene (*Col5a3*) during the osteoblast differentiation

Wu Yun-Feng, Noritaka Matsuo, Hideaki Sumiyoshi, Hidekatsu Yoshioka *

Department of Matrix Medicine, Faculty of Medicine, Oita University, 1-1 Idaigaoka Hasama-machi, Yufu, Oita 879-5593, Japan

ARTICLE INFO

Article history:
Received 26 February 2010
Available online 3 March 2010

Keywords:
Sp7/Osterix
Type V collagen
Bone
Osteoblast
Differentiation
Sp family

ABSTRACT

Type V collagen is a quantitatively minor collagen, but acts as critical regulator of fibril formation in the extracellular matrix. The purpose of this study is to clarify the mechanism responsible for the transcriptional regulation of the mouse *Col5a3* gene in osteoblastic cells. Sp7/Osterix is a transcription factor specifically expressed by osteoblasts and is important for osteoblast differentiation. The overexpression of Sp7/Osterix significantly increased the promoter activity and the endogenous mRNA level of the *Col5a3* gene in osteoblastic cells. Conversely, a reduction of Sp7/Osterix by siRNA treatment decreased the promoter activity and the endogenous mRNA level of the *Col5a3* gene. A CHIP assay confirmed that Sp7/Osterix interacted with the *Col5a3* core promoter *in vivo* at the Sp1 binding site. The data from the experiments using the osteoblast differentiation model and the co-overexpression of Sp7/Osterix with Sp1 suggest that Sp7/Osterix promotes the expression of the collagen gene, *Col5a3*, and thereby playing a role in bone formation.

1. Introduction

Collagens, which are the major constituents of extracellular matrices, are critical for the formation and function of the organs in the body [1]. Type V collagen is a member of the fibrillar collagen group, which is a minor subgroup based on the relative expression level and presence in tissues where type I collagen is expressed. Type V collagen is incorporated into the fibrils of the more abundant type I collagen, and acts as regulator of the size and the shape of the fibrils [2]. There are several type V isoforms that differ in chain composition. The major isoform is $[\alpha1(V)]_2\alpha2(V)$. Defects in the human *COL5A1* and *COL5A2* genes have been identified so far in half of the cases of classic Ehlers–Danlos syndrome (EDS, type I/II) [3,4] . In addition, the $[\alpha1(V)]_3$ homotrimer and the α1(V)α2(V)α3(V) heterotrimer have been also reported. The α1(V)α2(V)α3(V) isoform was isolated from human placenta as well as the uterus, skin, and synovial membranes [5–7]. The pro-α3(V) chain is also expressed in bone and the N-terminal globular domain influences bone formation by osteoblasts through proteoglycans on the cell surface [8].

Sp7/Osterix is a zinc finger-containing protein which belongs to the Sp/KLF (Kruppel like Factor) family of transcription factors [9]. The murine Sp7/Osterix protein contains 428 amino acids yielding a molecular weight of 44.7 kDa. It is distinctly expressed in all of the developing bones and is important for osteoblast differentiation. It binds to GC box elements and activates mRNA synthesis from the genes containing functional recognition sites. In null mice, endochondral and intramembranous bone formation does not occur.

Characterization of the core promoter of the human *COL5A3* gene has been demonstrated that the CBF/NF-Y acts as a transcriptional activator of the *COL5A3* gene in a human Schwannoma cell line [10]. In a previous study, a GC-rich domain was found in the core promoter of the mouse *Col5a3* gene [11]. The Sp1 bound to the GC-rich domain and activated the *Col5a3* gene in combination with CBF/NF-Y in osteoblastic cells. In this study, the involvement of Sp7/Osterix, which binds to the Sp1 binding site, in the activation of the *Col5a3* gene during osteoblast differentiation was examined.

2. Materials and methods

2.1. Cell culture

The cell lines used in this study was mouse preosteoblast MC3T3-E1 cells. Cells were cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum (Sanko Junyaku, Tokyo, Japan) at 37 °C in a 5% CO_2/air environment. For differentiation studies, MC3T3-E1 cells were fed at confluence (time 0) with the above medium additionally containing 10 μM

Abbreviation: CHIP assay, chromatin immunoprecipitation assay.

* Corresponding author. Fax: +81 97 586 5674.
E-mail address: hidey@med.oita-u.ac.jp (H. Yoshioka).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.02.171

β-glycerophosphate and 50 μg/mL of ascorbic acid. The medium for differentiation was changed every 2 days.

