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REVIEW

Pleiotropic function of SRY-related HMG box transcription factor 4 in regulation of tumorigenesis

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Abstract In addition to their critical roles in embryonic development, cell fate decision, and differentiation, members of Sox (Sry-related high-mobility group box) family of transcription factors including Sox4 have been implicated in various cancers. Multiple studies have revealed an increased expression along with specific oncogenic function of Sox4 in tumors, while others observed a reduced expression of Sox4 in different types of malignancies and suppression of tumor initiation or progression by this protein. More interestingly, the prognostic value of Sox4 is debated due to obvious differences between various reports as well as inconsistencies within specific studies. This review summarizes our current understanding of Sox4 expression pattern and its transcription-dependent, as well as transcription-independent, functions in tumor initiation or progression and its correlation with patient survival. We also discuss the existing discrepancies between different reports and their possible explanations.

Keywords Cancer · Sox4 · Oncogene · Tumor suppressor

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Introduction

Sox family of transcription factors

The Sox (SRY-related HMG box) gene family is found throughout the animal kingdom. In humans, at least 20 members of this family have so far been identified [1], showing a diverse and dynamic pattern of expression throughout embryogenesis and in a variety of adult tissue types [2]. Sry (for sex-determining region Y), the founding member of this family, was identified through searches for conserved sequences among translocated Y chromosomal DNA from XX male patients [3], and was later confirmed to be involved in male sex differentiation [4].

All Sox proteins are characterized by possession of a high mobility group (HMG) DNA-binding domain. The 79-amino-acid HMG domains bind to the consensus target sequence (A/T)ACAA(T/A) in the minor grooves of DNA [2] and modify the chromatin structure to generate a conformation that facilitates various DNA-dependent activities. This domain is shared with other DNA binding proteins, including those that bind DNA without sequence specificity, such as HMG-1 protein and ubiquitous binding factor (UBF), as well as several sequence-specific DNA-binding proteins, such as the T cell-specific factors TCF/LEF [5]. The DNA-binding domains of Sox proteins are at least 60 % similar or 50 % identical to the HMG box domain of SRY [6]. The nomenclature of this family is based on the order of gene discovery [7]. Nine of the 20 human *Sox* genes contain a single exon, likely reflecting the mechanism of expansion of this ancient gene family via non-tandem duplication and retroposition [7]. This family is sub-grouped into six distinct classes (A–F), based on homology within the HMG domain and other structural motifs as well as functional properties. These classes

include: A, Sry; B, Sox1, -2, -3, -14, -15, and -19; C, Sox4, -11, -12, and -20; D, Sox5, -6, and -13; E, Sox8, -9, and -10; F, Sox7, -17, and -18 [6].

Despite this common feature, each Sox protein selectively interacts with and regulates a unique set of target genes [8]. It appears that Sox proteins are able to bind to a large number of transcription factors [9] and on many occasions cooperate with them to exert their regulatory function. For instance, Sox6 was shown to suppress cyclin-D1 promoter activity by interacting with β-catenin and HDAC1 in pancreatic β-cells [10]. Recruitment of HDAC1 to the promoter regions of the target genes such as *DCT* and *MITF* by Sox5 and suppression of their expression was also observed in melanocytic cells [11]. On the other hand, Sox10 physically interacts with MEF2C and cooperatively activates the promoter of the *Mef2c* gene [12].

Since the discovery of SRY, many other Sox family members have also been implicated in the regulation of critical functions in various developmental processes, such as sex differentiation, neurogenesis, skeletogenesis, hematopoiesis, angiogenesis, cardiogenesis, melanogenesis, and hair development. Interested readers are referred to recent reviews on this topic [13–15].

Sox4

The human *Sox4* gene was first identified based on homology with its mouse homologue [5]. The location of *Sox4* was determined by metaphase fluorescence in situ hybridization (FISH) at chromosome 6p.23 [5]. Sox4 contains a single exon and its open reading frame (ORF) encodes a protein of 474 amino acids with a molecular weight of 47 kD [6]. The encoded protein is particularly rich in serine residues (18 % overall) with several poly-serine stretches. It also includes multiple stretches of glycine and alanine residues [5].

Sox4 belongs to the class C (SoxC), which contains two other members; Sox11 and Sox12 [1]. In 2- to 3-day-old mice, SoxC genes are expressed at high levels in the brain. Sox4 and Sox12 are also expressed at a high level in the heart and lung. While Sox11 is expressed in developing limbs, face, and kidneys of the mouse embryo, it is only detectable in considerable amounts in neuronal tissues [16] and is not expressed in detectable amounts in tissues of adult mouse, perhaps due to a general decrease in Sox11 expression during late embryogenesis [17].

All SoxC proteins have a high degree of similarity in the HMG domain. The HMG box is 84 % identical and 95 % similar among SoxC proteins across all vertebrates, whereas their TADs share 67 % identity and 94 % similarity [16]. The structure of the sequence-specific HMG domain of Sox4 provided some insight into its mode of function [18]. This domain has an L-shaped structure consisting of three α-helices connected by loop regions, which are stabilized by a highly conserved cluster of mainly aromatic residues. These hydrophobic cores are mostly conserved within the HMG box family. Helices I and II are positioned in an anti-parallel mode and form one arm of the HMG box, while helix III, which is less rigid, forms an average angle of 90° with the other two helices and constitutes the other arm of the molecule [18]. The N-terminus of the HMG box interacts with the first 6 base pairs of the target sequence, and binding of the HMG box to the minor groove of a straight DNA helix in this manner introduces a sharp bend (on the order of 90°) in the DNA helix and alters the local chromatin conformation [18], which is crucial for its transcriptional activity.

Another distinctive feature of the SoxC class is a conserved proline, serine, and acidic residues-rich transactivation domain (TAD) at the C-terminal [16]. Protein secondary structure modeling predicted that 20 residues in the Sox4 C-terminal domain form an α-helix which is interrupted in the middle of its sequence by three randomly coiled residues, and have the last two residues in extended conformation rather than in helical conformation [16]. This domain is crucial and sufficient for the activity of SoxC proteins and its deletion completely abrogates their transactivation capacity [16]. The Sox4 TAD shows stronger transactivating capacity than Sox12 but weaker than that of Sox11 [16, 17] due to the more stable α-helix structure of Sox11 TAD [16]. The transactivating function of Sox4 TAD is independent of its HMG domain which is responsible for DNA binding, as evident from its sustained activity upon grafting onto a GAL4 DNA-binding domain [19]. SoxC proteins seem to compete with each other to bind to their target sequences, since the activity of any full-length SoxC protein is inhibited by expressing equivalent amounts of Sox4, -11 or -12 proteins lacking the TAD [16]. The exact mechanism by which TAD regulates transcriptional activation of target genes is not fully understood. One possible mechanism is that it is achieved through direct interaction with other transcription factors such as p53 [20] and β-catenin/TCF4 complex [21]. In melanoma cells, Sox4 TAD also interacts with syntenin resulting in prevention of proteasomal degradation of Sox4, therefore enhancing Sox4 stability [22].

In addition to HMG (aa 57–136) and TAD (aa 441–474) domains, Sox4 also contains a glycine-rich region (aa 152–227) at the center and a serine-rich region (SRR, aa 333–397) adjacent to the TAD (Fig. 1). A study of HEK293 cells revealed that the central domain containing a glycine rich region has pro-apoptotic activity which is independent of the transcriptional activity of Sox4 [23].

Sox4 in development; jack of many trades

Similar to many other members of the family, the SoxC class has also been identified as a necessary factor in

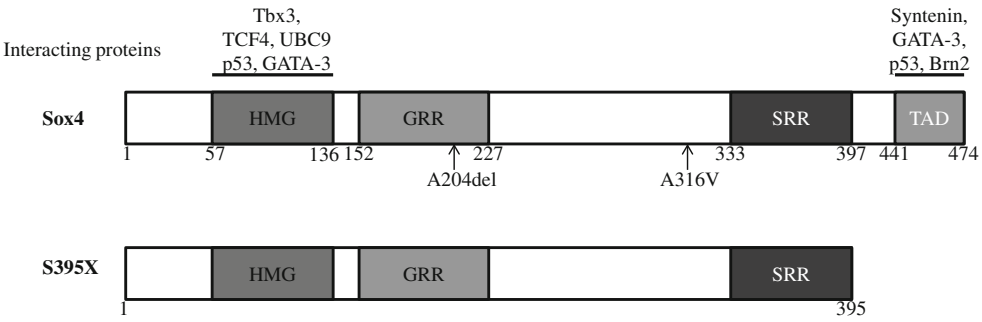

Fig. 1 Structure of the human Sox4 protein, its binding partners, mutations and truncated isoform (S395X) detected in human lung cancer. *GRR* glycine-rich region, *SRR* serine-rich region, *TAD* transactivation domain

embryonic development. In developing mice, Sox4, -11, and -12 are co-expressed at high levels in neuronal and mesenchymal tissues [16]. The absence of overt phenotype in Sox12$^{-/-}$ mice suggests that at least some functions of Sox4, -11, and -12 are redundant and Sox4 and -11 may compensate the loss of Sox12 during mouse development [24]. Consistently, Bhattaram and colleagues showed that concomitant loss of *SoxC* genes confers a worsened phenotype in mouse embryo than loss of each single gene [25]. Indeed, the more *SoxC* genes that are deleted, the more severe and widespread is organ hypoplasia [25]. SoxC proteins control neural and mesenchymal cell survival as shown by increased cell death in the neural tube, branchial arches, and somites of the *Sox4*$^{-/-}$*11*$^{-/-}$ embryos. The pro-survival role of SoxC proteins in these tissues is probably through direct activation of Tead2, a transcriptional mediator of the Hippo signalling pathway which can promote cell survival during organogenesis [25]. However, not all functions of the SoxC proteins seem to be redundant. Accordingly, *Sox4* knockout mice die halfway through gestation (E14) due to severe heart defect as a result of impaired development of the endocardial ridges into the semilunar valves and the outlet portion of the muscular ventricular septum [26] which indicates that Sox11 or -12 cannot compensate for the lack of Sox4 expression in this tissue. The critical role of Sox4 in development of cardiac tissues may at least partially be due to its role in regulation of expression of the gap junction protein, connexin 43 (Cx43), in cooperation with the Tbx3 transcription factor [27].

