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REVIEW

The Orphan Nuclear Receptor TLX/NR2E1 in Neural Stem Cells and Diseases

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Abstract The human *TLX* gene encodes an orphan nuclear receptor predominantly expressed in the central nervous system. *Tailess* and *Tlx*, the *TLX* homologues in *Drosophila* and mouse, play essential roles in body-pattern formation and neurogenesis during early embryogenesis and perform crucial functions in maintaining stemness and controlling the differentiation of adult neural stem cells in the central nervous system, especially the visual system. Multiple target genes and signaling pathways are regulated by TLX and its homologues in specific tissues during various developmental stages. This review aims to summarize previous studies including many recent updates from different aspects concerning TLX and its homologues in *Drosophila* and mouse.

Keywords TLX · Neural stem cell · Neurogenesis

Introduction

Nuclear receptors (NRs) are a group of transcriptional regulators with broad functions in physiological and developmental processes, including metabolism, xenobiotic defense, and reproduction [1]. So far, 18, 49, and 48 NRs in six subfamilies have been identified in *Drosophila melanogaster*, mice, and humans, respectively [2–4]. *TLX*, also known as *NR2E1* (nuclear receptor subfamily 2, group E, member 1), encodes an orphan NR with no identified ligand. *TLX* is the human homologue of the *Drosophila tailless* (*tll*) gene and is predominantly expressed in the developing brain and retina [5–7]. Human TLX and its homologues in *Drosophila*, zebrafish, and mouse have conserved functions in brain and retinal development [8–10]. TLX as well as its homologues in other species predominantly acts as a transcriptional repressor, although a function in transcriptional activation has also been suggested in some studies [11, 12].

The functional equivalence between human *TLX* and mouse *Tlx* has been supported by the finding that the expression of human *TLX* reverses the brain and eye defects and aggressive behavior of mice with a deletion of *Tlx* [13, 14]. Given the validated function of TLX in neural stem cells (NSCs) and brain development, it has been suggested that defects in *TLX* are associated with several human mental illnesses such as schizophrenia and bipolar disorders [15]. TLX has also been associated with NSC expansion and brain tumorigenesis [16].

Protein Structure of TLX

The human *TLX* gene was first cloned in a 24-kb region on the chromosomal band 6q21 [6]. This gene includes 9 exons and encodes a protein of 385 amino-acids. Like its homologues in *Drosophila*, zebrafish, and mouse, the human TLX protein contains a highly-conserved N-terminal DNA-binding domain (DBD), a hinge domain, and a moderately-conserved C-terminal ligand-binding domain (LBD) based on sequence homology analysis [5, 7, 17]. The TLX DBD contains two C4 zinc-finger motifs and includes a P Box, a D Box, and a T/A box involved in DNA binding and dimerization (Fig. 1) [11]. It is notable that TLX contains a ligand-dependent C-terminal activation function 2 (AF-2) domain but not a ligand-independent

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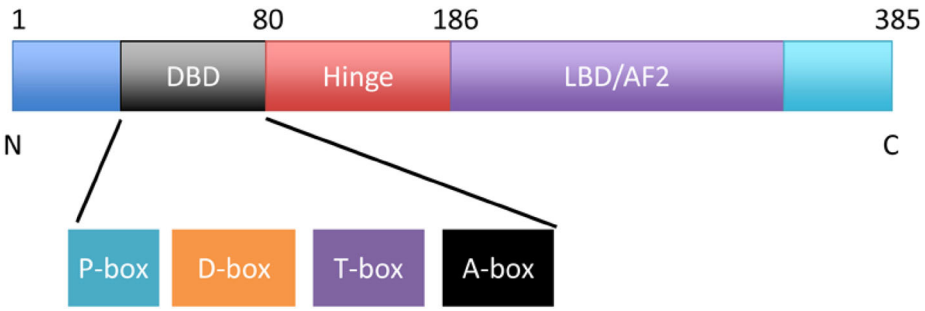

Fig. 1 Schematic diagram of basic TLX structure. *DBD* DNA-binding domain, *LBD* ligand-binding domain, *AF* activation function.

N-terminal activation function 1 (AF-1) domain, which is found in many NR family members [18]. In order to explore the ligand-binding potential of TLX, Benod *et al*. recently developed three homology models for the TLX LBD in a transcriptionally-active conformation using a chimera LBD template based on the crystal structures of two human NRs, COUP-TFII (COUP transcription factor II) and RXRα (retinoid X receptor α) [18–20]. This study confirmed that the TLX LBD structure resembles other NR LBD conformations as a canonical helical sandwich, and also revealed notable differences, in that the TLX LBD lacks the first helices α1 and α2 and contains a unique 5-amino-acid insertion between helices α8 and α9. In contrast to the previous description of the full-length TLX-forming monomer [21], Benod *et al*. suggested that the recombinant TLX LBD can form a homodimer in solution [18]. More interestingly, three compounds were shown to be able to bind the recombinant TLX LBD, indicating that TLX functions can be manipulated using selected compounds [18]. Most recently, Xu *et al*. have reported the crystal structure of the corepressor assembly in TLX. They found that TLX adopts an auto-repressed conformation in which its helix H12 occupies the coactivator-binding groove, and that, unexpectedly, H12 in this auto-repressed conformation forms a novel binding pocket with residues from helix H3 that accommodates a short helix formed by the conserved ALXXLXXY motif of the Atro box [22].