2.2. RT-PCR and real-time PCR

Total RNA was isolated from cultured cells using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. RNA was quantified by optical density (A260) and stored at −80 °C until used. Three micrograms of total RNA was reverse transcribed by random hexamer priming using SuperScript II reverse transcriptase (Invitrogen, CA, USA). The single-stranded cDNA was amplified by PCR using specific primer pairs (Table S1). PCR was carried out for 25–30 cycles using a step cycle of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 7 min. The PCR products were analyzed by electrophoresis on a 1.5% ethidium bromide-stained agarose gel. The amplified fragment was eluted from the gel and sequenced.

Real-time PCR was performed using a LightCycler TaqMan Master. The thermal cycling conditions included 1 cycle at 95 °C for 10 min, 40 cycles at 95 °C for 15 s, 60 °C for 1 min. The relative mRNA expression levels were normalized against that of *GAPDH* gene from the same RNA preparations, using a comparative threshold cycle method. The primer sets are listed in Table S1.

2.3. Construction of chimeric plasmids

The luciferase constructs, pGL4.10 −337/+92 and mut 1-Luc, and the pCMV-Sp1 expression plasmid were previously described [9]. The Sp7/Osterix expression vector, pMAM-BSD-Sp7, was generated by RT-PCR using RNA extracted from MC3T3-E1 cells. The set of primers are listed in Table S1. The PCR product was cloned into the pGEM-T Easy vector and sequenced. The correct plasmid was digested with KpnI/NotI, and then subcloned into the mammalian expression vector, pMAM-BSD.

2.4. Transient transfection and luciferase Assays

The cells were plated at a density of 2×10^5 per 35-mm dish ~18 h before transfection. For transient transfection, 0.5 μg of plasmid DNA was transfected into these cells by using the Lipofect-AMINE Plus reagent system. Plasmid pRL-TK vector (Promega) was always cotransfected as an internal control for transfection efficiency. After an additional cultivation for 48 h, the transfected cells were harvested, lysed, centrifuged to pellet the debris, and subjected to luciferase assay. The luciferase activities were measured as chemiluminescence in a luminometer (Lumat LB 9507, PerkinElmer Life Sciences) using the Dual Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol. The cotransfection experiments were performed using 1.5 μg of Sp1 or Sp7/Osterix expression plasmids. Mithramycin A was incubated with a concentration of 100 nM for 24 h before the luciferase assays.

2.5. Chromatin immunoprecipitation (CHIP) assay

The CHIP assays were performed using a chromatin immunoprecipitation assay kit (Upstate Biotechnology, Lake Placid, NY) according to the manufacturer's protocol. The anti-Sp1 and anti-Sp7/Osterix antibodies were purchased (Cosmo Bio, Tokyo, Japan). The primer sets are listed in Table S1. The procedure was described previously [11].

2.6. siRNA transfection

The siRNA cocktails targeting mouse Sp1 or mouse Sp7/Osterix were purchased (Santa Cruz Biotechnology Inc, Sant Cruz, CA, USA). The mouse MC3T3-E1 osteoblastic cells were transfected using Lipofectamine 2000 (Invitrogen) to achieve a final siRNA concentration of 50 nM. The specificity of Sp1 or Sp7/Osterix knockdown was confirmed by a RT-PCR reaction. For the promoter analysis, transfections, including luciferase reporter construct, were allowed to proceed for 48 h before the analysis. For the quantitative analysis of the expression levels of the *Col5a3* mRNA, a real-time PCR reaction was performed.

2.7. DNA sequencing

Nucleotide sequences were determined by automated DNA sequencing (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems).

3. Results

3.1. Up-regulation of the promoter activity of the Col5a3 gene by Sp7/Osterix

To characterize the effects of the Sp7/Osterix transcription factor on the mouse *Col5a3* gene, the Sp7/Osterix overexpression vector pMAM-BSD-Sp7 was generated. It was cotransfected into the MC3T3-E1 cells with the −337/+92-Luc promoter luciferase reporter gene, which contains the basic promoter region of the

