Published in final edited form as:

Curr Osteoporos Rep. 2016 February ; 14(1): 32–38. doi:10.1007/s11914-016-0296-1.

SOXC genes and the control of skeletogenesis

Véronique Lefebvre and Pallavi Bhattaram

Department of Cellular & Molecular Medicine, Orthopaedic and Rheumatologic Research Center, Cleveland Clinic Lerner Research Institute, 9500 Euclid Avenue, Cleveland, OH 44195, Phone: 1-216-445.0762, Fax: 1-216-444.9404

Véronique Lefebvre: lefebvv@ccf.org

Abstract

The SOXC group of transcription factors, composed of SOX4, SOX11 and SOX12, has evolved to fulfill key functions in cell fate determination. Expressed in many types of progenitor/stem cells, including skeletal progenitors, SOXC proteins potentiate pathways critical for cell survival and differentiation. As skeletogenesis unfolds, SOXC proteins ensure cartilage primordia delineation by amplifying canonical WNT signaling and antagonizing the chondrogenic action of SOX9 in perichondrium and presumptive articular joint cells. They then ensure skeletal elongation by inducing growth plate formation via enabling non-canonical WNT signaling. Human studies have associated *SOX4* with bone mineral density and fracture risk in osteoporotic patients, and *SOX11* with Coffin-Siris, a syndrome that includes skeletal dysmorphism. Meanwhile, in vitro and mouse studies have suggested important cell-autonomous roles for SOXC proteins in osteoblastogenesis. We here review current knowledge and gaps in understanding of SOXC protein functions, with an emphasis on the skeleton and possible links to osteoporosis.

Keywords

Bone; cartilage; cell fate; progenitor/stem cell; SOXC; transcription

Introduction

Osteoporosis is a highly prevalent bone disease, especially among the elderly [1–3]. Characterized by increased bone fragility, osteoporosis drastically increases the risk for morbid bone fractures. It typically develops due to imbalance arising between the bone-forming activity of osteoblasts and the bone-resorbing activity of osteoclasts. Major scientific progress has been made in recent years on multiple fronts of bone biology and pathology to identify the signaling pathways and master transcription factors that are instrumental in the regulation of these cells. Newly acquired knowledge has translated into

Correspondence to: Véronique Lefebvre, lefebvv@ccf.org.

Compliance with Ethics Guidelines

Conflict of Interest

V. Lefebvre and P. Bhattaram both declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent

All animal studies carried by the authors were performed as approved by the Cleveland Clinic Institutional Animal Care and Use Committee. No studies were conducted by the authors using human subjects or materials.

new and improved therapeutic strategies, but the disease is still far from being completely under control. Further research is thus warranted to fully decipher all the nuts and bolts of how osteoblasts and osteoclasts acquire their lineage identity, differentiate, and balance their specific actions to ensure bone homeostasis and repair throughout life. This review focuses on the SOXC proteins and recent studies that have associated their genes with osteoporosis and characteristic skeletal morphology in humans. Key roles that have been proposed for these factors in skeletal progenitor/stem cells and osteoblasts through in vitro and animal studies are also discussed.

SOXC, a unique group within the SOX family

The discovery of SRY in the early 90's was a major breakthrough, not only because it identified the gene defining the male sex-determining region on the Y chromosome, but also because it prompted uncovering of a whole family of essential regulatory genes, unique to the animal kingdom [4–8]. These genes encode transcription factors that govern cell fate determination in many lineages. They thereby allow the development of such critical entities as the cardiovascular system, the central and peripheral nervous system, the endocrine system, and of particular interest to this review, the skeletal system. These factors owe their SOX acronyms to a common, unique feature: an SRY-related high-mobility-group box-type domain. This domain mediates DNA binding, nuclear trafficking, and interactions with transcriptional partners and other regulatory proteins. Mammalian genomes possess twenty SOX genes. Based on sequence identity within and outside the SOX domain, SOX proteins are distributed into eight groups, A to H. For instance, SRY is a SOXA protein, while SOX2, a master regulator of pluripotent embryonic stem cells, is a SOXB protein. SOX9, a mandatory factor for chondrogenesis and multiple other processes, is a SOXE protein, whereas its chondrogenic partners, SOX5 and SOX6, are SOXD proteins. The SOXC group, highlighted in this review, is composed of SOX4, SOX11, and SOX12. The facts that the three proteins share unique structural features and that most invertebrates have only one SOXC-like member [9] strongly suggest that the mammalian SOXC proteins have arisen from a single ancestor. The three mouse and human proteins have conserved 84% identity in the SOX domain, compared to only 45 to 67% identity with SRY and other SOX proteins [10]. SOXC proteins avidly bind DNA in vitro to a SOX domain consensus motif (C^A/T^TTTGTT) [10–12], and genome-wide analyses have validated this motif as the preferential target site of SOX4 in developing B cells [13] and SOX11 in mantle cell lymphoma [14]. While having similar DNA-binding abilities, the mammalian SOXC proteins have evolved to activate transcription with differential forces. They share 67% identity in their C-terminus, a transactivation domain that has no relatives in other proteins [10, 12]. Unique features in this domain allow SOX11 to be the most potent transactivator of the three proteins, and SOX12 to be the weakest one, at least in vitro. As described below, these molecular properties concur with overlapping expression patterns to endow SOXC proteins with in vivo roles that are unique among those of all SOX proteins.

