Published in final edited form as:

Pediatr Nephrol. 2013 June ; 28(6): 843–854. doi:10.1007/s00467-012-2246-1.

The EYA-SO/SIX complex in development and disease

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Abstract

Eyes absent (EYA) and Sine oculis (SO/SIX) proteins function as transcriptional activation complexes and play essential roles in organogenesis during embryonic development in regulating cell proliferation and survival and coordination of particular differentiation programs. Mutations of the *Eya* and *So/Six* genes cause profound developmental defects in organisms as diverse as flies, frogs, fish, mice, and humans. EYA proteins also possess an intrinsic phosphatase activity, which is essential for normal development. Here, we review crucial roles of EYA and SO/SIX in development and disease in mice and humans.

Keywords

Transcription factor; Phosphatase; Sensory placode; Specification of metanephric mesenchyme; Induction of ureteric bud; Ureter; Cell proliferation; Cell survival

Introduction

The Eyes absent (*Eya*) and Sine oculis (*So/Six*) gene families were first identified in *Drosophila* as key coregulators for eye development and subsequently they were identified in a number of species ranging from worms, frogs, fish, birds, and to higher vertebrates [1–13]. *Eya* is also found in plants such as *Arabidopsis* and rice [14] and homologues of *So/Six* genes have been identified in sponges [15]. While *So/Six* genes encode transcription factors with direct DNA-binding capacity, *Eya* genes encode proteins that affect transcription indirectly by interacting with DNA-binding proteins such as SO/SIX as well as other DNA-binding proteins. The key role of EYA-SO/SIX during development and disease was first revealed by loss- and gain-of-function studies in fruit flies. In *Drosophila*, *eya* functions in a molecular network with the fly *Pax6* gene *eyeless* (*ey*), *so* and *dachshund* (*dach*) to regulate eye morphogenesis (Fig 1) [16]. The components of this network have been highly conserved during evolution with related genes in mammals regulating the development of multiple organ systems [17, 18]. When misexpressed, *eya* acts synergistically with *so* or *dach* to direct the formation of ectopic eyes [19, 20]. Genetic analyses indicate that *eya* is epistatic to *so* and both reside in a transcriptional network downstream of *ey* (Fig 1) [21, 22].

While there appears to be only a single *eya* or *so* gene in fruit flies, at least four *Eya* (*Eya1–4*) and six *So* (*Six1–6*) genes are present in the mammalian genome [2, 9, 23–25] (Fig 2).

EYA proteins are defined by the presence of a 271–273—amino acid conserved C-terminal domain, the Eya domain (ED). SO/SIX proteins are identified by the presence of two evolutionarily conserved domains, a 110–115—amino acid SO/SIX-specific domain (SD) and a 60—amino acid homeo-domain (HD). Crucial functions during organogenesis have been described for several EYA and SO/SIX family members in a variety of organisms. Recent studies have provided exciting data suggesting that EYA and SO/SIX proteins have roles at several stages during organ development, including cell fate specification, proliferation, differentiation, and maintenance. In this review, we provide a general background to the EYA-SO/SIX complex and focus on the roles of EYA-SO/SIX in inner-ear and renal development and diseases

EYA and SO/SIX family proteins and their functional domains

Eya gene products operate as both transcription cofactors and phosphatases. Transactivation assays identify the divergent N-terminal transcription activation domain in EYA proteins (Fig. 2) [26]. The conserved Eya domain not only interacts with other proteins but also possesses a catalytic motif belonging to the phosphatase subgroup of the haloacid dehalogenase (HAD) superfamily of enzymes [27–29]. Work from several groups has demonstrated that EYA protein has phosphatase capability in vitro either as a protein tyrosine phosphatase (PTP) when using recombinant Eya domain (the catalytic domain-containing region only) [27, 28] or as both protein tyrosine and serine-threonine phosphatase when using recombinant full-length EYA [29]. These findings suggest that EYA is likely to regulate the phosphorylation state of its transcriptional cofactors or itself to directly modify transcriptional output within a cell.