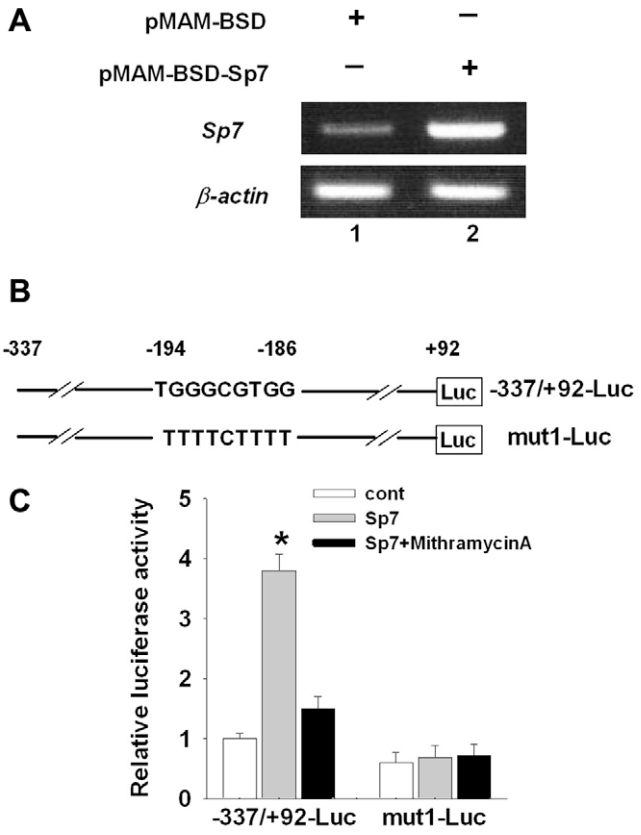

Fig. 1. Transactivation of the *Col5a3* by overexpression of Sp7/Osterix in MC3T3-E1. (A) An RT-PCR analysis of Sp7/Osterix transcripts from MC3T3-E1 cells transfected with the Sp7/Osterix expression construct (lane 2) or with the control empty plasmid (lane 1). (B) A schematic illustration of the luciferase reporter constructs. The mut1-Luc is mutated in the Sp1 binding site of pGL4.10 −337/+92-Luc construct. (C) The effect of Sp7/Osterix expression construct and mithramycin A (100 nM) on the activity of *Col5a3* promoter constructs. The relative luciferase activities were normalized against the activity of the pGL4.10 −337/+92-Luc construct without any additional treatment. Data are means ± S.D. of three independent experiments. The asterisks indicate statistically significant results ($p < 0.05$).

Col5a3 gene [11]. The overexpression of Sp7/Osterix was verified by a PCR reaction (Fig. 1A). Sp7/Osterix greatly activated the −337/+92 promoter luciferase reporter gene ($p < 0.05$) (Fig. 1B and C). This increase was cancelled by the addition of mithramycin A, which recognizes GC-rich promoter regions and interferes with the binding of the Sp family [12]. However, it had no effect on the cells that were cotransfected with mut1-Luc, in which the Sp1 binding site of the promoter of the luciferase reporter gene was mutated (Fig. 1B and C).

3.2. The effect of Sp7/Osterix on the promoter activity and endogenous expression of the Col5a3 gene

We previously demonstrated that Sp1 binds to the *Col5a3* promoter and that Sp1 increased the activity [11]. To compare the effect of Sp1 and Sp7/Osterix, we overexpressed Sp1 and Sp7/Osterix. The expression was detected by a RT-PCR reaction with primers that are specific for either Sp1 or Sp7/Osterix (Fig. 2A). The amount of overexpressed Sp1 and Sp7/Osterix gene was examined by real-time PCR and no difference was observed between both expressed amounts (data not shown). The overexpression of both Sp1 and Sp7/Osterix increased the promoter activities (Fig. 2B) and endogenous expression (Fig. 2C) of the *Col5a3* gene in MC3T3-E1 cells. The effect of Sp7/Osterix overexpression was more substantial than that of Sp1 overexpression. The overexpression of Sp7/osterix transactivated the *Col5a3* promoter by approximately 4-fold, while overexpression of Sp1 transactivate the *Col5a3* by approximately 2-fold. Similarly, the overexpression of Sp7/Osterix significantly increased the endogenous mRNA levels of *Col5a3* by approximately 3-fold, while

