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Runx2 is Involved in Regulating Osterix Promoter Activity and Gene Expression*

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Abstract Though Runx2 and Osterix are both key transcription factors in the pathway of osteoblast differentiation, whether Runx2 positively regulates Osterix being unknown. It was showed that Runx2 induced the gene expression of Osterix both in the non-osteoblastic cell lines, either pluripotent or differentiated, and in the osteoblastic cell lines. At the same time, the results also indicated that Runx2 up-regulated the activity of the 3.2 kb human Osterix promoter. Further experiments identified a highly conserved and functional Runx2 binding site "AGTGGTT" within the promoter. Thus the results support the hypothesis that Runx2 is involved in the regulation of the Osterix gene expression. Moreover, the transient transfection and dual-luciferase assay showed Osterix up-regulated the activity of the 2.3 kb type Ⅰ collagen promoter in the non-osteoblastic cells, but Runx2 did not. This difference implies that Osterix, the down stream transcription factor of Runx2 during osteoblast differentiation, is needed to stimulate the osteoblast-specific gene expression of type Ⅰ collagen.

Key words Runx2, Osterix, gene expression, regulation

Osteoblasts, the bone forming cells, synthesize and mineralize the bone extracellular matrix. Functional osteoblasts arise from pluripotent mesenchymal cells and *via* the intermittent osteoprogenitor and preosteoblast stages. Osteoblasts can further differentiate into bone-lining cells and osteocytes [1]. Many factors influence osteoblast differentiation, maturation and function, and therefore fundamentally affect the balance between bone formation and bone loss. Among these factors, osteoblast specific transcription factors should play a central regulatory role[2].

Runx2 (Runt related gene 2, also previously called Core binding factor α1) is the first identified osteoblast specific transcription factor. It plays a pivotal role in osteoblast differentiation and bone formation[3~6]. In mice, homozygous mutation of this gene results in a complete lack of osteoblastic cells and bone formation [3,4]. In humans, the skeletal development disorder cleidocranial dysplasia is caused by the haploinsufficiency of Runx2[5]. The study using transgenic mice that specifically over-express Runx2 in osteoblasts shows Runx2 inhibits late stage osteoblast differentiation, but accelerates early stage osteoblast differentiation [7]. More recently, another osteoblast specific transcription factor, Osterix, is identified. Homozygous mutation of this gene in mice also results in a complete lack of bone formation, and osteoblast differentiation is completely arrested[8]. The phenotypes of Osterix null mice are similar to that of Runx2 null mice in the disappearance of osteoblastic cells and bone. These mice, however, show normal chondrocyte differentiation, osteoclastogenesis and neovascularization, all of which are not observed in Runx2 null mice. Osterix is not detected in Runx2 null mice, whereas Runx2 is normally expressed in Osterix null mice. These phenotypic differences between Runx2 and Osterix null mice suggest that Osterix is a more restricted transcription factor than Runx2 in osteoblast differentiation and acts downstream of Runx2[8].

To further discover the relationships between

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Runx2 and Osterix, we performed some experiments including RT-PCRs, dual luciferase assays and so on. Our results show that Runx2 not only induces the Osterix gene expression, but also up-regulates the activity of the human Osterix promoter. Considering the ability of Runx2 to regulate the gene expression of Osterix and other osteoblast related genes [9] in non-osteoblastic cells, we suppose that Runx2 may be also involved in inducing Osterix during early osteoblast differentiation from pluripotent mesenchymal cells.

1 Materials and methods

1.1 Cell lines, antibodies and plasmids

C3H10T1/2, C2C12 and MC3T3-E1 were kindly provided by Dr. Komori. MLO-Y4 was the generous gift from Dr. Bonewald. NIH3T3, 293 and the above mentioned four cell lines were maintained in αMEM or IMDM. The media (Gibco) were supplemented with 10% fetal bovine serum (Hyclone). PEBP2aA M-70 rabbit polyclonal IgG was purchased from Santa Cruz Biotechnology. FITC conjugated goat anti-rabbit IgG was from Beijing Zhongshan Golden Bridge Biotechnology. Osterix C-12 rabbit polyclonal IgG was kindly provided by Dr. Fujita. The 2.3 kb type Ⅰ collagen promoter-firefly luciferase construct in pGL3-basic and the mouse Osterix expression vector in pFLAG-CMV2 were provided by Dr. Fujita. The Runx2 expression vector pCMV5-Osf2/Cbfa1 was provided by Drs. Ducy and Karsenty.

