Genes & Diseases (2014) 1, 149–161

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REVIEW ARTICLE

The versatile functions of Sox9 in development, stem cells, and human diseases

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Received 3 September 2014; accepted 6 September 2014
Available online 16 October 2014

KEYWORDS
Development;
Sox9;
Stem cells;
Transcription factor

Abstract The transcription factor Sox9 was first discovered in patients with campomelic dysplasia, a haploinsufficiency disorder with skeletal deformities caused by dysregulation of *Sox9* expression during chondrogenesis. Since then, its role as a cell fate determiner during embryonic development has been well characterized; *Sox9* expression differentiates cells derived from all three germ layers into a large variety of specialized tissues and organs. However, recent data has shown that ectoderm- and endoderm-derived tissues continue to express *Sox9* in mature organs and stem cell pools, suggesting its role in cell maintenance and specification during adult life. The versatility of Sox9 may be explained by a combination of post-transcriptional modifications, binding partners, and the tissue type in which it is expressed. Considering its importance during both development and adult life, it follows that dysregulation of Sox9 has been implicated in various congenital and acquired diseases, including fibrosis and cancer. This review provides a summary of the various roles of Sox9 in cell fate specification, stem cell biology, and related human diseases. Ultimately, understanding the mechanisms that regulate Sox9 will be crucial for developing effective therapies to treat disease caused by stem cell dysregulation or even reverse organ damage.

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Peer review under responsibility of Chongqing Medical University.

http://dx.doi.org/10.1016/j.gendis.2014.09.004

Introduction

Stem cells are undifferentiated biological cells that can self-renew or differentiate into one or more mature cellular lineages.[1] In mammals, stem cells can differentiate into specialized cells of every germ layer—ectoderm, endoderm and mesoderm—in a developing embryo, but can also maintain the normal turnover of regenerative tissues, such as in skin and intestines.[1] In adult organisms, stem and progenitor cells are kept in pools known as "niches," which act as a repair system to replenish damaged tissues.[2] A stem cell's decision between self-renewal and differentiation is a tightly regulated process that requires expression of cell type-specific transcription factors. Over the last few years, several such molecules have been implicated in stem cell biology, although their versatile functions in different tissues remain to be fully elucidated.

Sox family proteins are a group of transcriptional regulators containing a high mobility group (HMG) domain that is highly conserved.[3] The HMG domain was first identified in *Sry*, a crucial factor involved in mammalian male sex determination.[3,4] In general, proteins containing an HMG domain with 50% or higher amino acid similarity to the HMG are referred to as Sox proteins (*Sry*-related HMG box). Around 20 Sox proteins to this date has been identified in mice and humans, and are grouped A through H based on the structural homology outside of their HMG boxes. Notably, Sox-like proteins are identified in invertebrate lineages and in the unicellular organisms, suggesting that it is evolutionarily conserved.[5] Early insights into the function of Sox factors have involved cell fate determination during development, although recent findings reveal its crucial role in establishing and maintaining stem and progenitor cell pools.

In this review, we confine our discussion to one of the well-characterized SoxE proteins, Sox9. After discussing multiple levels of regulation and mechanisms, we review the versatile functions of Sox9 in germ layers and adult tissues as a stem cell regulator. We then discuss its function in disease pathogenesis while highlighting the Sox9-related pathology of fibrosis and cancer.

Molecular characteristics of Sox9

Structural domains of Sox9

Research on Sox9 began with its seminal discovery as the gene underlying campomelic dysplasia (CD), a haploinsufficiency disorder characterized by defective chondrogenesis and a high proportion of male-to-female sex reversals in XY males.[6] Along with Sox8 and 10, Sox9 belongs to the SoxE subgroup, and, characteristic of all Sox proteins, contains the HMG domain which induces significant bending at the consensus-binding motif (A/TA/TCAAA/TG) by forming an L-shaped complex in the minor groove of DNA.[3] Members of the SoxE subgroup share regions of significant homology outside the HMG domain, and constitute two additional functional domains: a self-dimerization domain and a transactivation domain at the C-terminus (Fig. 1).[3,7]

A recurring theme among Sox proteins, Sox9 shares functions redundant within the SoxE subgroup. This is well demonstrated in knockout mutants, where individual Sox9 mutants often have a starkly less severe phenotype than double or triple SoxE mutants. For instance, separate deletions of either Sox9 or Sox10 retain normal formation of oligodendrocytes, whereas the deletion of both results in widespread apoptosis.[8] However, depending on the tissue in question, the individual contribution of each member may differ temporally and in the amount of expression. While replacing Sox8 with Sox10 resumes normal development of glial cells and neurons in the sensory and sympathetic parts of the peripheral nervous system, only Sox10-deficient mice show defective melanocyte development.[9] Another report showed that, despite functional redundancy of Sox8 and Sox10 in oligodendrocyte development, Sox8 expression levels are significantly lower than those for Sox10.[10]

Posttranscriptional regulation of Sox9

Sox9 is subject to context-dependent regulation at multiple levels. One type of regulation is posttranscriptional modification, which modulates the stability, intracellular localization, and the overall activity of Sox9.[11–13] Phosphorylation by protein kinase A (PKA) enhances DNA-binding affinity of Sox9 and leads to its translocation into the nucleus in testis cells.[14] Interestingly, this same event is also required in the neural crest (NC) cells for the Sox—Snail interaction during NC delamination, and is necessary for parathyroid hormone-related peptide (PTHrP)-mediated regulation of chondrocyte maturation.[11,12]

SUMOlaytion, or post-transcriptional regulation by small ubiquitin-related modifier, has also been noted to influence Sox9-dependent transcription, although context determines whether it is activational or repressive. For example, co-transfection with a SUMO-expressing vector enhances the transcriptional activity of Sox9-dependent Col2a1 reporter.[15] On the other hand, covalent attachment of SUMO-1 to Sox9 by gene fusion dramatically compromises its transcription activity on the reporter gene.[16] In some situations, SUMOlyation of Sox9 acts as a switch to drive tissue differentiation one way or another. In *Xenopus*, non-SUMOlyated SoxE proteins promote NC development, whereas SUMOylated SoxE proteins promote inner ear development.[13]

MicroRNAs, small noncoding RNAs that control gene expression, inhibit Sox9 expression in lung development, during chondrogenesis and neurogenesis, and in developing mouse ovarian cells.[17–20] The ubiquitin-proteasome pathway represses Sox9 transcriptional activity by degrading Sox9 in hypertrophic chondrocytes.[21] The regions of Sox9 subject to these posttranslational modifications are shown in Fig. 1.