SOXC, decisive factors in progenitor cells from development to disease

Studies carried out by various teams worldwide have revealed that the SOXC genes are broadly expressed in the embryo, but primarily in progenitor cells. These cells derive from

all three germ layers and include neural cells, mesenchymal cells, pancreas progenitor cells, and pro-B and -T cells, to cite only a few [10, 12, 14–17]. Gene inactivation studies in mice and other animal species have revealed vital roles for the genes in development. *Sox4*^{-/-} mouse embryos die at day 14 of gestation (E14) from outflow tract malformation, but do not appear to have other major defects [18]. *Sox11*^{-/-} mice are born alive, but succumb within a few hours [19]. Their heart has malformations that resemble those of *Sox4*^{-/-} embryos, but whose milder impact is lethal only upon birth. In addition, these mice lack a spleen and have underdeveloped internal organs, such as the lungs, stomach, and pancreas. *Sox12*^{-/-} mice, in contrast, appear normal throughout life [12, 20]. These single knockout studies have thus revealed that SOX4 and SOX11 exert at least some of their developmental functions in a non-reciprocal manner, whereas SOX12 lacks non-reciprocal functions. Interestingly, we found that *Sox4*^{-/-}*Sox11*^{-/-} mouse embryos die around E10, with severe heart malformation and a virtual block in organogenesis, and that SOXC triple-null embryos are slightly more affected [20]. Thus, SOX4 and SOX11 act in redundancy in multiple pivotal developmental processes and SOX12 does bring its share to SOXC functions. Gene conditional inactivation studies have revealed essential redundant roles for *Sox4* and *Sox11* in the progenitor cell types mentioned above and in others [17, 21–25]. In most of these studies, the SOXC proteins were shown to have a primary role in ensuring cell survival. No evidence was found that the proteins directly control cell differentiation programs, except in neurogenesis, where SOX4 and SOX11 were described to be directly involved in the activation of cell type-specific genes [26, 27]. The mechanisms whereby SOXC proteins support cell survival in development remain largely unknown. We proposed that SOXC proteins mediate their survival function by directly activating *Tead2*, a gene that encodes a transcriptional mediator of the HIPPO pathway, but our data also called for additional, complementary mechanisms to mediate this important SOXC function [20]. SOXC genes likely remain important in many physiological processes postnatally, but this has been demonstrated so far only in a few processes, namely T and B cell development [18, 28] and hippocampal neurogenesis [29]. In contrast, many studies have demonstrated high expression and critical roles of SOX4 and SOX11 in many types of human cancer [30–33]. While *SOX4* and *SOX11* expression was generally found to correlate with poor prognosis, as for instance in breast cancer [34] and acute lymphoblastic leukemia [35], it has in a few instances been found to correlate with good prognosis, as for instance in ovarian cancer [36] and glioblastoma [33, 37]. These studies have unraveled many possible molecular actions of SOXC proteins, including promotion of epithelial-to-mesenchymal transition [38], and activation of genes essential in the AKT, p53, WNT, and NOTCH signaling pathways [35, 39–41]. Together, these studies indicate that SOXC proteins have central roles in a large spectrum of processes from early embryonic development to adult diseases. They achieve cell survival and other functions at least in part by activating genes involved in major intracellular and intercellular signaling pathways.

SOXC, defining boundaries, articulation and growth of endochondral anlagen

The mammalian skeleton is composed of a few hundreds of individual elements that are most often connected by joints and that shape and protect the head, trunk and limbs [42–44].

Most elements develop through endochondral ossification, a process whereby skeletal progenitors first form cartilage anlagen and later replace them with bone. As cartilage structures develop, perichondrium and presumptive joint cells entertain active crosstalk with chondrocyte neighbors to define tissue boundaries and to coordinate cartilage growth and ossification. Skeletal elements forming most of the skull vault form through intramembranous ossification, a process whereby skeletal progenitors do not form cartilage anlagen, but build an osteoid extracellular matrix that they later mineralize. These elements grow through tissue apposition, as progenitor cells located in sutures actively proliferate and differentiate into osteoblasts. SOXC genes are avidly expressed in vivo in the mesenchymal progenitors of endochondral and intramembranous bones [10, 20, 45–47]. They are then strongly downregulated in differentiating chondrocytes, but remain robustly expressed in surrounding presumptive joint and perichondrium cells. Reproducing these findings in vitro, Sekiya and colleagues in the Prockop group reported that *SOX4* is expressed in human bone marrow-derived mesenchymal stem cells and that its expression declines during chondrocyte differentiation [48].