1.2 Immunofluorescence examinations

Cells were cultured on cover slides and transfected by Fugene 6 transfection reagent (Roche). After transfection, cells were fixed in 4% paraformaldehyde/PBS and permeabilized with 0.5% Triton X-100/PBS for 20 min. Cells were further washed with 0.1% Tween20/PBS, and incubated in 10% fetal bovine serum/PBS for 30 min to block non-specific binding of the antibodies. Cells were then incubated with primary antibodies in 10% fetal bovine serum/PBS for 1 h at room temperature. After wash with 0.1% Tween20/PBS 3 times, 10 min each, cells were incubated with 2nd antibodies in 10% fetal bovine serum/PBS for 30 min at 37℃. After the incubation, cells were washed with 0.1% Tween20/PBS 3 times, 10 min each. To stain nucleus, cells were finally incubated in 0.1 mg/L DAPI.

1.3 Western blot analysis

Cells were harvested in direct lysis buffer (2% SDS, 10 mmol/L DTT, 10% glycerol, 2 mol/L urea, 1 mmol/L PMSF, 10 mmol/L Tris-HCl [pH 6.8], 0.002% bromophenol blue, and a proteinase inhibitor mixture), and nuclear extracts were prepared from the cell lysate. Nuclear extract proteins were separated on 10% SDS-polyacrylamide gel. Separated proteins were electrophoretically transferred to nitrocellulose membranes (Schleicher & Schuel, Keene, NH). The membranes were blocked with BSA (Roche) in TBST for 2 h at room temperature. Primary antibody (Runx2 antibody, or Osterix antibody) was used at a dilution of 1∶1 000. Secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG) was used at a dilution of 1∶4 000. Blots were incubated overnight at 4℃ with primary antibody in 3% BSA-TBST and subsequently with horseradish peroxidase-conjugated anti-rabbit secondary antibody for 2 h at room temperature. Immunoreactivity was determined using the ECL chemiluminescence reaction (Amersham).

1.4 RNA preparation and RT-PCR

For RT-PCR analysis, cells were transfected by Polyfect transfection reagent (Qiagen) according to the provided protocol. Total RNA extraction was done with Trizol reagent (Invitrogen) 48 h after transfection. RT-PCR was performed according to the instructions for ImProm-Ⅱ reverse transcriptase (Promega) and Taq polymerase premix (Takara). Together with the antisense primer, 5′ GTT CAG TGG AGG CTG AGC TG 3′, the following sense primers were used in the gene expression analysis of Osterix: 5′ GAG TAG GAT TGT AGG ATT GG 3′, for the isoform encoding the shorter polypeptide; 5′ ATG GCG TCC TCC CTG CTT GAG GAG GAA G 3′ for the one encoding the longer polypeptide; and 5′ TGC TTG AGG AGG AAG TTC ACT ATG GC 3′ for routine analysis. As an internal control, the primers for β-actin were used: 5′ CCA ACC GTG AAA AGA TGA CCC 3′ and 5′ ACC GCT CGT TGC CAA TAG TG 3′.

1.5 Cloning of the human Osterix promoter and construction of the promoter-reporter vectors

The human genomic DNA BAC clone RP11-680A11 containing the Osterix gene was purchased from Chori. A 3.2 kb 5′ flanking region was obtained by PCR using 5′ CTA TCT TTG GCG CTG ATC TCT G 3′ and 5′ AGG CAG ATG GAG AGA GCT GAG 3′. Serial 5′ truncation of this region was done by PCR with the sense primers (for the 1.9 kb, 5′ GGA GAG TGG AAA GGA TG 3′; for the 1.1 kb, 5′

