



SP7: from Bone Development to Skeletal Disease

Jialiang S. Wang¹ · Nicha Tokavanich^{1,2} · Marc N. Wein^{1,3,4}

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Abstract

Purpose of Review The purpose of this review is to summarize the different roles of the transcription factor SP7 in regulating bone formation and remodeling, discuss current studies in investigating the causal relationship between SP7 mutations and human skeletal disease, and highlight potential therapeutic treatments that targeting SP7 and the gene networks that it controls.

Recent Findings Cell-type and stage-specific functions of SP7 have been identified during bone formation and remodeling. Normal bone development regulated by SP7 is strongly associated with human bone health. Dysfunction of SP7 results in common or rare skeletal diseases, including osteoporosis and osteogenesis imperfecta with different inheritance patterns. SP7-associated signaling pathways, SP7-dependent target genes, and epigenetic regulations of SP7 serve as new therapeutic targets in the treatment of skeletal disorders.

Summary This review addresses the importance of SP7-regulated bone development in studying bone health and skeletal disease. Recent advances in whole genome and exome sequencing, GWAS, multi-omics, and CRISPR-mediated activation and inhibition have provided the approaches to investigate the gene-regulatory networks controlled by SP7 in bone and the therapeutic targets to treat skeletal disease.

Keywords SP7 · Bone development · Skeletal disease · Therapeutic approaches

Introduction

Bones are formed through either endochondral or intramembranous ossification. During early embryogenesis, migration and condensation of mesenchymal cells initiate skeletal specification. Bone remodeling is a life-long process involving osteocytes (the main orchestrator), bone-forming osteoblasts, and bone-resorbing osteoclasts [1]. As a highly dynamic tissue, both building the bone structure during embryonic development and maintaining bone homeostasis in adulthood require the regulation of transcription factors

(TFs) [2]. TFs are proteins that recognize and bind to specific DNA sequences to regulate gene expression. Their activities are controlled at multiple levels, including epigenetic mechanisms, co-factor association, gene regulatory elements, and environmental cues [3, 4]. Several master TFs are identified and examined with crucial roles in regulating the bone formation, resorption, and remodeling (reviewed in [5, 6], such as SOX9, RUNX2, OSX/SP7, ATF4, NF- κ B, MITF, and NFATc1 [7–13].

Osterix/SP7 (encoded by the *SP7* gene) is a zinc finger-containing transcription factor that contains three C2H2-type zinc fingers as other SP/XKLF family members [14]. The protein sequence of SP7 is highly conserved between humans and mice. The human *SP7* gene is consist of 3 exons and 2 introns and generates 3 alternative splicing transcripts (Type I, II, and III) [15]. The expression of SP7 is detected in chondrocytes, hypertrophic chondrocytes, osteoblasts, and osteocytes [16]. For the past decade, SP7 had been thought as the essential regulator of osteoblast differentiation and bone formation [9]. Recent studies suggest novel roles of SP7 in chondrocyte and osteocyte development and function [17, 18]. In this review, we will first summarize the function

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✉ Jialiang S. Wang
jwang101@mgh.harvard.edu

¹ Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

² Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA, USA

³ Broad Institute of Harvard and MIT, Cambridge, MA, USA

⁴ Harvard Stem Cell Institute, Cambridge, MA, USA

of SP7 in bone formation and remodeling (reviewed in [19]), and then will focus on recent studies on SP7 in human skeletal disease and will discuss the future therapeutic potential of SP7 for bone health.

Role OF SP7 in Different Cell Types During Bone Formation and Remodeling

The expression of Sp7 is identified in several tissues, including bone, tooth, brain, and the reproductive tract [9, 20–22]. In brain, Sp7-positive cells were reported in the mitral and granule cell layers in the olfactory bulb [20]. In bone, Sp7 is expressed at multiple stages throughout the mesenchymal bone lineage including in osteoblasts, osteocytes, pre-hypertrophic and hypertrophic chondrocytes. Since a unique suite of genes is required for the differentiation and function of each of these distinct cell types within the mesenchymal lineage, it is likely that this transcription factor utilizes unique, stage-specific mechanisms to control cell differentiation and function at each stage. Figure 1 summarizes distinct roles of Sp7 in different bone cells as evidenced by conditional deletion of this gene using Cre drivers active at different stages in mesenchymal differentiation.