Sox11$^{-/-}$ mice die at birth from ventricular septation defects and outflow tract malformations resulting in heart defects, in addition to malformation in other organs such as skeleton, lung, stomach, and pancreas [28]. The fact that Sox4 knockout embryos die at E14 and the *Sox11*$^{-/-}$ mice complete the fetal stage indicates that Sox4 has more important roles in early and Sox11 in late embryogenesis. Interestingly, *SoxC*-null embryos will arrest in development at E8.5 [25], earlier than the embryos lacking each individual SoxC member, further confirming that these genes have partial overlap in their function which results in exaggerated phenotype upon further loss of each gene.

Sox4 is expressed in the thymus, bone marrow, and gonads of adult mice [29], which highlights its possible function(s) in hematopoiesis. Early studies showed that Sox4 is highly expressed in thymocytes and induces their differentiation while promoting pro-B lymphocyte expansion [29, 30]. Study of hemopoiesis in lethally irradiated mice reconstituted with *Sox4*$^{-/-}$ fetal liver cells demonstrated that the absence of Sox4 specifically blocks development of B cells at the pro-B cell stage [26]. Consistently, Sox4-null fetal liver progenitors give rise to all hemopoietic lineages except the B-cell [29]. The pro-B cells in these mice are moderately reduced in number, but their capacity to proliferate in response to IL-7 is strongly abrogated [29]. Sox4 regulates expression of the *λ5* and *VpreB1* genes which form part of the surrogate light chain and are expressed in pro- and pre-B cells. λ5 and VpreB1 expression is initially regulated by Sox2 in the pluripotent cells of the inner cell mass (ICM) of the developing mouse blastocyst, which is then replaced by Sox4 as the cells differentiate toward a very early progenitor for the hematopoietic and endothelial lineages, the hemangioblasts [31].

The absence of Sox4 expression may also have some subtle effects on thymocyte development. In fact, thymi from Sox4-null embryos contain two- to four-fold fewer cells than thymi from their wild-type or heterozygous counterparts [29]. Furthermore, maturation in fetal thymic organ culture of mutant thymi is impaired, indicating that absence of Sox4 blocks development of T cells from their progenitors [29]. A recent study by Kuwahara and colleagues [32] revealed that Sox4 inhibits the function of GATA-3, the master regulator of T helper type 2 (Th2) cells, therefore suppressing their differentiation and Th2 cell-mediated inflammation. In these cells, Sox4 acts as a downstream factor of TGF-*β* and upon activation directly binds to GATA-3 and prevents its binding to GATA-3 consensus DNA sequences. In addition, Sox4 binds to the promoter region of the interleukin 5 (*IL-5*) gene and prevents binding of GATA-3 to this promoter, thereby suppressing its expression [32]. It had been previously observed that, during hematopoiesis, the IL-5 receptor activates Sox4 through syntenin-1 [33], perhaps by suppressing proteasomal degradation of Sox4 protein [22]. It is

not clear whether these observations, which were made in different cell types, could indicate the possible existence of a negative feedback loop between Sox4, IL-5, and IL-5R or could simply represent different modes of interaction between these factors in different cell types.

Sox4 is important for the development of the central nervous system. In fact, expression of Sox4 and Sox11 is critical for the establishment of pan-neuronal protein expression [34]. These proteins act as transcriptional activators downstream of proneural basic helix-loop-helix (bHLH) proteins to exert this function. Sox4 and Sox11 exhibit highly overlapping expression in the mouse hippocampal neurogenic lineage, and overexpression of either suffices to induce neuronal marker expression in adult neural stem cells. Consistently, loss of Sox4/Sox11 expression results in loss of expression of neuron-specific protein DCX, a microtubule-associated protein [35]. Sox4 and Sox11 are also critical for specification of corticospinal neurons partially due to the important role of their downstream transcription factor Fezf2 in this process [36]. Interestingly, this function of Sox4 and Sox11 is mediated by direct competition with Sox5 which acts as a repressor of Fezf2 expression [36]. It is also suggested that Sox4 expression in oligodendrocyte precursor cells may induce premature differentiation of these cells. In fact, prolonged expression of Sox4 in vivo reduces myelin gene expression in oligodendrocyte linage which consequently interferes with normal myelination in the central nervous system [37]. Interestingly, expression of Sox4 and Sox11 in neural progenitor cells is restricted by REST/NRSF [34]. REST/NRSF is a transcriptional repressor which restrains the neurogenic program in progenitor cells [38], and repression of Sox4 and Sox11 by this protein may contribute to the mechanism by which REST/NRSF prevents expression of neuronal proteins in these cells.

Other than the nervous system, Sox4 has a number of important roles in other developmental processes. During mouse development, Sox4 expression is more confined to the pancreatic epithelium and later islet cells along with Sox9 expression [39]. Sox4b (an isoform of the zebrafish homologue of Sox4) is also a key player in pancreatic alpha cell differentiation [40]. In adult mice, Sox4 is broadly expressed in the early pancreatic buds and in the nuclei of all islet cells [41]. Homozygous deletion of *Sox4* did not show any abnormalities in pancreas development up to embryonic day 12.5; however, a significantly reduced number of endocrine cells were found to be scattered through the culture site, causing failure to form normal islets [41]. This phenomenon significantly affects normal pancreatic function, as it has been proven that *Sox4* deletion in adult mouse results in a considerable defect in insulin secretion and leads to impaired glucose tolerance [42]. The other important role of Sox4 in the developmental process was described in the musculoskeletal system. Indeed, 3-month-old $Sox4^{+/-}$ mice have a 64 % lower bone formation rate compared to wild-type, probably due to significant defects in osteoblast proliferation, differentiation, and mineralization, which eventually lead to lower bone mass and to reduced trabecular and cortical thickness and growth plate in these mice [43].

The SoxC proteins, including Sox4, are also required for the survival of the multipotent neural and mesenchymal stem cells. These cells give rise to various differentiated cell types and are crucial for organogenesis and embryo growth [25]. Importantly, Sox4 is required for maintenance of the stemness of the glioma initiating cells (GICs) through integration of the Oct4/Sox2 axis [44]. The Oct4/Sox2 axis is crucial for the continuous self-renewal and developmental potential of normal stem cells, specially the embryonic stem cells [45]. However, it is not clear whether the Sox4-mediated regulation of stemness is a general phenomenon or is limited to the GICs and multipotent neural and mesenchymal cells.

Regulation of Sox4 expression

Regulation of Sox4 expression, especially in the pathological contexts, is a field that has remained largely unknown and requires further investigation. Initially, Clarke and colleagues [46] reported that Sox4 is a progesterone-regulated gene in breast cancer cells and that its expression is induced by progestins. Consistently, treatment of T-47D breast cancer cells with the synthetic progestin ORG-2058 increased Sox4 transcription within few hours of treatment. Notably, ORG-2058 had no detectable effect on the expression of other Sox genes, suggesting that in this system the observed phenomenon was specific to Sox4 [46]. In osteoblast-like cells, physiological concentrations of human parathyroid hormone stimulate Sox4 mRNA expression in a time-dependent manner, indicating involvement of the PTH/PTHrP receptor [47]. Prostaglandin A2 and delta12-PGJ2 also induce Sox4 mRNA expression in hepatocarcinoma cells [48]. Del Giacco and colleagues found that IFN-beta/all-trans retinoic acid treatment induced the expression of Sox4 in a thioredoxin reductase 1 (TrxR1) enzyme-dependent manner in hepatocarcinoma cells [49]. However, in none of these observations is it clear whether these hormones affect the activity of the Sox4 promoter or whether they exert their function at other levels (e.g., mRNA stability).