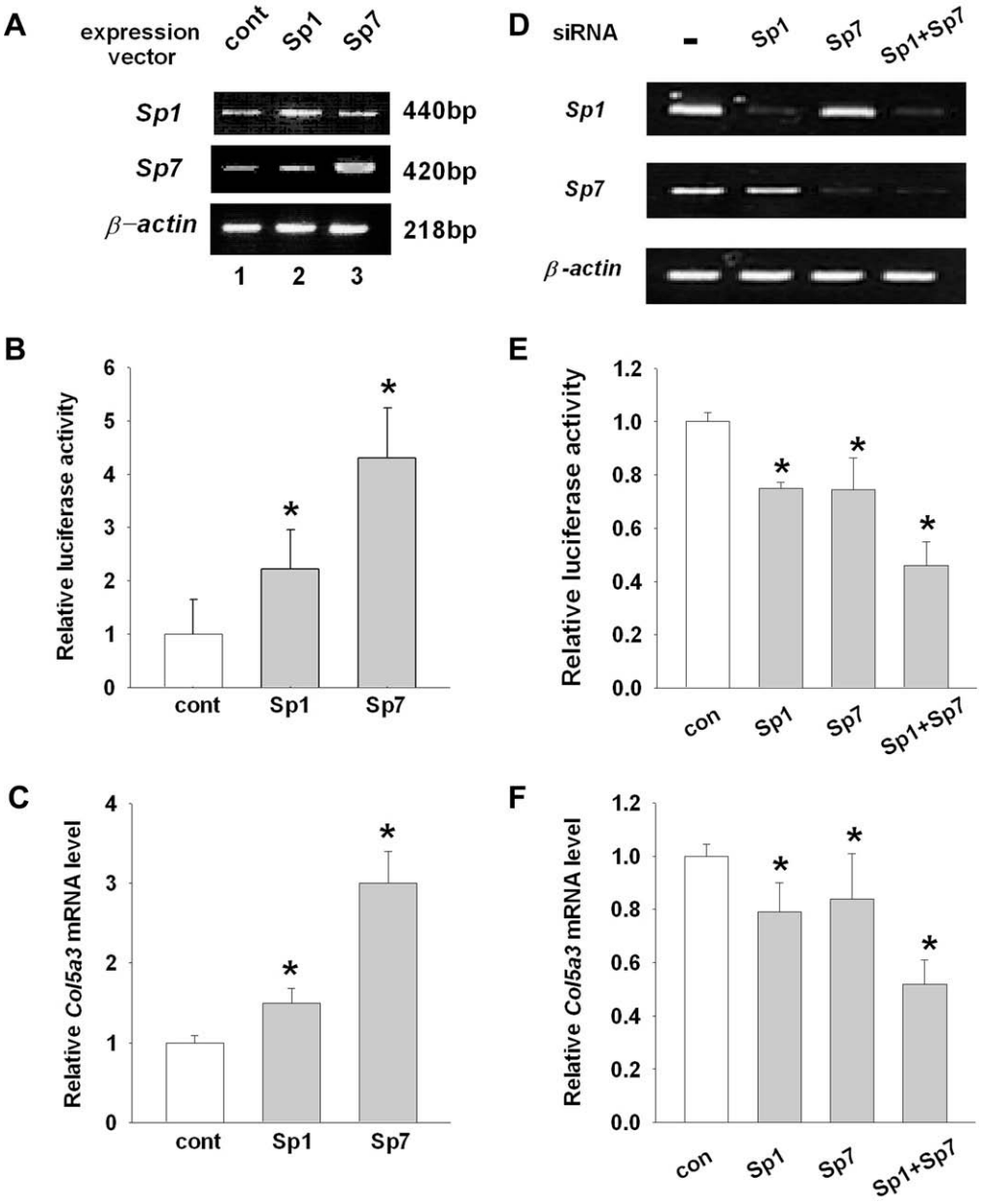

Fig. 2. (A) RT-PCR was performed with total mRNA from MC3T3-E1 cells which were transfected with overexpression vector of Sp1 (lane 2) and Sp7/Osterix (lane 3). The empty vector was transfected as a control. (lane 1). Comparison of effect of the Sp1 and Sp7/Osterix expression constructs on (B) the activity of *Col5a3* promoter using pGL4.10 −337/92-Luc construct and on (C) the endogenous mRNA levels of *Col5a3*. (D) RT-PCR was performed with total mRNA from the MC3T3-E1 cells, which were transfected with siRNA against Sp1 (lane 2), Sp7/Osterix (lane 3), and both (lane 4). A comparison of the effect of selective inhibition of Sp1 and Sp7/Osterix on (E) the activity of the *Col5a3* promoter using the pGL4.10 −337/92-Luc construct and (F) on the endogenous mRNA levels of *Col5a3*. Real-time PCR was performed to examine the amount of endogenous mRNAs. GAPDH were used to normalize *Col5a3* expression levels. Data are means ± S.D. of at least three independent experiments. $^{*}p < 0.05$ compared with the control.

overexpression of Sp1 increased the levels by approximately 1.5-fold.