SOXC gene inactivation in the mesenchymal precursors of appendicular skeletal elements (using a *Prx1Cre* transgene) or in the neural crest precursors of craniofacial skeletal elements (using a *Wnt1Cre* transgene) triggers cell death at a high rate, as seen in other types of progenitor cells [20]. We recently reported that surviving cells are able to undergo chondrocyte differentiation and form cartilage anlagen. Surviving perichondrium and presumptive joint cells are properly specified, but are unable to decline chondrogenesis, a defect that leads to massive fusion of cartilage anlagen [48]. Moreover, these cells are unable to entertain proper crosstalk with chondrocytes to induce cartilage growth plate formation and subsequent endochondral ossification [49]. Using cell type-specific conditional inactivation strategies, we obtained data that strongly suggest that SOXC proteins are required cell-autonomously to secure the non-chondrocytic fate of perichondrium and presumptive joint cells, and are required primarily non-cell-autonomously to induce and organize cartilage growth plates. At the molecular level, we showed that SOXC proteins efficiently stabilize β -catenin by directly interacting with the protein in its destruction complex [48]. Through this non-transcriptional action, SOXC proteins synergize with canonical WNT signaling to downregulate *Sox9* expression in perichondrium and presumptive joint cells and thereby to abort chondrogenesis of these cells. Our group and the Tabin group also proposed that SOXC proteins act as transcription factors in these cells to directly stimulate the expression of markers and signaling factors that critically control skeleton patterning and growth. For instance, the Tabin group suggested that SOX11 could upregulate the expression of the growth and differentiation factor-5 gene (*Gdf5*) in presumptive joint cells, favoring thereby articular cartilage development [46]. We proposed that SOXC proteins transactivate the gene for the non-canonical WNT5 ligand in perichondrium cells and thereby instrumentally contribute to cartilage growth plate formation and organization [49]. Interestingly, as we were assessing the contribution of each SOXC gene to skeletogenesis, we discovered that SOX12 acted as a rheostat of SOX4/SOX11 actions [49]. Its inactivation slightly worsened the phenotype of *Sox4/Sox11* mutant limb buds, implying that it adds a minor, but significant share to the powerful actions of its relatives in skeletal progenitors. In contrast, *Sox12* inactivation

improved rather than aggravated the cartilage growth plate phenotype of *Sox4/Sox11* single and double mutants, implying that SOX12 refrains rather than supports the actions of its relatives in this specific developmental process. Using molecular assays, we showed that when SOXC proteins are present in limiting amount for a transactivation function, the contribution of SOX12 adds up to those of SOX4 and SOX11. In contrast, when SOXC proteins are present in excess amount, they compete with each other to gain access to gene targets. Being a relatively weak transcriptional activator, SOX12 then lowers the overall transcriptional effect generated by SOXC proteins.

Overall, these findings have newly uncovered that SOXC genes are critical regulators of endochondral bone development. Strikingly, their cell-autonomous functions in perichondrium and joint precursor cells are anti-chondrogenic, whereas their non-cell-autonomous functions are pro-chondrogenic, allowing perichondrium cells to induce growth plate chondrocyte maturation. It is possible, and even likely, that these studies have only unearthed a small fraction of SOXC actions in this process. For instance, as these studies have only analyzed embryos, it remains unknown how important SOXC genes are in postnatal growth plates and in the overt development and adult homeostasis of articular joints. Kan and colleagues observed that the SOX11 protein is expressed in articular cartilage in healthy adult mice, but that its level is reduced in this tissue upon induction of osteoarthritis [46]. This finding thus supports a role for this SOXC protein and possibly for its relatives in adult joint maintenance. At the molecular level, it can be anticipated that like other major regulators SOXC proteins fulfill transcriptional and non-transcriptional actions that span a wide spectrum. Further studies are thus warranted to fully decipher the actions of SOXC proteins in cartilage and joints as well as to uncover the mechanisms that regulate them at gene, RNA, and protein levels.

SOXC, promoters of bone formation from development to adulthood

The first clue that SOXC genes could be important in the process of bone formation per se, independently of roles in endochondral skeletogenesis, was provided by the Wegner group, which showed that *Sox11*^{-/-} mice are born with undermineralized bones [19]. This defect was seen in both endochondral and intramembranous bones, but was most striking in the latter in the skull vault. A few years later, the Gautvik group showed that *Sox4*^{+/-} mice are osteopenic at both young and advanced adult ages [50]. These mice have thinner cortical and trabecular bones than control mice, and their bone formation rate is reduced, whereas their bone resorption rate appears normal. In 2011, this group provided evidence that SOXC genes may be important for bone formation not only in the mouse, but also in humans [51]. Since osteoporosis, bone mineral density, and many bone phenotypic variances and diseases are compounded by genetic inheritance, this group conducted a genome-wide association study to newly identify susceptibility candidate genes for these diseases. This led them to uncover that *SOX4* expression was significantly reduced in bone samples from osteoporotic Norwegian women compared to bones from healthy controls. *SOX4* expression correlated not only with osteoporosis, but also with bone mineral density at the level of hip and femoral neck, supporting the hypothesis that SOX4 is necessary for bone homeostasis throughout life. The same year, a research group led by Dr. Brown reported that single nucleotide polymorphisms (SNPs) within and around *SOX4* showed moderate association with bone