CTC AGA ACC TTC CAT G 3′; for the 0.5 kb, 5′ CAG ATG CCT ATG GGA CTG 3′) and the common antisense primer (5′ AGG CAG ATG GAG AGA GCT GAG 3′). To mutate the putative Runx2 binding sites in the 1.1 kb 5' flanking region, the mutant primers for "TGTGGT" (5′ GCT GGG GCG GGA ATT CTG CGT GTG 3′ and 5′ CAC ACG CAG AAT TCC CGC CCC AGC 3′) and that for "AGTGGTT" (5′ CTC TAA TTA GAA TTC TGG GGT TTG 3′, and 5′ CAA ACC CCA GAA TTC TAA TTA GAG 3′) were used in combination with the primers that were used for amplifying the normal 1.1 kb 5′ flanking region, and the two-step PCR mutagenesis method was used. An *Eco*R Ⅰ restriction site was introduced to replace each putative Runx2 binding site. All the PCR products were sequenced and subcloned into pREP4-luc [10], resulting in the several promoter-firefly luciferase constructs.

1.6 Transient transfections and dual luciferase assays

For luciferase assay in NIH3T3 and C2C12, the cells were cotransfected by Polyfect with 1.5 μg of the 3.2 kb promoter-firefly luciferase construct and 1.0 μg of pCMV5 or pCMV5-Osf2/Cbfa1. For some luciferase assays in 293, the cells were cotransfected by electroporation (Bio-Rad) with 5 μg of each construct and 2.5 μg of pCMV5 or pCMV5-Osf2/Cbfa1. For the other luciferase assays in 293, Fugene 6 was used instead of electroporation, and the total amount of DNA/well was added according to the provided protocol. Luciferase activities were measured 36～48 h after transfection, using the dual luciferase assay system (Promega) on a TD-20/20 luminometer (Turner Designs). 0.1 μg of pREP7-Rluc containing the *Renilla* luciferase cDNA[10] was always used as an internal control. For other cotransfection experiments, Fugene 6 was used according to the provided instructions. pREP7-Rluc was still used as an internal control in these experiments. 40～48 h after transfection, cells were harvested and analyzed for promoter-reporter activity by Dual-Luciferase Repoter Assay System (Promega).

1.7 Statistics

Data were shown as $\bar{x} \pm s$ in cotransfection experiments ($n \geqslant 3$), and $P < 0.05$ computed by one-way ANOVA was regarded as significantly different and marked as an asterisk in the figures.

2 Results

2.1 The over-expressed Runx2 is localized in the nucleus

To verify whether Runx2 can be over-expressed and localized in the nucleus, we transfected 293 cells with the Runx2 expression vector pCMV5-Osf2/Cbfa1. 48 h after transfection, immunofluorescence analysis shows Runx2 was expressed and localized in the nucleus and the fluorescence signal was strong. (Figure 1a) The transfection efficiency was estimated about 30% according to DAPI counter-staining (Figure 1a and 1b).

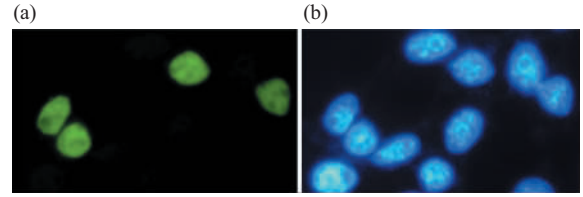

Fig. 1 The over-expressed Runx2 was localized in the nucleus

Immunofluorescence examination (a) shows that Runx2 was expressed and finally localized in the nucleus where is demarcated by DAPI counter-staining in (b).

2.2 Runx2 induces the gene expression of Osterix in the non-osteoblastic and osteoblastic cells

To address whether Runx2 regulates Osterix, the gene expression of Osterix in a number of different cells that were forced to express Runx2 was examined. These cell lines included two non-osteoblastic cell lines (293 and NIH3T3), two mesenchymal stem cell lines (C2C12 and C3H10T1/2), one osteoblastic cell line (MC3T3-E1) and one osteocytic cell line (MLO-Y4). Based on RT-PCR, the Osterix gene expression was only detected at an extreme low level in MC3T3-E1 that was transfected with the empty vector pCMV5. No Osterix gene expression was detected in the other five cell lines that were transfected with the empty vector. However, the Osterix gene expression was strongly detected in all the six cell lines that were transfected with the Runx2 expression vector pCMV5-Osf2/Cbfa1. This induction of the Osterix gene expression by Runx2 is probably cell type independent, since the cell lines are either differentiated (293, NIH3T3, MLO-Y4 and MC3T3-E1) or pluripotent (C2C12 and C3H10T1/2) (Figure 2a).