TLX Expression, Its Targets, and Related Signal Pathways

The expression patterns of *TLX* and its homologues in other species have been extensively investigated. The *Drosophila tll* mutant was identified during large-scale screening for genes associated with pattern-formation in the early embryo [23]. *Tll* plays important roles in establishing the segmentation pattern during early embryogenesis. It is expressed at both ends of early embryos and functions to suppress the expression of other genes such as *Krüpple* and *Knirps* in both the anterior and posterior caps of early embryos; this is indispensable for the appropriate specification of terminal cells [9, 24, 25]. Most of the posterior structures and some portions of the anterior structures including the brain are missing in *tll* mutant embryos [9, 26–28]. Embryonic cells expressing *tll* in the head region develop an optic lobe instead of a Bolwig's organ because tll antagonizes EGFR signaling, which is required for cells to develop into Bolwig's organ [29]. *Tll* is also expressed in undifferentiated proliferating neural precursor cells at the third instar larval stage, the last larval stage before eclosion into adult flies, indicating that tll may have an essential function in neuronal stem cells [11]. Consistent with the findings in *Drosophila*, mouse *Tlx* is expressed in the developing central nervous system (CNS) from embryonic day 8 (E8) until E13.5 and in retinal progenitor cells (RPCs) from E11.5 until E17.5 during retinal development [5, 30–32]. At the embryonic stage, *Tlx* expression presents a graded pattern in the telencephalon along the dorsal–ventral axis with a high level in the dorsal-lateral region and a low level in the ventral-medial region [33]. *Tlx* is also expressed in postnatal NSCs and may activate neurogenesis in the subventricular zone of the lateral ventricle based on the findings that *Tlx* mutant brains have severely reduced hippocampal dentate gyri and greatly expanded lateral ventricles in adult mice [34–36]. *Tlx* expression is also detectable in the ventricular zone enriched with NSCs at the postnatal stage [30, 37, 38]. In adults, TLX is detectable in the subependymal zone of the CNS [38]. *Tlx* overexpression promotes NSC expansion and results in the development of gliomas in mouse [16]. Interestingly, that study also found that *TLX* expression is elevated in human glioblastomas. These studies suggest that the fruit fly and mouse homologues of human *TLX* are predominantly expressed in NSCs or neuronal precursor cells and are important for the proliferation and self-renewal of NSCs. Human *TLX* expression is detectable in multiple organs and tissues including the adrenal gland, brain, cerebellum, testis, placenta, and bone marrow [6, 15, 39]. According to the RNA-seq data from the Illumina Human Body Map 2.0 project (http://www.ensembl.org/info/genome/genebuild/rnaseq_annotation.html), *TLX* expression in brain is

significantly higher than in other organs and tissues, indicating its evolutionarily conserved function in the CNS.

Studies of *Drosophila* embryogenesis have suggested that Tll acts as a transcriptional repressor to directly suppress the expression of target genes such as *knirps*, *ems*, and *otd*, which are required for the formation of segmented regions in early embryonic development [28, 40, 41]. *Prospero*, which encodes a homeodomain transcription factor associated with the differentiation of ganglion mother cells after asymmetric stem cell division of neuroblasts in the central brain [42, 43], is the only target gene that has been found to be directly regulated by Tll in post-embryonic stages [44]. In the mouse, multiple genes involved in the development of the CNS and visual system have been identified as Tlx targets, such as *Gsh2*, *Pax2, Pten*, *p21*, *p57*, *S100β*, *Aqp4*, *Plce1*, *Wnt7a*, *microRNA-9*, *Bmp4*, and *GFAP* [21, 32–37, 45–50] (Table 1). Interestingly, Tlx may act as a transcriptional activator for the expression of *sirt1*, *Wnt7a*, *Mash1*, and *Oct-3/4* based on *in vitro* luciferase assays and chromatin immunoprecipitation assays [16, 21, 51–53]. A consensus Nr2e1 binding sequence within the promoter region of its target gene has been proposed to contain AAGTCA, which has been identified in the promoters of *Wnt7a*, *GFAP*, *S100β*, *Oct3/4*, *p21*, *Pax2*, *Plce1*, and *Pten* [21, 32, 34, 45, 46, 53, 54]. Given the functional equivalence and the highly-conserved DBDs between human TLX and mouse Tlx [13, 14], TLX may have the capacity to bind a similar consensus promoter sequence and suppress or activate the expression of multiple human counterparts of the identified mouse Tlx targets. In addition, a recent study using human neuroblastoma cell lines showed that human TLX, through binding the von Hippel-Lindau protein and stabilizing hydroxylated hypoxia-inducible factor, may bind the *VEGF* (vascular endothelial factor) promoter and induce *VEGF* expression to promote angiogenesis under hypoxic conditions [55]. A recent study [55] also reported that TLX binds the promoters of *MMP-2* (matrix metalloproteinase-2) and *Oct-4* and activates the expression of these genes in neuroblastoma cells [56]. This study further identified two TLX-binding sites with an AAGTCA sequence 1.2-kb upstream of the *MMP-2* transcription start site. Apparently, *TLX* has either a positive or a negative effect on target gene expression in different types of cells and tissues.