mineral density of the hip [52]. The cohorts of patients used by this group were different from that used by the Gautvik group, thus strengthening the possibility of a link between *SOX4*, bone mineral density and osteoporosis. *SOX11* and *SOX12* have not been associated with any human adult skeletal disease yet, but *de novo* *SOX11* heterozygous mutations were recently discovered in a fraction of patients born with the congenital disorder known as Coffin-Siris syndrome (CSS) [53]. CSS is characterized by absence of the nails of the fifth fingers and toes, severe mental retardation, and features that, we predict, could directly or indirectly result from improper skeletogenesis [54]. These features include postnatal growth deficiency, microcephaly, brachymorphism, and facial dysmorphism. The *SOX11* mutations causing CSS are located in the SOX domain and were shown to disrupt SOX11 DNA binding and hence transactivation. CSS in these patients is thus likely the result of *SOX11* haploinsufficiency. Together, these animal and human studies support the notion that *SOX4* and *SOX11* are likely essential to promote bone formation from development to advanced adult ages.

SOXC and the control of osteoblastogenesis

While establishing invaluable links between SOXC genes and bone formation, the human and mouse studies described above did not inform on whether SOXC proteins cell- or non-cell-autonomously control bone-forming cells. Several groups have shown that the SOXC genes are expressed in osteogenic progenitors in the developing mouse and in bone marrow- or adipose-derived mesenchymal stem cells [10, 50, 55–57]. *SOX11* expression was shown to be downregulated as osteoblasts differentiate, whereas *SOX4* expression was shown to be either maintained or downregulated during this process. Such evidence supports the possibility of cell-autonomous roles for SOXC proteins in osteoblasts and their progenitors, but does not prove it, especially when one considers the wide spectrum of expression of SOXC genes in vivo.

The Gautvik group was first to conduct in vitro studies supporting the proposition that *SOX4* may directly control osteoblastogenesis [50]. Knockdown or heterozygous inactivation of mouse *Sox4* in primary osteoblastic cells was found to reduce proliferation and delay differentiation of osteoblast progenitors. These data are consistent with the smaller fraction of bone surface occupied by osteoblasts that they detected in *Sox4*^{+/-} mice. A question that remains to be addressed is whether *SOX4* is directly involved in the expression of osteoblast differentiation genes or whether its roles in cell proliferation or survival may secondarily affect the ability of progenitor cells to differentiate. While this group indicated that they found no sign that *SOX4* promotes cell survival, we found that *SOX4* and *SOX11* combine efforts to promote the survival of osteoblast progenitors in vivo as well as in primary cultures [20]. It is possible that the Gautvik group did not observe cell death in their study because they inactivated *Sox4* only partially and did not manipulate *Sox11* expression.

More recently, the Lim group conducted in vitro experiments to help address the question of the involvement of *SOX11* in osteoblastogenesis [56]. In agreement with our previous findings in vivo and in primary cells [20], this group showed that knockdown of *Sox11* in the mouse MC3T3-E1 osteoblastic cell line or in the mouse C3H10T1/2 mesenchymal cell line led to reduced cell numbers, likely because of increased cell death and at least in part

because of *Tead2* downregulation. In agreement with findings from the Gautvik group for SOX4 [50], the Lim group observed a significant delay in osteoblast differentiation of *Sox11*-deficient mesenchymal cells. Through chromatin immunoprecipitation and reporter assays in vitro, they suggested that SOX11 might be directly involved in upregulating the genes for the osteoblast master transcription factors RUNX2 and OSX. Genome-wide and complementary assays in vivo will be necessary to validate these interesting findings. Choi and colleagues in the Kim group recently added a new component to our nascent understanding of SOXC-regulated osteoblastogenesis [57]. Studying the ability of mouse adipose-derived mesenchymal stem cells to undergo osteoblast differentiation in vitro, this team uncovered that downregulation of *Sox11* was necessary to allow this process to occur efficiently. This conclusion was drawn from results obtained using both gain-of-function and loss-of-function approaches to manipulate *Sox11* expression. Furthermore, by forcing the expression of SOX11 chimeric proteins harboring a potent VP16 transactivation domain or an engrailed transrepression domain, this group proposed that SOX11 actively promotes expression of genes antagonizing osteoblast differentiation. We previously showed that SOXC proteins promote canonical WNT signaling in skeletogenic progenitors through non-transcriptional actions. As the latter pathway is highly conducive for osteoblastogenesis, it will be interesting in future studies to discern between permissive and non-permissive actions of SOXC proteins in osteoblastogenesis as well as to discern between transcriptional and non-transcriptional actions. It will also be important to determine whether SOX12 contributes to osteoblastogenesis and, last but not least, to validate in vitro findings in vivo. Towards this end, the Guo group recently isolated rat mesenchymal stem cells from bone marrow and transduced them with lentiviruses to force SOX11 overexpression [58]. The group reported that this strategy significantly enhanced the ability of mesenchymal stem cells to differentiate into osteoblasts, chondrocytes and adipocytes in vitro, to generate bone in a subcutaneous ectopic assay in the rat, as well as to heal an open femur fracture in vivo. Although the authors showed data that suggest that SOX11 might directly stimulate expression of such important genes for cell differentiation as *Runx2*, it remains possible, as discussed by the authors, that SOX11 might confer these important advantages primarily by promoting mesenchymal stem cell survival.