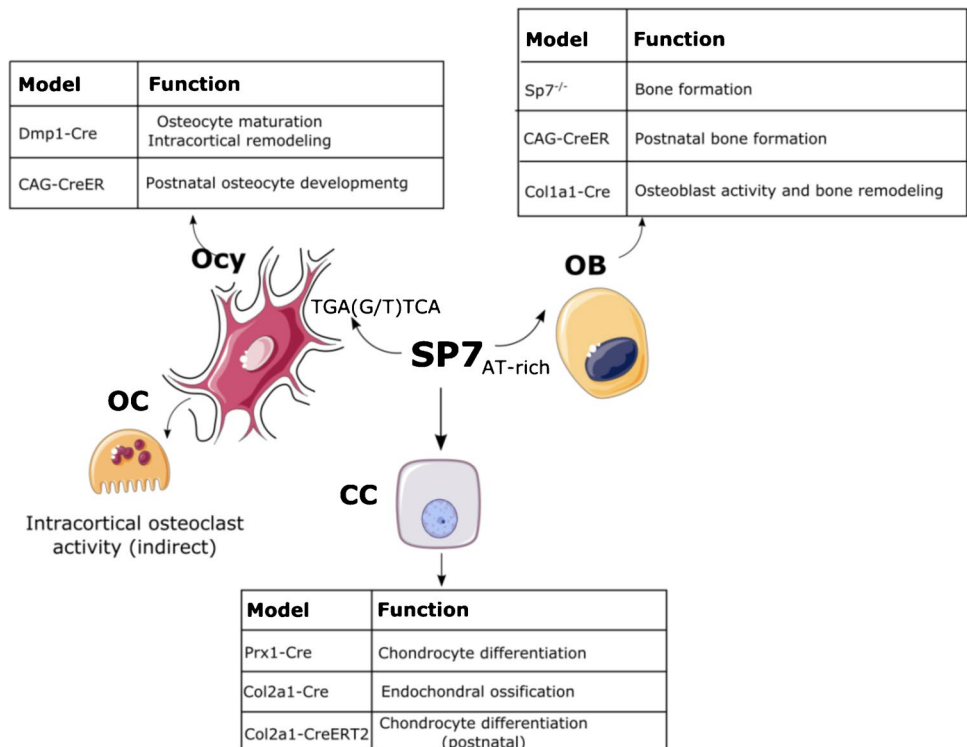
Osteoblast

Runx2 and Sp7 are crucial regulators for osteoblast differentiation. *Runx2-null* mice completely lack ossification, which

suggests that both endochondral and intramembranous ossification are blocked [8]. No bone formation and no bone matrix deposition occur in *Sp7-null* mice [9], which suggests that osteoblast differentiation is blocked during embryogenesis in the absence of this transcription factor. Sp7 acts downstream of Runx2 since Runx2 is expressed in the pre-osteoblasts of *Sp7-null* mice while Sp7 is not expressed in *Runx2-null* mice. When *Sp7* was conditionally deleted at different time points postnatally (*CAG-CreER*), mutant mice showed reduced bone formation, severely altered bone structure, and accumulation of calcified cartilage. This suggests that Sp7 is required for osteoblast differentiation and bone formation in the postnatal skeleton as well [23]. Several studies reported the osteoblast-specific role of Sp7 by applying different Cre/LoxP systems. Mice exhibited osteopenia, including reduced bone formation rate and short bones, when *Sp7* was conditionally deleted in osteoblasts with *Col1a1-Cre* [24], suggesting that Sp7 inactivation in growing bones delays osteoblast maturation. Further studies using *Col1a1-CreERT2* to delete *Sp7* in formed bones [25] showed that inducible Sp7 deletion leads to decreased mineralized surface and bone formation rate. These data support a role for Sp7 in maintaining osteoblast function in adult bone remodeling.

Like other Sp family members, Sp7 was previously reported to be a transcription factor that binds GC-box DNA elements in osteoblasts [26]. However, de novo motif analysis revealed that osteoblast-specific Sp7-bound enhancer regions are significantly enriched in AT-rich motifs [19].

Fig. 1 Schematic of SP7 functions in chondrocytes, osteoblasts, and osteocytes. SP7 regulates osteoblast differentiation and bone formation through osteoblast target genes by recognizing AT-rich region. In osteocytes, SP7 regulates osteocyte maturation and intracortical remodeling through osteocyte target genes by recognizing TGA(G/T)TCA TCA motif. SP7 also regulates chondrocyte differentiation and endochondral ossification. CC, chondrocyte; OB, osteoblast; Ocy, osteocyte; OC, osteoclast. Part of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (<https://creativecommons.org/licenses/by/3.0/>)



Interestingly, Sp7 does not directly bind to AT-rich DNA sequences but rather acts with Dlx factors (that directly bind AT-rich motifs) to regulate the expression of osteoblastic genes. This suggests that Sp7 forms transcriptional complexes with other co-factors to control cell-specific gene expression. Several osteoblast-related genes have Sp7-dependent expression and show direct regulation by Sp7. One group of genes are regulated via direct binding of Sp7 to GC-rich regions, such as *Bsp*, *Sost*, *Colla1*, *Colla2*, *Mmp13*, and *Enpp1* [26–31]. The other group of genes are regulated via the complex formed by Sp7 and other factors, including *Runx2* (Sp7-Dlx5-Msx2), *Notch2* (Sp7-Dlx5), and *Colla1* (Sp7-NFATc1) [32–34].