The identification of target genes regulated by *TLX* homologues in fruit fly, mouse, and other species indicates that TLX has important functions in various cellular and developmental processes. Recent studies by Qin *et al*. showed that mouse *Tlx* controls the timing of postnatal astrogenesis by regulating the BMP-SMAD rather than the JAK-STAT signaling pathway [48]. The expression of BMP signaling genes such as *Bmp4*, *Hes1*, and *Id3* and astrocyte marker genes such as *GFAP* and *S100β* is up-regulated in the postnatal brain of the *Tlx*-deleted mouse [48]. Tlx has been reported to interact with BMP7 and/or Sonic Hedgehog (SHH) and relieve the repression of *Pax2* expression in mouse retinal astrocytes [50]. Mouse Tlx also regulates the Wnt-Frizzled signaling pathway by directly activating *Wnt7a* expression [21]. Niu *et al*. showed that mouse Tlx is involved in regulating DNA replication and cell-cycle signaling in postnatal NSCs [49]. They found that Tlx directly suppresses the expression of *p21*, a critical cell-cycle gene, *via* a p53-dependent mechanism [49]. This is consistent with the previous finding of a Tlx-binding site in the *p21* promoter region [46]. In contrast to the antagonizing effect of *tll* on EGFR signaling in *Drosophila* embryonic optic lobe development, overexpression of mouse *Tlx* promotes EGFR signaling in cells within the subventricular zone of the adult brain [16]. *Tlx* may also modulate the mitogen-activated protein kinase pathways to regulate RPC differentiation [45]. In addition, mammalian target of rapamycin signaling and transforming growth

Table 1 Candidate target genes of Tlx

Target genes	Effect of Tlx	Conserved	DNA binding assay			Reference
		AAGTCA motif	Luciferase	EMSA	ChIP	
Pten	Repress	+	+	+	+	Sun *et al*. 2007 [46]
P21	Repress	+	+	+	+	Sun *et al*. 2010 [54]
Pax2	Repress	+	+	+	+	Yu *et al*. 2000 [32]
BMP4	Repress			+	+	Qin *et al*. 2014 [48]
miR-9/miR-137	Repress	+				Zhao *et al*. 2009 [47]
Gfap	Repress			+		Shi *et al*. 2004 [34]
Oct3/4 (Pou5f1)	Promote	+	+	+	+	Chavali *et al*. 2011 [53]
MMP-2	Promote	+	+			Chavali *et al*. 2014 [56]
SIRT1	Promote	+				Iwahar 2009 [52]
AscL1 (Mash1)	Promote		+			Elmi *et al*. 2010 [51]
Wnt7a	Promote	+	+			Qu *et al*. 2010 [21]

factor-β signaling are significantly perturbed in the retina of the *Tlx*-null mouse [45].

These distinct findings from different types of cells/tissues at different developmental stages suggest the temporal and spatial specificity of the Tlx regulation of gene expression and signaling pathways. Such temporal and spatial specificity probably depends on other interactive proteins that function together with Tlx (or TLX in humans) to form a transcriptional complex in specific cells at certain developmental stages. Atrophin1, BCL11A, LSD1 (a histone demethylase), histone deacetylase 3 (HDAC3), and HDAC5 have been reported to interact with Tlx to regulate the expression of target genes [45, 54, 57, 58]. The interactions among TLX, LSD1, and Atrophin1 have been validated in human Y79 retinoblastoma cells [57]. In that study, TLX, LSD1, CoREST, and HDAC1/2 were suggested to form a transrepression complex at the *Pten* promoter to suppress *Pten* expression in retinal cells. *Pax6* also genetically interacts with *Tlx* to regulate the dorsal–ventral patterning of the mouse telencephalon [33].