3.3. Selective inhibition of Sp7/osterix by siRNA

To examine the relative contribution of Sp1 and Sp7/Osterix to *Col5a3* transcription in MC3T3-E1 cells, selective inhibition by specific siRNA was performed. Before inhibition, the expression of Sp1 was approximately five times higher than that of Sp7/Osterix in the cells (data not shown). The siRNA treatment of Sp1 or Sp7/Osterix decreased the promoter activity and the endogenous mRNA levels of the *Col5a3* gene ($p < 0.05$) (Fig. 2D–F). The inhibition of Sp1 or Sp7/Osterix, singly and combination, decreased the promoter activity as well as the

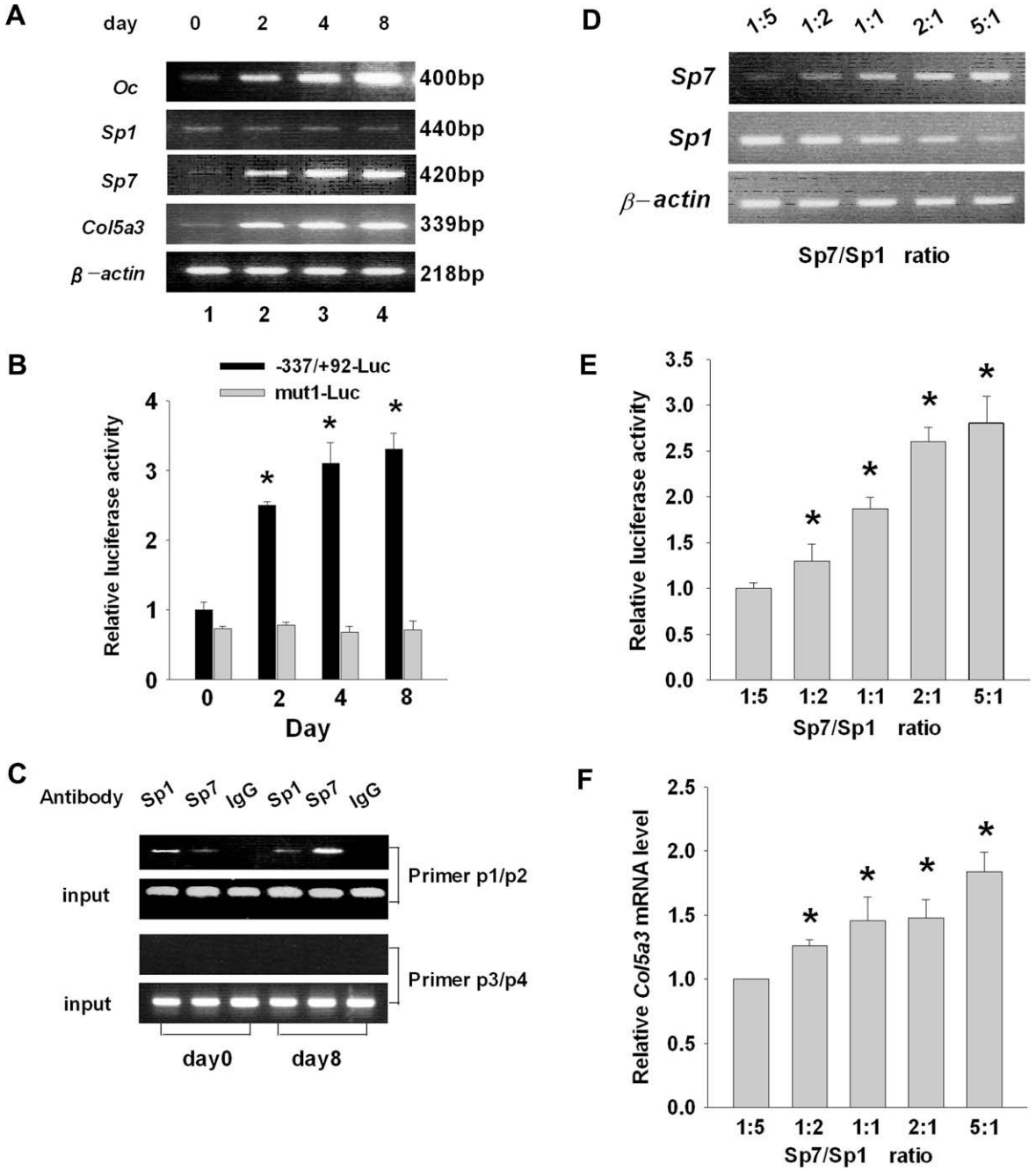

Fig. 3. (A) Expression of Sp1 and Sp7/Osterix during MC3T3-E1 osteoblast differentiation. An RT-PCR analysis for *Osteocalcin (Oc)*, *Sp1*, *Sp7/Osterix* and *Col5a3* gene on 0, 2, 4 and 8 days of differentiation. β-Actin was included as a positive control for the PCR. PCR was carried out for 25 cycles. (B) The relative luciferase activity of *Col5a3* promoter using pGL4.10 –337/92-Luc and mut1-Luc. Data are means ± S.D. of three independent experiments. Asterisks indicate statistically significant results ($p < 0.05$). (C) A CHIP assay was performed to examine the binding of Sp1 and Sp7/Osterix to the *Col5a3* promoter at 0 and 8 days. All of the immunoprecipitated DNA fragments were analyzed by a PCR reaction with primer sets, p1 and p2, for the −293 to −68 in the proximal promoter region of the *Col5a3* gene and another set of control primers, p3 and p4, which amplify the segments that were located approximately 4 kb upstream. IgG was used as a control. (D) The co-overexpression of Sp1and Sp7/Osterix in MC3T3-E1. RT-PCR analysis after the transfection with different ratios of Sp7(Osterix)/Sp1. (E) The relative luciferase activity