Nonetheless, other studies have revealed details on the regulation of the Sox4 promoter. In hematopoietic progenitor cells, Sox4 expression is regulated at transcription level by the HOXB4 transcription factor [50]. The HOX gene family members are important regulators of hematopoiesis. In T cells, expression of Sox4 is upregulated at

both mRNA and protein levels by TGF-β. This regulation is mediated by downstream mediators of TGF-β, Smad2 and Smad3, which bind to the regulatory region of the *Sox4* gene [32]. Overexpression of the Sox4 transcript upon binding of the Smad2/3 complex to its promoter after TGF-β treatment has also been observed in glioma cell lines [51]. On the other hand, inhibitors of differentiation 2 (ID2) and REST/NRSF repress expression of Sox4 in neural progenitor cells [34]. Since REST/NRSF is a transcriptional repressor, it is pertinent to assume that it could suppress Sox4 promoter activity, although this assumption requires further experimental verification. The retinoic acid receptor-related orphan receptor α (RORα) is also suggested to upregulate Sox4 transcription [52]. RORα plays a critical role in regulation of the circadian clock and enhances Sox4 transcription by direct binding to the promoter sequences after activation by its agonist [52].

Sox4 is transcriptionally regulated by Sox7 in endometrial cancer cells [53]. Accordingly, overexpression of Sox7 activates the Sox4 promoter, leading to the increased expression at both mRNA and protein levels, seemingly due to the presence of Sox7-responsive elements in the promoter region of Sox4 gene. Interestingly, Sox7 inhibits the activity of its own promoter [53], further confirming that the same Sox protein can simultaneously enhance and suppress the activity of different promoters in the same cell, consistent with the context-dependent manner of function for Sox proteins. Sox4 expression may further be influenced by other members of the SoxC class. In fact, whereas ablation of *Sox4* in the sympathoadrenal lineage did not have any significant effect on expression of Sox11, knockdown of Sox11 markedly reduced Sox4 protein expression [54], although it is not clear whether or not this effect of Sox11 is exerted through direct binding to the Sox4 promoter. In addition, Sox4 may regulate its own expression as is evident by binding of the Sox4 protein to its promoter sequence in prostate cancer cells [55].

Other mechanisms also contribute to the regulation of Sox4 expression at post-transcriptional and post-translational levels, which are summarized in Fig. 2 and will be discussed in detail throughout this review.

Role of Sox4 in tumorigenesis

In retrospect, many genes that are involved in developmental processes have also been found to play key roles in the formation of malignancies. For instance, the hedgehog pathway was originally discovered in *Drosophila*, in which its members encode segment polarity proteins responsible for determining the anterior–posterior orientation of the *Drosophila* embryo and larva [56], and was later found to be implicated in tumorigenesis [57, 58]. Involvement of the HMG protein family in cancer was already known [59, 60] before the emergence of evidence that the developmentally critical Sox proteins may also play a role in some cancers. Several lines of evidence pointed to the fact that Sox proteins may be implicated in cancer. For instance, amplification of Sox2 at chromosome 3q has been detected in prostate cancer [61]. The chromosomal region 20q13, which contains Sox18 coding gene, is also amplified in some cases of breast [62] and colon cancers [63]. Deregulated expression of other members of the Sox family in malignancies has also been demonstrated [64–66]. Keeping in mind that in many cases the expression status of *Sox* genes differs in various cancers, it would be implausible to assume a ubiquitous oncogenic or tumor suppressive function for any given *Sox* gene. This kind of inconsistency in the expression pattern and mode of function (oncogenic vs. tumor suppressive) in different types of cancer exists for several members of the Sox family, especially Sox4, which will be described below in more detail.

Oncogenic functions of Sox4

Several lines of evidence suggest an oncogenic role for Sox4 in a wide range of cancers. Some of the earliest indirect evidences highlighting a role for Sox4 in cancer came from studies on colon carcinoma by Miyamoto and colleagues [67]. In this study, Sox4 was found to enhance transcription of p56lck, a member of the family of Src tyrosine kinases that is aberrantly expressed in colon and small lung carcinoma cell lines. p56lck is also expressed in T cells and is among the first signaling molecules to be activated downstream of the T cell receptor and plays a significant role in T cell differentiation, survival, and activation [68]. In Jurkat and HeLa cells, expression of p56lck is controlled by Myb, in synergy with Ets factors [69]. However, in colon carcinoma, aberrant expression of p56lck arises from transcriptional activation mediated by cooperation between Ets-1 and Sox4 [67]. Amplification of chromosomal region 6p22.3 in at least some tumor types [70, 71] was another indirect evidence for a tumorigenic function of Sox4. Nevertheless, in some cases, there is no significant correlation between expression of Sox4 and amplification of 6p22.3 [73, 74], suggesting that amplification of Sox4 coding sequences might be a bystander effect and not a growth advantage for some cancer cells. This suggestion is in line with existence of important proto-oncogenes such as E2F3 [72], ID4 [73] and, PRL [74] in the 6p22.3 chromosomal region, which might provide the actual benefit to the cancer cells upon amplification. This notion is consistent with an independent observation in bladder cancers. In this cancer, 30.8 % of the patients had chromosomal amplification of this region. The mean E2F3 expression of amplified tumors was a 1.8-fold increase compared with not-amplified tumors, whereas no

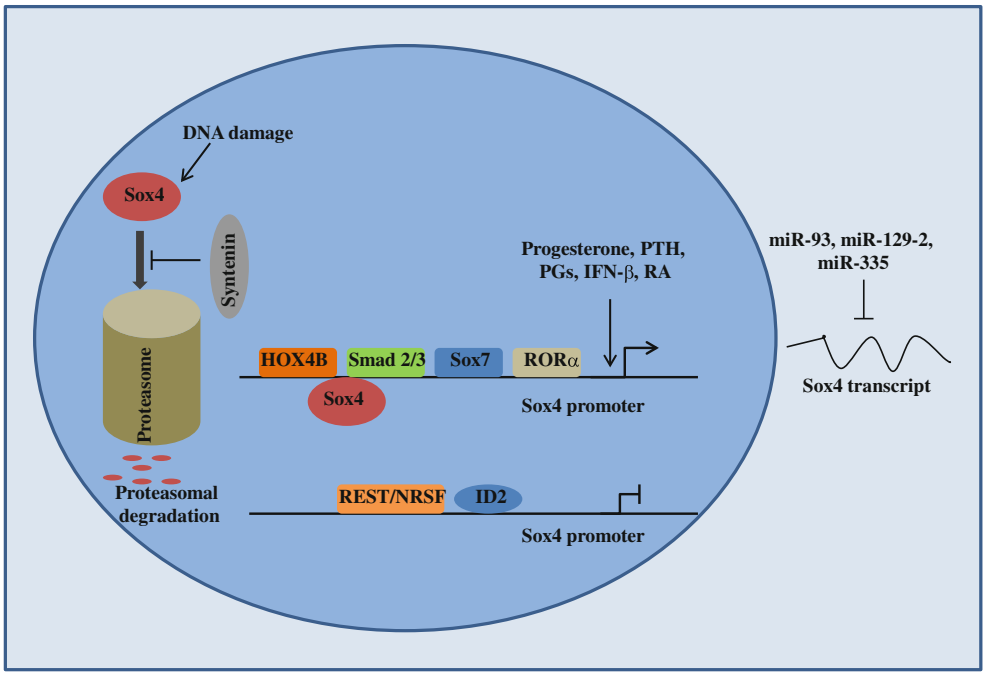

Fig. 2 Regulation of Sox4 expression. Expression of Sox4 is regulated at three distinct levels; transcriptional, post-transcriptional, and post-translational. *PGs* prostaglandin A2 and delta12-prostaglandin J2

correlation was found between chromosomal amplification and Sox4 expression [75]. Nonetheless, the inconsistency between 6p amplification and Sox4 expression may not be a ubiquitous phenomenon. In fact, another study by Medina et al. [76] found an increased expression of Sox4 in lung primary tumors and lung cancer cell lines with chromosome 6p amplification. Interestingly, they also reported identification of a somatic mutation at the 395 residue of Sox4 that introduces a premature stop codon at the C-terminal domain, producing a shorter Sox4 protein, S395X, which is devoid of the region immediately after the serine-rich domain and does not possess transactivation potential (Fig. 1). While neither wild-type Sox4 nor its truncated isoform could induce oncogenic transformation of the NIH3T3 mouse fibroblasts, wild-type Sox4 enhances tumorigenicity of the NIH3T3 cells expressing the activated HRAS. On the other hand, the truncated isoform significantly reduced the tumorigenicity of the HRAS-expressing cells [76], which suggests that transcriptional activity of Sox4 supports the HRAS-transforming ability in lung cancer cells. However, it is not clear why cancer cells with a truncated Sox4 that could reduce the tumorigenicity would have a selective advantage.

Increased expression of Sox4 at mRNA or protein levels in several types of epithelial and hematopoietic cancers further confirmed its putative oncogenic function (Table 1). However, this observed correlation between Sox4 overexpression and cancer progression does not seem to always be consistent with patient survival outcome or even functional role of Sox4 in those cells. For instance, Sox4 protein is overexpressed in human hepatocellular carcinomas (HCC) [77], yet there is an inverse correlation between expression of Sox4 and recurrence of cancer as well as a positive correlation between Sox4 expression and overall patient survival which does not fit into a possible oncogenic role for Sox4 in this type of cancer. This study also found that the HMG box domain of Sox4 interacts with p53. This interaction, which happens at the p53-responsive promoters, results in inhibition of p53-mediated activation of Bax promoter, leading to repression of p53-induced Bax expression and subsequent repression of p53-mediated apoptosis induced by gamma irradiation. Therefore, Sox4 suppresses p53-mediated apoptosis induced by gamma irradiation in HCC cells [77]. Of note, this function of Sox4 in HCCs is in sharp contrast with its role in colorectal cancer in which Sox4 interacts with and stabilizes p53 activity and enhances p53-mediated apoptosis, cell cycle arrest, and inhibits tumorigenesis [20].