Roles of TLX Homologues in the Regulation of NSCs and Brain Development

Evidence from the *TLX* expression profile, TLX target genes, and signaling pathways involving TLX clearly demonstrated that *TLX* plays important roles both in embryonic neurogenesis and in maintaining the stemness of adult NSCs. As noted above, *Drosophila tll* is required for both anterior and posterior body pattern formation during early embryogenesis and is important for maintaining the proliferating and undifferentiated state of neuroblasts, *Drosophila* NSCs, in the late larval stage [9, 12, 25, 28]. Appropriate *tll* expression is crucial for the suppression of *prospero* expression and maintenance of the proliferation capacity in neuroblasts. In contrast, in the ganglion mother cells derived from neuroblasts through asymmetric division, a lack of *tll* expression and elevated *prospero* expression promote differentiation [42, 43]. It has been reported that ectopic expression of *tll* in ganglion mother cells inhibits apoptosis, promotes cell-cycle progression, and results in tumor formation in the *Drosophila* brain [44].

As noted above, mouse *Tlx* is expressed mainly in the CNS and NSC niche during all developmental stages. *Tlx* functions to maintain the self-renewal capacity of NSCs in mouse brain from the embryonic to the adult stage [11, 33, 49, 59]. Studies of *Tlx*-knockout mice have shown that *Tlx* is required for neurogenesis in the superficial cortical layers and for the normal development of zinc-containing cortical circuits [60, 61]. *Tlx* is also important for normal development of the cerebral hemispheres [62]. Roy *et al*. found that *Tlx* regulates the timing of neurogenesis in the cortex [38]. The temporal and regional requirements for *Tlx* expression in neuronal progenitor cells have been reported. In *Tlx*-deficient mice, CNS structures that are generated at a later developmental stage seem to be affected more severely than structures formed during early- or mid-stage neurogenesis. This timing effect may be related to the consequence of a shortened cell cycle due to loss of *Tlx*. Li *et al*. also found elevated *p21* expression, decreased *cyclin D1* expression, prolonged cell cycles, and increased cell-cycle exit in *Tlx*-null embryonic brains, showing that *Tlx* functions to regulate the cell cycle of NSCs in the developing mouse brain [37].

In addition to its functions in NSCs in embryonic brains, *Tlx* also maintains adult NSCs in an undifferentiated, proliferative state [34]. In that study, *Tlx*-null cells isolated from mutant adult mouse brains presented proliferation defects that were rescued by reintroducing *Tlx* expression. The essential role of mouse *Tlx* in activating neurogenesis from adult NSCs has been validated by several studies [35, 49]. Zhang *et al*. further showed that *Tlx* is indispensable for stem-cell proliferation in the adult mouse brain and for certain cognitive functions such as spatial learning [36]. A later study using cultured adult rat hippocampus-derived progenitors showed that Tlx not only inhibits glial differentiation but also promotes neuronal lineage commitment by activating expression of the pro-neural gene *Mash1* in these adult progenitor cells [51]. Recently, Tlx has been shown to restrain senescence in NSCs and cancer cells [63, 64]. As discussed above, *Tlx* may maintain the stemness of mouse adult NSCs by activating autonomous Wnt/beta-catenin signaling [21].

Evidence supporting the function of human *TLX* in brain development is limited. However, the rescue effect of human *TLX* on brain abnormalities and fierce behavior in *Tlx*-null mice suggests that human *TLX* has functions similar to those of mouse *Tlx* in normal brain development [13, 14].

Roles of TLX Homologues in Regulation of Retinal Progenitor Cell Differentiation and Visual System Development

Another essential function of *TLX* homologues is regulating RPC differentiation during visual system development. *Drosophila tll* drives embryonic cells into the fate of developing into optic lobes but not Bolwig's organ *via* antagonizing EGFR signaling [29]. Mouse Tlx is involved in eye development from early RPC differentiation to later astrocyte maturation [31]. It controls the generation of proper numbers of RPCs by activating cyclin-D1 and p27 during early retinal layer formation [31]. Further, Tlx

expression has been found in Müller glial cells and mature astrocytes of the inner nuclear layer after birth and temporarily in immature astrocytes before migration from the optic nerve to the inner nuclear layer [31]. Consistently, reduced retinal size and cell number, optic nerve degeneration, and visual impairment have been reported in *Tlx*-deficient mice [32, 45, 65]. It has been suggested that Tlx regulates retinogenesis either through direct suppression of GFAP expression or by modulating upstream BMP signaling [34, 48].

Again, human TLX may serve a function similar to that of mouse Tlx in eye development based on rescue experiments in which retinal defects in *Tlx*-deficient mice are corrected after introduction of the human *LTX* gene [14].

