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Review

# TLX: An elusive receptor

Cindy Benod*, Rosa Villagomez, Paul Webb

*Department of Genomic Medicine, Houston Methodist Research Institute (HMRI), 6670 Bertner Avenue, Houston, TX 77030, USA*

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ABSTRACT

TLX (tailless receptor) is a member of the nuclear receptor superfamily and belongs to a class of nuclear receptors for which no endogenous or synthetic ligands have yet been identified. TLX is a promising therapeutic target in neurological disorders and brain tumors. Thus, regulatory ligands for TLX need to be identified to complete the validation of TLX as a useful target and would serve as chemical probes to pursue the study of this receptor in disease models. It has recently been proved that TLX is druggable. However, to identify potent and specific TLX ligands with desirable biological activity, a deeper understanding of where ligands bind, how they alter TLX conformation and of the mechanism by which TLX mediates the transcription of its target genes is needed. While TLX is in the process of escaping from orphanhood, future ligand design needs to progress in parallel with improved understanding of (i) the binding cavity or surfaces to target with small molecules on the TLX ligand binding domain and (ii) the nature of the TLX coregulators in particular cell and disease contexts. Both of these topics are discussed in this review.



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* Corresponding author. Fax: +1 713 441 2178.
*E-mail address:* ccbenod@houstonmethodist.org (C. Benod).

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## 1. Introduction

Nuclear receptors (NR) control a myriad of biological and disease processes involved in homeostasis, reproduction, metabolism, tumor development and progression [1]. While activity of many NR is controlled by hormones or other small lipophilic molecules, others belong to a class called "orphan receptors" for which no physiological ligands and, sometimes, no synthetic ligands have been identified [2]. TLX (Tailless Homolog, NR2E1) belongs to this category. TLX was first identified as an orphan of unknown function that was highly expressed in the vertebrate forebrain and adult brain [3,4]. It is now known that TLX plays a crucial role in Neural Stem Cell (NSC) self-renewal and in maintenance of the undifferentiated state of this cell population [3,5,6]. Further, TLX plays a crucial, but incompletely defined, role in brain tumor stem cells (BTSC) and various brain tumors including glioblastoma multiforme (GBM) [7–12]. Thus, it is crucial to understand whether TLX can accommodate ligands and how such ligands can be used to control its biological activities.

TLX is a member of the NR subfamily 2 (NR2), which also includes HNF4s (Hepatocyte Nuclear Factor 4, NR2A), RXRs (Retinoic X Receptors, NR2B), COUP-TFs (COUP Transcription Factor 2, NR2F), TR2/4 (Testicular Receptor 2 and 4, NR2C), PNR (photo-receptor cell specific nuclear receptor, NR2E3) and EAR2 (V-ErbA-Related protein 2, NR2F6). Except for RXRs, for which ligands have been identified and studied for many years [13], the other members of this subfamily are orphans or have only been very recently "adopted" (such as HNF4s [14] and COUP-TFs [15]). In this review, we present evidence that TLX is a druggable target [16] and highlight substantial gaps in knowledge of TLX function that need to be addressed in creation of rational strategies to develop novel ligands to modulate its activity.

## 2. TLX biological roles

### 2.1. TLX expression and function

TLX expression is limited to the mammalian central nervous system (CNS) [5]. During development, TLX is predominantly expressed in the periventricular neurogenic zone (PVN) from E8 to E16, peaking at E13.5 [4]. TLX can also be detected in the optic processes of the developing mouse eye as early as E9 [4]. In adult mouse brain, TLX is restricted to two highly neurogenic areas; the subgranular zone of the hippocampal dendate gyrus and the subventricular zone (SVZ) of lateral ventricles [5]. TLX knockout does not lead to obvious defects in brain development during normal embryogenesis [17]. Adult mice, however, display reduced cerebral hemispheres and retinopathies along with severe defects in the limbic system, including reduced size of the olfactory, infrarhinal and entorhinal cortex, amygdala and dentate gyrus [18]. TLX knockout mice also display notable behavioral defects, including progressively worsening violent behavior, reduced copulation and lack of normal maternal instincts in females [17,19].