The Sox9-partner complexes

Sox proteins generally exhibit their gene regulatory functions by forming complexes with partner transcriptions factors, which can be transcription factors from another protein family, homologous Sox protein or heterologous Sox protein (Fig. 2A). Binding of either a single Sox protein or the partner protein alone to a DNA site does not elicit transcriptional activity.[22] Target genes often have binding sites for a partner protein adjacent to a functional

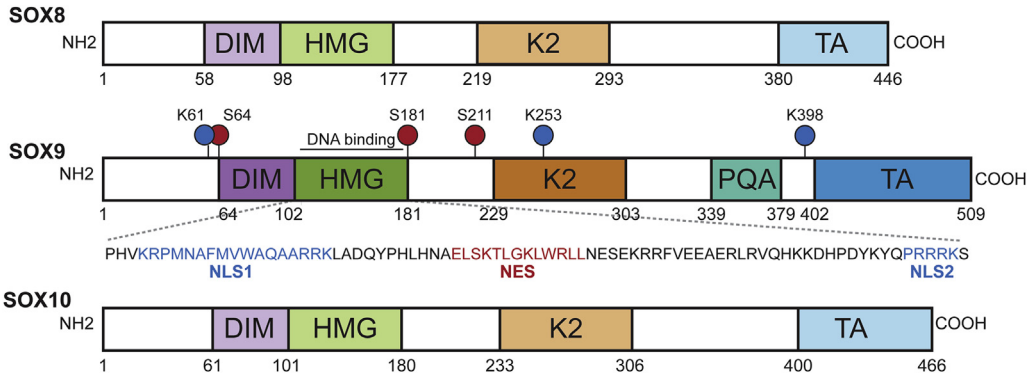

Figure 1 Schematic structures of SoxE proteins. In all SoxE proteins, the dimerization domain (DIM) precedes the DNA-binding high mobility group (HMG) domain and two separate transactivation domains are located in a central position (K2) and at the C-terminus (TA). For Sox9, two independent nuclear localization sequences (NLS) and the nuclear export sequences (NES) in the HMG domain, phosphorylation sites (red), and ubiquination/sumolyation sites (blue) are highlighted.[3,7].

Sox-binding site, as in the case with the homodimer-binding sequences on the enhancer regions of chondrogenic genes.[22,23] It is inferred that a Sox-partner complex forms first, and then recognizes target DNA sites as a complex.[23]

Whether Sox9 elicits transcriptional activation or repression depends on the target site, partner factors, and the subsequent recruitment of either co-activators or repressors (Fig. 2B). During hypertrophic chondrocyte maturation, Sox9 recruits *Gli* protein as the partner factor, and the complex represses the gene transcription of *Col10a1*, the gene that is required for chondrocyte maturation.[24] On the other hand, a Sox9 dimer recruits SoxD (Sox5/6) dimers to activate *Col2a1*, which is required for chondrogenic differentiation and extracellular matrix (ECM) deposition.[25]

One of the advantages of Sox-partner interactions is that they allow for stepwise progression of developmental processes (Fig. 2C). For instance, Sox-partner complexes can activate a second Sox gene that acts downstream, employing the same partner factor. In male gonad, Sry and steroidogenic factor-1 (Sf1, also known as *AD4BP*) form a complex to induce Sox9 expression, and this newly transcribed Sox9 partners with Sf1 to promote subsequent development processes. This self-pertetuating pathway helps maintain continued Sox9 expression, even after that

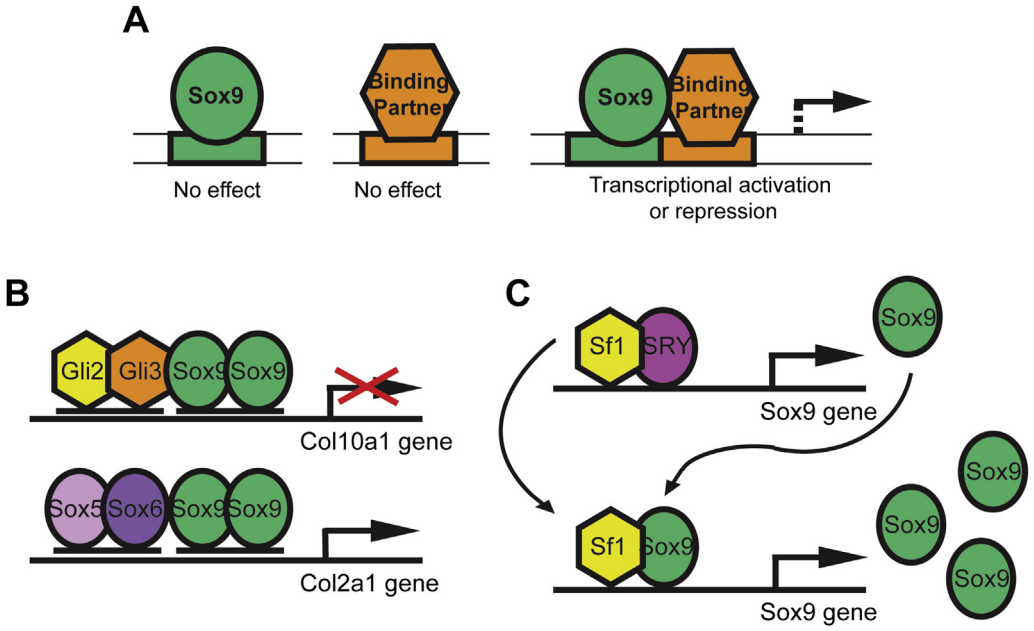

Figure 2 Regulation by Sox9-partner complexes. A) Sox9 requires a binding partner to elicit either transcriptional activation or repression.[22,23] B) Sox9 can function to activate or repress transcription, depending on the partner factor and on the tissue in which it is expressed. During earlier chondrogenesis, Sox9-Gli2/3 complex represses Col10a1 while the "sox trio," Sox9-Sox5/6 complex, activates Col2a1.[24,25] C) Sox-partner complexes form a feedforward, self-reinforcing pathway. During male gonad genesis, Sf1 and SRY cooperatively upregulate Sox9 and then, together with Sf1, Sox9 maintains its own expression.[26].

of SRY has ceased.[26] Knowing how such binding partners work is important for the ensuing discussion on diverse functions of Sox9 in different organs and tissue.