A tissue microarray analysis of 2,360 samples revealed an increased expression of Sox4 protein in bladder tumors, suggesting an oncogenic role for Sox4 in this type of tumor [75]. Nevertheless, this notion is in contrast with survival studies in the same set of patients where a significant correlation between strong Sox4 expression and increased patient survival was found, probably due to the role of Sox4 in induction of apoptosis in bladder carcinoma cells. Indeed, in these cells, Sox4 overexpression impairs cell viability and enhances cell death [75]. This study also

Table 1 Expression pattern and biological functions of Sox4 in different types of malignancies and its correlation with patient survival

Type of tumor	Sox4 expression	Method of detection	Suggested function	Correlation with survival	Reference
Acute myeloid leukemia	↑	Western blot	Promotion of cell growth	ND	[96]
Adenoid cystic carcinoma	↑	cDNA microarray	ND	ND	[82]
Adenoid cystic carcinoma	↑	Tissue microarray	Suppression of apoptosis	ND	[81]
Bladder carcinoma	↑	cDNA microarray/tissue microarray	Induction of apoptosis	Positive	[75]
Colon adenocarcinomas	↓	cDNA microarray	ND	ND	[131]
Colorectal cancer	↑	Tissue microarray	ND	ND	[112]
Colorectal cancer	↑	cDNA microarray	ND	Negative	[112]
Cutaneous melanoma	↓	Tissue microarray	Suppression of invasion and migration	Positive	[104]
Endometrial cancer	↑	Tissue microarray	Promotion of cell growth	ND	[85]
Hepatocellular carcinoma	↑	Tissue microarray	Suppression of apoptosis	Positive	[77]
Gallbladder carcinoma	↓	IHC	ND	Positive	[110]
Gastric cancer	↑	Tissue microarray	Suppression of apoptosis	Negative	[86]
Glioblastoma	↑	Parallel signature sequencing/ RT-PCR/IHC	ND	ND	[99]
Lung cancer	↑	RT-PCR/western blot	Induce transformation of NIH3T3 cells	ND	[76]
Medulloblastoma	↑	RT-PCR/ISH	ND	ND	[65]
Medulloblastoma	↑	RT-PCR	ND	ND	[132]
Pediatric medulloblastoma	↑	cDNA microarray	ND	Positive	[111]
Prostate cancer	↑	cDNA microarray	ND	ND	[78]
Prostate cancer	↑	Tissue microarray/cDNA microarray	Suppression of apoptosis	ND	[79]
Uveal melanoma	↓	Suppressive subtractive hybridization	ND	ND	[105]

ND not determined, *ISH* in situ hybridization, *IHC* immunohistochemistry

found that, in bladder cancer cells, Sox4 regulates a plethora of genes including transcription factors, such as MEF2C and zinc finger protein 6 (ZNF6), and signaling molecules such as phosphoinositide-3-kinase regulatory subunit polypeptide 3 (PIK3R3), that promotes cell cycle arrest, and mitogen-activated protein kinase kinase 5 (MAP2K5) [75].

An oncogenic role for Sox4 in prostate cancer and its progression is well established [78, 79]. Moreno and colleagues reported that Sox4 is the only member of the Sox family that is overexpressed in prostate cancer, and its knockdown induces apoptosis in the LNCaP prostate cancer cell line. Consistently, Sox4 overexpression induced transformation of RWPE-1 prostate cancer cells. Furthermore, depletion of Sox4 expression resulted in an increased expression of p53 protein and loss of survivin expression [79], possibly explaining the mechanism by which Sox4 suppresses apoptosis and enhances transformation of prostate cancer cells. However, the mechanism by which Sox4 regulates p53 expression in these cells is not understood. Further analysis by cDNA microarray of LNCaP cells treated with Sox4 siRNAs or overexpressing Sox4 revealed 466 genes which were consistently responsive both to Sox4-knockdown and overexpression. Among the Sox4 target genes identified in this study were genes with roles in Wnt signaling (TLE-1), apoptosis (BCL10 and PUMA), and inflammation (CSF1) [79].

The same group later performed a genome-wide promoter analysis of the Sox4 transcriptional network in prostate cancer cells and identified 3,470 different genes whose promoter sequences contain direct binding sites of Sox4. However, cDNA microarray analysis revealed that, among these genes, expression of only 282 of the 3,470 binding targets is significantly influenced by Sox4 [80].

This observation suggests that a change in expression of Sox4 is not sufficient to alter the expression status of the other 3,188 genes, reflecting the requirement for other regulatory components such as binding partners in this process. Among the genes which were shown to be directly regulated by Sox4 in this study are imminent proto-oncogenes such as Tenascin-C, Frizzled-3, 5 and 8, Patched-1, Delta-like1, and EGFR, which are positively regulated by Sox4 and reduced after Sox4-knockdown in prostate cancer cells [80]. The extent of the Sox4 network encompasses various cancer-related pathways and mechanisms including the TGF-β, Wnt, hedgehog, and Notch pathways, as well as growth factor signaling and tumor metastasis. Notably, this study revealed differential regulation of expression of target genes by Sox4 in different prostate cancer cell lines. For instance, Sox4 binds to the promoter sequences of EGFR and enhances its expression in RWPE-1 cells, yet it does not bind to these sequences in LNCaP cells [80]. Interestingly, Sox4 overexpression increases expression of the transcription factor SON by 1.8-fold, whereas its knockdown increases expression of purine biosynthetic enzyme GART by threefold. These two genes, located on chromosome 21, are regulated by a common bidirectional promoter but transcribed in opposite directions. Differential regulation of expression of these two genes by Sox4 suggests that it regulates the directionality of this promoter [80]. This study also identified co-occurring binding sites of several transcription factors such as E2F1, E2F4, PAX5, LEF1/TCF1, and MYC along with Sox4 in its target promoter [80], which highlights the possibility of cooperation between Sox4 and one or more of these factors in regulation of the expression of target genes.

Sox4-knockdown causes a considerable reduction in adenoid cystic carcinoma (ACC) cell viability due to induction of apoptosis in a caspase-dependent manner [81]. In concert with this observation, both Sox4 transcript and protein were found to be upregulated in ACC [81, 82]. Microarray gene expression profiling of Sox4-knockdown ACC cells confirmed Sox4-modulated expression of critical genes involved in apoptosis and cell cycle control such as cyclin G2 (CCNG2), DUSP4, and BNIP3, indicating the contribution of Sox4 to the malignant phenotype of ACC cells by promoting cell survival. This study, along with other cDNA microarray analyses in human prostate cancer [79, 80], bladder cancer [75], and small cell lung cancer [83] cells, identified a large number of genes, the expression of which is affected by Sox4. Although each study identified a considerable number of target genes, the overlaps between these datasets are almost negligible (Fig. 3). In fact, there is no gene whose expression is at least two-fold changed upon overexpression or knockdown of Sox4 in all five datasets. Even more surprisingly, comparison of two studies in the same prostate cancer cell line (LNCap) only returned three common genes (Fig. 3). Bearing in mind that these comparisons should be considered carefully due to differences in the technical aspects, such as the type of microarray platforms or the time points after overexpression or knockdown of Sox4 at which the RNA samples were collected, these results are also another indication of the highly tissue- and cell-specific network of Sox4.

As mentioned earlier, expression of Sox4 is regulated at post-transcriptional level. For instance, miR-129, which is overexpressed in bladder carcinoma and inversely correlated with patient survival, targets Sox4 in these cells [84]. Similarly, expression of miR-129-2 is lost in the majority of primary endometrial tumors, probably due to hypermethylation of the miR-129-2 regulatory regions, with a concomitant gain of Sox4 expression [85]. Restoration of miR-129-2 expression in these cells leads to decreased Sox4 expression and reduced proliferation of cancer cells. Consistently, Sox4 protein is overexpressed in endometrial tumors [85], and its knockdown suppresses endometrial cancer cell growth. Downregulation of Sox4 by miR-129-2 as well as hypermethylation-mediated suppression of this miRNA is also described in gastric cancers, in which both Sox4-knockdown and overexpression of miR-129-2 induce apoptosis [86].

In a search for miRNAs which regulate cancer growth and metastasis, Tavazoie et al. [87] found that expression of miR-335 is lost in the majority of primary breast tumors, and that this loss of expression is associated with poor distal metastasis-free survival. In this type of cancer, miR-335 regulates a set of genes, including Sox4 and the extracellular matrix component Tenascin C, whose expression in a large cohort of human breast tumors is associated with risk of distal metastasis. Consistently, knockdown of Sox4 or Tenascin C significantly abolishes lung colonization by LM2 breast cancer cells. Interestingly, in the genome-wide promoter analysis in prostate carcinoma cells, Sox4 was shown to bind to the promoter sequences of Tenascin C [80]. The study by Tavazoie and colleagues showed that miR-335 directly targets Sox4. However, it is not clear whether downregulation of Tenascin C by miR-335 is a direct event or is achieved through initial downregulation of Sox4, resulting in reduced expression of Tenascin C.