of *Col5a3* core promoter using pGL4.10 −337/92-Luc construct. (F) The endogenous mRNA levels of *Col5a3*. Real-time PCR was performed to examine the amount of endogenous mRNAs. GAPDH were used to normalize *Col5a3* expression levels. Data are means ± S.D. of at least three independent experiments. $^{*}p < 0.05$ compared with the ratios 1:5 of Sp7(Osterix)/Sp1.

endogenous mRNA levels by approximately 20% and 50%, respectively.

3.4. Activation of Col5a3 gene during the process of MC3T3-E1 cell differentiation by Sp7/Osterix

To further examine the activation of *Col5a3* gene by Sp7/Osterix in osteoblast cells, the cell culture model of osteoblast differentiation was used. Preosteoblast cells, MC3T3-E1, were differentiated for this purpose. As shown in Fig. 3A, osteocalcin, an osteoblast differentiation marker, was observed to markedly increase in the process of the differentiation of MC3T3-E1 cell. The Sp7/Osterix expression was markedly increased, while that of Sp1 constantly demonstrated a small amount. The expression of *Col5a3* gene also increased during osteoblast differentiation. The activity of *Col5a3* promoter with −337/+92-Luc construct increased approximately 3.5-fold, but it did not change with mut1-Luc (Fig. 3B). Consistent with these data, CHIP assay revealed that Sp7/Osterix increasingly bind to the *Col5a3* core promoter sequence *in vivo* during differentiation (Fig. 3C).

Finally, to confirm that Sp7/Osterix is responsible for activation of the *Col5a3* gene rather than Sp1, the co-overexpression of the Sp7Osterix and Sp1 with the different ratios was performed (Fig. 3D–F). The activity of *Col5a3* core promoter as well as the endogenous mRNA levels increased along with the change in the ratio of Sp7(Osterix)/Sp1from 1:5 to 5:1 (Fig. 3E and F).