It has been noted that there are at least two isoforms of Osterix mRNA in human, mouse and rat ([11], and GenBank accession Nos. BK001413, BK001412, AF184902, and AK077375). The two isoforms are alternatively spliced and different at their 5′ termini: one encodes a longer polypeptide (starts with "MASSLL"), and the other encodes a shorter polypeptide (starts with "MLTAA") (Figure 2b). Using RT-PCR, we found that only the isoform encoding the longer polypeptide was induced by Runx2 in C2C12 and C3H10T1/2 (Figure 2c). This is consistent with the previous report[8]. Further study is therefore needed to investigate the differences between the two isoforms in the temporal and spatial expression and function.

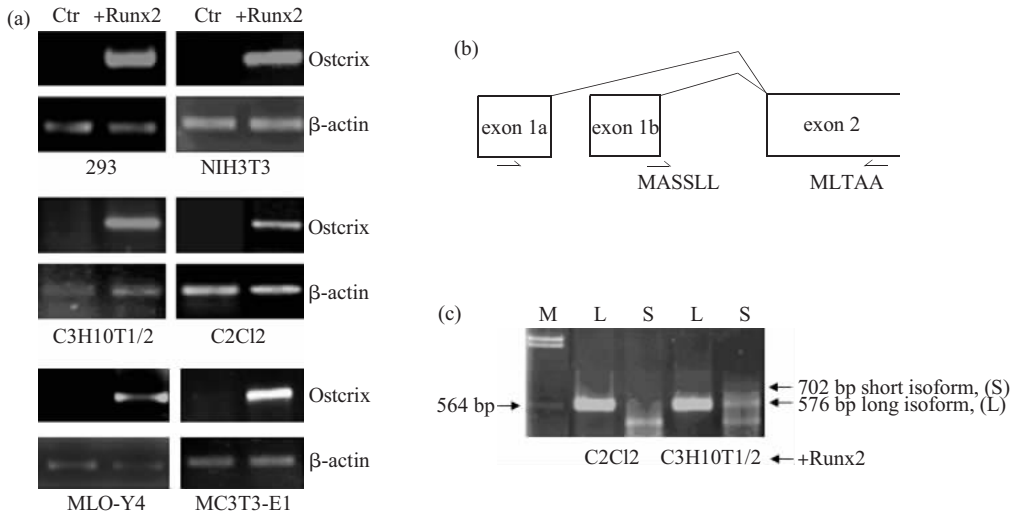

Fig. 2 Runx2 induced the gene expression of Osterix *in vitro*

(a) The RT-PCR experiments show that transient transfection with the Runx2 expression vector pCMV5-Osf2/Cbfa1 induced the Osterix gene expression in 293, NIH3T3, C2C12, C3H10T1/2, MC3T3-E1 and MLO-Y4. Representative data from two independent transfection experiments are shown. The PCR product clearly appeared at 564 bp. Ctr, transfection with pCMV5; +Runx2, with pCMV5-Osf2/Cbfa1. (b) The schematic alternative splicing pattern of Osterix in human, mouse and rat. Exons are shown as boxes. "MASSLL" and "MLTAA" represent the first several amino acid residues of the longer polypeptide and the shorter polypeptide, respectively. The primers specific for each mRNA isoform encoding either the longer polypeptide (L) or the shorter polypeptide (S) are marked as arrows. (c) Identification of the isoforms of the Osterix mRNA by RT-PCR in C2C12 and C3H10T1/2 that were forced to express Runx2. M stands for the lambda *Hin*dⅢ marker. The expected size for each isoform (L or S) is shown by arrows with base pairs.

2.3 Cloning of the human Osterix promoter

To further investigate the role of Runx2 in inducing the gene expression of Osterix *in vitro*, a 3.2 kb fragment of the human Osterix promoter was cloned and sequenced (The sequence data was submitted to GenBank with the accession No. AY496272). This region was first theoretically analyzed for putative regulatory *cis*-elements, using the online tool "TESS" with "TRANSFAC" database. Computer analysis reveals the presence of multiple putative *cis*-elements for transcription factors, such as GC box, Ap1, CCAAT, MyoD and Runx2. This promoter lacks a canonical TATA box, but has two GC boxes in the proximity in stead that may mediate initiation of transcription (data not shown).