In addition to miR-335, in breast cancer cells miR-93 also targets Sox4. This effect may be important for the observed induction of mesenchymal–epithelial transition (MET) associated with downregulation of TGF-β signaling in breast cancer cells and resulting in cancer stem cell depletion [88]. In line with this observation, Sox4 may contribute to invasion and metastasis of breast cancer cells by induction of epithelial-mesenchymal transition (EMT). In fact, overexpression of Sox4 in immortalized human

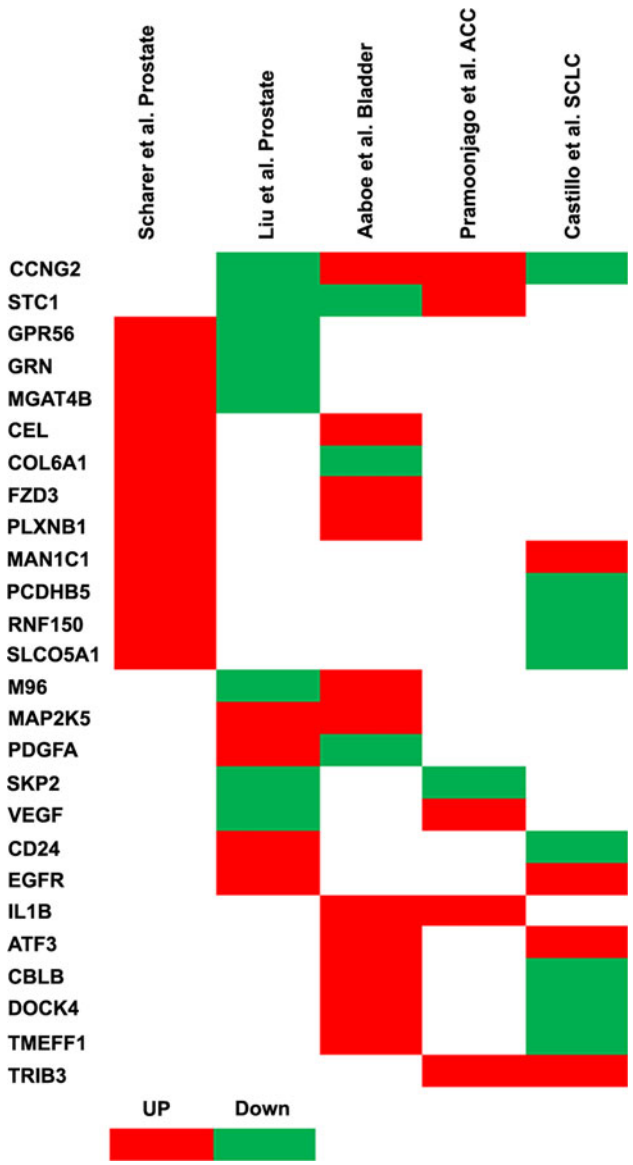

Fig. 3 Comparative analysis of cDNA microarray studies of Sox4 target genes in prostate cancer, adenoid cystic carcinoma, bladder, and small cell lung cancer cell lines. Target genes whose expression was >two-fold changed after Sox4 overexpression [75, 79, 80] or knockdown [81, 83] and represented in at least two datasets were included in this analysis

mammary epithelial cells is sufficient for acquisition of mesenchymal traits, enhanced cell migration, and invasion [89]. Moreover, based on tumorigenesis assay in mice, Sox4 cooperates with activated Ras to promote tumorigenesis in vivo [89]. In addition to breast cancer, Sox4 also augments the migration, invasion, and metastasis of hepatocellular carcinoma cells [90]. This function of Sox4 is probably exerted by upregulation of two downstream factors, neuropilin 1 (NRP1) and semaphorin 3C (SEMA3C), knockdown of which drastically reduces cell migration [90].

By a sophisticated approach, using oncogenic retrovirus-induced insertional mutagenesis, Copeland and colleagues [91] showed that replication-defective retrovirus carrying Sox4 can induce myeloid leukemias after transplanting into mice bone marrow cells in cooperation with Mef2c, a transcription factor involved in regulation of homing and invasiveness of leukemic cells. Sox4 can integrate at the site of Sfpi1, an Ets1-related transcription factor essential for normal myeloid and lymphoid development, and reduce Sfpi1 expression [92]. The same group later revealed that Sox4 represses Sfpi1 transcription by binding to a critical Sfpi1 upstream DNA element [93]. Additionally, by analyzing 285 acute myeloid leukemia (AML) patient samples, they found a significant negative correlation between Sox4 and Sfpi1 mRNA expression providing convincing evidence for the involvement of Sox4 in promotion of at least a subset of myeloid malignancies [93]. *Sox4* is the site of the Evi1 (ectopic viral integration site 1) proviral insertions in myeloid leukemias. This insertion results in transcriptional activation and increased Sox4 expression [91]. Interestingly, Sox4 expressing myeloid cells have a higher level of transcripts associated with proliferation including Evi1, despite no measurable effect on cell proliferation or differentiation after overexpression of Sox4 [91].

Sox4 is also reported to be a common retrovirus insertion site in myeloid and monocytic cells [94], suggesting a role of Sox4 in promoting malignant disease in these cells. Consistently, retroviral tagging strategy in combination with high throughput inverse-PCR identified Sox4 as one of the most common retroviral integration sites in a tumor panel composed primarily of B cell lymphomas [95]. In myeloid cells, Sox4 binds to the promoter sequences of CREB and enhances its expression [96]. Transduction of CREB transgenic mouse bone marrow cells with a Sox4 retrovirus increases the in vitro survival and self-renewal. In addition, leukemic blasts from the majority of AML patients have higher CREB, phosphorylated CREB, and Sox4 protein expression, indicating that Sox4 and CREB cooperate and contribute to increased proliferation of hematopoietic progenitor cells and myelogenesis.

Sox4 also binds to and activates the promoter of CD56 in primary myeloma cells [97]. CD56 is a member of the immunoglobulin superfamily that was initially characterized in the cells of the nervous system and is overexpressed in more than 80 % of myeloma patients as well as some other types of malignancies [98]. Thus, it would be of interest to investigate the possible role of Sox4 in regulation of CD56 expression in other types of cancer.

Promotion of tumorigenesis by sustainment of stemness

In another study delineating the molecular details of Sox4's involvement in tumorigenesis, Ikushima et al. [51] found

that Sox4 is highly expressed in GICs and required for the TGF-β-induced expression of Sox2 that is essential for retention of stemness in GICs. Consistently, Sox4-knockdown cells show less sphere-forming ability and self-renewal capacity, two characteristic features of GICs. Sox4 regulates Sox2 expression at transcriptional level by binding to an enhancer element located at its 3′ flanking region. This activity is increased after TGF-β stimulation. In addition, Sox4 mRNA expression is immediately induced after TGF-β stimulation through binding of Smad2/3, the DNA-binding mediators of TGF-β signaling. Consistently, inhibitors of TGF-β signaling drastically deprived tumorigenicity of GICs by promoting their differentiation, while these effects are attenuated in GICs overexpressing Sox2 or Sox4 [51]. The same group later demonstrated that Oct4 physically interacts with Sox4 and cooperatively activates the Sox2 enhancer region to maintain stemness properties of GICs [44]. Supporting this notion, consensus sequences of Sox proteins and Oct4 exist in close proximity of each other in the Sox2 enhancer region, and ChIP re-IP (back-to-back immune-precipitation of chromatin with two different antibodies) assay demonstrated that Sox4 and Oct4 exist in the same transcription complex in the Sox2 enhancer region and enhance the activity of Sox2 promoter [44], further confirming the role of Sox4 in regulation of Sox2 expression and maintenance of GICs' tumorigenicity. A separate study identified elevated expression of Sox4 and TGF-β in human glioblastoma compared with normal brain tissues at both RNA and protein levels, and confirmed that Sox4 expression is increased by TGF-β stimulation [99].

In breast cancer, Sox4 overexpression induces EMT, resulting in a cancer stem cell-like phenotype in breast cancer cells. These cells exhibited an increase in the CD44 high/CD24 low sub-population and enhanced size and number of mammospheres, indicating that the Sox4-induced EMT generates mesenchymal cells with stem cell-like phenotype [89]. This function of Sox4 may also be mediated by the TGF-β pathway as is evident by increased expression of TGF-β1 and TGF-β2 mRNAs and the phospo-Smad2 protein in Sox4-expressing cells [89].