The roles of Sox9 in mesoderm development

Sox9 in chondrogenesis and skeletal development

During chondrogenesis and endochondral ossification, mesenchymal cells condense and differentiate into chondrocytes in a pattern that will define the eventual shape of the skeletal elements.[27,28] In this process, Sox9 is essential for mesenchymal condensation prior to chondrogenesis, and for inhibiting hypertrophy. Inactivation of *Sox9* in chondrocytes at different stages of differentiation suggests that its expression is essential for the survival of chondrocytes to progress to hypertrophy.[25] Upon hypertrophy, the chondrocytes down-regulate *Sox9* expression to allow for vascular invasion and bone marrow formation.[29]

Sox9 activates many genes in proliferating chondrocytes, including the ECM genes *Col2a1*, *Col9a1*, *Col11a2* and *Acan* (aggrecan).[30] Sox9 directly trans-activates *Col2a1*, the collagen II gene that is expressed most strongly in proliferating chondrocytes, *in vivo* via a conserved enhancer sequence within the first intron.[31] In addition to trans-activating genes expressed in non-hypertrophic chondrocytes, Sox9 directly represses expression of *Col10a1* just prior to the onset of hypertrophy.[24] Given the importance of Sox9 in chondrogenesis, it was reported that Sox9 may be explored as an important biofactor to treat or prevent intervertebral disc degeneration.[32] The versatile functions of Sox9 in developmental and homeostatic processes are shown in Fig. 3, and the related signaling pathways are summarized in Table 1.

Sox9 in male gonad genesis

In mammals, *Sry* on the Y chromosome initiates the testis differentiation program, and Sox9 carries out the process by specifying the Sertoli cell lineage. The role of Sox9 in testis formation and subsequent sex determination was first recognized by genetic analysis of human campomelic dysplasia, in which about 75% of XY males with one mutant Sox gene exhibit male-to-female sex reversal.[42] Similarly, duplicate Sox9 genes have been linked with male gonad genesis even in karyotypically XX subjects.[43] In the male gonad, the combination of Sry and Sf1 initiates Sox9 expression, which is continued even after *Sry* expression disappears in positive auto-regulatory feedback loops.[26] In the female gonad, on the other hand, Sox9 expression disappears due to the lack of *Sry* expression.[42] Sox9-axis signaling induces ovary—testis transition in zebrafish, suggesting that its role in sex reversal is conserved.[44]

To complete gonad genesis, Sox9 recruits different binding partners to elicit two separate trans-activating functions.[45,46] In the former, Sox9 homodimerizes to activate prostaglandin D synthase (*Ptdgs*), the gene that encodes an enzyme responsible for producing prostaglandin D2 (Pgd2). Pgd2 then recruits cells of the supporting lineage to become Sertoli cells.[45] In the latter, the Sox9-Sf1 complex upregulates anti-Mullerian hormone (AMH) in a cyclic AMP-dependent manner, which inhibits the development of the female Mullerian ducts.[46] Sox8 is also important for testis cord differentiation. In mice, an experiment using *Sox9* conditional knockout on a *Sox8* mutant background showed that Sox8 expression follows that of Sox9, being required for the maintenance of testicular function at a later stage.[47] However, the regulation of AMH by SoxE proteins is not conserved in mice and chickens. In the developing chicken, AMH is expressed one day before Sox9, suggesting that another AMH activating factor exists, and Sox8 is expressed at similar levels in both sexes during the sex-determining period.[48,49]

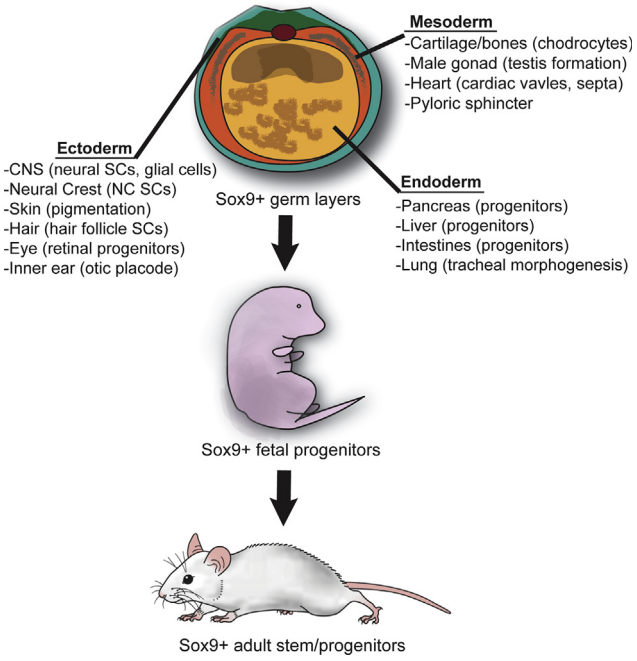

Figure 3 Sox9 expression in pluripotent, fetal, and adult stem and progenitor cells. Sox9 is expressed throughout development, initially in pluripotent founder cells and subsequently in ectodermal, endodermal, and mesodermal derivatives. Sox9 expression is maintained in fetal and adult tissues derived from Sox9+ fetal progenitor cells and also in differentiated cells in some cases.[26—32,39,40,42—101].