Osteocyte and Osteoclast

Some osteoblasts become buried within mineralized bone matrix and become osteocytes [35]. We and others have shown that Sp7 plays an important role in osteocyte differentiation and maturation. Sp7 acts upstream and directly regulates the osteocyte-specific gene *Sost*. Both in vivo and in vitro models showed that Sp7 specifically binds and activates the *Sost* promoter [23, 28]. Sp7 is required for postnatal osteocyte development. Global postnatal inactivation of Sp7 (*CAG-CreER*, *Sp7^{fl/fl}*) leads to deformed osteocytes and reduced expression levels of *Dmp1*, *Phex*, and *Sost* [23]. We deleted Sp7 using *Dmp1-Cre*, which targets mature osteoblasts and osteocytes [36]; these mutant mice showed increased cortical porosity, abnormal intracortical bone remodeling, osteocyte dendrite defects, and increased osteocyte apoptosis [18]. Among osteocyte-specific Sp7 target genes, we identified osteocrin (encoded by the *Ostn* gene) as a secreted factor that promotes osteocyte dendrite formation and maintenance. Sp7 ChIP-seq followed by de novo motif binding analysis between osteocytes and osteoblasts revealed that the osteocyte-specific Sp7 binding sites are enriched for the TGA(G/T)TCA motif bound by activator protein 1 (AP-1) family members. Therefore, Sp7 may use distinct binding factors in osteocytes versus osteoblasts. Single-cell RNA sequencing identified discrete populations of cells undergoing the osteoblast-to-osteocyte transition, and dramatic osteocyte differentiation defects in this normal process in the absence of Sp7. In addition to loss-of-function studies, overexpression of Sp7 under the control of the 2.3 kb *Colla1* promoter [37•] led to porous cortical bone, decreased number of osteocyte dendrites, disrupted lacunar-canalicular network and increased *Sost* expression upon skeletal unloading. Taken together, these findings raise the interesting hypothesis that proper expression level of Sp7 is required in osteocyte development.

Integrin $\beta 3$ (encodes by the *Itgb3* gene) associates with αV and is expressed in osteocyte dendrites where it plays an important role in mechano-transduction [38, 39]. *Itgb3-null*

mice have significantly reduced femoral length and decreased cortical thickness [40]. Chromatin immunoprecipitation (ChIP) assays in osteoblastic MC3T3-E1 cells showed that Sp7 binds to the promoter of the *Itgb3* gene (encodes integrin $\beta 3$) [40]. Since both Sp7 and *Itgb3* are expressed in osteocytes and Sp7 plays a critical role in osteocyte dendrite development and maintenance, more studies are needed to explore the specific role of Sp7/*Itgb3* axis in dendrite formation, osteocyte maturation and mechano-sensing.

Sp7 is not expressed in osteoclasts. However, several studies showed that Sp7 may affect osteoclast activity in vivo via indirect mechanisms. Conditional deletion of Sp7 in osteoblasts and osteocytes (*Dmp1-Cre*; *Sp7^{fl/fl}*) does not affect osteoclasts on trabecular bone. *Dmp1-Cre*; *Sp7^{fl/fl}* mice, on the other hand, have increased intracortical osteoclast activity confirmed by the elevated level of TRAP staining [18]. This increased osteoclast activity may be caused by increased Rankl expression triggered by apoptotic osteocytes [41•]. When Sp7 was ablated postnatally by *CAG-CreER*, primary spongiosa osteoclast density is reduced [23] while more osteoclasts are noted in porous cortical bone in *Sp7* mutant (*CAG-CreER*; *Sp7^{fl/fl}*) mice. It is possible that reduced hypertrophic chondrocyte Rankl expression drives osteoclast defects in the primary spongiosa in global/inducible Sp7 mutant mice. Thus, Sp7 likely controls bone resorption indirectly through different cell type-specific mechanisms.

Chondrocyte

There is no cartilage phenotype identified in *Sp7-null* embryos [9]. However, when Sp7 expression was reduced in chondrocyte-like ATDC5 cells, the expression of several chondrocyte genes is down-regulated, including *Sox9*, *Dlx5*, *Alpl*, and *Col10a1* [42]. Chondrocyte-specific deletion of Sp7 (*Col2a1-Cre*; *Sp7^{fl/+}*) in mice results in impaired endochondral bone formation including delayed chondrocyte differentiation, increased hypertrophic chondrocytes, reduced formation of trabecular bone, and reduced skeletal growth [43]. Another study used both *Col2a1-Cre* and *Prx1-Cre* to ablate Sp7 expression [44]. Both models showed arrested endochondral ossification with chondrocytes blocked at the hypertrophic stage. *Col2a1-Cre*; *Sp7^{fl/fl}* mice also show blocked calcification of cartilage matrices and reduced *Mmp13* expression. ChIP assays suggest that Sp7 regulates *Mmp13* during chondrocyte maturation. Conditional deletion of Sp7 during postnatal growth (*Col2a1-CreERT2*; *Sp7^{fl/fl}*) results in impaired secondary ossification by delaying chondrocyte hypertrophy and conversion to osteoblasts [17]. Importantly, more studies are needed to identify the direct targets of Sp7 during chondrocyte differentiation and what co-factors are involved in this process. Ultimately, detailed analysis of how Sp7 regulates key stage-specific factors in

bone lineage cells will identify novel co-factors required for this transcription factor to exert multiple roles throughout the mesenchymal lineage.