Sox4 as a tumor suppressor

The first evidence to pinpoint a possible role for Sox4 in suppression of tumorigenesis came from a study by Ahn et al. [48]. Using mRNA differential display and northern blot analysis, they revealed that expression of Sox4 mRNA is enhanced during apoptosis induced by prostaglandin (PG)A2 and Delta12-PGJ2 in human hepatocellular carcinoma cells, Hep3B, suggesting the possible involvement of Sox4 in the process of apoptosis. Nevertheless, Sox4 was also highly expressed in subcutaneous tumors grown in nude mice as a xenograft from Hep3B cells [48], which is not consistent with a tumor suppressor role for Sox4. Unfortunately, the authors did not investigate this contradiction in their results in further detail. The same group later observed that overexpression of Sox4 induces significant apoptosis in Hep3B and HepG2 cell lines, probably through the caspase-mediated pathway [100]. In addition, Sox4-knockdown blocks the apoptosis induced by PGA(2) and delta(12)-PGJ(2) in these cells [100]. The caspase-dependent manner of Sox4 for induction of apoptosis was later confirmed by Kim et al. [101]. Interestingly, although several prominent apoptosis-related factors such as Bax and PUMA are among the direct transcriptional targets of Sox4 (Table 2), the pro-apoptosis function of Sox4 can be dissociated from its transcriptional activity [23]. Indeed, the central domain (aa 166–342) of Sox4 is critical for induction of apoptosis in HEK293 cells. Accordingly, deletion of the DNA-binding domain or trans-activation domain in Sox4 does not significantly affect its pro-apoptotic activity in these cells, whereas overexpression of a construct containing the central domain induces the apoptotic activity comparable to that of the full-length protein [23].

As an indirect link between Sox4 and regulation of apoptotic cell death, human ubiquitin-conjugating enzyme 9 (Ubc9) interacts with HMG-box of Sox4 and represses Sox4 transcriptional activity in HEK293T cells [102]. The C93S mutant of Ubc9, which abrogates SUMO-1 conjugation activity, does not abolish its ability to inhibit Sox4 activity. Elevated Ubc9 expression has been detected in at least some types of malignancies, such as primary and metastatic melanomas in which Ubc9 plays a crucial role in suppression of apoptosis [102]. On this note, in some malignancies such as breast cancer [103], the ability of Ubc9 to promote cancer initiation and progression is independent of its sumoylation function. The possible involvement of Sox4 inactivation in the sumoylation-independent oncogenic function of Ubc9 remains to be uncovered.

Our own studies on cutaneous malignant melanoma have revealed that Sox4 expression is significantly reduced in metastatic melanomas [104]. Furthermore, Sox4 expression is positively correlated with better patient survival. Consistently, Sox4 suppresses melanoma cell migration and invasion ability through inhibition of NF-κB p50 expression by binding to sequences upstream of the NF-κB p50 promoter [104]. A recent study reported a reduced expression of Sox4 mRNA in uveal melanoma [105]. Overexpression of several members of the NF-κB pathway has also been observed in metastatic uveal melanoma [106]. However, it is not clear whether or not Sox4-mediated inhibition of NF-κB can play a role in suppression of this type of melanoma.

Table 2 Validated direct transcriptional targets of Sox4

Tissue/cell type	Gene symbol	Mode of regulation	Function	Assay	References
Breast cancer	ZEB1	↑	Transcription repressor	Reporter assay	[89]
Breast cancer	Snail	↑	Transcription repressor	Reporter assay	[89]
Colon carcinoma	p56lck	↑	Protein tyrosine kinase	Reporter assay	[67]
COS1	Tubb3	↑	β-tubulin isotype III	EMSA	[34]
Endometrial carcinomas	TCF4	↑	Transcription factor	Reporter assay	[53]
Glioma	Sox2	↑	Transcription factor	ChIP	[51]
HCC	SLC2A1	↑	Agmatine transport	ChIP	[90]
HCC	Bax	↓	Apoptosis	ChIP	[77]
HCC	CREB3L1	↑	Axon guidance	ChIP	[90]
HCC	MYEF2	↑	Axon guidance	ChIP	[90]
HCC	NAV3	↑	Axon guidance	ChIP	[90]
HCC	NRP1	↑	Axon guidance	ChIP	[90]
HCC	SEMA3C	↑	Axon guidance	ChIP	[90]
HCC	NEIL3	↑	Base excision DNA repair	ChIP	[90]
HCC	CHFR	↑	Cell cycle checkpoints	ChIP	[90]
HCC	NPNT	↑	Extracellular matrix protein	ChIP	[90]
HCC	GPRC5B	↑	G-protein signaling pathway	ChIP	[90]
HCC	HUNK	↑	Kinase	ChIP	[90]
HCC	PFKFB4	↑	Kinase	ChIP	[90]
HCC	AKR1B10	↑	Metabolism	ChIP	[90]
HCC	ALDH18A1	↓	Metabolism	ChIP	[90]
HCC	DHRS13	↑	Metabolism	ChIP	[90]
HCC	GYG	↑	Metabolism	ChIP	[90]
HCC	PAM	↑	Metabolism	ChIP	[90]
HCC	RCC2	↓	Metabolism	ChIP	[90]
HCC	MAP4	↑	Microtubule-associated protein	ChIP	[90]
HCC	SMG5	↑	mRNA decay	ChIP	[90]
HCC	HSPBAP1	↓	Protein folding	ChIP	[90]
HCC	LCK	↑	Protein tyrosine kinase	ChIP	[90]
HCC	CSPG2	↑	Proteoglycan	ChIP	[90]
HCC	CTSC	↑	Proteolysis	ChIP	[90]
HCC	RBM10	↓	RNA binding	ChIP	[90]
HCC	SERPINE2	↑	Serine protease inhibitor	ChIP	[90]
HCC	INST8	↑	Small nuclear RNAs processing	ChIP	[90]
HCC	HIC2	↓	Transcription factor	ChIP	[90]
HCC	VGLL4	↑	Transcription factor	ChIP	[90]
HCC	FOXQ1	↑	Transcription factor	ChIP	[90]
HCC	TEAD2	↑	Transcription factor	ChIP	[90]
HCC	CCDC97	↓	Unknown	ChIP	[90]
HCC	UBAP2L	↓	Unknown	ChIP	[90]
HCC	DKK1	↑	Wnt signaling pathway	ChIP	[90]
Heart	Connexin 43	↑	Gap junction protein	ChIP	[27]
Hemangioblasts	$\lambda 5$	↑	Component of precursor of BCR	ChIP	[31]
Hemangioblasts	VpreB1	↑	Component of precursor of BCR	ChIP	[31]
Melanoma	NF-κB p50	↓	Inflamation, cell growth	ChIP	[104]
Melanoma	Dicer	↑	miRNA biogenesis	ChIP	[107]
Mouse embryonic mesenchymal cells	Tead2	↑	Transcription factor	ChIP	[25]
Myeloid Leukemia	CREB	↑	Transcription factor	ChIP	[96]

Table 2 continued

Tissue/cell type	Gene symbol	Mode of regulation	Function	Assay	References
Myeloid leukemias	Sfpi1	↓	Transcription factor	ChIP	[93]
Myeloma	CD56	↑	Immunoglobulin superfamily	ChIP	[97]
Neural cells	DCX	↑	Microtubule-associated protein	ChIP	[35]
Neural cells	GnRH	↑	Sexual development	ChIP	[133]
Neural cells	FEZF2	↑	Transcription factor	ChIP	[36]
Prostate	PUMA	↑	Apoptosis	ChIP	[79]
Prostate	MLL	↑	Histone methyl-transferase	ChIP	[80]
Prostate	ADAM10	↑	Metalloproteinase	ChIP	[80]
Prostate	AGO1	↑	miRNA biogenesis	ChIP	[80]
Prostate	DHX9	↑	miRNA biogenesis	ChIP	[55, 80]
Prostate	Dicer	↑	miRNA biogenesis	ChIP	[55, 80]
Prostate	DLL1	↑	Notch signaling	ChIP	[80]
Prostate	HES2	↑	Notch signaling	ChIP	[80]
Prostate	RBL1	↑	Regulation of cell cycle	ChIP	[80]
Prostate	EGFR	↑	Signaling	ChIP	[80]
Prostate	ELF5	↓	Transcription factor	ChIP	[80]
Prostate	Sox4	↑	Transcription factor	ChIP	[55]
Prostate	ZNF281	↑	Transcription repressor	ChIP	[80]
Prostate	TLE-1	↑	Transcription repressor	ChIP	[79, 80]
Prostate	FZD3	↑	Wnt receptor	ChIP	[80]
Prostate	FZD5	↑	Wnt receptor	ChIP	[80]
Prostate	FZD8	↑	Wnt receptor	ChIP	[80]
Prostate	AXIN2	↑	Wnt signaling	ChIP	[55]
SCLC	VASH2	↑	Angiogenic factor	ChIP	[83]
SCLC	PCDHB	↑	Protocadherin	ChIP	[83]
SCLC	Tead2	↑	Transcription factor	ChIP	[83]
SCLC	MYB	↑	Transcription factor	ChIP	[83]
SCLC	Tubb3	↑	β-tubulin isotype III	ChIP	[83]
T-cells	Interleukin 5	↓	Cytokine	ChIP	[32]
T-Cells	CD2	↑	Surface antigen	EMSA	[134]

EMSA electrophoretic mobility shift assay, *ChIP* chromatin immunoprecipitation, *HCC* hepatocellular carcinoma

In addition, Sox4 may recruit miRNA machinery to negatively regulate melanoma invasion. In cutaneous melanoma cells, Sox4 regulates transcription of Dicer by binding to its promoter and enhancing its activity which is critical for expression of a considerable number of cancer-related miRNAs [107]. Interestingly, Dicer and Drosha have recently been identified to be necessary to activate the DNA repair mechanism upon exogenous DNA damage and oncogene-induced genotoxic stress [108], although this function appears to be independent from the canonical miRNA-mediated translational repression mechanisms regulated by Dicer. This mechanism may also be responsible for Sox4-mediated suppression of tumorigenesis, considering the regulation of Dicer expression by Sox4 and the fact that Sox4 has also been reported to be a DNA damage sensor and is required for activation of p53 tumor suppressor in response to DNA damage (discussed in further details below).