Sox9 in other mesoderm tissues: cardiac valves/septa, and pyloric sphincter

In the heart, Sox9 is highly expressed in cardiac cushion cells, and is required for the normal development of valves and septa.[50] Furthermore, Sox9 is required for precursor cell expansion and ECM organization during mouse heart development.[51] In these instances, Sox9 seems to promote epithelial-mesenchymal transition (EMT) after delamination and initial migration of endocardial endothelial cells.[50] Given the significance of EMT in fibrosis and cancer prognosis, there is much consideration about the relevance of Sox9 in these diseases.[52]

In the pyloric sphincter, a structure that demarcates the stomach from the duodenum, Sox9 is important in specifying its epithelium. Misexpression of Sox9 in the mesoderm of the stomach inhibits the differentiation of the gastric

Table 1 Signaling pathways that regulate Sox9 during development and in human diseases.

Key factors	Mesoderm	Ectoderm	Endoderm
Hh	Sonic hedgehog (Shh) upregulates *Sox9* to generate chondrogenic precursors[33]; Indian hedgehog (Ihh) upregulates Sox9 for proliferation and maturation of chondrocytes[34]	N/A	Upregulates *Sox9* to modulate *OPN* in liver fibrosis[35]
Wnt/β-catenin	Wnt5 upregulates *Sox9* during early stages of chondrogenesis and inhibits it during chondrocyte maturation[36,37]; Sox9 interacts with β-catenin to inhibit its transcription[38]	Phosphorylates Sox9 for NC cell delamination along with BMP[12]	Upregulates *Sox9* for intestinal SC proliferation and Paneth cell differentiation[41]; upregulates Sox9 to inhibit villus maturation[96]
Notch	Inhibits *Sox9* expression *in vivo* and *in vitro*[102]; upregulates *Hes1* and *Hey1*, which compete for Sox9 binding of the *Col2a1* enhance to prevent Sox9-mediated activation[103]	Induces Sox9 expression for stem cell maintenance and strogliogenesis[55]; regulates Sox9 and Hes1 in the developing and mature retina[67]	Regulates *Sox9* in the liver development[81]; regulates Sox9 in dose-dependent manner to induce *Ngn3* for pancreatic endocrine and ductal cell differentiation[75]
TGF-β	Upregulates Sox9 and *Smad3*,[36] and activates Sox9 *in vitro* to mediate chondrogenic commitment[104]	N/A	Induces *Sox9* expression to inhibit hepatogenic differentiation potential of ADHLSCs[105]
NFκB	Reduces Sox9 activity and cartilage gene expression by converging with RAR pathway[106]; RelA activates *Sox9* for chondrogenic differentiation[107]	N/A	Epigenetically regulates *Sox9* in pancreatic cancer stem cells[108]
BMP	BMP2 induces chromatin remodeling, and modifies the *Sox9* promoter[109]; BMP4 upregulates Sox9 in semilunar valve cells[110]; upregulates *Sox9* and *Nkx2.5* to determine the pyloric sphincter epithelium[54]	Phosphorylates Sox9 with Wnt for NC cell delamination along with Wnt[12]	Induces Sox9 expression in endoderm and pancreatic lineage differentiation along with Activin and FGF pathway[111]
Fgf	Fgf9 upregulates *Sox9* to induce endochondral ossification[112]	Activates Sox9-Sox10 pathway for branching morphogenesis of mouse ocular glands[113]; upregulates Sox9[140]	Creates feed-forward loop to maintain pancreatic organ identity[114]

epithelium into pyloric sphincter-like epithelium.[53] Similarly, another finding showed that Sox9 is regulated by BMP signaling in the pyloric sphincter, a pathway involved in epithelial-mesenchymal interactions for organ-specific morphogenesis of the gut tube.[54]

The roles of Sox9 in ectoderm development

Sox9 in neural stem cells (NSCs), gliogenesis, and neural crest (NC) stem cells

Sox9 regulates wide-ranging aspects of development in the central nervous system (CNS) and in neural crest (NC). Gain- and loss-of-function studies indicated that, during the CNS development, Sox9 is necessary and sufficient to initiate the induction of embryonic and adult neural stem cells.[55,56] Moreover, Sonic Hedgehog (SHH) induces *Sox9* expression, which in return stimulates precocious generation of NSCs.[56]

In the CNS, Sox9 drives the differentiation program away from neurogenesis and towards gliogenesis.[8,55,57,58] Sox9 expression in NSCs continues in glial cells, but not in neurons. A study using *Cre/loxP* recombination system that ablates *Sox9* expression showed that, in the developing spinal cord, Sox9 elicits the specification of myelin-forming oligodendrocytes and astrocytes, the two main types of glial cells in the CNS.[59] For glial initiation, Sox9 recruits the transcription factor *NFIA* as a binding partner to co-regulate migratory and metabolic genes in astrogliogenesis, such as *Apcdd1* and *Mmd2*.[57] Importantly, Sox9 and Sox10 play redundant functions in survival and migration of oligodendrocyte precursors.[8] Notch1 seems to be a part of the upstream pathway in astrogliaogenesis and stem cell maintenance, as demonstrated in the studies involving transient activation and knockdown of Notch1 during neuroectodermal differentiation.[55]

Neural crest is a population of multipotent stem cells derived from dorsal neural folds at the border between neural and non-neural ectoderm in the vertebrate embryo. Once induced, neural crest cells undergo epithelial-mesenchymal transition (EMT), delaminate from the neural tube, and migrate into the periphery to give rise to multiple differentiated cell types.[60] Sox9 plays a crucial role in NC development, and is required for NC progenitor

specification. Forced expression of Sox9 promotes neural crest-like properties in neural tube progenitors at the expense of CNS neuronal differentiation, and in migratory NC cells, SoxE expression guides NC stem cells towards a glial cell fate.[61] As in the heart, Sox9 is important for EMT (Fig. 4A). In avian neural tube, Sox9 is essential for BMP signal-mediated induction of *Snail2* and subsequent EMT, and cotransfection of Sox9 and Snail2 is sufficient to induce ectopic EMT.[12,39] In *Xenopus*, however, Sox9 is required only for neural crest specification but not migration,[40] implying that the fates of NC progenitors are not conserved between species.