TLX and Human Diseases

Given the wide range of mutant phenotypes such as brain abnormalities, visual impairments, violent behavior, and learning disabilities, it is reasonable to suspect that human *TLX* mutations may be associated with some pathophysiological conditions. Kumar *et al*. conducted a series of studies to investigate whether human *TLX* mutations are associated with abnormal brain-behavior conditions such as microcephaly, bipolar disorder, schizophrenia, and aggression [15, 66]. These studies showed that *TLX* mutations are associated with bipolar disorder [15], and four candidate mutations in the regulatory region of *TLX* have been associated with microcephaly [66].

Tlx has been reported to interact with Pax6 during mouse brain development [33]. Although human *PAX6* mutations are known to cause several disorders including aniridia, a recent study did not find *TLX* mutations in patients with this condition [67].

There is still a major lack of evidence establishing an association between loss-of-function mutations of *TLX* and human disorders. On the contrary, elevated expression of *TLX* has been reported in various types of human glioma [68–73] and glioma cell lines [74], and *TLX* overexpression seems to be associated with a poor prognosis for patients with glioma [74]. A recent study further reported that TLX promotes the progression of neuroblastoma and is correlated with poor survival [56]. These studies suggested that uncontrolled *TLX* expression contributes to tumorigenesis. On the other hand, *TLX* could be a therapeutic target for cancer treatment.

Summary and Future Prospects

TLX and its homologues in other species have been increasingly investigated during the past 15 years. The recent discovery of the involvement of TLX in NSC self-renewal elucidated its functions at the molecular and genetic levels. It is notable that TLX and its homologues, as a key component of a transcription complex, regulate various target genes and modulate multiple signaling pathways in specific tissues from embryonic stages through to adulthood. TLX may have distinct regulatory effects on target genes or signaling pathways depending on the specific internal molecular environment and external stimuli. Thus, the molecular mechanisms of TLX function identified *in vitro* should be cautiously examined *in vivo* with the consideration of unique temporal and spatial conditions. It is encouraging that the functional conservation between mouse Tlx and human TLX has been validated by the finding that the ectopic expression of the genomic region of human *TLX* can correct brain, retinal, and behavioral abnormalities in *Tlx*-null mice [13, 14]. However, recent initial screening for *TLX* mutations in several human disorders resulted in only limited findings. Nevertheless, we should remain optimistic regarding the discovery of pathological *TLX* mutations in the near future with increasing applications of whole-exon and whole-genome sequencing in identifying etiologic mutations in patients with genetic disorders. On the other hand, future studies will focus on how TLX or its regulators such as stem cell-specific transcriptional factors contribute to brain tumorigenesis and provide novel insights into the histogenesis and molecular pathogenesis of primary brain tumors. Moreover, further resolving the structure of TLX protein may help better understand its functional mechanisms and develop potential drugs to modulate them.

References

1. Pardee K, Necakov AS, Krause H. Nuclear Receptors: Small Molecule Sensors that Coordinate Growth, Metabolism and Reproduction. Subcell Biochem 2011, 52: 123–153.
2. Germain P, Staels B, Dacquet C, Spedding M, Laudet V. Overview of nomenclature of nuclear receptors. Pharmacol Rev 2006, 58: 685–704.
3. King-Jones K, Thummel CS. Nuclear receptors—a perspective from Drosophila. Nat Rev Genet 2005, 6: 311–323.
4. Polvani S, Tarocchi M, Tempesti S, Galli A. Nuclear receptors and pathogenesis of pancreatic cancer. World J Gastroenterol 2014, 20: 12062–12081.
5. Yu RT, McKeown M, Evans RM, Umesono K. Relationship between Drosophila gap gene tailless and a vertebrate nuclear receptor Tlx. Nature 1994, 370: 375–379.
6. Jackson A, Panayiotidis P, Foroni L. The human homologue of the Drosophila tailless gene (TLX): characterization and mapping to a region of common deletion in human lymphoid leukemia on chromosome 6q21. Genomics 1998, 50: 34–43.
7. Abrahams BS, Mak GM, Berry ML, Palmquist DL, Saionz JR, Tay A, *et al*. Novel vertebrate genes and putative regulatory elements identified at kidney disease and NR2E1/fierce loci. Genomics 2002, 80: 45–53.