Odontoblast

Sp7 is expressed in many cell types during tooth development, including dental papilla, odontoblasts, alveolar bone osteoblasts, and dental follicle cells [45]. Sp7 plays an important role in tooth development including direct control of dentin sialophosphoprotein (Dspp), the key protein for postnatal odontogenesis [45]. Constitutive lineage tracing revealed that Sp7-labeled cells actively contribute to dental development, especially tooth root formation [46]. Sp7 is strongly detected in the crown in early tooth development, but then down-regulated after root formation [47]. Tamoxifen-dependent lineage-tracing further showed that Sp7 is expressed in dental mesenchymal progenitors and contributes to all relevant cell types during dental root morphogenesis. In *Sp7*-null embryos, mineralization was absent in the mandible, maxilla, and craniofacial bones [9, 48]. Though these mice died soon after birth, the examination of perinatal pups showed that both maxillary and mandibular incisors were smaller in *Sp7*^{-/-} mice compared to control littermates [48]. The alveolar bone that surrounds incisors was completely absent in *Sp7*-null mice. The formation of alveolar bone is not absolutely needed for tooth morphogenesis since initial tooth morphogenesis is normal in *Sp7*^{-/-} mice. Sp7 is crucial for the normal architecture and organization of dental tubules in the root. *Sp7*^{-/-} mice showed disorganized odontoblasts and ameloblasts around the pre-dentin-like layers in incisors, suggesting that Sp7 promotes the differentiation and maturation of odontoblasts and ameloblasts. The deletion of Sp7 specifically in odontoblasts (*Col1-Cre; Sp7*^{fl/fl} and *OC-Cre; Sp7*^{fl/fl}) showed short molar root, reduction of dentin, and malformed dentinal tubule of tooth roots [49–51]. Sp7 can also regulate cementum formation by promoting cementoblast differentiation and mineralization [51]. Overexpression of Sp7 (3.6 kb *Colla1-Sp7*) accelerated the formation of cellular cementum, while deletion of Sp7 (*Col1-Cre; Sp7*^{fl/fl} and *CAG-CreER; Sp7*^{fl/fl}) dramatically reduced cementogenesis and mineralization rate. These studies stimulate much more interest in investigating the role and the mechanism of Sp7 in tooth formation and provide potential therapeutics for periodontal regeneration and alveolar bone healing.

SP7 and Human Skeletal Disease

A primary goal of human genetics is to identify DNA sequence variants that influence biomedical traits, particularly those related to the onset and progression of human

disease, such as osteoporosis. Genetic discoveries have substantially improved our understanding of the molecular and genetic mechanisms responsible for many common and rare skeletal diseases and driven the development of novel preventative and therapeutic strategies [52–55]. SP7 plays fundamental roles in different cell types during bone formation and remodeling. Recently, many exciting studies have investigating connections between SP7 and skeletal disease.

Common Skeletal Disease: Osteoporosis

Genome-wide association studies (GWAS) on skeletal polygenic disorders have made great achievements in the past two decades and identified hundreds of susceptibility genes/loci for the pathophysiology of common skeletal disease [56]. Osteoporosis is a common bone disease characterized by low bone mass, deteriorative microarchitecture, loss of bone strength, and increased risk of bone fracture. Bone mineral density (BMD) is used as the diagnosis of osteoporosis and serves as a strong predictor of fracture risk [57].

Several studies demonstrated that *SP7* is a locus associated with BMD at the GWS level (Table 1). GWAS analysis of BMD and related traits were performed in children (age 9) from the Avon Longitudinal Study of Parents and Children (ALSPAC) [58]. 4 polymorphisms (rs2016266, rs4759021, rs6580842, and rs10876432) residing in a linkage disequilibrium block near the *SP7* gene were identified with significant associations to total body BMD. The meta-analysis of two existing studies (a European women population and an Icelandic population) [59, 60] further suggested a strong association between these SNPs and adult lumbar spine BMD. Styrkarsdottir and colleagues performed extended GWAS analysis of BMD among the European descendent subjects and identified one significant locus near the *SP7* gene (rs10876432) [61]. Within the Genetic Factors of Osteoporosis (GEFOS) consortium, a large-scale GWAS meta-analysis was performed on spine and femoral neck BMD in Northern European descent subjects [62]. This confirmed *SP7* (12q13, rs2016266) as a locus significantly associated with BMD. Another genome-wide meta-analysis was performed on the lumbar spine and femoral neck BMD in European and east Asian ancestry individuals [63]. *SP7* (rs7108738) was further proved as a BMD-associated locus which was also associated with fracture risk. Taken together, these findings clearly demonstrate that *SP7* polymorphisms in humans are associated with BMD variation and subsequent fracture susceptibility. At present, the precise mechanisms connecting *SP7* polymorphisms with BMD variation remains under-explored. For example, it is possible that non-coding *SP7* polymorphisms may regulate *SP7* expression levels in bone cells, a possibility that requires exploration in large numbers of clinical bone biopsy samples from “genotyped” individuals and genome editing in human bone cell culture models

Table 1 Genome-wide association studies reported *SP7* as the locus associated with BMD and fracture risk

Studies	Traits	SNPs	Sample number and ancestry	Design
Timpson et al. [58]	BMD (femoral head, total body)	rs2016266, rs4759021, rs6580842, rs10876432	1518 European children	GWAS
Timpson NJ, Richards J, Stykarsdottir U [58–60]	BMD (lumbar spine)	rs2016266, rs4759021, rs6580842, rs10876432	2094 European women; 5861 Icelandic population	Meta-GWAS
Stykarsdottir et al. [61]	BMD (hip, spine)	rs10876432	6865 European descendants	GWAS
Rivadeneira et al. [62]	BMD (lumbar spine, femoral neck)	rs2016266	19,195 Northern European descendants	Meta-GWAS
Estrada et al. [63]	BMD (lumbar spine, femoral neck)	rs7108738	32,961 European and east Asian descendants (elderly)	Meta-GWAS

to explore consequences of non-coding *SP7* polymorphisms on *SP7* mRNA levels.