Sox9 in hair follicle stem cells (HF-SCs)

The function of Sox9 in HF-SCs was first noted in the HF bulge, an adult-specific stem cell niche that provides an appropriate microenvironment to preserve the proliferative potential of hair follicles.[62] Although the study by Vidal et al in 2005, which will be discussed in the later section, established the importance of HF-SCs in adult life, whether SCs exist or function earlier during development was largely unknown.[63] However, the findings by Nowak et al in 2008 demonstrated that HF-SCs are formed at earlier stages and that the niche formation dependent on Sox9. In this newer study, embryonic ablation of *Sox9* using *Sox9-Cre* genetic marking and K14-*Cre* led to a reduced number of Sox9-expressing cells in all skin epithelial lineages. In the absence of early SCs, hair follicle and sebaceous gland morphogenesis is blocked and epidermal wound repair is compromised.[64]

Sox9 in other ectodermal tissues: retinal progenitor cells (RPCs) and otic placode

In the retina, Sox9 maintains a multipotent pool of retinal progenitor cells (RPCs), playing a role similar to that in the CNS. In multipotent murine RPCs, *Sox9* is expressed throughout retinogenesis, and is continuously expressed in Muller glial cells into adulthood.[65] *Sox9* is induced by Notch signaling during retinal development, and once expressed, it recruits binding partners such as microphtahlmia-associated transcription factor (MITF) and OTX2 to maintain retinal pigment epithelium (RPE).[66,67] During this process, another SoxE protein, Sox8, and a SoxB protein, Sox2, play compensatory roles.[68]

In inner ear, Sox9 plays essential functions, although its roles vary among species. In *Xenopus* and zebrafish, Sox9 is required for initial specification of the otic placode. A morpholino antisense oligonucleotide-mediated depletion of Sox9 in *Xenopus* results in loss of early otic markers and failure of otic vesicle development, and overexpression of Sox9 leads to enlarged or ectopic otic vesicles.[13,69] Similarly in zebrafish, loss of Sox9a and Sox9b results in absence or severe reduction of the otic vesicle.[12] On the other hand, in mice, Sox9 is not required for initial specification of the otic placode but instead controls adhesive properties and invagination of placodal cells.[70] Interestingly, covalent attachment of SUMO to Sox9 by gene fusion inhibits expression of neural crest markers but increases expression of markers of inner ear development, suggesting that the posttranslational SUMOlyation may act as a switch.[13]

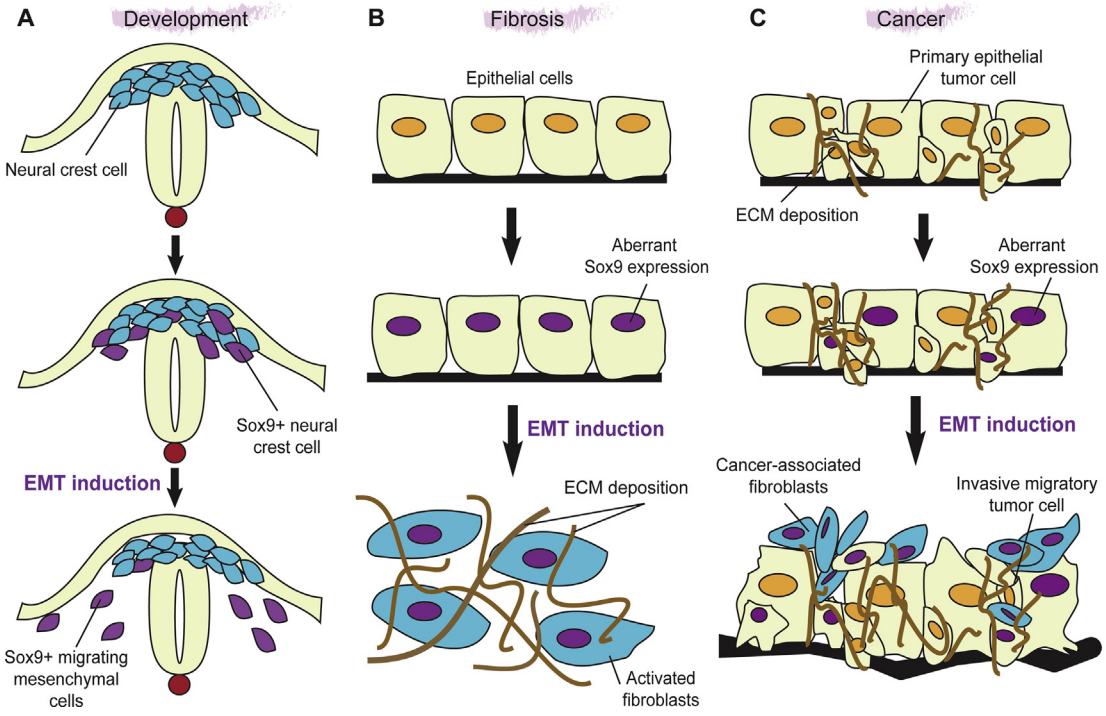

Figure 4 EMT induction by Sox9 in acquired diseases. A) Sox9 is involved in epithelial-mesenchymal transition (EMT) for neural crest delamination during development.[12,39,40] B) Sox9 plays a role in excess extracellular matrix (ECM) deposition and EMT,[25] which may be related to fibrosis. C) Sox9 is important for ECM deposition and EMT,[12,25,39,40] which implies its role in tumor formation and invasive metastasis.