8. Kitambi SS, Hauptmann G. The zebrafish orphan nuclear receptor genes nr2e1 and nr2e3 are expressed in developing eye and forebrain. Gene Expr Patterns 2007, 7: 521–528.
9. Pignoni F, Baldarelli RM, Steingrimsson E, Diaz RJ, Patapoutian A, Merriam JR, *et al*. The Drosophila gene tailless is expressed at the embryonic termini and is a member of the steroid receptor superfamily. Cell 1990, 62: 151–163.
10. Young KA, Berry ML, Mahaffey CL, Saionz JR, Hawes NL, Chang B, *et al*. Fierce: a new mouse deletion of Nr2e1; violent behaviour and ocular abnormalities are background-dependent. Behav Brain Res 2002, 132: 145–158.
11. Gui H, Li ML, Tsai CC. A tale of tailless. Dev Neurosci 2011, 33: 1–13.
12. Moran E, Jimenez G. The tailless nuclear receptor acts as a dedicated repressor in the early Drosophila embryo. Mol Cell Biol 2006, 26: 3446–3454.
13. Abrahams BS, Kwok MC, Trinh E, Budaghzadeh S, Hossain SM, Simpson EM. Pathological aggression in "fierce" mice corrected by human nuclear receptor 2E1. J Neurosci 2005, 25: 6263–6270.
14. Schmouth JF, Banks KG, Mathelier A, Gregory-Evans CY, Castellarin M, Holt RA, *et al*. Retina restored and brain abnormalities ameliorated by single-copy knock-in of human NR2E1 in null mice. Mol Cell Biol 2012, 32: 1296–1311.
15. Kumar RA, McGhee KA, Leach S, Bonaguro R, Maclean A, Aguirre-Hernandez R, *et al*. Initial association of NR2E1 with bipolar disorder and identification of candidate mutations in bipolar disorder, schizophrenia, and aggression through resequencing. Am J Med Genet B Neuropsychiatr Genet 2008, 147B: 880–889.
16. Liu HK, Wang Y, Belz T, Bock D, Takacs A, Radlwimmer B, *et al*. The nuclear receptor tailless induces long-term neural stem cell expansion and brain tumor initiation. Genes Dev 2010, 24: 683–695.
17. Bain DL, Heneghan AF, Connaghan-Jones KD, Miura MT. Nuclear receptor structure: implications for function. Annu Rev Physiol 2007, 69: 201–220.
18. Benod C, Villagomez R, Filgueira CS, Hwang PK, Leonard PG, Poncet-Montange G, *et al*. The human orphan nuclear receptor tailless (TLX, NR2E1) is druggable. PLoS One 2014, 9: e99440.
19. Haffner CD, Lenhard JM, Miller AB, McDougald DL, Dwornik K, Ittoop OR, *et al*. Structure-based design of potent retinoid X receptor alpha agonists. J Med Chem 2004, 47: 2010–2029.
20. Kruse SW, Suino-Powell K, Zhou XE, Kretschman JE, Reynolds R, Vonrhein C, *et al*. Identification of COUP-TFII orphan nuclear receptor as a retinoic acid-activated receptor. PLoS Biol 2008, 6: e227.
21. Qu Q, Sun G, Li W, Yang S, Ye P, Zhao C, *et al*. Orphan nuclear receptor TLX activates Wnt/beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. Nat Cell Biol 2010, 12: 31–40.
22. Zhi X, Zhou XE, He Y, Searose-Xu K, Zhang CL, Tsai CC, *et al*. Structural basis for corepressor assembly by the orphan nuclear receptor TLX. Genes Dev 2015, 29: 440–450.
23. Nusslein-Volhard C, Wieschaus E, Kluding H. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. Wilhelm Roux's Arch Dev Biol 1984, 193: 267–282.
24. Rudolph KM, Liaw GJ, Daniel A, Green P, Courey AJ, Hartenstein V, *et al*. Complex regulatory region mediating tailless expression in early embryonic patterning and brain development. Development 1997, 124: 4297–4308.
25. Steingrimsson E, Pignoni F, Liaw GJ, Lengyel JA. Dual role of the Drosophila pattern gene tailless in embryonic termini. Science 1991, 254: 418–421.
26. Casanova J. Pattern formation under the control of the terminal system in the Drosophila embryo. Development 1990, 110: 621–628.
27. Strecker TR, Merriam JR, Lengyel JA. Graded requirement for the zygotic terminal gene, tailless, in the brain and tail region of the Drosophila embryo. Development 1988, 102: 721–734.
28. Younossi-Hartenstein A, Green P, Liaw GJ, Rudolph K, Lengyel J, Hartenstein V. Control of early neurogenesis of the Drosophila brain by the head gap genes tll, otd, ems, and btd. Dev Biol 1997, 182: 270–283.
29. Daniel A, Dumstrei K, Lengyel JA, Hartenstein V. The control of cell fate in the embryonic visual system by atonal, tailless and EGFR signaling. Development 1999, 126: 2945–2954.
30. Monaghan AP, Grau E, Bock D, Schutz G. The mouse homolog of the orphan nuclear receptor tailless is expressed in the developing forebrain. Development 1995, 121: 839–853.
31. Miyawaki T, Uemura A, Dezawa M, Yu RT, Ide C, Nishikawa S, *et al*. Tlx, an orphan nuclear receptor, regulates cell numbers and astrocyte development in the developing retina. J Neurosci 2004, 24: 8124–8134.
32. Yu RT, Chiang MY, Tanabe T, Kobayashi M, Yasuda K, Evans RM, *et al*. The orphan nuclear receptor Tlx regulates Pax2 and is essential for vision. Proc Natl Acad Sci USA 2000, 97: 2621–2625.
33. Stenman J, Yu RT, Evans RM, Campbell K. Tlx and Pax6 cooperate genetically to establish the pallio-subpallial boundary in the embryonic mouse telencephalon. Development 2003, 130: 1113–1122.
34. Shi Y, Chichung Lie D, Taupin P, Nakashima K, Ray J, Yu RT, *et al*. Expression and function of orphan nuclear receptor TLX in adult neural stem cells. Nature 2004, 427: 78–83.
35. Liu HK, Belz T, Bock D, Takacs A, Wu H, Lichter P, *et al*. The nuclear receptor tailless is required for neurogenesis in the adult subventricular zone. Genes Dev 2008, 22: 2473–2478.
36. Zhang CL, Zou Y, He W, Gage FH, Evans RM. A role for adult TLX-positive neural stem cells in learning and behaviour. Nature 2008, 451: 1004–1007.
37. Li W, Sun G, Yang S, Qu Q, Nakashima K, Shi Y. Nuclear receptor TLX regulates cell cycle progression in neural stem cells of the developing brain. Mol Endocrinol 2008, 22: 56–64.
38. Roy K, Kuznicki K, Wu Q, Sun Z, Bock D, Schutz G, *et al*. The Tlx gene regulates the timing of neurogenesis in the cortex. J Neurosci 2004, 24: 8333–8345.
39. Nishimura M, Naito S, Yokoi T. Tissue-specific mRNA expression profiles of human nuclear receptor subfamilies. Drug Metab Pharmacokinet 2004, 19: 135–149.
40. Hartmann B, Reichert H, Walldorf U. Interaction of gap genes in the Drosophila head: tailless regulates expression of empty spiracles in early embryonic patterning and brain development. Mech Dev 2001, 109: 161–172.
41. Pankratz MJ, Hoch M, Seifert E, Jackle H. Kruppel requirement for knirps enhancement reflects overlapping gap gene activities in the Drosophila embryo. Nature 1989, 341: 337–340.
42. Bello B, Reichert H, Hirth F. The brain tumor gene negatively regulates neural progenitor cell proliferation in the larval central brain of Drosophila. Development 2006, 133: 2639–2648.
43. Betschinger J, Mechtler K, Knoblich JA. Asymmetric segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. Cell 2006, 124: 1241–1253.
44. Kurusu M, Maruyama Y, Adachi Y, Okabe M, Suzuki E, Furukubo-Tokunaga K. A conserved nuclear receptor, Tailless, is required for efficient proliferation and prolonged maintenance of mushroom body progenitors in the Drosophila brain. Dev Biol 2009, 326: 224–236.
45. Zhang CL, Zou Y, Yu RT, Gage FH, Evans RM. Nuclear receptor TLX prevents retinal dystrophy and recruits the corepressor atrophin1. Genes Dev 2006, 20: 1308–1320.
46. Sun G, Yu RT, Evans RM, Shi Y. Orphan nuclear receptor TLX recruits histone deacetylases to repress transcription and regulate