Rare Skeletal Dysplasia: Osteogenesis Imperfecta

To date, five *SP7* mutations have been reported that cause rare skeletal disease including osteogenesis imperfecta (OI) (Table 2). Although the majority of OI cases are caused by mutations in the *COL1A1* and *COL1A2* genes, a large number of genes that are crucial for osteoblast function, have been identified to cause skeletal fragility and a phenotype similar to collagen-mutated OI [64, 65]. Other clinical signs and symptoms may be found including dentinogenesis imperfecta (DI), blue sclera, hearing loss, growth deficiency, and joint laxity [66–68]. DI is a heredity disorder of dentin formation. DI type I, which is mostly linked to OI, is the oral symptom of inadequate collagen production [69]. In addition to DI, significant delay of tooth eruption can be found in young OI patients who had been treated with bisphosphonate [70, 71] since osteoclasts are crucial for tooth eruption and resorption of the primary teeth [72, 73].

The first *SP7* mutation was reported in an 8-year-old Egyptian child [74] with recurrent fractures, below-normal height, bowing of upper and lower limbs, mild scoliosis, delayed tooth eruption, and normal hearing. Bone Densitometry (DEXA) results showed low BMD in the spine and hip. A homozygous single base pair deletion (c.1052delA) was identified in *SP7* by a combination of homozygosity mapping and candidate gene approach. This deletion causes a frameshift, introduces 18 novel residues at codon 351, and results in premature termination (p.E351GfsX19). The mutant transcript is translated into a truncated *SP7* protein lacking the 3rd zinc-finger domain. The altered zinc-finger structure may affect the binding affinity of *SP7* to target regions. Further studies are needed to examine the mechanisms that changes the binding affinity and identify the target genes that are affected by this frameshift mutation that

eventually causes the OI-like phenotypes. This same rare deletion was also reported in a 17-year-old Chinese boy with recurrent long bone fractures, vertebral collapse, tooth eruption delay, and maxillary hypoplasia [75]. He had a dental manifestation of dentinogenesis imperfecta in both primary and permanent dentition. Enamel hypoplasia, discoloration, and severely worn primary dentition were reported. His permanent dentition phenotype included bulbous crown, short roots, and impacted dentition which necessitating combined surgical and orthodontic treatment.

A 32-year-old Pakistani man with recurrent fractures, short stature, lower limb bowing, craniofacial dysmorphism, and normal hearing was found to have a *SP7* homozygous missense mutation (c.824G > A) [76]. This c.824G > A mutation is located within exon 2 of *SP7* and causes a cysteine to tyrosine amino acid change at position 275 (p.C275Y). At present, future studies are needed to understand how this mutation, outside the zinc finger domain of *SP7*, affects its function.

A 13-year-old child from Iraq with recessive pattern OI was found to have a homozygous *SP7* (c.946C > T) mutation [77•]. The patient presented with short stature, low-trauma fracture, low BMD, mild scoliosis, hearing loss, facial dysmorphism, and delayed tooth eruption. The proband is from a family of eight siblings. One younger brother and one sister are homozygous for the same mutation. His younger brother was short, had low-trauma hip fracture and hearing loss. His sister had a history of low-trauma fractures, low bone density, normal height, and no hearing loss. A trans-iliac bone biopsy was obtained from the proband patient which showed high cortical porosity and high trabecular bone turnover. The c.946C > T mutation locates in exon 2 of *SP7* gene and results in a substitution from arginine to cysteine (R316C). The arginine position 316 is located in the 1st zinc finger domain. The substitution of arginine with cysteine may change the affinity of *SP7* to the genome or other co-factors