The roles of Sox9 in endoderm development

Sox9 in pancreas, liver, and intestine

During embryonic development in mammals, the upper digestive tract organs—the liver, pancreas, and duodenum—are derived from the primitive foregut endoderm, and share Sox9 expression in their progenitor populations. The pancreas has two different secretory structures—endocrine and exocrine—that originate from different sources of progenitors.[71] Sox9 is necessary for normal pancreas development, as pancreas-specific Sox9 depletion result in severe pancreas hypoplasia.[72] In line with abundant cases of pancreatic endocrine impairments in patients with campomelic dysplasia (CD), the endocrine lineage seems to be more sensitive to Sox9 than the exocrine lineage.[73] Experimentally reducing *Sox9* gene expression to 50% in mouse pancreatic progenitors led to a reduction in endocrine progenitors expressing neurogenin 3 (*Ngn3*), a gene necessary and sufficient for establishing an endocrine cell fate.[74] During this process, *Notch/Ngn3/Hes1* signaling regulates Sox9 in a dose-dependent manner. Too little or too much Notch results in tight regulation of Sox9: activated Ngn3 downregulates *Sox9* expression in the endocrine cell compartment, and with high Notch activity, the transcription factor hairy and enhancer of split-2 (Hes1) represses *Ngn3*.[75] In exocrine pancreatic development, a similar mechanism seems to be at work, with pancreas-specific transcription factor 1a (Ptf1a) substituting for Ngn3.[76]

Bile ducts are structures within the liver that produce and secrete bile, and are divided into intrahepatic (within the liver) or extrahepatic (outside the liver).[71] During liver development, Sox9 is expressed not in hepatocytes, the cells that secrete bile, but instead in cholangiocytes and mucin-producing cells that line the extrahepatic bile duct.[77] Notably, studies using lineage tracing with *Sox9-IRES-Cre* knock-in mice and BAC *Sox9-CreER* transgenic mice demonstrated that embryonic Sox9+ cholangiocytes could differentiate into hepatocytes.[78,79] Evidence suggests that Sox9 determines the timing of bile duct morphogenesis: after a maturation step, the biliary tube is entirely composed of Sox9+ cholangiocytes, and embryonic liver-specific inactivation of *Sox9* results in delayed duct maturation.[80] In addition, Notch seems to regulate *Sox9* in this process, as seen in the etiology of Alagille syndrome, a genetic disorder in the liver, heart, kidney, and other systems of the body caused by mutations in Notch pathway.[81]

In normal intestinal epithelium, Sox9 is localized to the nuclei of crypt cells, including terminally differentiated Paneth cells, stem cells, and a subset of transit-amplifying (TA) cells.[82] Functionally, Sox9 suppresses proliferation in mouse intestinal epithelium *in vivo*, and inactivation of *Sox9* results in increased proliferation.[82,83] A recent report demonstrated that Sox9 regulates insulin-like growth factor (IGF)-binding protein 4 (*IGFBP-4*), an inhibitor of the IGF/IGF-receptor pathway in cell proliferation.[84]

Sox9 in lung

The discovery of *Sox9* expression in bronchial epithelium, and neonatal deaths of CD patients due to respiratory distress, first hinted at the significance of Sox9 in lung development.[85] However, there are conflicting results regarding the role of Sox9 in the lung epithelium. Specific inactivation of Sox9 in respiratory epithelial cells of the mouse lung using a doxycycline-inducible *Cre/loxP* system leads to normal lung structure, postnatal survival, and repair following oxygen injury.[86] However, other studies suggest that Sox9 is required for proper lung morphogenesis; loss of Sox9 leads to extracellular matrix defects, cytoskeletal disorganization and aberrant epithelial movement.[87,88] Another finding suggests that the role of Sox9 is crucial in tracheal development, as transgenic mice lacking Sox9 expression have morphological defects in the trachea, are unable to breathe, and die at birth.[89] These contrasting reports on Sox9 regulation of lung epithelial lung branching may be due to the different genetic backgrounds of the mice.

The roles of Sox9 in adult tissues

To maintain homeostasis of an adult organ, either in the physiological state or a regenerative state after injury, an orchestrated mechanism ensures correct cell type and tissue architecture. *Sox9* expression during development continues in adult stem and progenitor cells, and seems to be crucial in adult tissues. Here, we review recent data linking Sox9 with adult stem/progenitor cell maintenance and specification.

Sox9 in ectoderm-derived tissues: NPCs, retina, HF-SCs, and skin pigmentation

In the CNS, Sox9 continues to play a necessary role in the maintenance of multipotent NPCs throughout adult life, as shown by *in vivo* fate mapping experiments in the adult subependymal zone and olfactory bulbs.[56] In the retina, Sox9 is crucial for retinogenesis, but continues to maintain these differentiated Muller glial cells postnatally as a result of Notch regulation, shown in a conditional knockout approach.[65,67] In addition, Sox9 expression in Muller glial cells persists in the adult tissues.[65]

In mature retinal pigment epithelium (RPE) cells, Sox9 acts synergistically with transcription factors orthodenticle homeobox 2 (OTX2) and the LIM homeobox family (LHX) to activate visual cycle genes by common miRNAs.[90]

Epithelial hair follicle stem cells (HF-SCs) reside in the "bulge" of the outer root sheath (ORS), and are essential for cyclic bouts of adult hair growth.[91] Sox9 is crucial in maintenance and differentiation of adult skin by HF-SCs; postnatal conditional ablation of Sox9 results in mice born with fragile, atrophic hair shafts, suggesting that Sox9 is expressed by adult HF-SCs in the bulge and also may be required for their survival.[63] Moreover, a recent study with conditional Sox9 targeting in adult HF-SCs demonstrated that Sox9 elicits an inhibitory function on epidermal differentiation in the SC bulge. While Sox9-defiecent HF-SCs transition from quiescence to proliferation and launch the subsequent hair cycle, they differentiate into epidermal cells rather than remaining as HF-SCs.[92]

Although many findings position Sox9 in stem cell homeostasis and regeneration during adult life, some

evidence reveals its role in cell fate specification. One such case is in skin pigmentation. In *Xenopus*, along with Sox10, Sox9 induces NC stem cells to differentiate into melanocytes.[93] Another finding showed that *Sox9* is upregulated by ultraviolet B exposure in adult and neonatal melanocytes. This regulation results in activation of microphthalmia-associated transcription factor, dopachrome tautomerase, and tyrosinase promoters, all of which contribute to increases in key melanogenic proteins and subsequent pigmentation.[94]

Sox9 in endoderm-derived tissues: intestines, liver and pancreas

Developmentally derived Sox9+ progenitors in upper digestive tract organs from primitive foregut—the liver, pancreas, and duodenum—carry over as imprints into adult life.[71] However, the extent of Sox9's effects within each organ varies considerably. While it is well established that Sox9+ progenitor zone serves as a continuous source of new tissues intestines, whether it has any physiological function in the adult liver and pancreas is still debated.