Regulation of expression of miRNA-related factors such as Dicer, Argonaute 1, and RNA helicase A by Sox4 had been previously reported in prostate cancer cells by Scharer et al. [80]. Although there were some inconsistencies in the pattern of Sox4 target genes between different cell lines, they were able to confirm the regulation of Dicer expression by Sox4 at both transcript and protein levels [80], which was consistent with our observation in melanoma cells [107]. It is noteworthy that, while we observed that Dicer expression is lost in metastatic melanoma and it is required for Sox4-mediated suppression of melanoma cell invasion, expression of Dicer is upregulated in prostate adenocarcinomas [109]. Although the expression status of Dicer is not known in other subtypes of prostate cancer,

this observation may be related to overexpression of Sox4 [79] and highlights the importance of Sox4-regulated Dicer expression in these two different types of malignancies.

In primary gallbladder carcinoma, Sox4 expression is reduced compared with the normal epithelium of the gallbladder [110]. Reduced Sox4 expression is associated with high histological grade, high pathologic T stage, and late clinical stage of this malignancy. In addition, expression of Sox4 in tissues with positive nodal metastasis is lower than those without metastasis. In line with these observations, Sox4 expression is positively correlated with a better overall and disease-free patient survival [110]. It is intriguing that, in the majority of studies that investigated the correlation between Sox4 expression and patient survival, Sox4 has been found as a positive marker for survival. For instance, despite an enhanced expression of Sox4 in bladder carcinomas, there is a positive correlation between strong Sox4 expression and increased patient survival [75]. Similarly, there is a trend toward favorable prognosis with increasing Sox4 expression levels in patients with medulloblastoma, despite the enhanced expression of Sox4 in medulloblastoma compared with normal cerebellum [111]. Also, Sox4 is overexpressed in hepatocellular carcinoma, but its expression correlates with diminished risk of recurrence and improved overall survival in HCC patients [77]. Nevertheless, none of these studies addressed the observed discrepancy in the expression level or function of Sox4 and its correlation with survival. In contrast to these reports that suggest Sox4 as a marker for improved prognosis, at least in some cases Sox4 expression was found to be inversely correlated with survival (Table 1). For instance, Sox4 expression inversely correlates with overall patient survival of gastric cancer [86] and recurrence-free survival of microsatellite-stable stage II (no lymph node or distant metastases) colorectal carcinoma [112].

As mentioned earlier, in HCC cells, the HMG box domain of Sox4 interacted with p53, resulting in the inhibition of p53-induced Bax expression and the p53-mediated apoptosis induced by gamma irradiation [77]. However, it is not clear how Sox4 expression would confer a survival advantage to the patient while it can suppress the p53-mediated apoptosis. Also, the observed suppression of p53 activity by Sox4 is in contrast to another report by Pan et al. [20], who demonstrated that Sox4 induces p53 activity in colon cancer cell lines. In these cells, expression of Sox4 protein but not mRNA is increased upon treatment with doxorubicin (DOX) and Sox4 is required for activation of p53 in response to DOX-induced DNA damage. In fact, in these cells, Sox4 interacts with and stabilizes p53 protein by repressing Mdm2-mediated ubiquitination and degradation of p53. Furthermore, Sox4 interacts with p300/CBP and enhances p300/CBP/p53 complex formation and p53 acetylation which increases p53 stability. In this system, Sox4 promotes cell cycle arrest and apoptosis, and inhibits tumorigenesis in a p53-dependent manner [20]. In line with these observations, ionizing radiation induces Sox4 expression, in parallel with induction of p53 protein in medulloblastoma cell lines [113]. In addition, Sox4-knockdown dramatically blocks the radiation-induced increases in p53 (S-15) phosphorylation and XRCC1 protein expression along with an increase in levels of phospho-γ-H2AX compared with cells subjected to radiation alone [113], suggesting that Sox4 functions upstream of p53-mediated DNA repair, an important process in prevention of mutations and tumorigenesis.

Role of Sox4/β-catenin axis in regulation of tumorigenesis

Wnt signaling has long been implicated in tumorigenesis and deregulated expression of its components is a common phenomenon in many types of tumors [114]. One of the main mediators of the wnt signaling pathway is β-catenin, abnormal expression and/or mutation of which is a common feature of almost all types of human malignancies [115]. In HEK293 cells, overexpression of Sox4 enhances β-catenin/TCF activity by increasing the stability of β-catenin, which induces wnt signaling pathway activity and expression of its target genes, cyclin D1 and c-myc. The enhanced β-catenin/TCF activity by Sox4 is caused by stabilization of the β-catenin protein, through induction of CK2, a kinase involved in regulation of β-catenin stability, suggesting that Sox4 may act as an activator of the wnt signaling pathway [116]. In a study that revealed a novel non-transcription related mechanism by which Sox4 may act as an oncogene, Sinner et al. [21] observed that, unlike Sox17 that represses β-catenin/TCF activity and inhibits proliferation of SW480 colon carcinoma cells, Sox4 enhances β-catenin/TCF activity and cell proliferation. In fact, both Sox17 and Sox4 physically interact with TCF/LEF family members via their HMG-box domains and while Sox17 promotes their degradation, Sox4 stabilizes β-catenin and TCF/LEF proteins [21].

γ-catenin, also known as junction plakoglobin (JUP), is a Sox4 binding protein in prostate cancer cells [55]. Interaction between these two proteins is enhanced by wnt3A treatment. However, this interaction could inhibit Sox4 binding to downstream target promoters and may repress its transcriptional activity in addition to modulation of the β-catenin-mediated transcription. These observations led to the suggestion that γ-catenin may compete with β-catenin for binding to Sox4, downregulating wnt-responsive transcription [55]. This model is also consistent with the observations that γ-catenin expression is lost in human prostate cancer through epigenetic and genetic pathways

[117], as is its potential to suppress cell migration and tumorigenic potential [118]. In malignant melanoma, Sox4 is reported to activate the wnt/β-catenin signaling pathway [119]. Accordingly, Sox4 knockdown leads to a decreased β-catenin protein expression, resulting in inhibition of wnt/β-catenin signaling pathway activity and reduced expression of survivin as well as attenuated cell proliferation [119]. However, In contrast to this observation, our own studies have shown that knockdown of Sox4 slightly increases melanoma cell growth [104].

As opposed to the aforementioned role of the Sox4/β-catenin axis in the promotion of tumorigenesis, a recent report has revealed that Sox4 could indeed enhance β-catenin/TCF4 transcription, through upregulation of TCF4 at the transcription level, without any direct β-catenin association, resulting in a significant decrease in the proliferation rate, along with increases in expression of p21, as well as of TCF4, in endometrial carcinoma cells [53]. Consistently, Sox4-knockdown increased cell growth in this model. These observations provide evidence that at least in some types of malignancy the Sox4/β-catenin axis may contribute to suppression of tumorigenesis. In concert with this notion, although debated [120], β-catenin is implicated in suppression of melanoma invasion and tumorigenesis [121, 122]. In addition, at least in some cases expression of β-catenin is reduced during melanoma progression [123–125] and decreased β-catenin expression is linked to worsened patient survival [126, 127]. Considering the loss of expression of Sox4 in melanoma and the considerable amount of evidence that Sox4 may regulate β-catenin expression and/or activity, it would be interesting to identify the possible role of Sox4 in regulation of β-catenin in melanoma cells and its significance in tumor progression. It should also be noted that in some cases overexpression of Sox4 may suppress β-catenin expression. In fact, overexpression of Sox4 induces a significant reduction of β-catenin expression in breast cancer cell lines [89], which further complicates the mutual relationship between Sox4 and β-catenin and their functional outcome in different types of tissue.

Concluding remarks

In recent years, numerous publications have described the expression pattern and possible functional significance of Sox4 in various types of malignancies. However, despite this considerable progress, some outstanding questions regarding the mechanistic details of Sox4 functionality and its significance in cancer initiation and progression remain to be answered. For instance, in several types of cancer, while the expression pattern of Sox4 is known to be different than their normal counterparts, the mechanism(s) by which Sox4 contributes to the tumorigenesis (or suppression of tumorigenesis) in those cancers is not known. Also, whereas differential and tissue-dependent regulation of transcription is customary in Sox family, in some cases it is not understood whether or not the mode of Sox4-mediated regulation of activity, but not expression, of other proteins can also be tissue-dependent. For instance, in hepatocellular carcinoma cells, Sox4 interacts with p53, resulting in the inhibition of p53 activity and apoptosis [77], whereas, in colon cancer cell lines, Sox4 interaction results in enhanced p53 activity and apoptosis [20]. Several members of the Sox family have been implicated in tumorigenesis, and in a number of cases some siblings compete with Sox4 in binding to and regulating the activity of the target promoters or binding partners [16, 21, 36]. Nevertheless, except in a few studies, the possible roles of other Sox proteins in Sox4 networks and their putative influence on Sox4's oncogenic or tumor suppressive functions remain unknown. Despite the recent progress in understanding the mechanisms which regulate expression of Sox4 (Fig. 2), little is known about the mechanisms responsible for the aberrant expression or function of Sox4 in cancers.

In this review, we summarized the progress made over more than two decades after the discovery of Sox4 in defining its role in tumorigenesis, with an emphasis on the tissue-dependent function of Sox4 as well as occasional discrepancies between various reports. Although the actual reason for these discrepancies is not known, in some cases it may be due to the remarkable short half-life of the Sox4 protein and the inability of mRNA expression studies to accurately reflect the level of protein expression. Indeed, it seems that Sox4 mRNA expression may not be able to mirror the expression level of its translation product [22], and is insufficient to permit the drawing of a conclusion regarding the expression levels of the Sox4 protein in different types of cancer. This notion is especially critical in the studies which use RT-PCR or cDNA microarray. It is worth emphasizing that Sox4 contains a single exon and, due to the lack of introns, it is difficult to distinguish between the sequences of the genomic DNA and the complementary DNA (cDNA) in studies that investigate the expression levels of the Sox4 transcript. Therefore, more precautionary measures such as DNase treatment of the extracted mRNAs are required to avoid false positive signals due to genomic DNA contamination in these types of studies.

At least one study has identified three variants, including a nonsense mutation in the coding region of Sox4 that could result in expression of a truncated isoform [76]. Although it is not known whether similar truncated isoforms of Sox4 are also expressed in other types of tissues, the majority of studies regarding the expression status of Sox4 in cancer did not consider its mutation status and possible influences of these mutations in stability and/or

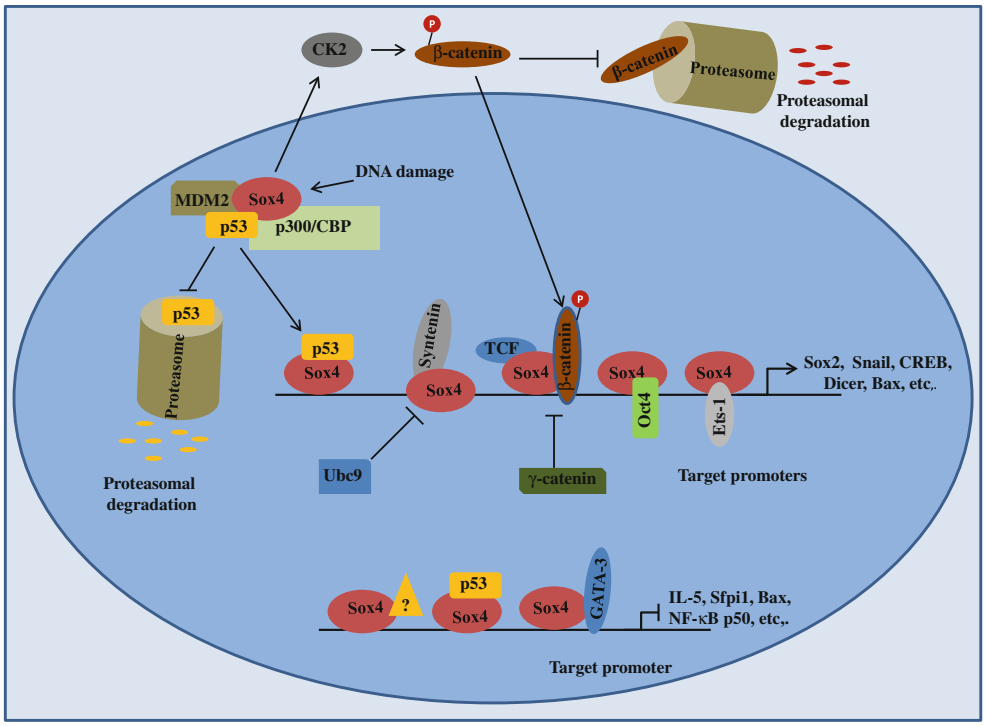

Fig. 4 Regulation of gene expression by Sox4. So far, two modes of regulation of gene expression by Sox4 have been identified; transcriptional and post-translational. At the transcriptional level, Sox4 can act both as a transcription factor and repressor, depending on the gene or cell type of interest. This differential regulation of gene expression, which is discussed in the text, plays a very important role in oncogenic or tumor suppressive functions of Sox4. It should be noted that not all of the illustrated complexes are present in every type of cell

functional properties of the Sox4 protein in the tissues of interest. It is intuitive that, due to increased mortality associated with late stages of cancer, factors whose expression positively correlates with tumor progression should have an inverse correlation with patient survival. Nevertheless, in some cases, we observe a contradiction between Sox4 expression pattern and its correlation with survival (Table 1). It is plausible that these contradictions may be due to expression of multiple isoforms and/or mutant forms of Sox4 in these malignant tissues, particularly the mutants that lack the C-terminal domain and are more stable [76], perhaps due to the absence of the degron located in this domain [22]. Future studies are required to empirically validate the expression or lack of expression of such isoforms in other types of cancer and determine what proportion (if any) of the Sox4 staining could be due to these isoforms.

At first glance, the inconsistency in the role of Sox4 in various tumors might seem puzzling. Sox4 expression gives a selective edge to some types of tumors, consistent with an oncogenic role, while it can suppress initiation or progression of other types of cancer, fitting to the definition of a tumor suppressor. As mentioned earlier, this feature is characteristic of Sox family whose function relies on the context of the host cells. In addition, in the same type of cells, Sox4 can impede or augment activity of target promoters. For instance, in melanoma cells, we observed that Sox4 inhibits NF-κB p50 [104] but induces Dicer [107] promoter activity. At the molecular level, this discrepancy might be explained by the requirement for specific partners at the site of the target promoters for Sox4 to either enhance or inhibit their activity. So far, several factors, such as POU proteins Oct4 [44] and Brn2 [16], as well as other factors like Tbx3 [27], p53 [20], GATA-3 [32], TCF4 [21], and JUP [55], have been identified to cooperate with Sox4 in the regulation of target promoters. These binding partners can have a crucial impact on the expression profile of Sox4 downstream targets and its overall function in each type of cell (Fig. 4). The expression pattern of many of these partners is differentially regulated in various cell types or different developmental stages of the cells. For instance, Oct4 is a well-known marker of pluripotency which is expressed in embryonic stem cells, some adult stem cells, and cancer progenitor cells, but not differentiated cells [128]. This phenomenon would restrict the effect of Sox4 on the expression of those targets only to the cell types that express both Sox4 and Oct4. Nevertheless, in most cases, the putative binding partners of Sox4 are not known, which beckons further attention in future studies to this important aspect of the Sox4 network.

Sox4 regulates the expression of a large set of genes in different tissues as evidenced by genome-wide cDNA microarray or promoter binding profiling studies. Despite this large number, there seems to be very limited overlap between the Sox4 targets identified in different studies (Fig. 3). This negligible overlap further indicates the existence of distinct Sox4 downstream pathways in each cell type. Also, in the majority of cases, it is not clear whether regulation of target gene expression by Sox4 is achieved by direct binding to their promoters and acting as a transcription factor or via indirect modes such as activation of a mediator. We compiled a list of genes whose expression is demonstrated to be either up- or downregulated in a direct manner by Sox4 (Table 2). This list contains a wide range of proteins involved in different cellular functions, including but not limited to inflammation, axon direction, differentiation, cell growth, and proliferation. But one major group of validated targets consists of prominent transcription factors such as CREB, Sox2, and TCF4. Considering the potential targets of each of these transcription factors, there is an immense network of genes whose regulation is at some level controlled by Sox4. Furthermore, miRNA biogenesis factors are also among the direct transcriptional targets of Sox4. miRNAs are major regulators of gene expression, and it is estimated that at least one-third of all protein-coding genes in the human genome are regulated by miRNAs [129]. These notions highlight the immense number of potential Sox4 target genes, and also indicate that many of the genes whose expression status is changed by loss or overexpression of Sox4, as identified by cDNA microarray studies, may in fact be indirect targets.

Many miRNAs have opposite effects on the biological or pathological state of the cells depending on the type of tissue and the expression profile of their target protein-coding mRNAs. Therefore, regulation of their maturation by Sox4 [80, 107] may explain, at least in part, the pleiotropic role of Sox4. It is notable that regulation of miRNAs expression by Sox4 may not only be limited to their post-transcriptional maturation. Indeed, it is plausible that Sox4, as a transcription factor, can directly regulate the transcription of certain miRNAs. In addition, some downstream targets of Sox4, such as p53, have been shown to control expression of a number of prominent miRNAs involved in tumorigenesis [130]. These notions may contribute to delineating the complexity of Sox4-mediated regulation of normal and pathological processes.

Finally, the pleiotropic and tissue-dependent functions of Sox4 may have some clinical significance. Accordingly, this demands the utmost attention to consider this feature of Sox4 in future studies to design therapeutic strategies involving suppression or activation of Sox4 for treatment of a specific type of malignancy, as the desired effect of the modulation of Sox4 in one tissue may also end up as a major side effect in another type of tissue.

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