Table 2 Rare skeletal dysplasia caused by *SP7* mutations

	Lapunzina et al. [74] and Tung et al. [75]	Hayat et al. [76]	Fiscaletti et al. [77•]	Ludwig et al. [78•]	Whyte et al. [79]	Lui et al. [80••]
Patients (age)	Egyptian (8y) and Chinese (17y)	Pakistani (32y)	Iraq (13y)	Haitian (20y)	Race not provided (15y)	Race not provided (3y)
Diagnosis	Recessive OI	Recessive OI	Recessive OI	Dominant OI	Juvenile Paget's disease	Neomorphic mutation
Mutations	c.1052delA (p.E351GfsX19)	c.824G>A (p.C275Y)	c.946C>T (p.R316C)	c.1019A>C (p.E340A)	c.926C>G (p.S309W)	c.926C>G (p.S309W)
Alterations	Absence of 3rd zinc-finger domain	No change to zinc-finger domain	Altered 1st zinc-finger domain	Altered 2nd zinc-finger domain	Altered 1st zinc-finger domain	Altered 1st zinc-finger domain
Height	Below normal	Short	Short	Normal	Normal	NA
Fractures	Yes	Yes	Yes	Yes	Yes	Yes
Bowing	Upper and lower limbs	Lower limbs	NA	NA	Lower limbs	Lower limbs
Scoliosis	Mild	Severe	Mild	Severe	Mild	Severe
Bone mineral density	Low at lumbar spine and femur	NA	Low at lumbar spine	Low at lumbar spine	High lumbar spine areal BMD	High lumbar BMD
Cortical porosity	NA	NA	High	High	NA	NA
Bone turnover	Normal ALP	Normal ALP	High trabecular BFR	Low BFR	High ALP and bone turnover	High ALP and bone turnover
Serum calcium and phosphorus	Normal	Normal	NA	Normal	Normal	Normal
Tooth morphology and eruption	Delayed [74, 75], DI, primary dentition, enamel hypoplasia, discoloration, obliterated pulp chamber, permanent dentition, bulbous crowns, short roots [75]	NA	Delayed, No DI	Broken teeth, No DI	DI, Short roots, Thin or no pulp	NA
Hearing	Normal	Normal	Loss	Loss	Loss	Normal
Face	Dysmorphism	Dysmorphism	Dysmorphism	Malocclusion	Dysmorphism	Craniosynostosis
Treatment	Bisphosphonate	NA	Zoledronate	NA	NA	Bisphosphonate

by altering the zinc-finger cysteine-histidine ratio. More recently, we analyzed osteocyte morphology in non-decalcified iliac crest bone biopsies from two R316C patients (the proband and his younger brother) and two age- and sex-matched healthy patients. In this analysis, we noted reduced osteocyte dendrite length and number in patients versus healthy controls [18]. Sp7 binds an enhancer ~ 110 kB upstream of the *Ostn* transcription start site. The transcriptional activity of this putative *Ostn* enhancer was reduced when HEK293T cells were transfected with R316C mutated *SP7* compared to wild-type *SP7* [18]. As discussed above, *SP7* may utilize different DNA-binding cofactors in osteocytes compared to osteoblasts. Further studies are needed to investigate whether the *SP7* R316C mutation affects the binding activity and the cofactor assembly of *SP7* to osteocyte-specific target genes.

One heterozygous mutation of *SP7* (c.1019A>C) was identified in young adult siblings of Haitian descent (20-year-old female and 17-year-old male) who presented with dominant-pattern OI [78•]. One female patient had fractures, poor bone healing, severe scoliosis, hearing loss, normal height, and reduced cortical BMD in long bones. Though most surface parameters were within the normal range on skeletal histomorphometry, she had low mineral apposition rate and low bone turnover. The male patient shared a similar OI phenotype as his sister, including fragility fractures, low cortical volumetric BMD, and high cortical porosity. The c.1019A>C mutation is located in exon 2 of the *SP7* gene and causes a glutamic acid to alanine amino acid change (E340A). The E340A mutation may alter the 2nd zinc-finger domain of *SP7*. When HEK293T cells were co-transfected with *DLX5*- and *SP7*-expression plasmids,

the luciferase activity of *SP7* promoter was significantly reduced when transfected with mutant *SP7* (E340A) compared to wild-type *SP7*.

Two unrelated individuals were identified with the same heterozygous de novo *SP7* mutation (c.926C>G) [79, 80••]. Whyte and colleagues reported a 15-year-old girl with Juvenile Paget's disease caused by c.926C>G mutation [79]. She had recurrent fractures in lower limbs, skull deformity, scoliosis, generalized osteosclerosis, hyperostosis, short roots with very thin or no pulp teeth, and hearing loss. DXA revealed high lumbar spine areal BMD and wrist total BMD. Her serum ALP and urinary total hydroxyproline levels were elevated. Lui and colleagues reported a 3-year-old boy bearing the same *SP7* c.926C>G mutation with similar phenotypes, including recurring fractures, severe scoliosis, osteosclerosis, elevated alkaline phosphatase, increased lumbar BMD, and high bone turnover [80••]. The c.926C>G mutation locates in exon 3 of *SP7* gene and results in a serine to tryptophan mutation (S309W) in the 1st zinc-finger domain. Notably, knock-in mice bearing the S309W mutation were generated. Both homozygous and heterozygous knock-in mice died shortly after birth with a complex skeletal phenotype that is dissimilar to *Sp7* knockout mice. The human and mouse phenotypes shared similarities and showed some differences. For example, clavicles in the knock-in mice showed decreased length and increased thickness, which resembles the clavicle osteosclerosis in the patient. Cranial hyperostosis in the patient, however, was not observed in the knock-in mouse. Further mechanistic studies revealed that the mutation alters the binding specificity of *SP7* from AT-rich motifs to a GC-consensus sequence (typical of other *SP* family members) [33] and produces an aberrant gene expression profile, including increased expression of *Colla1* and endogenous *Sp7*, but decreased expression of genes involved in matrix mineralization.