In intestinal epithelium, the most rapidly self-renewing tissue in adult mammals, a continuous supply of new cells and elimination of old cells preserves homeostasis at the top of the intestinal villi.[95] In colon epithelium-derived cells, Sox9 transcriptionally represses the *CDX2* and *MUC2* genes, normally expressed in the mature villus cells, and may therefore contribute to the Wnt-dependent maintenance of a progenitor cell phenotype.[96] In addition, Sox9 is required for differentiation of Paneth cells, which reside adjacent to the crypt's niche as post-mitotic, differentiated cells, and are important for maintaining epithelial cell renewal.[83] Taken together, Sox9 maintains the homeostasis of the intestinal epithelium both directly and indirectly.

In adult liver, tamoxifen-related toxicity in lineage tracing studies has complicated the interpretation of Sox9 expression hepatocytes.[79,97] More recently, tamoxifen-independent tracing experiments argued against physiologically functioning progenitors in ducts. They revealed that hepatocytes labeled with this virus-mediated induction method were maintained solely through proliferation, and that Sox9+ duct cells do not participate in maintaining adult organ homeostasis.[98] Interestingly, Sox9+ cells in the liver can be reprogrammed into insulin-secreting duct cells, implying that developmentally related cells can be modified to be used in a potential therapy for diabetes.[99]

In the adult pancreas, although Sox9 expression persists throughout the pancreatic ductal tree,[79] it is not clear whether these Sox9+ cells are physiologically active. Lineage-tracing experiments using BAC *Sox9-CreER* transgenic mice show that Sox9+ duct cells lose their differentiation ability within a few days after birth,[100] suggesting that adult Sox9+ duct cells do not function as stem/progenitor cells. A similar result was obtained from another lineage-tracing experiment, in which targeted adult ductal β cells failed to differentiate into functioning acinar/endocrine cells.[101] Moreover, pulse and chase experiments support the notion that adult pancreatic β cells and acinar cells are maintained by the self-duplication of preexisting cells rather than by differentiation from progenitors.[115,116] Taken together, most of these results refute the existence of stem/precursor cells in the adult pancreatic duct.

Sox9 in developmental disorders

Campomelic dysplasia (CD)

Campomelic dysplasia (CD) refers to a rare autosomal dominant skeletal dysmorphology syndrome characterized by congenital bowing of the limb long bones, a small, bell-shaped thoracic cage, and hypoplastic scapulae.[85] Other features not related to chondrogenesis include respiratory deficiencies with softening of the laryngo-tracheal cartilages, male-to-female sex reversal in XY patients, and a variety of congenital heart defects.[117] CD is caused by haploinsufficiency of *Sox9* due to deletions or mutations in or around the Sox9 gene.[6,42] Furthermore, disrupting the homodimerizing capacity of Sox9 has been linked to CD but not male-to-female sex reversal, indicating that homodimerization of Sox9 is required for proper cartilage formation but not for gonad formation.[23]

XY gonad dysgenesis

The role of Sox9 in gonad dysgenesis was first speculated due to a high proportion of male-to-female sex reversals in XY males with CD, as mentioned above.[85] This is logical considering that Sox9 is downstream of *Sry*, the gene that encodes a crucial factor in triggering Sertoli cell development. Ectopic expression of Sox9 in the female gonad of XX mice causes complete female-to-male sex reversal, demonstrating that Sox9 is sufficient to trigger testis differentiation in the absence of Sry.[118]

Hypertrichosis and alopecia areata

Sox9 has been implicated in hereditary disorders of hair growth. Hypertrichosis is a rare syndrome defined as excessive hair growth in a particular body area that is not hormone dependent.[119] Evidence suggests that *Trps1*, a gene associated with hypertrichosis in mice and humans, directly represses *Sox9*, and the absence of this gene activity results in premature proliferation of HF-SCs.[120] In a family with a history of hypertrichosis, a copy number variation upstream of *Sox9* showed decreased expression of HF genes.[120] On the other end of the spectrum is alopecia areata, a condition that causes characteristic patches of hair loss.[121] In mice, skin-specific knockout of Sox9 leads to the loss of hair shaft stem cells and causes similar bald patches.

Sox9 in acquired diseases

Sox9 in fibrosis, sclerosis and related disorders

One of the common characteristics among fibrosis, sclerosis, and related disorders is excessive, inappropriate extracellular matrix (ECM) deposition, and subsequent destruction of tissue architecture and function in response to injury.[122] Considering its role in ECM deposition, evidenced in

chondrogenesis, it seems logical that Sox9 has been implicated in the pathology of fibrotic diseases (Fig. 4B).

When damage occurs in the liver, a Sox9-dependent process causes hepatic stellate cells (HSCs) to proliferate into myofibroblasts, migrate to the surrounding parenchymal cells, and secrete ECM components for repair. In human fetal hepatocytes, aberrant induction of Sox9 causes ectopic expression of genes that encode the ECM components, *Col2a1* and *Comp1*, which are normally expressed during chondrogenesis. Inducing transforming growth factor-β (TGF-β) signaling in activated HSCs leads to Sox9 expression, and causes type1 collagen production.[123] Moreover, *in vivo* experiments using culture-activated HSCs posited Sox9 as a critical regulator of Osteopontin (OPN), an ECM component that is a biomarker for the severity of liver fibrosis. However, the same study also suggested that it is Hedgehog signaling, not TGF-β, that lies upstream of Sox9.[35]

In the kidney, high Sox9 expression is correlated with glomerulosclerosis. A microarray gene expression profiling diseased glomeruli showed strongly upregulated expression of Sox9.[124] In addition, highly elevated expressions of OPN and other TGF-β pathway-related genes were observed, suggesting that Sox9 activity is similar in both glomerulosclerosis and liver fibrosis.[124] In keeping with this finding, Sox9 appears to function downstream of TGF-β1 to activate *Col4a2* transcription in mesangial cells, the specialized cells that surround blood vessels in the kidney.[125]

Sox9 in tumorigenesis and cancer

Dysregulation of tissue differentiation pathways and stem cell homeostasis can contribute to the development and progression of cancer. Sox9 has been implicated in the formation and growth of tumors in prostate, the CNS, skin, pancreas, ovary, and esophagus.[126–135] It seems logical that the role of Sox9 in controlling progenitor cells, to either proliferate or differentiate during development and adult life, could actually promote neoplasia if dysregulated (Fig. 4C).