TLX is required for NSC self-renewal [3,5,6,20–23]. An ingenious mouse model, in which a β-galactosidase marker was knocked into the TLX locus, was used to show that TLX expression co-localized with the NSC marker nestin and sites of cell division, as judged by incorporation of bromodeoxyuridine (BrDU) [3]. Cell sorting approaches reveal that TLX positive cells can divide, self-renew and differentiate into all neural cell types and later double labeling studies showed that almost all self-renewing NSC express TLX [3]. While equivalent TLX null cells lack the capacity for division, reintroduction of TLX rescues the capacity for proliferation and self-renewal [3]. In further support of the idea that TLX is required for NSC self-renewal, TLX null mice showed reduced cell proliferation and nestin staining in neurogenic brain areas [3]. Moreover, forced expression of TLX in NSC under control of the nestin promoter in a transgenic mouse showed enhanced neurogenesis and cognition with increased capacity for learning and memory and rescued defects in TLX knockout mice [3,24,25]. TLX was shown to directly repress an astrocyte marker, GFAP (glial fibrillary acidic protein), pointing to role in suppression of differentiation [3,24]. Interestingly, endogenous TLX mRNA and protein levels were inversely affected by manipulations of miR-9 levels in cultured mouse NSC [26]. Reducing mature miR-9 levels led to increased TLX expression and NSC proliferation [26]. Thus, TLX may be subject to regulation by transcriptional networks that involve microRNAs.

### 2.2. Gliomagenesis

TLX also appears to play an important role in BTSC and GBM [6,9,11,12]. Strikingly high levels of TLX expression are detected in glioma cell lines, glioma stem cell lines [8] and patient samples where TLX expression correlates with a poor prognosis [9,27–32]. Further, we analyzed potential roles of TLX in GBM using a recently published database which links prevalence of transcription factor binding events defined by ChIP-seq or consensus transcription factor binding sites with cancer transcript profiles in an unbiased fashion, while controlling for confounding events such as copy number alteration and somatic mutation of transcription factors [33]. This method reveals strong links between proximity of the TLX consensus binding site and gene signatures that are characteristic of GBM, but not a panel of other types of human cancer (data not shown); suggestive of a functional link between TLX and GBM gene expression profiles. Human brain tumors in general, and GBM in particular, are frequently comprised of heterogeneous cell populations that include BTSC which fuel cancer growth and recurrence after therapy. In this context, Zhu et al. [7] demonstrated that TLX is expressed in slow-dividing BTSC by using a mouse somatic brain tumor model. Three lines of evidence indicate that TLX is important for BTSC self-renewal and cancer emergence [10]. First, NSC-specific overexpression of TLX induces NSC expansion and glioma-like lesions in adult mouse brain, which progress to invasive glioma when p53 function is lost [34]. Second, TLX maintains cell proliferation via repressor actions at the p21 and pten genes [34]. In BTSC, TLX may also control cell proliferation by repressing CDKN2A, CDKN2B, PML tumor suppressor genes and differentiation markers such as SMACC1, Dlx2, TGFβR1 [7,34]. We emphasize that these genes are markers of changes of TLX activity and it is not yet clear whether all are direct TLX targets. Third, a BTSC-specific knock-out of TLX in primary mouse tumors leads to loss of BTSC self-renewal which is associated with induction of senescence, neurogenic differentiation and prolongation of animal survival, accompanied by induction of cell-cycle arrest, cell death and neural differentiation [7]. TLX is also overexpressed in other forms of glioma including astrocytoma, oligodendroglioma, hemangiopericytoma and neuroblastoma [8,10,34]. Thus, TLX regulates BTSC proliferation and expansion and may play a crucial role in mature GBM and other forms of brain cancer.

### 2.3. TLX and mental illnesses

TLX may be involved in human mental illness [35]. Bipolar disorder is a highly heritable multifactorial psychiatric disorder linked to abnormalities in the 6q21–22 chromosomal locus [36]. *Tlx* is located within this region [36] and mutations in the regulatory regions of the human tlx gene have been positively correlated with bipolar disorder and schizophrenia.