**Derepression of *Mmp* gene transcription and an emphysema-like phenotype in *Zbtb7c* knockout mouse lungs**

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(Running title: Zbtb7c degradation causes emphysema-like phenotype)

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**Abstract**

Dysregulated *MMP* expression is a major cause of the degradation of lung tissue that is integral to emphysema pathogenesis. Cigarette smoking (CS) increases *MMP* gene expression, a major contributor to emphysema development. We previously reported that Zbtb7c is a transcriptional repressor of several *Mmp* genes (*Mmps-*8, -10, -13, and -16). Here, we show that *Zbtb7c* knockout mice have mild emphysema-like phenotypes, including alveolar wall destruction, enlarged alveoli, and upregulated *Mmp* genes. Alveolar size and *Mmp* gene expression, in *Zbtb7c-/-* mouse lungs, were increased more severely upon exposure to CS, compared to those of *Zbtb7c+/+* mouse lungs. These observations suggest that Zbtb7c degradation or absence may contribute to the pathogenesis of emphysema.

**Keywords:**

Emphysema, MMP (matrix metalloproteinases), Zbtb7c (Kr-pok), Cigarette smoking

**Abbrevation:**

MMPs, matrix metalloproteinases; CS, cigarette smoking; IHC, histology and immunohistochemistry; qChIP, quantitative chromatin immunoprecipitation

**Introduction**

Emphysema is the most prevalent member of chronic obstructive pulmonary disease (COPD), the third-leading cause of worldwide death, causing over 3.1 million deaths annually in the U.S.A [1-4]. Emphysema is a long-term, progressive disease in which lung alveolar walls are destroyed by elevated proteolytic activity, oxidative stress, cellular senescence (without adequate cell replacement), autoimmune responses, and apoptosis of epithelial cells, endothelial cells, and macrophages of the alveolar spaces  [1-4]. Emphysema is most often caused by cigarette smoking, genetic factors, inflammation, and air pollution, which can influence the proteinase-antiproteinase balance [5-10]. Some matrix metalloproteinases (MMPs) are also implicated in the protease and antiprotease balance, important for lung tissue homeostasis (and disturbance of which can lead to emphysema), and can degrade the extracellular matrix [11,12]. Consequently, overexpression of MMPs, or underexpression of TIMPs (tissue inhibitors of metalloproteinases), can lead to emphysema [1,4,7,8,13-16]. Thus, the expression of *MMP* and *TIMP* genes is tightly regulated, and shows cell-and tissue-specific expression patterns. Despite their differential expression, a subset of *MMP* genes share a similar *cis*-acting AP-1-binding promoter element [17,18], and inflammatory cytokines and CS alter the molecular events at this element by replacing transcriptionally repressive c-Jun/NCoR complexes with transcriptionally activating p-c-Jun/p300 coactivator complexes  [19-22].

To investigate whether specific genetic variants identified in human studies actually increase emphysema risk, significant efforts have been made to find naturally occurring spontaneous or transgenic mouse emphysema models [23-28]. For example, a genetically deficient alpha 1-antitrypsin (A1AT) mouse develops emphysema from increased elastase activity [26-28]. However, only about 10~15% of all emphysema patients actually have A1AT deficiency [1,6]. Also, while *Klotho* and *Fgf23* (fibroblast growth factor 23) knockout mice similarly develop spontaneous emphysema, the roles of these genes in the human lung are not well established [1].

Recently, we found that ZBTB7c (KR-POK) is a proto-oncoprotein that stimulates cell proliferation  [29,30]. In the current study, we show that upon aging or tobacco exposure *Zbtb7c-/-* mice exhibit an emphysema-like lung phenotype, with enlarged alveolar structure and upregulation of *Mmp* genes. Our further investigation of Zbtb7c shows its critical role in the maintenance of lung alveolar structure, acting by repression of *Mmp* genes.