Studies in human and mice place Sox9 as a key player in prognosis of prostate cancers. In phosphatase and tensin homolog (PTEN)+/− mice, overexpression of Sox9 in adult mouse prostate epithelia induces an early high-grade prostate intraepithelial neoplasia (PIN) lesion, indicating that Sox9 augments the loss of PTEN to promote disease.[136] Furthermore, Sox9 levels are found to be increased in advanced lesions of human prostate cancer, and overexpression of *Sox9* in LNCaP prostate cancer xenografts enhances growth, angiogenesis, and tumor invasion.[134,137] One possible mechanism by which Sox9 functions here is by trans-activating the androgen receptor, as some prostate cancers are androgen-dependent.[135] However, one study showed that Sox9 suppresses growth and tumorigenesis in the prostate tumor cell line M12.[127]

Sox9 is also implicated in nervous system tumors. In glioma cell lines, siRNA knockdown of Sox9 reduced cell proliferation *in vitro*.[132] *In vivo*, Sox9 production is increased in malignant nerve sheath tumors, and repressing this expression by small hairpin RNA causes cell death in culture.[129]

In skin, Sox9 is expressed in basal cell carcinomas, and is detected in over 80% of melanomas.[130,133] Sox9 is thought to lie downstream of Sonic hedgehog (Shh) and Gli2 transcription factor both of which have been implicated in skin tumors.[63,138,139] However, another study demonstrated an inhibitory function of Sox9 in melanomas, as vector-derived Sox9 in both melanoma cell lines and xenografts decreased cell proliferation and tumor growth by direct upregulation of the cell cycle arrest gene, *p21*.[130]

In the pancreatic ductal system, clinical adenoma and carcinoma samples showed Sox9 overexpression localized to the bottom part of the crypts, suggesting that dysregulation of stem cell homeostasis may be responsible.[131] In addition, Sox9 accelerates the formation of precursor lesions of pancreatic ductal adenocarcinoma (PDA) when co-expressed with a PDA-initiating *Kras* mutation.[140] In adenocarcinomas, a potential NF-κB binding site was found in the *Sox9* promoter with NF-κB subunits up-regulating Sox9 expression, indicating that Sox9 is epigenetically regulated by NF-κB signaling pathway.[108]

Taken together, these data present opposing roles for Sox9 in tumors, either inducing or potentially inhibiting cell proliferation. It should be kept in mind that the difference between these studies could be attributed, in part, to the differences in cell lines and levels of Sox9. These factors should be controlled for in future experiments.

Concluding remarks and future directions

Most insight into the biological properties of Sox9 has come from developmental studies, particularly involving chondrogenesis and male gonad genesis. Recent molecular and functional analyses of Sox9 have documented an additional role in stem cell biology of mesoderm-, ectoderm-, and endoderm-derived tissues and organs. While Sox9 maintains adult stem and progenitor cells with high turnover, as in intestine and hair follicles, it is also crucial for postnatal injury repair in endodermic and ectodermic organs. Identifying partner factors, signaling pathways, and post-transcriptional modifications have provided a better understanding of Sox9's versatility in different tissues and at different stages in mammalian life. The availability of appropriate mouse models and the ability to maintain rare stem cell populations in culture, combined with genome-wide technologies, should now enable researchers to further address fundamental questions at the mechanistic level.

In human diseases, mutations in Sox9 can cause birth defects in skeletal deformity, male-to-female sex reversals, and hair growth, and has been implicated in fibrosis and cancer. In addition, recent findings regarding fibrosis and cancer correlate Sox9 with developmental roles in cell proliferation, extracellular matrix (ECM) deposition, and epithelial-to-mesehchymal (ETM) transition. However, conflicting results position Sox9 with opposing roles in tumorigenesis, as evidenced in melanoma studies. These discrepancies may be due to the differences in individual cancer cell lines or mouse models, with further investigation being warranted.

Immunostaining for Sox9 carries prognostic value in a wide range of tumors, including neurofibromatosis,

meduloblastoma, pancreatic cancer, and protstate cancer, and can aid in diagnosis. Moreover, Sox9 can be a potential target for novel therapeutic intervention that might compensate for the current lack of effective anti-fibrotic therapies and cancer treatments. However, Sox9 expression in many tissue types complicates cell-specific effects, which is why investigating this protein's diverse mechanisms and pathways is so important. Another challenge of using Sox9 therapeutically is modulating transcription factor levels. One potential way of reducing Sox9 could be to use small peptides or neutralizing antibodies to modify its function and expression. The manipulation of Sox9 levels might also be possible indirectly by modulating key molecules involved in upstream signaling pathways, such as TGF-β1, Wnt, and Hh signaling, all three of which have been linked to cancer and fibrosis.

In summary, accumulating evidence implicates Sox9 in pluripotent and multipotent stem cell biology and tissue regeneration, in addition to its role in cell fate decisions. A better understanding of the mechanisms by which Sox9 induces and maintains these stem cell populations should provide important insights into how tissue stem cells are regenerated and maintained, and might lead to new strategies for treating degenerative diseases and cancer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The investigators who reported the work were supported in part by the research grant from the National Institutes of Health (AR50142 to RCH). This work was also supported in part by The University of Chicago Core Facility Subsidy grant from the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health through Grant UL1 TR000430. SD was a recipient of The University of Chicago Pritzker Fellowship and AOA Carolyn L. Kuckein Fellowship.

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