In summary, there is a strong correlation between *SP7* mutations and rare skeletal dysplasia (mutation locations are summarized in Fig. 2). Abnormal skeletal developmental

processes caused by dysfunction of *SP7* result in skeletal disease. Thus, future directions should emphasize on the establishment of both in vivo and in vitro systems to characterize these disease-causing *SP7* mutations, including generating mouse models that carry *SP7* mutations as well as introducing these mutations to organoids and cell lines by gene editing. These models will provide optimal systems to investigate the mechanism and the causal relationship between these mutations and the function of *SP7* in bone.

Bone Cancer: Osteosarcoma

SP7 is expressed in nearly all osteoblastic tumors but not expressed in giant cell tumor, chondroblastoma, and chondromyxoid fibroma [81]. Osteosarcoma is the most common bone sarcoma and the 3rd most common malignancy in children and adolescents [82]. Not knowing the exact cause, osteosarcoma could be due to DNA mutations of oncogenes and tumor suppressor genes in the primitive mesenchymal bone-forming cells and produces malignant osteoid. *SP7* expression is significantly reduced in both murine and human osteosarcoma cells (murine: K7, K7M2; human: HOS, Krib, MG) compared with its expression in normal murine and human osteoblastic cells (MC3T3 and HOB) [83]. Overexpression of *SP7*, on the other hand, can inhibit tumor cell growth, suppress osteolytic lesions, and suppress lung metastasis. *SP7* down-regulates expression of the cytokine IL-1 α and reduces lytic activity in osteosarcoma cells by targeting the GC-rich region of the IL-1 α promoter [84]. Suppression of *SP7* resulted in up-regulation of both IL-1 α promoter activity and IL-1 α protein level. Site-directed mutagenesis and chromatin immunoprecipitation showed that *SP7* was unable to repress the IL-1 α promoter activity in osteosarcoma cells after mutating the GC-rich *SP1*-binding site. p53 is a tumor suppressor regulating cell proliferation and apoptosis. *Trp53* (encodes p53) knockout mice were characterized by a denser skeleton compared to the wild-type littermates and the *Trp53-deficient* bone marrow-derived mesenchymal progenitor cells had a higher

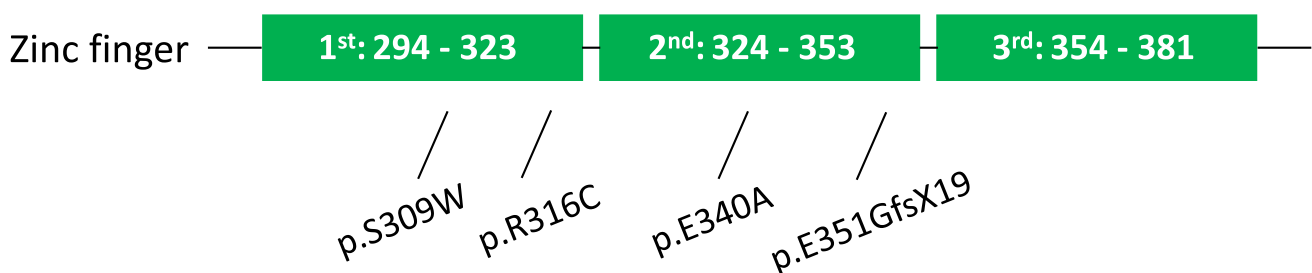


Fig. 2 The sequence of human *SP7* zinc finger domains. Four *SP7* mutations located within zinc finger domains are marked. Green boxes: 3 zinc finger domains

capacity to differentiate towards the osteoblastic fate [85, 86]. p53 represses the osteogenic transcriptional network of SP7 by blocking SP7-DNA binding and SP7-DLX5 interaction [87]. In summary, SP7 regulates the normal formation of mature osteoblasts and abnormal SP7 expression may trigger mesenchymal stem/stromal cells and/or osteoblastic precursors to form osteosarcoma microenvironment by producing excessive osteoid matrix. Future studies are necessary to examine the important roles of SP7 with oncogenes and tumor suppressor genes in osteosarcomas, and evaluate the correlation between SP7 to growth factors, cytokines, chemokines, and metalloproteinases in osteosarcoma microenvironment.

Bone Metastasis

Skeletal metastases are common in patients with advanced breast cancer. Interestingly, SP7 expression is elevated in human breast cancer cell lines and tumors of some breast cancer patients [88, 89]. This finding suggests that epithelial breast cancer cells may adapt an osteogenic SP7-dependent gene expression program to promote skeletal metastasis. Patients with high SP7 expression had poorer survival rates and SP7 expression was significantly associated with lymph node metastasis. SP7 knockdown inhibited breast cancer invasion and osteolytic metastasis, whereas overexpression of SP7 promoted the invasiveness. SP7 facilitates bone metastasis of breast cancer by upregulating the expression of genes (*MMP9*, *MMP13*, *VEGF*, *IL-8*, and *PTHrP*) that contribute to the metastatic cascade. Particularly, SP7 directly targets the GC-rich promoter of *MMP9* and mediates the SP7-driven breast cancer invasion in the bone metastatic niche.