Conclusions

Commendable efforts by many teams of basic and clinician scientists over the world have started to provide solid and exciting evidence that SOXC proteins comprise a group of unique, essential regulatory factors in many processes. Expressed mainly in multipotent progenitor cells, these proteins encourage cell survival and also have, at least in several lineages, differentiation and other roles. They act both as transcriptional activators and as protein interacting factors and they can thereby decisively modulate pivotal intracellular and intercellular signaling pathways. As of today, it is clear that SOXC proteins have chief roles in endochondral and intramembranous skeletogenesis, and that they may also be implicated in bone formation throughout life in mice and humans. These emerging findings motivate further studies to fully uncover the importance, the precise cellular and molecular actions, and the modes of regulation of SOXC proteins in the skeleton from early to advanced ages and in both physiological and disease processes. As new knowledge becomes available, we

predict that SOXC proteins, their downstream mediators or their upstream regulators may become key targets in novel strategies to improve the prevention and treatment of osteoporosis as well as many other skeletal and non-skeletal diseases.

Acknowledgments

Work in the Lefebvre lab was supported by the NIH grants AR54513, AR46249, and AR60016 (to VL) and an Arthritis National Research Foundation grant (to PB).

References

Papers of particular interest, published recently, have been highlighted as:

* Of importance

1. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. *Lancet*. 2011; 377(9773): 1276–1287. [PubMed: 21450337]
2. Binkley N, Adler R, Bilezikian JP. Osteoporosis diagnosis in men: the T-score controversy revisited. *Curr Osteoporos Rep*. 2014; 12(4):403–409. [PubMed: 25255867]
3. Jackson RD, Mysiw WJ. Insights into the epidemiology of postmenopausal osteoporosis: the Women's Health Initiative. *Semin Reprod Med*. 2014; 32(6):454–462. [PubMed: 25321423]
4. Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for Sry. *Nature*. 1991; 351(6322):117–121. [PubMed: 2030730]
5. Schepers GE, Teasdale RD, Koopman P. Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. *Dev Cell*. 2002; 3(2):167–170. [PubMed: 12194848]
6. Lefebvre V, Dumitriu B, Penzo-Méndez A, Han Y, Pallavi B. Control of cell fate and differentiation by Sry-related high-mobility-group box (Sox) transcription factors. *Int J Biochem Cell Biol*. 2007; 39(12):2195–2214. [PubMed: 17625949]
7. Kamachi Y, Kondoh H. Sox proteins: regulators of cell fate specification and differentiation. *Development*. 2013; 140(20):4129–4144. [PubMed: 24086078]
8. Sarkar A, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. *Cell Stem Cell*. 2013; 12(1):15–30. [PubMed: 23290134]
9. Phochanukul N, Russell S. No backbone but lots of Sox: Invertebrate Sox genes. *Int J Biochem Cell Biol*. 2010; 42(3):453–464. [PubMed: 19589395]
10. Dy P, Penzo-Méndez A, Wang H, Pedraza CE, Macklin WB, Lefebvre V. The three SoxC proteins--Sox4, Sox11 and Sox12--exhibit overlapping expression patterns and molecular properties. *Nucleic Acids Res*. 2008; 36(9):3101–3117. [PubMed: 18403418]
11. van Beest M, Dooijes D, van De Wetering M, Kjaerulff S, Bonvin A, Nielsen O, Clevers H. Sequence-specific high mobility group box factors recognize 10–12-base pair minor groove motifs. *J Biol Chem*. 2000; 275(35):27266–27273. [PubMed: 10867006]
12. Hoser M, Potzner MR, Koch JM, Bösl MR, Wegner M, Sock E. Sox12 deletion in the mouse reveals nonreciprocal redundancy with the related Sox4 and Sox11 transcription factors. *Mol Cell Biol*. 2008; 28(15):4675–4687. [PubMed: 18505825]
13. Mallampati S, Sun B, Lu Y, Ma H, Gong Y, Wang D, Lee JS, Lin K, Sun X. Integrated genetic approaches identify the molecular mechanisms of Sox4 in early B-cell development: intricate roles for RAG1/2 and CK1epsilon. *Blood*. 2014; 123(26):4064–4076. [PubMed: 24786772]
14. Kuo PY, Leshchenko VV, Fazzari MJ, Perumal D, Gellen T, He T, Iqbal J, Baumgartner-Wennerholm S, Nygren L, Zhang F, Zhang W, Suh KS, Goy A, Yang DT, Chan WC, Kahl BS, Verma AK, Gascoyne RD, Kimby E, Sander B, Ye BH, Melnick AM, Parekh S. High-resolution chromatin immunoprecipitation (ChIP) sequencing reveals novel binding targets and prognostic role for SOX11 in mantle cell lymphoma. *Oncogene*. 2015; 34(10):1231–1240. [PubMed: 24681958]