neural stem cell proliferation. Proc Natl Acad Sci USA 2007, 104: 15282–15287.
47. Zhao C, Sun G, Li S, Shi Y. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. Nat Struct Mol Biol 2009, 16: 365–371.
48. Qin S, Niu W, Iqbal N, Smith DK, Zhang CL. Orphan nuclear receptor TLX regulates astrogenesis by modulating BMP signaling. Front Neurosci 2014, 8: 74.
49. Niu W, Zou Y, Shen C, Zhang CL. Activation of postnatal neural stem cells requires nuclear receptor TLX. J Neurosci 2011, 31: 13816–13828.
50. Sehgal R, Sheibani N, Rhodes SJ, Belecky Adams TL. BMP7 and SHH regulate Pax2 in mouse retinal astrocytes by relieving TLX repression. Dev Biol 2009, 332: 429–443.
51. Elmi M, Matsumoto Y, Zeng ZJ, Lakshminarasimhan P, Yang W, Uemura A, *et al*. TLX activates MASH1 for induction of neuronal lineage commitment of adult hippocampal neuroprogenitors. Mol Cell Neurosci 2010, 45: 121–131.
52. Iwahara N, Hisahara S, Hayashi T, Horio Y. Transcriptional activation of NAD + -dependent protein deacetylase SIRT1 by nuclear receptor TLX. Biochem Biophys Res Commun 2009, 386: 671–675.
53. Chavali PL, Saini RK, Matsumoto Y, Agren H, Funa K. Nuclear orphan receptor TLX induces Oct-3/4 for the survival and maintenance of adult hippocampal progenitors upon hypoxia. J Biol Chem 2011, 286: 9393–9404.
54. Sun G, Alzayady K, Stewart R, Ye P, Yang S, Li W, *et al*. Histone demethylase LSD1 regulates neural stem cell proliferation. Mol Cell Biol 2010, 30: 1997–2005.
55. Zeng ZJ, Johansson E, Hayashi A, Chavali PL, Akrap N, Yoshida T, *et al*. TLX controls angiogenesis through interaction with the von Hippel-Lindau protein. Biol Open 2012, 1: 527–535.
56. Chavali PL, Saini RK, Zhai Q, Vizlin-Hodzic D, Venkatabalasubramanian S, Hayashi A, *et al*. TLX activates MMP-2, promotes self-renewal of tumor spheres in neuroblastoma and correlates with poor patient survival. Cell Death Dis 2014, 5: e1502.
57. Yokoyama A, Takezawa S, Schule R, Kitagawa H, Kato S. Transrepressive function of TLX requires the histone demethylase LSD1. Mol Cell Biol 2008, 28: 3995–4003.
58. Estruch SB, Buzon V, Carbo LR, Schorova L, Luders J, Estebanez-Perpina E. The oncoprotein BCL11A binds to orphan nuclear receptor TLX and potentiates its transrepressive function. PLoS One 2012, 7: e37963.
59. Wang Y, Liu HK, Schutz G. Role of the nuclear receptor Tailless in adult neural stem cells. Mech Dev 2013, 130: 388–390.
60. Land PW, Monaghan AP. Abnormal development of zinc-containing cortical circuits in the absence of the transcription factor Tailless. Brain Res Dev Brain Res 2005, 158: 97–101.
61. Land PW, Monaghan AP. Expression of the transcription factor, tailless, is required for formation of superficial cortical layers. Cereb Cortex 2003, 13: 921–931.
62. Monaghan AP, Bock D, Gass P, Schwager A, Wolfer DP, Lipp HP, *et al*. Defective limbic system in mice lacking the tailless gene. Nature 1997, 390: 515–517.
63. O'Loghlen A, Martin N, Krusche B, Pemberton H, Alonso MM, Chandler H, *et al*. The nuclear receptor NR2E1/TLX controls senescence. Oncogene 2015, 34: 4069–4077.
64. Wu D, Yu S, Jia L, Zou C, Xu Z, Xiao L, *et al*. Orphan nuclear receptor TLX functions as a potent suppressor of oncogene-induced senescence in prostate cancer *via* its transcriptional coregulation of the CDKN1A (p21(WAF1) (/) (CIP1)) and SIRT1 genes. J Pathol 2015, 236: 103–115.
65. Uemura A, Kusuhara S, Wiegand SJ, Yu RT, Nishikawa S. Tlx acts as a proangiogenic switch by regulating extracellular assembly of fibronectin matrices in retinal astrocytes. J Clin Invest 2006, 116: 369–377.
66. Kumar RA, Everman DB, Morgan CT, Slavotinek A, Schwartz CE, Simpson EM. Absence of mutations in NR2E1 and SNX3 in five patients with MMEP (microcephaly, microphthalmia, ectrodactyly, and prognathism) and related phenotypes. BMC Med Genet 2007, 8: 48.
67. Corso-Diaz X, Borrie AE, Bonaguro R, Schuetz JM, Rosenberg T, Jensen H, *et al*. Absence of NR2E1 mutations in patients with aniridia. Mol Vis 2012, 18: 2770–2782.
68. Modena P, Lualdi E, Facchinetti F, Veltman J, Reid JF, Minardi S, *et al*. Identification of tumor-specific molecular signatures in intracranial ependymoma and association with clinical characteristics. J Clin Oncol 2006, 24: 5223–5233.
69. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, *et al*. An integrated genomic analysis of human glioblastoma multiforme. Science 2008, 321: 1807–1812.
70. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, *et al*. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006, 9: 157–173.
71. Sharma MK, Mansur DB, Reifenberger G, Perry A, Leonard JR, Aldape KD, *et al*. Distinct genetic signatures among pilocytic astrocytomas relate to their brain region origin. Cancer Res 2007, 67: 890–900.
72. Sim FJ, Keyoung HM, Goldman JE, Kim DK, Jung HW, Roy NS, *et al*. Neurocytoma is a tumor of adult neuronal progenitor cells. J Neurosci 2006, 26: 12544–12555.
73. Taylor MD, Poppleton H, Fuller C, Su X, Liu Y, Jensen P, *et al*. Radial glia cells are candidate stem cells of ependymoma. Cancer Cell 2005, 8: 323–335.
74. Park HJ, Kim JK, Jeon HM, Oh SY, Kim SH, Nam DH, *et al*. The neural stem cell fate determinant TLX promotes tumorigenesis and genesis of cells resembling glioma stem cells. Mol Cells 2010, 30: 403–408.