Cancer-associated fibroblasts (CAFs) are cells of the mesenchymal lineage involved in supporting tumorigenesis from growth to metastasis [90]. SP7 expression in CAFs with pro-tumorigenic characteristics was reported using *Osx-Cre; TdTomato (TdT^{Osx})* reporter mice [91•]. Most of these TdTomato-positive cells were positive for CD45 and had tumor-infiltrating immune cell-related genes expressed. SP7 is expressed in a subset of hematopoietic stem cells (HSC) that give rise to TdT^{Osx+};CD45⁺ tumor-infiltrating immune populations. This study supports that a subset of CAFs, derived from *Osx* + cells in the bone marrow, contributes to extracellular matrix (ECM) production at the tumor site, thereby creating a tumor-supporting stroma. These findings emphasize the importance of SP7 in the tumor microenvironment and progression. Future studies are needed to identify the mechanisms that regulate *Osx* + mesenchymal cells during extracellular matrix remodeling and examine how SP7 contributes to this process.

Therapeutic Approaches

Targeting SP7-Dependent Genes: *Ostn*

Long-term glucocorticoid (GC) treatment is associated with skeletal side effects including bone loss, fracture, osteoporosis, and osteonecrosis [92]. GCs induce osteocyte apoptosis and cause loss of osteocyte dendrites [93]. Our previous work demonstrated that the Sp7 target gene *ostecrin (Ostn)* regulates osteocyte dendrite formation and maintenance [18]. Therefore, we tested whether *Ostn* overexpression might restore osteocyte defects in prednisolone-treated mice [94]. Though not able to rescue the reduced cortical thickness caused by prednisolone, *Ostn* treatment led to modest protection at the level of osteocyte morphology from the deleterious effects of GC treatment. These findings support a modest therapeutic potential for systemic osteocrin delivery in preserving osteocyte morphology during disease. Since SP7 level is reduced in the setting of GC treatment, it remains possible that other cell type-specific SP7 target genes may represent attractive therapeutic targets for bone loss due to GC excess.

Targeting Post-Transcriptional Modifications of SP7: Ubiquitination

Melatonin is a neurohormone mainly secreted by pinealocytes in the pineal gland and plays an important role in bone-related diseases by promoting bone formation and preventing bone destruction [95, 96]. Melatonin stabilized the SP7 protein production by blocking the ubiquitin–proteasome pathway and promoted osteoblast differentiation via the PKA and PKC signaling pathways [97]. The same group previously showed that the E3 ligases Cbl-b and c-Cbl induced SP7 ubiquitination and degradation [98]. Melatonin treatment partially prevented SP7 degradation by inhibiting these ubiquitin ligases. This suggests that melatonin may be a potent osteogenic agent targeting mature osteoblast differentiation and bone formation in the treatment of osteoporosis. Future research identifying targeted approaches to manipulate SP7 degradation may be beneficial in the setting of osteoporosis and metastatic bone diseases.

Targeting at the Transcriptional and Translational Level: lncRNAs

Recent study showed that inflammatory osteoclasts (iOCLs) exosomes specifically target osteoblasts via ephrinA2/EphA2 [99]. Exosomal lncRNA LIOCE enriched in iOCL exosomes promoted osteogenic activity by being incorporated into osteoblasts. lncRNA LIOCE directly binds and stabilizes SP7 protein and reduces its ubiquitination, and

thus, exosomes bearing lncRNA LIOCE may be an effective strategy to increase bone formation in osteoporosis and other bone metabolic disorders [99]. The metastasis-associated lung adenocarcinoma transcript-1 (MALAT1) non-coding RNA is induced during osteoblast differentiation and is expressed at lower levels in cells from osteoporosis patients versus controls [100]. MALAT1 positively regulates the expression of SP7 by binding to miR-143 and miR-196 and inhibiting the binding of these inhibitory miRNAs to the SP7 mRNA [101]. Therefore, multiple non-coding RNAs (LIOCE, MALAT, miR-143, and miR-196) all control SP7 at the post-transcriptional level. These non-coding RNAs provide an additional opportunity for future diagnostics and therapeutics to modulate SP7 activity for diseases including osteoporosis and metastatic bone disease.

Future Directions and Conclusions

SP7 plays important regulatory roles in different mesenchymal lineage bone cell types during bone formation and remodeling, including osteoblasts, osteocytes, and chondrocytes. As discussed in this review, common *SP7* polymorphisms are linked to BMD variation and fracture risk, rare *SP7* mutations cause skeletal dysplasia, and *SP7* may contribute to bone metastasis. Novel genomic and proteomic methods should enable the discovery of cell type-specific gene regulatory elements and cofactors for *SP7* to help explain how this single transcription factor accomplishes so many important roles throughout the mesenchymal bone cell lineage. Improved understanding of the mechanisms underlying *SP7*-causing skeletal diseases will provide novel therapeutic targets for the treatment of skeletal disorders.

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Compliance with Ethical Standards

Conflict of interest MNW receives research funding from Radius Health and holds equity in and is a scientific advisory board member of Relation Therapeutics.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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