15. van de Wetering M, Oosterwegel M, van Norren K, Clevers H. Sox-4, an Sry-like HMG box protein, is a transcriptional activator in lymphocytes. *EMBO J.* 1993; 12(10):3847–3854. [PubMed: 8404853]
16. Lioubinski O, Müller M, Wegner M, Sander M. Expression of Sox transcription factors in the developing mouse pancreas. *Dev Dyn.* 2003; 227(3):402–408. [PubMed: 12815626]
17. Sun B, Mallampati S, Gong Y, Wang D, Lefebvre V, Sun X. Sox4 is required for the survival of pro-B cells. *J Immunol.* 2013; 190(5):2080–2089. [PubMed: 23345330]
18. Schilham MW, Oosterwegel MA, Moerer P, Ya J, de Boer PA, van de Wetering M, Verbeek S, Lamers WH, Kruisbeek AM, Cumano A, Clevers H. Defects in cardiac outflow tract formation and pro-B-lymphocyte expansion in mice lacking Sox-4. *Nature.* 1996; 380(6576):711–714. [PubMed: 8614465]
19. Sock E, Rettig SD, Enderich J, Bösl MR, Tamm ER, Wegner M. Gene targeting reveals a widespread role for the high-mobility-group transcription factor Sox11 in tissue remodeling. *Mol Cell Biol.* 2004; 24(15):6635–6644. [PubMed: 15254231]
20. Bhattaram P, Penzo-Méndez A, Sock E, Colmenares C, Kaneko KJ, Vassilev A, Depamphilis ML, Wegner M, Lefebvre V. Organogenesis relies on SoxC transcription factors for the survival of neural and mesenchymal progenitors. *Nat Commun.* 2010; 1:9. [PubMed: 20596238]
21. Thein DC, Thalhammer JM, Hartwig AC, Crenshaw EB 3rd, Lefebvre V, Wegner M, Sock E. The closely related transcription factors Sox4 and Sox11 function as survival factors during spinal cord development. *J Neurochem.* 2010; 115(1):131–141. [PubMed: 20646169]
22. Potzner MR, Tsarovina K, Binder E, Penzo-Méndez A, Lefebvre V, Rohrer H, Wegner M, Sock E. Sequential requirement of Sox4 and Sox11 during development of the sympathetic nervous system. *Development.* 2010; 137(5):775–784. [PubMed: 20147379]
23. Lin L, Lee VM, Wang Y, Lin JS, Sock E, Wegner M, Lei L. Sox11 regulates survival and axonal growth of embryonic sensory neurons. *Dev Dyn.* 2011; 240(1):52–64. [PubMed: 21117150]
24. Jiang Y, Ding Q, Xie X, Libby RT, Lefebvre V, Gan L. Transcription factors SOX4 and SOX11 function redundantly to regulate the development of mouse retinal ganglion cells. *J Biol Chem.* 2013; 288(25):18429–18438. [PubMed: 23649630]
25. Paul MH, Harvey RP, Wegner M, Sock E. Cardiac outflow tract development relies on the complex function of Sox4 and Sox11 in multiple cell types. *Cell Mol Life Sci.* 2014; 71(15):2931–2945. [PubMed: 24310815]
26. Bergsland M, Werme M, Malewicz M, Perlmann T, Muhr J. The establishment of neuronal properties is controlled by Sox4 and Sox11. *Genes Dev.* 2006; 20(24):3475–3486. [PubMed: 17182872]
27. Bergsland M, Ramsköld D, Zaouter C, Klum S, Sandberg R, Muhr J. Sequentially acting Sox transcription factors in neural lineage development. *Genes Dev.* 2011; 25(23):2453–2464. [PubMed: 22085726]
28. Schilham MW, Moerer P, Cumano A, Clevers HC. Sox-4 facilitates thymocyte differentiation. *Eur J Immunol.* 1997; 27(5):1292–1295. [PubMed: 9174623]
29. Mu L, Berti L, Masserdotti G, Covic M, Michaelidis TM, Doberauer K, Merz K, Rehfeld F, Haslinger A, Wegner M, Sock E, Lefebvre V, Couillard-Despres S, Aigner L, Berninger B, Lie DC. SoxC transcription factors are required for neuronal differentiation in adult hippocampal neurogenesis. *J Neurosci.* 2012; 32(9):3067–3080. [PubMed: 22378879]
30. Penzo-Méndez AI. Critical roles for SoxC transcription factors in development and cancer. *Int J Biochem Cell Biol.* 2010; 42(3):425–428. [PubMed: 19651233]
31. Jafarnejad SM, Ardekani GS, Ghaffari M, Li G. Pleiotropic function of SRY-related HMG box transcription factor 4 in regulation of tumorigenesis. *Cell Mol Life Sci.* 2013; 70(15):2677–2696. [PubMed: 23080209]
32. Lu TX, Li JY, Xu W. The role of SOX11 in mantle cell lymphoma. *Leuk Res.* 2013; 37(11):1412–1419. [PubMed: 24001358]
33. Vervoort SJ, van Boxtel R, Coffey PJ. The role of SRY-related HMG box transcription factor 4 (SOX4) in tumorigenesis and metastasis: friend or foe? *Oncogene.* 2013; 32(29):3397–3409.