The serial 5′-truncation of the promoter and the following dual luciferase assays reveal the minimum region responsible for both the basal and the Runx2 up-regulated promoter activities (Figure 3b). Overall, our dual luciferase assays show that Runx2 up-regulated the 3.2 kb promoter activity by three times, four times and six times in NIH3T3, C2C12 and 293 cells, respectively (Figure 3c).

2.4 Identification of a functional Runx2 binding site

Computer analysis shows that, within 1.1 kb to 0.5 kb upstream of the start codon of the longer polypeptide, i.e. the above mentioned minimum region, there are two putative canonical Runx2 binding sites, "TGTGGT" and "AGTGGTT", both of which are previously documented [12,13]. The Blast analysis of the human 5′ flanking region against mouse

and rat genomes indicates that the Runx2 binding site is highly conserved (Figure 4a), but the putative Runx2 binding site, “TGTGGT”, which is also canonical, is not conserved. We generated two more 1.1 kb promoter-reporter constructs, each contains a single mutant Runx2 binding site (gTGTGGT to gAATTCT,

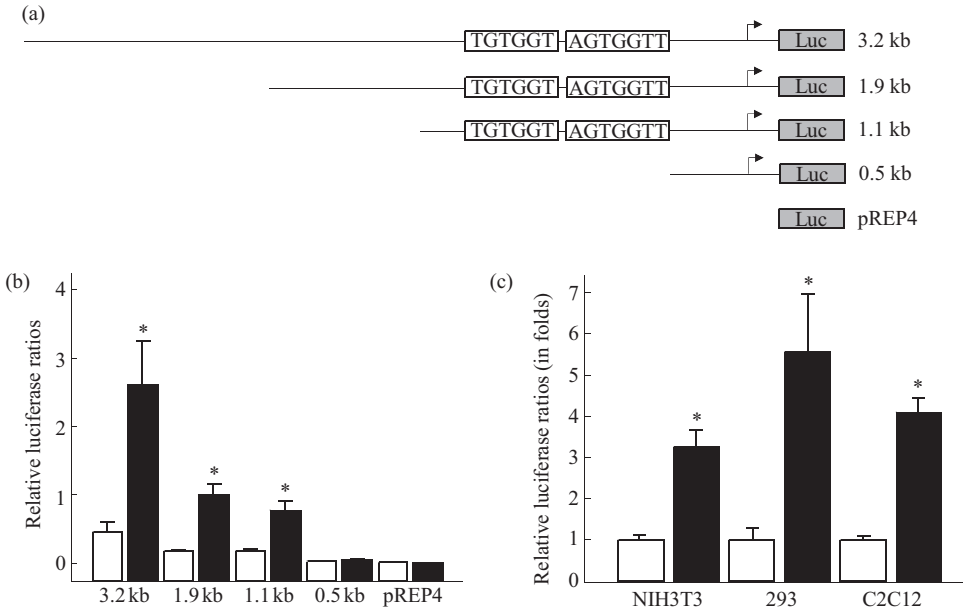

Fig. 3 Up-regulation of the human Osterix promoter activity by Runx2

(a) Schematic diagram representing the constructs of the serial 5-truncated promoters-firefly luciferase reporter. (b) 293 cells were cotransfected with these constructs and the empty vector pCMV5 or the Runx2 expression vector pCMV5-Osf2/Cbfa1. (c) In addition to 293, C2C12 and NIH3T3 were also transfected with the 3.2 kb construct and with pCMV5 or pCMV5-Osf2/Cbfa1. Arrows show the transcription start site[14]. Luc stands for the firefly luciferase gene. Values are firefly luciferase to *Renilla* luciferase ratios (b) or ratios in folds (c). □: Basal; ■: Runx2.

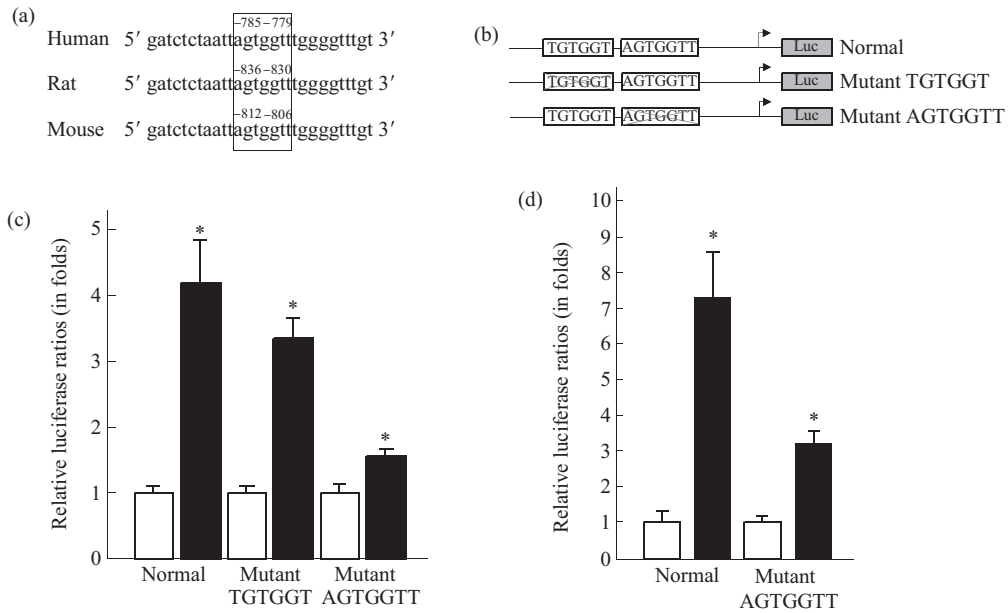

Fig. 4 Identification of the functional Runx2 *cis*-element

(a) Blast analysis of the 3.2 kb 5′-flanking region against mouse and rat genomes. Numbers indicate the relative distance of the conserved element from the translation start codon of “MASSLL”. (b) Schematic diagram that represents the normal and the mutant 1.1 kb constructs. Arrows show the transcription start site[14]. (c) 293 cells were cotransfected with these 1.1 kb constructs and the empty vector pCMV5 or the Runx2 expression vector pCMV5-Osf2/Cbfa1. (d) The identified functional Runx2 binding site in 293 “AGTGGTT” was further confirmed in MC3T3-E1 cells. Luc stands for the firefly luciferase gene. Values are firefly luciferase to *Renilla* luciferase ratios in folds. □: Basal; ■: Runx2.

or AGTGGTT to AGAATTC. Underlined is an *Eco*R Ⅰ restriction site. See Figure 4b). Only the mutation in "AGTGGTT" dramatically decreased the efficacy of Runx2 in up-regulating the Osterix promoter activity (Figure 4c). These results indicate that "AGTGGTT" is a functional Runx2 binding site involved in the up-regulation of the Osterix promoter activity by Runx2. In order to further confirm that the mutation in "AGTGGTT" affects the ability of Runx2 in up-regulating the Osterix promoter activity, we transfected MC3T3-E1 osteoblastic cells with the normal or the mutant 1.1 kb promoter and found that the mutation led to about 4 folds decrease of the efficiency of Runx2 in up-regulating the Osterix promoter activity (Figure 4d). In summary, the results demonstrate "AGTGGTT" is a conserved and functional binding site for Runx2 within the Osterix promoter.

2.5 Osterix up-regulates the type Ⅰ collagen promoter activity, whereas Runx2 does not

As 90% of the bone matrix protein is composed of type Ⅰ collagen, and in the bone research field, the popularly used 2.3 kb promoter of type Ⅰ collagen contains all necessary *cis*-elements that enable it to direct tissue specific gene expression in bone[7, 15], we next used this promoter to further characterize the relationship between Runx2 and Osterix in the regulation of bone related genes. The cotransfection experiments indicate that Osterix up-regulated the type Ⅰ collagen promoter activity in 293 and C3H10T1/2, however, Runx2 had no obvious stimulating effect on the promoter in the two cell lines. In 293, the up-regulation of the type Ⅰ collagen promoter activity by the over-expressed Osterix is more than 2 times compared with the empty vector (Figure 5a), and more than 4 times in C3H10T1/2 (Figure 5b). Nevertheless, the cotransfection with Runx2 and Osterix didn't result in a synergistic effect on the up-regulation of the promoter activity, on the contrary, it seemed that Runx2 interfered with the function of Osterix in stimulating type Ⅰ collagen promoter.