4. Discussion

Sp7/Osterix is a recently identified zinc finger-containing transcription factor that belongs to Sp family [9]. It is expressed in all of the developing bones, and it is essential for osteoblast differentiation and bone formation. Therefore, Sp7/Osterix was thought to be involved in regulating the *Col5a3* gene in osteoblastic cells. In this study, we compared the contribution of both Sp1 and Sp7/Osterix to the regulation of the *Col5a3* gene. For this purpose, the osteoblast differentiation model was used. The results showed the activation of the *Col5a3* gene was dependent on increasing amounts of amount of Sp7/Osterix but not Sp1 during osteoblast differentiation (Fig. 3A–C). This result was confirmed with experiments in which the cells overexpressed different ratios of Sp7(Osterix)/Sp1 (Fig. 3D–F). Conversely, selective inhibition by specific siRNA of Sp1 and Sp7/Osterix showed similar degree of inhibition although the expression of Sp1 was dominant in MC3T3-E1 cells (Fig. 2D–F). These data therefore showed that Sp7/Osterix promotes the expression of the collagen gene, *Col5a3*, in comparison to Sp1 in osteoblastic cells.

Sp7/Osterix activated *Col1a1* promoter activity by forming a complex with NFAT (nuclear factor of activated T cells) [13]. Goto et al. showed that the transcription factors, Sp1, Sp3, and Sp7/Osterix, regulate *COL11A2* gene expression through its proximal promoter in osteoblastic cells [14]. Other α chains of type V collagen, α1(V) and α2(V), are expressed in osteoblastic cells [15]. The Sp1 site was identified in the mouse *Col5a1* gene [16]. Therefore, Sp7/Osterix might bind to the Sp1 site of the *Col5a1* gene and thereby regulate it in osteoblastic cells. Sp7/Osterix should be involved in the regulation of various collagen genes which are expressed in bone.

Gene transcription is controlled by a series of complex interactions of many transcription factors. The inhibition with siRNA (Fig. 2D–F) and deletions and/or mutations that removed the binding sites of Sp1 and CBF/NF-Y [11] did not completely eliminate the promoter activity of the *Col5a3* gene. This suggested that other factors or mechanisms are involved in the activation of the *Col5a3* gene in bone tissues. Runx2/Cbfa1 is also identified as a master transcription factor for osteoblast differentiation. It acts upstream

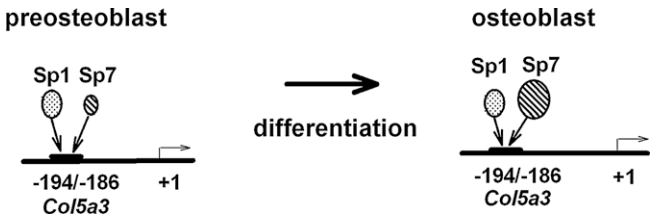

Fig. 4. A schematic illustration of the activation of the *Col5a3* gene by the Sp family during osteoblast differentiation.

of Sp7/Osterix and is needed for an early step of osteoblast differentiation [17]. Runx2/Cbfa1 contributes to the osteoblast-specific expression of both type I collagen genes. It binds to the regions at around −1347 bp in the mouse α1(I) and +12 bp in the mouse α2(I) promoters [18]. ATF4 and β-catenin also act downstream of Runx2/Cbfa1 [17]. ATF4 is a ubiquitous protein that is expressed in many cells, but it also interacts with Runx2/cbfa1 to regulate the transcriptional activity of osteocalcin. One Wnt protein, Wnt10b, was found to induce the expression of Runx2/cbfa1 and Sp7/Osterix. Runx2/Cbfa1 could be responsible for the activation of the *Col5a3* promoter in the early stages of osteoblast differentiation. The factors, such as ATF4 and β-catenin, that are ubiquitously expressed but which also interact with specific factors in osteoblastic cells may also be involved in the regulation of the *Col5a3* gene. In conclusion, Sp7/Osterix was observed to participate in the activation of the *Col5a3* promoter during osteoblast differentiation (Fig. 4). This finding should provide some clues regarding the mechanism of bone formation.

Acknowledgments

We thank Ms. S. Adachi for her valuable technical support, and the staff members of Research Promotion Project, Oita University. This work was supported by Grants-In-Aid for Scientific Research (No. 20390402 to H.Y.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.02.171.

References

[1] M. van der Rest, R. Garrone, Collagen family of proteins, FASEB J. 5 (1991) 2814–2823.
[2] D.E. Birk, Type V collagen: heterotypic type I/V collagen interactions in the regulation of fibril assembly, Micron 32 (2001) 223–237.
[3] H.V. Toriello, T.W. Glover, K. Takahara, P.H. Byers, D.E. Miller, J.V. Higgins, D.S. Greenspan, A translocation interrupts the COL5A1 gene in a patient with Ehlers–Danlos syndrome and hypomelanosis of Ito, Nat. Genet. 13 (1996) 361–365.
[4] K. Michalickva, M. Susic, M.C. Willing, R.J. Wenstrup, W.G. Cole, Mutation of the α2(V) chain of type V collagen impair matrix assembly and produce Ehlers–Dalos syndrome type I, Hum. Mol. Genet. 7 (1998) 249–255.
[5] R.K. Rhodes, E.J. Miller, Evidence for the existence of an α1(V)α2(V)α3(V) collagen molecule in human placental tissue, Collagen Relat. Res. 1 (1981) 337–343.
[6] M.Z. Abedin, S. Ayad, J.B. Weiss, Isolation and native characterization of cysteine-rich collagens from bovine placental tissues and uterus and their relationship to types IV and V collagens, Biosci. Rep. 2 (1982) 493–502.
[7] J.H. Fessler, L.I. Fessler, Structure and function of collagen types, in: R. Mayne, R.E. Burgeson (Eds.), Academic Press, Orland, FL, 1987, pp. 81–103.
[8] K. Yamaguchi, N. Matsuo, H. Sumiyoshi, N. Fujimoto, K-I. Iyama, S. Yanagisawa, H. Yoshioka, Pro-α3(V) collagen chain is expressed in bone and its basic N-terminal peptide adheres to osteosarcoma cells, Matrix Biol. 24 (2005) 283–294.
[9] K. Nakashima, X. Zhou, G. Kunkel, Z. Zhang, J.M. Deng, R.R. Behringer, B. de Crombrugghe, The novel zinc finger-containing transcription factor osterix is required for osteoblastdifferentiation and bone formation, Cell 108 (2002) 17–29.

[10] H. Nagato, N. Matsuo, H. Sumiyoshi, K. Sakata-Takatani, M. Nasu, H. Yoshioka, The transcription factor CCAAT-binding factor CBF/NF-Y and two repressors regulate the core promoter of the human pro-α3(V) collagen gene (*COL5A3*), J. Biol. Chem. 279 (2004) 46373–46383.
[11] Y.-F. Wu, N. Matsuo, H. Sumiyoshi, H. Yoshioka, The Sp1 and CBF/NF-Y transcription factors cooperatively regulate the mouse pro-α3(V) collagen gene (*Col5a3*) in osteoblastic cells, Acta Med. Okayama 64 (2010) 95-108.
[12] S.W. Blume, R.C. Snyder, R. Ray, S. Thomas, C.A. Koller, D.M. Miller, Mitamysin inhibits SP1 binding and selectively inhibits transcriptional activity of the dihydrofolate gene in vitro and in vivo, J. Clin. Invest. 88 (1991) 1613–1621.
[13] T. Koga, Y. Matsui, M. Asagiri, T. Kodama, B. de Crombrugghe, N. Nakashima, H. Takayanagi, NEAT and Ostrrix cooperatively regulate bone formation, Nat. Med. 11 (2005) 880–885.
[14] T. Goto, Y. Matsui, R.J. Fernandes, D.A. Hanson, T. Kubo, K. Yukata, T. Michigami, T. Komori, T. Fujita, L. Yang, D.R. Eyre, N. Yasui, Sp1 family of transcription factors regulates the human α2(XI) collagen gene (COL11A2) in Saos-2 osteoblastic cells, J. Bone Miner. Res. 21 (2006) 661–673.
[15] Y. Hamada, H. Sumiyoshi, N. Matsuo, Y-F. Wu, M. Nakashima, S. Yanagisawa, H. Yoshioka, The pro-α2(XI) collagen gene is expressed in osteoblasts, Biochem. Biophys. Res. Commun. 392 (2010) 166–170.
[16] K. Sakata-Takatani, N. Matsuo, H. Sumiyoshi, T. Tsuda, H. Yoshioka, Identification of a functional CCAAT-like motif in the core promoter of the mouse pro-α1(V) collagen gene (*Col5a1*), Matrix Biol. 23 (2004) 87–99.
[17] P.J. Marie, Transcription factors controlling osteoblastogenesis, Arch. Biochem. Biophys. 473 (2008) 98–105.
[18] B. Kern, J. Shen, M. Starbuck, G. Karsenty, Cbfa1 contributes to the osteoblast-specific expression of type I collagen genes, J. Biol. Chem. 276 (2001) 7107.