The physiologically relevant substrates remain largely unknown. In vitro studies have identified RNA polymerase II [29] and EYA itself [28] but neither has been validated in vivo. Recent studies have identified DNA damage-related histone H2AX as EYA's first in vivo relevant substrate [30, 31]. However, it is not understood whether the two functions depend on each other and how they operate during development in mammals. Future studies involving isolation of more binding partners and in vivo substrates of EYA will likely reveal the nature of how its functions as both transcriptional coactivator and phosphatase are interconnected and used throughout development.

The SO/SIX family represents a divergent group of the homeodomain (HD) superfamily of transcription factors and it contains the HD and the SO/SIX-specific domain (SD) involved in protein-protein interactions with EYA and other proteins (Fig. 2) [20]. SO/SIX family members have been divided into three subfamilies (SIX1/2, SIX4/5, and SIX3/6) on the basis of *Drosophila* SO, which belongs to the same subfamily as vertebrate SIX1/2 (Fig. 2). The SO/SIX HD binds to a TGATAC consensus sequence, differing from the classic TAAT core sequence because it lacks two highly conserved amino acid residues typical of most HDs [1, 32–34]. However, the HD of SIX3/6 can also bind to a common TAATGTC motif [35]. Depending on the context, SO/SIX proteins can act as either transcriptional activators [36, 37] or repressors [38]. When both EYA and SO/SIX are coexpressed, EYA-SO/SIX interactions lead to the formation of a bipartite transcription factor, with the homeodomain of SO/SIX providing the DNA-binding activity and the N-terminus of EYA providing the

transactivation potential, which leads to potent transcriptional activation—even under conditions in which SO/SIX alone acts as a repressor [36–39].

Factors that interact physically with EYA and SO/SIX proteins and regulate their transcriptional activity have been identified by yeast two-hybrid screens and immunoprecipitation. Recent findings have demonstrated that SOX2 physically interacts with EYA1 or SIX1 both in vitro and in vivo to synergize transcriptional output of EYA1-SIX1 during sensory cell development [39] and neurogenesis [40] in the inner ear. In addition to SOX2, components of the SWI/SNF chromatin-remodeling complex and the helix-loop-helix (bHLH) transcription factors NEUROG1 and NEUROD1 are found to be binding partners of EYA1 and SIX1 during inner-ear neurogenesis [40]. G protein subunits [41–43] and two novel EYA1-binding partners Sipl1 (Shank-interacting protein-like 1) and Rbck1 (RBCC protein interacting with PKC1) have been identified through yeast two-hybrid screens [44], while the T-box transcription factor TBX18 is found to physically interact with SIX1 protein both in vitro and in vivo [45]. However, how these protein-protein interactions modify transcriptional activation and repression activities remain to be elucidated. Below, specific functions of EYA-SO/SIX during development and disease will be discussed and common themes will be emphasized.

Nuclear and cytoplasmic roles of EYA

While the EYA-SO/SIX transactivation activity clearly represents a nuclear function, the phosphatase activity functions in either the nucleus or cytoplasm. The *Drosophila* EYA is primarily localized to the nucleus, both in cultured cells and in flies [46]. It contains two putative classical nuclear localization signals, at residues 18–23 and 457–465, which are absent in vertebrate EYA family proteins [6]. In contrast, the vertebrate EYA is strongly distributed in the cytoplasm in vivo [47] and in cultured cells. Studies in cultured cells revealed that vertebrate EYA1–4 require coexpression of SIX1/2 or SIX4/5 subgroups to translocate into the nucleus [36,48, 49], which has not been demonstrated in vivo during development. Although we currently do not fully understand how EYA localization is regulated during development and how such regulation affects its dual trans-activation and phosphatase function, a recent study in *Drosophila* has shown that the Abelson tyrosine kinase-mediated phosphorylation relocates EYA from the nucleus to the cytoplasm, where its phosphatase function is required in vivo [50]. Other studies have found that cytoplasmic EYA4 plays a role in innate immunity via its threonine phosphatase activity [51] and that cytoplasmic EYA3 tyrosine phosphatase activity promotes cell motility [52]. However, the function of the EYA tyrosine phosphatase in DNA repair [30] is clearly a nuclear activity. Additional cytoplasmic roles have been suggested for EYA family proteins, as previous studies isolated cytoplasmic G proteins as EYA2-binding partners [42].

Eya and So/Six genes in vertebrate development and disease

EYA and SO/SIX have emerged as important regulators of organogenesis in all species. These genes are generally coexpressed during development with different combinations between different vertebrates. One notable feature of their expression is in the cranial ectodermal placodes that give rise to specialized paired sense organs and cranial ganglia in

the vertebrate head. In vertebrates, *Eya1/2* and *Six1/2* and *Six4/5* subfamily genes are induced at early neural-plate stages in a common preplacodal region and continue to be expressed in the different placodes and their derivatives, including eye, nose, and ear, as well as in all cranial sensory ganglia [2, 6, 53]. *Eya4* is also expressed in cranial placodes and their derivatives [23], while *Six3* is expressed in lens placode and the developing eye [54, 55].

The importance and critical roles of Eya and So/Six genes in sensory placodal development in the vertebrate head have been demonstrated by loss-of-function studies in mutants of *Eya1* and *Six1* in mice, humans, fish, and frogs, and recent gain-of-function analysis in frogs, fish, and mice [3, 18, 29, 56–67]. Mutants of *Eya1* and *Six1* exhibit a very similar spectrum of deficiencies reflecting their coexpression and synergistic interaction in different tissues. In humans, mutations in *EYA1* or *SIX1* cause branchio-oto-renal (BOR) syndrome, an autosomal dominant disorder with incomplete penetrance and variable expressivity characterized by combinations of branchial, otic, and renal anomalies [3, 62, 68]. *EYA1* or *SIX1* mutations also account for some cases of branchio-oto (BO) syndrome, a second disorder lacking the renal defects observed in BOR syndrome [69]. Mutations of *EYA1* were also detected in patients with congenital cataracts and ocular anterior segment anomalies [70]. In addition, a frameshift mutation of *EYA1* has been linked to cardiofacial syndrome characterized by the combination of an asymmetric crying face and heart defect [71]. *SIX1* mutations isolated from human patients have been characterized and are found to disrupt the ability of SIX1 to bind EYA1, or to bind DNA [62]. Four mutations in the *SIX5* gene were also identified from BOR/BO patients and these mutations are also found to affect EYA1-SIX5 interaction [68]. Similarly, *EYA1* point mutations identified within the conserved Eya domain that cause a single amino acid substitution abolish EYA1-SIX interaction [48]. In addition, *EYA1* mutants that are defective in their intrinsic phosphatase activity have been identified in BOR patients [72]. These data indicate that both EYA1's interaction with SIX1 and its phosphatase activity are crucial for normal development.

Many placodal-derived sensory structures in *Eya1* or *Six1* mutant mice are similarly affected [18, 57, 59, 60, 73]. There is much evidence that EYA1 or SIX1 is required not only for normal cell proliferation but also for survival. In the absence of either gene, progenitor cells fail to proliferate normally and undergo programmed cell death, thus leading to reduced size or absence of organ development [60, 73]. Recent studies have suggested that SIX1 may promote proliferation by interaction with cell cycle control genes such as *CyclinA1*, *CyclinD1*, and *c-Myc* [29, 74–76], and that EYA1 may modulate apoptosis and survival decisions by dephosphorylating histone H2AX [30].

New insights into the critical roles of EYA1-SIX1 in triggering particular developmental programs have recently been obtained. Recent results from loss- and gain-of-function analyses indicate that EYA1/SIX1 are likely to operate through different mechanisms to induce neuronal vs. sensory fate in the otic placode, with the former induced several days earlier than the latter during development [39]. During sensory hair cell development, EYA1-SIX1 is likely to physically interact and cooperate with SOX2 to synergistically activate the hair cell differentiation bHLH gene *Atoh1* transcription via direct binding to the conserved Sox- and Six-binding sites in *Atoh1* enhancers to specify hair cell fate [39]. In

contrast, during neurogenesis, EYA1-SIX1 appears to interact with SWI/SNF chromatin-remodeling complex to specifically mediate the transcriptional activities of the bHLH family genes *Neurog1* and *Neurod1* to trigger the neuronal developmental program. SOX2 also appears to synergize with EYA1-SIX1 to induce neuronal development [40].

Six4 and *Eya4* are also expressed early in placodes but their mutant mice do not show early placodal phenotype [77]. This may be due to the ability of SIX1 or EYA1 to functionally compensate for the loss of SIX4 or EYA4 to some degree, as suggested by enhanced placodal phenotypes observed in *Six1/Six4* double-knockout mice [78, 79]. Mutations in the *EYA4* gene cause late-onset autosomal dominant hearing loss at the DFNA10 locus [80–82]. This suggests that *Eya4* plays an important role in inner-ear function. A recent study has shown that EYA4 and SIX3 interact both physically and functionally, and both genes have been reported to be mutated in some cases of holoprosencephaly [83–85].

In addition to cranial sensory structures, the *Eya* and *So/Six* genes are widely coexpressed in renal and urogenital ridge region, branchial arches and craniofacial region, dorsal root ganglion, skeletal muscles, heart, thymus, and parathyroid. Data from the analysis of *Eya* and *So/Six* mutants have demonstrated that these genes play important roles in the development of these organs/tissues as well as in tumorigenesis (Table 1) [52, 86–89]. During skeletal muscle development, several direct targets for SO/SIX proteins, including Myogenin [34], aldolase A [90, 91], and Myf5 [92] have also been identified. Below, the roles of EYA and SIX in renal development and diseases will be discussed.

EYA-SIX in renal development and diseases

Eya and *So/Six* family genes are known regulators of kidney development. In mammals, kidney development occurs in three stages that are all characterized by the mesenchyme-to-epithelial transformation within the intermediate mesoderm. The development of pronephros, the first kidney, is initiated at embryonic day 8 (E8) in mice when the nephric (Wolffian) duct (WD) forms from the intermediate mesoderm of the anterior region of the embryos [93, 94]. The mesonephros appears at E9.5 when the WD grows caudally and induces the adjacent nephrogenic mesoderm to aggregate and form mesonephric tubules. Both the pro- and mesonephros are transient organs and regress shortly after their formation. The development of metanephros or permanent kidney initiates at E10.5 when the ureteric bud (UB) appears as a thickening of the WD at its caudal end [95]. Its development involves several distinct processes: First, formation of the metanephric mesenchyme (MM) from intermediate mesoderm; second, induction and outgrowth of the UB; third, branching of the UB and differentiation of the mesenchyme into renal epithelial cells.

The MM appears as an aggregate of cells at the caudal end of the nephrogenic cord at around E10.5 in mice and the establishment of a functional MM is a central step in kidney organogenesis as this tissue has the specialized capability of inducing the growth and branching of the UB in its characteristic pattern [96–98]. The signals from the MM that regulate UB formation have been extensively reviewed [93, 99–101]. Among many of the signaling molecules, GDNF, which acts as a ligand that binds to the Ret receptor tyrosine kinase and the *Gfra1* coreceptor expressed in the WD, is a major inducer [96–99]. Genetic

studies have identified many other transcription factors, including HOX11, PAX2, OSR1, EYA1, WT1, SIX1, SIX4, and SALL1, which are important for stimulating and maintaining GDNF expression in the MM. *Eya1* appears to be important for the expression of *Gdnf*, *Six1*, *Six2*, and *Pax2*, and it also genetically interacts with *Six1* and *Pax2* as kidney phenotype is enhanced in their compound mutants [17, 102].

Prior to UB formation, the caudal WD swells to form a pseudostratified domain and previous data from *Osr1*^{-/-} mutants in which the WD fails to become pseudostratified suggests that an inductive signal from MM may act on the WD to induce pseudostratification [103]. However, GDNF does not appear to be required as the WD becomes pseudostratified in *Ret*^{-/-} animals [104]. Deletion of *Eya1* results in renal agenesis due to a complete absence of the MM (Fig. 3) [102]. The WD, pro-, and mesonephric tubules still form and the mutant WD is functionally competent for UB outgrowth as recombinant GDNF is able to induce UB formation from *Eya1*^{-/-} WD. However, the caudal WD is not pseudostratified and remains as a narrow epithelial tube in *Eya1*^{-/-} mutants [102] (J. Xu and P-X. Xu, unpublished), similar to that observed in *Osr1*^{-/-} mutants. Since *Eya1* and *Osr1* are coexpressed in the MM but not in the WD, the two genes might act together to regulate mesenchymal signals to induce pseudostratification. Further studies are required to define the nature of induction and what roles EYA1 plays to mediate pseudostratification.

In contrast to about 93 % of human BOR patients exhibiting hearing loss, only about 6 % of BOR patients reveal renal defects. This includes polycystic, hypoplastic, or totally absent kidneys and malformation of the collecting system, such as duplication or absence of the ureter, megaureter, blunted or distorted calyces, and extra or bifid pelvis [105–107]. These anomalies were observed in *Eya1* heterozygous animals [57]. Since *Eya1* is highly expressed in the undifferentiated cap mesenchyme and its expression is largely decreased in renal vesicles, it may play a critical role in regulating cell proliferation/renewal of the cap mesenchyme.

Among the nine members of the mammalian So/Six gene family, *Six1*, *Six2*, and *Six4* are expressed in the mesenchyme, which overlaps with *Eya1* [17, 108]. Deletion of *Six1* or *Six2* leads to abnormal kidney development [17, 57, 62, 108, 109]. *Six4* appears to act synergistically with *Six1* during normal development of the renal system, as the MM is not normally specified (J. Xu and P-X. Xu, unpublished) and *Gdnf* expression is not maintained in *Six1*;*Six4* double mutants [108]. *Six1* expression in the MM, which disappears after the initiation of UB branching, appears to mediate the expression of *Six2* and *Pax2*. Inactivation of *Six2* in mice leads to hypo-dysplastic kidney due to premature differentiation of metanephric mesenchymal progenitors [109, 110]. Mutations in the human *SIX2* have been linked to renal hypodysplasia, which is characterized by maldevelopment of renal tissue and/or reduced kidney size [111]. Expression and genetic studies have shown that *Eya1* and *Six2* are coexpressed in the cap mesenchyme and that *Eya1* is upstream of *Six2* in the MM as *Six2* expression is lost in *Eya1*^{-/-} mutants. Future studies will likely reveal that *Eya1* and *Six2* also act together to regulate cell proliferation/renewal of the cap mesenchyme.

More recently, *Six1* is found to be expressed in ureteral mesenchyme and it is essential for UB patterning by regulating BMP4-GREM1 interaction [45]. The balance between the

levels of BMP4 activity in the mesenchyme surrounding the nascent UB tip and GDNF production in the MM is crucial for UB development into either ureter or collecting system. BMP4 is a mesenchymal regulator promoting ureter development and is expressed in the mesenchyme surrounding the WD [112, 113]. The BMP inhibitor GREM1 is initially expressed in the mesenchyme around the caudal WD and nascent UB and its expression gradually becomes restricted in the MM to negatively regulate BMP4 activity to induce UB branching (Fig. 4). In *Six1*^{-/-} mutants, UB grows out normally and elongates to differentiate into ureter but fails to form collecting system caused by loss of *Grem1* expression in the MM, which leads to retention of BMP4 levels [17, 29, 114, 115] (Fig. 4). GREM1-soaked beads implanted into *Six1*^{-/-} kidney primordia in culture are sufficient to promote UB branching and removal of only one copy of the *Bmp4* gene in *Six1*^{-/-} embryos restores UB patterning and kidney organogenesis [115]. These data indicate that lowering of mesenchymal BMP activity is likely to relieve repression of UB outgrowth and enable its invasion into the mesenchyme in response to GDNF signaling. *Six1* might be specifically required for upregulating the expression of *Grem1* in the MM to locally restrict BMP4 activity in the mesenchyme to initiate ampulla formation and branching morphogenesis during urinary tract morpho-genesis (Fig. 4). How SIX1 acts to regulate *Grem1* expression and whether it interacts with EYA1 to trans-activate *Grem1* remain to be characterized.

Although ureter forms in *Six1*^{-/-} mutants, the differentiation of smooth muscle cells is impaired [45, 114]. Genetic and in vitro analyses have shown that *Six1* interacts with *Tbx18* to regulate ureteral smooth muscle cell development [45]. However, the direct downstream targets of these two proteins remain to be identified.

Downstream targets of and transcriptional regulation by the EYA-SO/SIX complex

In the *Drosophila* eye, *eya-so* target genes include *lozenge* (*Iz*), *hedgehog* (*hh*), *ey*, *so*, *string*, and *atonal* [116–118]. Only two of them, *hh* and *Iz*, have been elegantly shown to be transcriptionally regulated by EYA-SO complex in combination with other signaling pathways [116, 118]. In vertebrates, while expression studies have reported that the expression of many genes were affected in *Eya*- or *Six*-null mice, SO/SIX-binding DNA elements were found in *Na⁺/K⁺ ATPase α 1* subunit, *myogenin*, *Igfbp5*, *aldolase A*, *c-myc*, *Gdnf*, *cyclin A1*, *cyclin D1*, *Slc12a2*, *p27Kipl*, *muscle creatine kinase*, *ezrin*, *Six2*, and *Atoh1* [29, 32, 34, 38, 39, 75, 76, 91, 119–121]. Among these putative SO/SIX direct targets, combinatorial and direct transcriptional activation by the EYA-SO/SIX complex in combination with other factors has only been demonstrated for *Myogenin* during muscle development [34, 91] and *Atoh1* during cochlear hair cell induction [39].

Conclusions and future directions

The studies summarized here have begun to elucidate the important functions that Eya and So/Six genes play during multiple stages of development. These multiple functions such as maintaining proliferative state of cells and triggering differentiation programs, suggest a diversity of activity at the cellular level. This diversity is reflected at the molecular level by complex modes of interaction with chromatin, multiple binding partners and sophisticated

transcriptional activation and repression activities, as well as dephosphorylation and phosphorylation activities. However, details about how the EYA-SO/SIX network has been evolutionarily coopted and modified during distinct organ development, and their precise mode of action in mediating self-renewal of metanephric mesenchymal progenitors and mesenchyme-to-epithelial differentiation in the kidney, as well as other specific developmental programs, have much left to reveal. Future studies of conditional/tissue or cell type-specific knockouts, genetic lineage tracing, and ChIP-seq analysis are essential for fully understanding how this complexity is regulated in different biological processes and disease states.

Acknowledgments

The author wishes to thank all of the past and present members of my lab for their many contributions and valuable discussions. Some of the work presented was supported by grants from the National Institutes of Health to P-X. X (NIH RO1 DC005824 and DK064640).

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Fig. 1. The EYA-SO/SIX regulatory network. **a** The transcriptional hierarchy among the key regulators in the fly eye discs. Feedback loops and protein-protein interactions (*double-headed arrows*) are indicated. **b** EYA and SIX form transcriptional activation complexes to control cell proliferation and survival, as well as to trigger particular differentiation programs. EYA-SIX also interact with other proteins (X), which may vary depending on distinct developmental and cellular contexts and modify the specificity of EYA-SIX function

Fig. 2.

Structure of the EYA and SO/SIX family proteins. **a** Schematic representations of the fly EYA and mouse EYA1–4 proteins. The percentage of amino acid identity between the mouse and fly EYA are indicated. **b** Schematic representations of the fly SO and mouse SIX1–6 proteins showing their characteristic structural features. In addition to the Six-specific domain (SD) and the homeodomain (HD), SIX2, SIX4, and SIX5 possess a transcriptional activation domain (AD). The percentage of amino acid identity between the mouse SIX and fly SO proteins are indicated

Fig. 3. *Eya1*^{-/-} embryos lack metanephric mesenchyme (mm) and UB induction. **a, b** H&E-stained sections from posterior metanephric regions of E10.5 normal embryos showing the MM in wild-type embryos. *Arrow* points to no MM in *Eya1*^{-/-} embryos (**b**). **c–h** Whole-mount in situ hybridization showing *C-ret* expression in the WD and developing UB in wild-type embryos, but in *Eya1*^{-/-} mutant, the caudal WD is not induced for UB development (*arrows*). **i, j** Whole-mount in situ hybridization showing *Pax2* expression in the induced

MM and ureteric epithelium, but the UB development is not induced in *Eya1*^{-/-} embryos (*arrow*)

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Fig. 4.

Six1 is required for *Grem1* expression in the MM. **a, b** A section stained by X-gal showing *Six1* expression in the MM at E10.5 (**a**), but its expression disappears at E11.5 (**b**). **c, d** Section in situ with *Grem1* probe showing its expression in the MM surrounding the UB tip in normal (**c**) but its expression is largely reduced in *Six1*^{-/-} embryos (**d**). **e** Schematic drawing to show that upregulation of GREM1 by SIX1 in the MM is essential to locally restrict BMP4 signaling for the initiation of branching morphogenesis. In mice, the UB formation and outgrowth is induced by GDNF-RET signaling, which is initially expressed at normal levels in *Six1*^{-/-} embryos. During this inductive period, *Six1* is expressed in the MM

(*green*). Bmp4 (*red*) is expressed in the mesenchyme enveloping the WD and nascent UB. *Grem1* transcripts are upregulated in the MM around the UB tip region, which may locally antagonize BMP4 to enable ampulla formation of the UB and its invasion into the MM. *Gdnf* expression is also upregulated during this period to stimulate ampulla formation and establish WNT11-GDNF signaling for branching of the ampulla. In *Six1*^{-/-} embryos, the UB outgrowth is normal but the tip cells fail to be induced for ampulla formation. Instead, the tip is wrapped up by Bmp4-expressing mesenchyme, which is the cellular source for ureteral mesenchyme [122]. By responding to the signaling in the Bmp4-positive mesenchyme, the tip is induced for ureter differentiation. Our results show that the balance between the levels of BMP4 activity in the Bmp4⁺ mesenchyme and GDNF production in the MM may also be critical for UB patterning, as excessive GDNF can restore branching as well as kidney formation in *Six1*^{-/-} kidney rudiments in culture and lowering BMP4 activity in vivo can rescue branching morphogenesis and nephron formation [115] (used with permission)

Table 1

The Eya and Six gene families in mammals

Gene	Target organs/tissues	Associated human diseases	Cancers
EYA1	Ear, branchial arches and craniofacial, sensory neurons, thymus and parathyroid, kidney, ribs, skeleton, muscle	Branchio-oto-renal (BOR) syndrome Branchio-oto (BO) syndrome Cardiofacial syndrome	Acute leukemia (MLL)
EYA2	Muscle defects enhanced in <i>Eya1;Eya2</i> double mutants		Ovarian cancer Desmoid tumors Colorectal cancer
EYA3	Decreased body size, respiratory, muscle and heart function		Breast cancer cell line
EYA4	Ear	Late-onset deafness (DFNA10) tumors (MPNSTs) Cardiomyopathy Holoprosencephaly (HPE)	Malignant peripheral nerve sheath tumors (MPNSTs) Sporadic and colitic neoplasia Colorectal cancer Barrett's esophagus and esophageal adenocarcinoma
SIX1	Ear, olfactory, taste buds, branchial arches and craniofacial, sensory neurons, thymus and parathyroid, renal system, skeletal muscle	BOR or BO syndrome	Breast cancer Wilms' tumor Rhabdomyosarcoma Hepatocellular cancer Ovarian and cervical cancer Lung cancer
SIX2	Kidney	Renal hypodysplasia (RHD)	Lung cancer
SIX3	Forebrain, lens, diencephalon	HPE Aprosencephaly/atelencephaly (AP/AT)	Extraskelatal myxoid chondrosarcomas
SIX4	No obvious defect in <i>Six4</i> -null mice. Defects enhanced in <i>Six1;Six4</i> double mutants		
SIX5	Eye, heart, testis	Myotonic dystrophy (DM), BOR	Borderline ovarian tumors
SIX6	Eye, pituitary gland	Anophthalmia with pituitary defects	Acute T-cell leukemia