Because Runx2 induced Osterix gene expression but failed to up-regulate the type Ⅰ collagen promoter activity, whereas Osterix did stimulate the type Ⅰ collagen promoter, we supposed that the Osterix protein translated from the induced Osterix mRNA by Runx2 probably remained at an extremely low level. In fact, we failed to detect any Osterix immunofluorescent signal in the nucleus of Runx2 over-expressing 293 cells 42 h after transfection (Figure 5c). And the Western blot in 293 or C3H10T1/2 still didn't detect Osterix protein when transfected with Runx2 expression vector (Figure 5d).

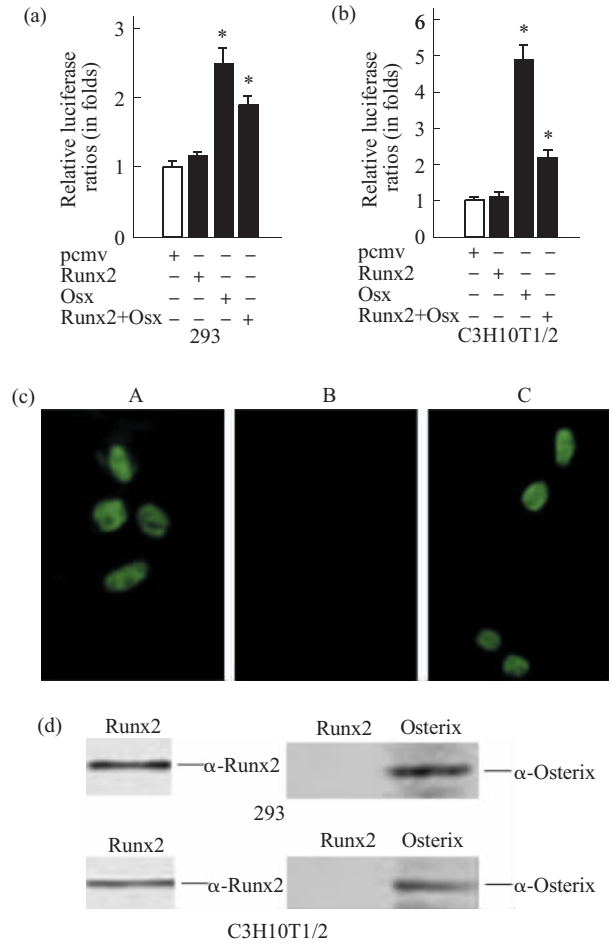

Fig. 5 Osterix, but not Runx2, up-regulated the type Ⅰ collagen promoter activity

Osterix up-regulated type Ⅰ collagen promoter activity in 293 (a) and in C3H10T1/2 (b), whereas Runx2 barely affected the promoter activity in 293 (a) and in C3H10T1/2 (b). In both two types of cells the cotransfection of Runx2 and Osterix upregulated the lower promoter activity than Osterix alone (a and b). (c) Immunofluorescence examination in 293 cells shows Runx2 was unable to induce detectable Osterix protein 42 h after transfection. A and B transfected with the Runx2 expression vector and detected by Runx2 antibody A and Osterix antibody B. C transfected with the Osterix expression vector and detected by Osterix antibody. (d) Western blot analysis in 293 and C3H10T1/2 cells. Both cell lines were transfected with Runx2 or Osterix expression vector and nuclear extracts were prepared and immuno-blotted. The results confirmed that Runx2 failed to induce detectable Osterix protein.

3 Discussion

We cloned the human Osterix promoter and functionally identified a highly conserved *cis*-element

by which Runx2 up-regulated the promoter activity. We also found that Runx2 induced the Osterix gene expression in various cells. And it was previously reported that Osterix is not expressed in mice without Runx2[8]. All these results support the hypothesis that Osterix is a target of Runx2 and can be positively regulated by Runx2. The cells used in this study range from non-osteoblastic cells, including mesenchymal stem cells, to osteolastic cells and even to osteocytic cells. It is thus possible that the induction is in a cell type independent manner.

Since Runx2 is predominantly expressed during chondrocyte hypertrophy and osteoblast differentiation[16～18], the biological significance of the observed regulation of Osterix by Runx2 in this study seems to be restricted in the pathway of osteoblast differentiation only, because Osterix is not detected in the hypertrophic zone of the growth plate cartilage where Runx2 is highly expressed[8]. Our results provide the preliminary evidence for the possible bone specific regulation of Osterix, especially during the early stage of osteoblast differentiation, since its regulator, Runx2, is so far the earliest osteoblast specific transcription factor and can commit the early stage osteoblast differentiation of mesenchymal stem cells or other non-osteoblastic cells[18].

Runx2 is essential for many bone related genes, such as type Ⅰ collagen, osteopontin and osteocalcin. However, the expression of many of the bone related genes still require Osterix, regardless of the presence of Runx2 [8,18]. It is thus difficult to accurately characterize the roles of the two transcription factors in regulating bone related genes. Though Runx2 and Osterix are both positive regulators of the osteoblast-specific expression of type Ⅰ collagen[8, 18], in this study, however, only Osterix can up-regulate the type Ⅰ collagen promoter activity. Therefore, it is possible that in terms of stimulating some bone related genes, such as type Ⅰ collagen, Osterix rather than Runx2 is needed. This *in vitro* result further suggests that: 1) Runx2 may unfortunately contribute partially but still weakly in the Osterix gene expression, and/or 2) there may be a special posttranscriptional mechanism governing the final Osterix production *in vivo*, which is essential for the Osterix dependent osteoblast differentiation and function, and such mechanism is absent in *in vitro* system. Finally, to stimulate the type Ⅰ collagen gene expression, enough high amount of Osterix is necessarily generated, probably through multiple Runx2 dependent and independent pathways. In fact, for example, BMP2 regulates Osterix through Dlx5 rather than Runx2[19].

There are different isoforms of Runx2, including the type Ⅰ-, type Ⅱ- and type Ⅲ isoform. Both *in vitro* and *in vivo* studies have demonstrated that these Runx2 isoforms exert different functions in regulating Runx2 target genes, such as the bone matrix genes, in osteoblast differentiation[20, 21]. In the current study, we showed that type Ⅲ Runx2 can regulate Osterix gene expression. In the other studies, others also have noticed that type Ⅱ Runx2 can regulate Osterix[19, 22]. Further, Xiao *et al*.[21] have found that type Ⅰ Runx2 is also involved in maintaining 50% Osterix gene expression in the type Ⅱ Runx2 deficient mouse. These results indicate that all the three Runx2 isoforms are involved in regulating Osterix gene. According to the results from this study and the other studies, we further noticed that transient but not stable overexpression of Runx2 is able to induce Osterix gene expression [19, 22, and this study]. The reason for this difference is unclear and needs future study. It seems that continuous overexpression of Runx2 inhibits Osterix gene expression. This suggests that Runx2 may transiently induce Osterix gene expression at an early stage of osteoblast differentiation.

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Runx2 参与调控 Osterix 启动子活性及其基因表达 *

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摘要 尽管 Runx2 和 Osterix 都是成骨细胞分化途径中关键的转录因子，但是 Runx2 是否能够调控 Osterix，还不为所知. 研究发现，在非成骨细胞系，无论是间充质干细胞还是已分化的细胞，以及成骨细胞系中，Runx2 都能诱导 Osterix 的表达. 同时 Runx2 能够上调 3.2 kb 人的 Osterix 基因启动子活性. 进一步实验证明，在这一段启动子中存在 Runx2 功能性的结合位点. 因而，实验结果有力地支持了这样一个假设，即 Runx2 参与了 Osterix 基因的表达调控. 瞬时转染和荧光素酶双报告分析结果显示，在非成骨细胞中，Osterix 明显上调 2.3 kb 的Ⅰ型胶原蛋白启动子活性，但 Runx2 却不能. 这样的差别暗示，在成骨细胞分化过程中位于 Runx2 下游的转录因子 Osterix 是刺激Ⅰ型胶原蛋白基因表达所必需的.

关键词 Runx2，Osterix，基因表达，调控
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