[PubMed: 23246969] *This paper reviews current knowledge on SOXC proteins in various types of cancer.*

34. Song GD, Sun Y, Shen H, Li W. SOX4 overexpression is a novel biomarker of malignant status and poor prognosis in breast cancer patients. *Tumour Biol.* 2015; 36(6):4167–4173. [PubMed: 25592378]
35. Ramezani-Rad P, Geng H, Hurtz C, Chan LN, Chen Z, Jumaa H, Melnick A, Paietta E, Carroll WL, Willman CL, Lefebvre V, Müschen M. SOX4 enables oncogenic survival signals in acute lymphoblastic leukemia. *Blood.* 2013; 121(1):148–155. [PubMed: 23152540]
36. Brennan DJ, Ek S, Doyle E, Drew T, Foley M, Flannelly G, O'Connor DP, Gallagher WM, Kilpinen S, Kallioniemi OP, Jirstrom K, O'Herlihy C, Borrebaeck CA. The transcription factor Sox11 is a prognostic factor for improved recurrence-free survival in epithelial ovarian cancer. *Eur J Cancer.* 2009; 45(8):1510–1517. [PubMed: 19272768]
37. Hide T, Takezaki T, Nakatani Y, Nakamura H, Kuratsu J, Kondo T. Sox11 prevents tumorigenesis of glioma-initiating cells by inducing neuronal differentiation. *Cancer Res.* 2009; 69(20):7953–7959. [PubMed: 19808959]
38. Tiwari N, Tiwari VK, Waldmeier L, Balwierz PJ, Arnold P, Pachkov M, Meyer-Schaller N, Schübeler D, van Nimwegen E, Christofori G. Sox4 is a master regulator of epithelial-mesenchymal transition by controlling Ezh2 expression and epigenetic reprogramming. *Cancer Cell.* 2013; 23(6):768–783. [PubMed: 23764001]
39. Pan X, Zhao J, Zhang WN, Li HY, Mu R, Zhou T, Zhang HY, Gong WL, Yu M, Man JH, Zhang PJ, Li AL, Zhang XM. Induction of SOX4 by DNA damage is critical for p53 stabilization and function. *Proc Natl Acad Sci U S A.* 2009; 106(10):3788–3793. [PubMed: 19234109]
40. Moreno CS. The Sex-determining region Y-box 4 and homeobox C6 transcriptional networks in prostate cancer progression: crosstalk with the Wnt, Notch, and PI3K pathways. *Am J Pathol.* 2010; 176(2):518–527. [PubMed: 20019190]
41. Zhang J, Jiang H, Shao J, Mao R, Liu J, Ma Y, Fang X, Zhao N, Zheng S, Lin B. SOX4 inhibits GBM cell growth and induces G0/G1 cell cycle arrest through Akt-p53 axis. *BMC Neurol.* 2014; 14:207. [PubMed: 25366337]
42. Lefebvre V, Bhattaram P. Vertebrate skeletogenesis. *Curr Top Dev Biol.* 2010; 90:291–317. [PubMed: 20691853]
43. Long F, Ornitz DM. Development of the endochondral skeleton. *Cold Spring Harb Perspect Biol.* 2013; 5(1):a008334. [PubMed: 23284041]
44. Kozhemyakina E, Lassar AB, Zelzer E. A pathway to bone: signaling molecules and transcription factors involved in chondrocyte development and maturation. *Development.* 2015; 142(5):817–831. [PubMed: 25715393] *This paper provides a comprehensive overview of current knowledge of molecular mechanisms controlling skeletogenesis.*
45. Cameron TL, Belluoccio D, Farlie PG, Brachvogel B, Bateman JF. Global comparative transcriptome analysis of cartilage formation in vivo. *BMC Dev Biol.* 2009; 9:20. [PubMed: 19272164]
46. Kan A, Ikeda T, Fukai A, Nakagawa T, Nakamura K, Chung UI, Kawaguchi H, Tabin CJ. SOX11 contributes to the regulation of GDF5 in joint maintenance. *BMC Dev Biol.* 2013; 13:4. [PubMed: 23356643]
47. Bhattaram P, Penzo-Méndez A, Kato K, Bandyopadhyay K, Gadi A, Taketo MM, Lefebvre V. SOXC proteins amplify canonical WNT signaling to secure nonchondrocytic fates in skeletogenesis. *J Cell Biol.* 2014; 207(5):657–671. [PubMed: 25452386] *This paper demonstrates the importance of SOXC proteins and their functional interactions with canonical WNT signaling in early skeletogenesis.*
48. Sekiya I, Vuorio JT, Larson BL, Prockop DJ. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. *Proc Natl Acad Sci U S A.* 2002; 99(7):4397–4402. [PubMed: 11917104]