**Materials and Methods**

**Cell cultures and animals**

MRC5 human lung fibroblast cells were cultured in media recommended by ATCC (Manassas, VA, USA). *Zbtb7c+⁄+* and *Zbtb7c-⁄-* mouse embryonic fibroblasts were prepared as reported elsewhere [29]. *Zbtb7c+/+* and *Zbtb7c-/-* mice were kept in a laminar airflow cabinet maintained at 24 ± 2°C, with 40-70% humidity, under a 12-h light/dark cycle, and specific pathogen-free conditions. For smoke-induced lung response tests, 8 week-old mice were exposed to the smoke of 10 filtered commercial cigarettes, each containing 8.5 mg tar and 0.9 mg nicotine (Eighty Eight Lights, KT&G, Korea) per day for 5 days per week, for a period of 4 months [5,21]. Mice were exposed to CS, using a smoking apparatus with the chamber adapted (50 x 40 x 30 cm) for a group of mice. The lung tissues were then embedded in paraffin, and tissue sections stained by H&E. Airspace enlargement was quantified using ten randomly selected fields of tissue sections. All animal experimental devices were approved by the Association for Assessment and Accreditation of Laboratory Animal Care, and all animal experiments were conducted under the institutional guidelines of the Panel on Laboratory Animal Care of Asan Medical Center (Department of Pulmonary and Critical Care Medicine, and Clinical Research Center for Chronic Obstructive Airway Diseases).

**Antibodies and reagents**

The following antibodies were used: GAPDH (FL-335, sc-25778), p-c-Jun (Ser 63/73, sc-16312), from Santa Cruz Biotechnology (Santa Cruz, CA, USA); Mmp-8 (ab81286), -9 (ab38898), -10 (ab59437), -12 (ab52897), and -16 (ab73877), from Abcam (Cambridge, MA, USA); Mmp-13 (Mab13424; lv1583450), from Millipore (Billerica, MA, USA); TNF (#3707) and p-Jnk (#9251), from Cell Signaling Technology (Danvers, MA, USA). To obtain a rabbit polyclonal antibody against Zbtb7c, a white rabbit was immunized by subcutaneous injection with recombinant polypeptide GST-POZ (a.a. 1-120) eight times, at 2-week intervals. Blood was collected, incubated at 37°C for 90 min, and centrifuged. The supernatant was incubated with Affi-Gel 10 beads cross-linked to a recombinant Zbtb7c POZ domain (Bio-Rad, Hercules, CA, USA.). The precipitated beads were washed with PBS, and the antibody was eluted (1.0 M Tris pH 7.6). Most of the chemical reagents were purchased from Sigma (St. Louis, MO, USA).

**Histology and Immunohistochemistry (IHC)**

Lung tissues were fixed in 4% paraformaldehyde and embedded in paraffin. 4-µm tissue slices were stained with hematoxylin and eosin (H&E) reagent. Diaminobenzidine (DAB) staining of human lung tissues was performed according to the manufacturer’s instructions (R.T.U. VECTASTAIN® ABC Kit, Vector Laboratories, Burlingame, CA, USA), after incubating tissue slices with anti-ZBTB7c or anti-CD16-2/FCGR4 antibodies. For immunofluorescence staining of mouse lung tissues, after antigen retrieval, tissue slices were blocked in 5% BSA, and incubated with antibodies against Zbtb7c or Mmps-8, -10, -13, and -16. The slices were further incubated with IgG-conjugated to Alexa Fluor 488 (FITC), and counterstained with DAPI. Microscopic images were obtained using a confocal microscope (LSM 510 META, Carl Zeiss AG, Oberkochen, Germany). The histology data were confirmed by pathologist.

**Quantitative real-time RT-PCR (qRT-PCR)**

Total RNA was isolated from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was prepared using total RNA, random hexamers, and Superscript reverse transcriptase II (Promega, Madison, WI, USA). qRT-PCR was conducted in an ABI PRISM 7300 RT-PCR System using a SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and gene-specific primers (see Supplementary Table). GAPDH mRNA was used as a normalization control.

**Western blot analysis**

Cells were washed, pelleted, and resuspended in RIPA buffer supplemented with protease inhibitors. Total cellular protein was quantified by Bradford assay, separated by 12% SDS-PAGE gel electrophoresis, transferred to Immun-Blot™ PVDF membranes (Bio-Rad, Hercules, CA, USA), and blocked with 5% skim milk (BD Biosciences, Franklin Lakes, NJ, USA) or BSA. Blotted membranes were then incubated with primary antibodies overnight at 4°C, followed by incubation with secondary antibodies conjugated to HRP (Thermo Scientific, Rockford, IL, USA) at RT for 2 h. Protein bands were visualized by ECL solution (Thermo Scientific). GAPDH protein was used as a normalization control.

**Quantitative chromatin immunoprecipitation (qChIP)**

DNA-protein interactions with Zbtb7c and c-Jun, within endogenous *Mmp* gene promoters, were analyzed by antibody pull-down and standard ChIP assay protocols, as reported elsewhere [31], with quantification carried out by amplification of the ChIP’ed DNA in an ABI PRISM 7300 RT-PCR System, using a SYBR Green PCR Master Mix, and gene-specific primers (Supplementary Table). IgG was used as negative control.

**Statistical analysis**

Student’s t-test was used for all statistical comparisons.

**Results**

**Zbtb7c-/- mice show enlarged alveolar structure and Zbtb7c represses transcription of Mmp genes**

To investigate the function(s) of Zbtb7c *in vivo*, we prepared *Zbtb7c-/-* mice by homologous recombination, with lung tissue histology revealing phenotypes of enlarged alveoli of old mice (8 months). Tissue histology of aged *Zbtb7c-/-* mouse lungs showed emphysema-like phenotypes with enlarged alveoli (Fig. 1A; Supplementary Fig. S1). Additionally, the MLI (mean linear intercept) was increased in lung tissues of 8 month-old *Zbtb7c-/-* mice. Mean airspace enlargement indices (%) of *Zbtb7c-/-* mice lungs increased by 2.22-fold, compared to those of *Zbtb7c+/+* mouse lungs (Fig. 1B). Because alveolar development is mainly a postnatal process and the lungs of young mice (2 months) showed no enlarged alveolar structure, the phenotype observed in 8-month old *Zbtb7c-/-* mice was not likely caused by developmental defects.

Dysregulated *MMP* expression is a major cause of the degradation of lung tissue that is integral to emphysema pathogenesis [1,7,13-16]. Because we previously reported that Zbtb7c represses transcription of *Mmp*s-8, -10, -13, and -16 [31], we investigated Mmp expression in lung tissues of *Zbtb7c+/+* and *Zbtb7c-/-* mice. Immunohistochemical staining of mouse lung tissue showed derepressed Mmp (-8, -10, -13, and -16) protein expression in *Zbtb7c-/-* mice (Fig. 1C). These data suggest that *Mmp* upregulation, in association with *Zbtb7c* gene knockout, might contribute to emphysema-like phenotypes.

**Zbtb7c knockout mice exposed to cigarette smoking have enlarged alveoli, a severe emphysema-like phenotype**

CS exposure is a major component of emphysema development though increasing *MMP* genes expression [21]. We investigated whether CS exposure affects alveolar destruction in *Zbtb7c+/+* and *Zbtb7c-/-* mouse lungs. 8 week-old mice were exposed to the smoke for a period of 4 months. Alveolar size of *Zbtb7c-/-* mouse lungs was larger than those of *Zbtb7c+/+* mice, and *Zbtb7c-/-* mouse lung alveoli were disrupted more severely upon exposure to CS (alveolar destruction index, airspace enlargement area: a 2-fold increase in *Zbtb7c+/+* mice vs a 4.3-fold increase in *Zbtb7c-/-* mice exposed to CS) (Fig. 2A; Supplementary Fig. S2).

We next examined the levels of p-Jnk, Zbtb7c, p-c-Jun, and Mmp expression in *Zbtb7c+/+* and *Zbtb7c-/-* mouse lung tissues exposed to CS. In *Zbtb7c+/+* lungs, TNF, p-Jnk, p-c-Jun, and Mmp expression were all upregulated, while Zbtb7c expression was downregulated by CS. In the absence of CS, Mmpexpression in *Zbtb7c-/-* mouse lung tissues remained relatively high, as compared to those of *Zbtb7c+/+* mouse lung tissues. TNF and p-Jnk levels in *Zbtb7c-/-* mouse lung tissues were similar to those in *Zbtb7c+/+* mouse lungs; however, p-c-Jun and Mmp expression levels were higher in *Zbtb7c-/-* mouse lung tissues, following CS exposure (Fig. 2B). Immunohistochemistry also showed higher expression of Mmp-13 protein in lung tissues of *Zbtb7c-/-* mice, compared to *Zbtb7c+/+* mice (Fig. 2C). Abnormal alveolar structure such as observed in 6-month old *Zbtb7c-/-* mice recapitulated the progressive alveolar destruction that is severely augmented by CS, resembling an emphysema-like phenotype [32]. *Mmp* genes were correspondingly upregulated, as seen in smokers with emphysema [32]. Our recent study showed that Zbtb7c is phosphorylated by p-Jnk (induced by TNF) and it (Zbtb7c) is subsequently degraded by the ubiquitin-mediated proteasomal pathway [31]. Consequently, these *in vivo* mouse data demonstrate that age- or CS-induced *Zbtb7c* degradation leads to loss of repression (and thus upregulation) of multiple *Mmp* genes, concomitantly leading to alveolar wall disruption and an emphysema-like phenotype.

**Differential expression of emphysema-related genes in Zbtb7c+/+ and Zbtb7c-/- MEF cells, and immunohistochemical analysis of macrophages in Zbtb7c+/+ and Zbtb7c-/- mouse lung tissues exposed to cigarette smoking**

Elastin is a protein that provides elasticity to allow the lung to resume its shape after stretching or contracting, and proteolytic degradation of elastin has been considered a primary cause of emphysema pathogenesis [33-35]. Accordingly, MMP-9, which degrades elastin and gelatin, is overexpressed in the lungs of smokers with emphysema [7,36]. Moreover, in animal emphysema models, increased Mmp-9 expression is accompanied by increased expression and activity of Mmp-12, a major metalloelastase found in human lung parenchyma [37]. Suggestive of a role in lung disease, mouse *Mmp-12* knockout was protective against CS-induced emphysema [7,38]. Thus, both Mmps-9 and -12, induced by inflammatory cytokines, may play important roles in the degradation of extracellular matrix proteins, thus leading to emphysema [33-35,37-39]. We therefore investigated whether Zbtb7c regulates expression of Mmp-9 and -12, by comparison of *Zbtb7c+/+* vs. *Zbtb7c-/-* MEF cells. TNFα, an inflammatory cytokine released by lung immune cells, increased Mmps-9 and -12 mRNA and protein levels, although such induced levels were unaffected by the *Zbtb7c* knockout (Fig. 3A and B). The expression of p-Jnk was upregulated by TNFα, while *Zbtb7c* knockout did not affect p-Jnk expression. In *Zbtb7c-/-* MEF cells, p-Jnk levels were similar to those in *Zbtb7c+/+* MEF cells; however, p-c-Jun expression levels were higher in *Zbtb7c-/-* MEF cells, following TNFα as we reported (Fig. 3B) [31]. Mechanistically, ChIP assays showed that Zbtb7c alone bound to neither the *Mmp-9* nor *-12* promoters, regardless of TNF treatment, nor did it regulate the transcription of either gene (Fig. 3C). Although Mmps-9 and -12 may be important for emphysema pathogenesis, as reported previously [7], our data indicates the potential importance of other *Mmp* genes in progression to an emphysema-like phenotype, in inverse correlation with depletion of *Zbtb7c*.

It was also reported that inflammation due to chronic exposure to cigarette smoke may lead to macrophage infiltration of alveoli, resulting in the release of the elastolytic proteinases that cause emphysema [1,4,13,16,40]. Accordingly, we by histochemical analysis we investigated macrophage infiltration in lung tissues of *Zbtb7c+/+* and *Zbtb7c-/-* mice exposed to CS, using a macrophage-specific antibody against CD16-2/FCGR4. The assay showed that the percentage of lung cells with macrophage infiltration increased in mice exposed to CS for 4 months, compared to 2 months. However, the percentage of cells with macrophage infiltration was similar in both *Zbtb7c+/+* and *Zbtb7c-/-* mice exposed to CS (Fig. 3D; Supplementary Fig. S3). Accordingly, the emphysema-like lung phenotype of *Zbtb7c-/-* mice is likely caused by upregulated expression of Mmps-8, -10, -13, and -16 in lung cells rather than Mmps secreted by infiltrating macrophages.

**ZBTB7c regulates transcription of MMP genes in human lung cells, and is decreased in human emphysema patients**

To evaluate the relevance of all these findings to human emphysema, we investigated whether ZBTB7c affects *MMP* gene expression in human fetal lung MRC5 fibroblasts. Ectopic *ZBTB7c* expression by infection with recombinant adenovirus (dl324-ZBTB7c) repressed transcription of *MMP* genes. Alternatively, *ZBTB7c* knockdown by adenovirus (dl324-shZBTB7c), resulted in increased *MMP* -8, -10, -13, and -16 mRNA and protein levels (Fig. 4A and B). We also examined a possible correlation between ZBTB7c expression and alveolar destruction in normal (n=3) and emphysema patients’ tissues (n=5). Immunohistochemistry analysis of nuclear ZBTB7c expression in human lung tissues showed drastically reduced ZBTB7c expression in emphysema patients (3% cell positivity) vs. normal controls (37% cell positivity) (Fig. 4C). Although limited numbers of emphysema tissues were analyzed, due to the difficulty of acquiring human lung tissue samples, this analysis strongly suggests that ZBTB7c expression in the lung is important not only for normal lung homeostasis (by maintaining a balance of proteases and antiproteases), but also that ZBTB7c downregulation correlates with an emphysema-like phenotype. Interestingly, previous studies showed that the expression levels of *MMPs* -8, -10, and -13 were increased in emphysema patients.

**Discussion**

In this study, we showed that *Zbtb7c* knockout mice (in particular, those exposed to CS) exhibit an emphysema-like phenotype in their lung tissue structure, correlated with overexpression of matrixmetalloproteinases (Mmps). Analogously, our immunohistochemistry analysis showed that ZBTB7c expression was hardly detectable in human emphysema alveoli (Fig. 4C). These data suggest that Zbtb7c expression in the lung is important not only for normal lung homeostasis, but also that long-term cigarette smoking and consequent downregulation of Zbtb7c expression may be an important factor in emphysema-like phenotypes.

We also showed that expression of MMPs-8, -10, -13, and -16 were elevated by *ZBTB7c* knockdown in human fetal lung MRC5 fibroblasts (Fig. 4B), while ectopic ZBTB7c potently repressed those MMPs. Likewise, we also investigated the expression of ZBTB7c in tissue from human emphysema patients by immunohistochemistry, showing no detectable protein. Although we could not perform immunohistochemistry of MMPs, several published reports indicate elevated expression of these MMPs in lungs of emphysema patients [41-43]. Also in accord with our findings, mouse MMP10 was recently shown to play a pathophysiological role in the development of cigarette smoke-induced lung disease, in *Mmp10* KO mice [44].

Elastin is a protein that provides elasticity to allow the lung to resume its shape after stretching or contracting, and proteolytic degradation of elastin has been considered a primary cause of emphysema pathogenesis [33-35]. Although alveolar wall destruction by elastin proteolysis has been well studied, several reports have also emphasized a role for collagenase-mediated collagen breakdown in emphysema [45-48]. MMP-8 and MMP-13 are both collagenases, whose substrates are collagens-I, -II, and -III, and both were previously shown to be upregulated in mice by long-term CS exposure, and in the lungs of COPD patients  [13,15,43,49-51]. Similarly, our study of *Zbtb7c* knockout mice showed good correlation between upregulated collagenases (Mmp-8 and Mmp-13) and the emphysema-like phenotype (Fig. 1 and 2).

Our recent study revealed that Zbtb7c is critical for the formation of a repressor complex composed of Zbtb7c, c-Jun, NCoR, and HDAC3 on *Mmp* gene promoters, and that this repressor complex is not established without Zbtb7c. The molecular interaction between c-Jun and Zbtb7c also prevents phosphorylation of c-Jun by p-Jnk, However, Zbtb7c phosphorylation by p-Jnk (induced by TNF), and its (Zbtb7c) subsequent degradation by the ubiquitin-mediated proteasomal pathway, leads to c-Jun phosphorylation by p-Jnk. Phosphorylated c-Jun activates *Mmp* genes expression by recruiting coactivators like p300 [31]. The absence or degradation of Zbtb7c may be responsible for derepression of *Mmp* genes and thus for the resulting emphysema-like phenotype caused by inflammatory cytokines or smoking. Hence, absent or decreased Zbtb7c expression results in a cascade of molecular events, including a coregulator switch, expression of *Mmp* genes, and destruction of alveolar walls.

Our previous [31]and current study, using *Zbtb7c* knockout mice, revealed several molecular targets for possible intervention in the pathogenic processes leading to emphysema-like phenotypes, *e.g.*, blocking phosphorylation of Zbtb7c by p-Jnk, blocking p-c-Jun recruitment of coactivator complexes, *etc*.

**Acknowledgements**

This work was supported by JeonRack-HooSok Grant 2016R1E1A1A02921938 (to M.-W. H), Do-Yak Research Grant 2011-0028817 (to M.-W.H.), and MRC Research Grant 2011-0030086 (to M.-W.H.) from the National Research Foundation of Korea (NRF) of the Korean Government (MSIP).

**Author Contributions**

B.-N.J., J.-Y.S. and M.-W.H. designed and performed experiments, analyzed data and wrote the manuscript. J.W.H. provided key material and established the CS-induced emphysema models in mice. W.-I.Y. analyzed and confirmed histology data.

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**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**1. Supplementary figures and legends**

**Fig. S1.** Old Zbtb7c knockout mice show enlarged alveolar structure.

**Fig. S2.** Cigarette smoke causes a severe emphysema-like phenotype in 6 month-old Zbtb7c-/- mice exposed to CS for 4 months.

**Fig. S3.** Immunohistochemical analysis of macrophages in Zbtb7c+/+ and Zbtb7c-/- mouse lung tissues exposed to cigarette smoke.

**2. Supplementary table of oligonucleotide sequence of qPCR primers**

**Figure Legends**

**Fig. 1.** Aged *Zbtb7c* knockout mice show enlarged alveolar structure. Zbtb7c represses transcription of *Mmp* genes. (A) H&E staining of lung tissues of *Zbtb7c+/+* and *Zbtb7c-/-* mice at 2, 4, and 8 months of age. (B) Top histogram: MLI (mean linear intercept, a microscopic measure of inter-alveolar wall distance) of lungs from *Zbtb7c+/+* and *Zbtb7c-/-* mice. Bottom histogram: quantitative analysis of alveolar airspace enlargement (%). Average airspace area, i.e., total airspace area divided by the total number of alveoli in three randomly selected microscopic fields of each tissue slide. \*P<0.05; n.s., not significant. (C) Immunofluorescence analysis (excitation wavelength, 488 nm) of Zbtb7c and Mmps-8, -10, -13, -16 expression in paraffin-embedded formalin-fixed lung tissues of *Zbtb7c+/+* and *Zbtb7c-/-* mice (magnification x400).

**Fig. 2.** Cigarette smoke decreases Zbtb7c expression and causes a severe emphysema-like phenotype in *Zbtb7c-/-* mice. (A) H&E staining of lung tissues of mice exposed to CS for 4 months starting at age 8 weeks, as described in methods. The histogram shown at the right represents quantitative analysis of alveolar airspace enlargement. Average airspace area was determined as the total airspace area divided by the total number of alveoli in three randomly selected microscopic fields in each section (magnification x400). \*P<0.05. (B) Western blot analysis of the expression of TNF, p-Jnk, Zbtb7c, c-Jun, and Mmp proteins in lung tissues of 6-month old *Zbtb7c+/+* and *Zbtb7c-/-* mice exposed to CS for 4 months in smoking chambers. (C) Immunofluorescence analysis of Zbtb7c and Mmp-13 expression in lung tissues of 6-month old *Zbtb7c+/+* and *Zbtb7c-/-* mice that were treated as in A.

**Fig. 3.** Differential expression of emphysema-related genes in *Zbtb7c+/+* and *Zbtb7c-/-* MEF cells, and immunohistochemical analysis of macrophages in *Zbtb7c+/+* and *Zbtb7c-/-* mouse lung tissues exposed to cigarette smoking. (A, B) Cells were treated with or without TNF and analyzed for *Zbtb7c*, *Mmp-9* and -*12* mRNA (A) and protein (B) expression levels. (C) ChIP assay of Zbtb7c and c-Jun binding to *Mmp-9* and *-12* gene promoters in *Zbtb7c+⁄+* and *Zbtb7c-/-* MEF cells treated with vehicle or TNFα. Values represent percentages of total DNA input. (D) DAB staining (left) of macrophages in lung tissues of *Zbtb7c+/+* and *Zbtb7c-/-* mice exposed to CS for 2 or 4 months in smoking chambers. Histogram (right) showing the percentage of macrophages (immunoratio analysis of CD16-2/FCGR4-positive cells / total cells) was similar in both *Zbtb7c+/+* and *Zbtb7c-/-* mice after 2- or 4-month CS exposure. \*P<0.05; n.s., not significant.

**Fig. 4.** ZBTB7c represses *MMP* genes in MRC cells, and ZBTB7c is not expressed in the lung tissues of emphysema patients. (A, B) qRT-PCR (A) and western blot (B) analyses of the expression of multiple *Mmp* genes in human lung MRC5 cells infected with the recombinant adenoviruses (20 m.o.i.) dl324 (empty vector), dl-ZBTB7c (overexpression vector) or dl-shZBTB7c (knockdown vector). \*P<0.05. (C) DAB staining of ZBTB7c expression in lung tissues of three normal human (N1 ~ N3) and five emphysema patients (E1 ~ E5). The histogram (lower left) shows the percentages of cells with nuclear ZBTB7c expression. \*P<0.05.