49. Kato K, Bhattaram P, Penzo-Méndez A, Gadi A, Lefebvre V. SOXC Transcription Factors Induce Cartilage Growth Plate Formation in Mouse Embryos by Promoting Noncanonical WNT Signaling. *J Bone Miner Res*. 2015; 30(9):1560–1571. [PubMed: 25761772] *This paper demonstrates the importance of SOXC proteins in establishing cartilage growth plates and identifies non-canonical WNT signaling as a key mechanism whereby the proteins mediate this function.*
50. Nissen-Meyer LS, Jemtland R, Gautvik VT, Pedersen ME, Paro R, Fortunati D, Pierroz DD, Stadelmann VA, Reppe S, Reinholt FP, Del Fattore A, Rucci N, Teti A, Ferrari S, Gautvik KM. Osteopenia, decreased bone formation and impaired osteoblast development in Sox4 heterozygous mice. *J Cell Sci*. 2007; 120(Pt 16):2785–2795. [PubMed: 17652162]
51. Jemtland R, Holden M, Reppe S, Olstad OK, Reinholt FP, Gautvik VT, Refvem H, Frigessi A, Houston B, Gautvik KM. Molecular disease map of bone characterizing the postmenopausal osteoporosis phenotype. *J Bone Miner Res*. 2011; 26(8):1793–1801. [PubMed: 21452281]
52. Duncan EL, Danoy P, Kemp JP, Leo PJ, McCloskey E, Nicholson GC, Eastell R, Prince RL, Eisman JA, Jones G, Sambrook PN, Reid IR, Dennison EM, Wark J, Richards JB, Uitterlinden AG, Spector TD, Esapa C, Cox RD, Brown SD, Thakker RV, Addison KA, Bradbury LA, Center JR, Cooper C, Cremin C, Estrada K, Felsenberg D, Glüer CC, Hadler J, Henry MJ, Hofman A, Kotowicz MA, Makovey J, Nguyen SC, Nguyen TV, Pasco JA, Pryce K, Reid DM, Rivadeneira F, Roux C, Stefansson K, Styrkarsdottir U, Thorleifsson G, Tichawangana R, Evans DM, Brown MA. Genome-wide association study using extreme truncate selection identifies novel genes affecting bone mineral density and fracture risk. *PLoS Genet*. 2011; 7(4):e1001372. [PubMed: 21533022]
53. Tsurusaki Y, Koshimizu E, Ohashi H, Phadke S, Kou I, Shiina M, Suzuki T, Okamoto N, Imamura S, Yamashita M, Watanabe S, Yoshiura K, Kodera H, Miyatake S, Nakashima M, Saitsu H, Ogata K, Ikegawa S, Miyake N, Matsumoto N. De novo SOX11 mutations cause Coffin-Siris syndrome. *Nat Commun*. 2014; 5:4011. [PubMed: 24886874] *This paper is the first one to demonstrate that SOX11 mutations can cause a congenital disease in humans.*
54. Verloes A, Bonneau D, Guidi O, Berthier M, Oriot D, Van Maldergem L, Koulischer L. Brachymorphism-onychodysplasia-dysphalangism syndrome. *J Med Genet*. 1993; 30(2):158–161. [PubMed: 8445623]
55. Billiard J, Moran RA, Whitley MZ, Chatterjee-Kishore M, Gillis K, Brown EL, Komm BS, Bodine PV. Transcriptional profiling of human osteoblast differentiation. *J Cell Biochem*. 2003; 89(2):389–400. [PubMed: 12704802]
56. Gadi J, Jung SH, Lee MJ, Jami A, Ruthala K, Kim KM, Cho NH, Jung HS, Kim CH, Lim SK. The transcription factor protein Sox11 enhances early osteoblast differentiation by facilitating proliferation and the survival of mesenchymal and osteoblast progenitors. *J Biol Chem*. 2013; 288(35):25400–25413. [PubMed: 23888050] *This paper and the next two papers are the first ones to suggest that SOX11 has key functions in skeletal progenitors and the osteoblast lineage.*
57. Choi MK, Seong I, Kang SA, Kim J. Down-regulation of Sox11 is required for efficient osteogenic differentiation of adipose-derived stem cells. *Mol Cells*. 2014; 37(4):337–344. [PubMed: 24722414] *See the comment made for reference 56.*
58. Xu L, Huang S, Hou Y, Liu Y, Ni M, Meng F, Wang K, Rui Y, Jiang X, Li G. Sox11-modified mesenchymal stem cells (MSCs) accelerate bone fracture healing: Sox11 regulates differentiation

and migration of MSCs. FASEB J. 2015; 29(4):1143–1152. [PubMed: 25466891] *See the comment made for reference 56.*

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript