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TCF7l2, a nuclear marker that labels premyelinating oligodendrocytes and promotes oligodendroglial lineage progression

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Abstract

Clinical and basic neuroscience research is greatly benefited from the identification and characterization of lineage specific and developmental stage-specific markers. In the glial research community, histological markers that specifically label newly differentiated premyelinating oligodendrocytes are still scarce. Premyelinating oligodendrocyte markers, especially those of nuclear localization, enable researchers to easily quantify the rate of oligodendrocyte generation regardless of developmental ages. We propose that the transcription factor 7-like 2 (TCF7l2, mouse gene symbol *Tcf7l2*) is a useful nuclear marker that specifically labels newly generated premyelinating oligodendrocytes and promotes oligodendroglial lineage progression. Here, we highlight the controversial research history of TCF7l2 expression and function in oligodendroglial field and discuss previous experimental data justifying TCF7l2 as a specific nuclear marker for premyelinating oligodendrocytes during developmental myelination and remyelination. We conclude that TCF7l2 can be used alone or combined with pan-oligodendroglial lineage markers to identify newly differentiated or newly regenerated oligodendrocytes and quantify the rate of oligodendrocyte generation.

Keywords

histological markers; myelinating oligodendrocytes; oligodendrocyte progenitor cells; oligodendrocytes; premyelinating oligodendrocytes

1 | INTRODUCTION

Myelination provides physical, trophic, and metabolic support for neurons and is required for activity-induced brain plasticity. Central nervous system (CNS) myelin sheath is formed by mature myelinating oligodendrocytes (OLs), which are differentiated from oligodendrocyte progenitor cells (OPCs) and undergo a series of maturation stages including

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AUTHOR CONTRIBUTIONS

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the intermediate short-lived premyelinating OLs. Maturation stage-specific protein markers with distinct expression dynamics (Figure 1) have been used as invaluable tools for studying the lineage progression under physiological and pathological conditions. For example, the cell surface markers PDGFRα and NG2 are widely used to label OPCs both in vivo and in vitro. The commonly used OLs markers, for instance clone CC1 (or CC1), ASPA, GST-π, label the accumulative population of all OLs regardless of their maturation stages. With modern technology advancement, 12 oligodendroglial populations of distinct nuanced maturation stages have been proposed based on the mRNA signatures derived from single cell RNA sequencing and bioinformatic analysis (Marques et al., 2016).

The terminal differentiation of OPCs into immature premyelinating OLs is an essential step for developmental myelination, experience-dependent adaptive myelination or remyelination after myelin damage. The number of premyelinating OLs is a surrogate for the rate or the extent of oligodendrocyte differentiation at a specific time in a CNS anatomical region. There is an imminent need to identify premyelinating oligodendrocyte-specific markers, which is of broad interest to researchers in the neuroscience community. In this article, we review previous data on the expression and function of the transcription factor TCF7l2 in the CNS. We conclude that TCF7l2 is a useful nuclear marker that can be used to specifically premyelinating OLs and quantify the rate of oligodendrocyte generation.

2 | DEFINITION OF PREMYELINATING OLS AND HISTOLOGICAL MARKERS

There is no consensus on the definition of premyelinating OLs. In this review, we define premyelinating OLs as the intermediate stages of oligodendrocytes between OPCs and mature myelinating OLs along the lineage progression. They are referred to as immature OLs in the literatures. Phenotypically, premyelinating OLs are post-mitotic, lose the expression of the prototypic cell surface markers of OPCs (PDGFRα and NG2), start to express major myelin-enriched genes (for instance PLP, MBP, and CNP) in the cell bodies and proximal processes, and have not yet formed compact myelin sheaths (Figure 1). Depending on the balance of positive and negative cues intrinsically and extrinsically, short-lived premyelinating OLs are either eliminated or further matured into stable long-lived myelinating OLs (Tripathi et al., 2017) to form compact myelin sheath. In the glial research field, premyelinating OLs are also termed as newly formed (generated) OLs with myelin gene expression (Zhang et al., 2014) or without myelin gene expression yet (Marques et al., 2016) depending on different criteria. In this review, we use the term premyelinating OLs to include the intermediate stages of OLs that express myelin gene and have not yet form myelin sheaths.

In the oligodendroglial research, *Gpr17*, *Enpp6*, *Bcas1*, and *Tcf7l2* have been shown to be upregulated in newly formed premyelinating OLs (Figure 2), providing new tools to label newly formed OLs. Despite of upregulation in premyelinating OLs, the cell membrane tethered GPR17 is initially expressed in OPCs (Chen et al., 2009), thus making it unlikely to distinguish premyelinating OLs from OPCs by immunohistochemical staining when used alone. Xiao and colleagues identified *Enpp6* mRNA transcript as a specific marker

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for newly formed premyelinating OLs (Xiao et al., 2016). scRNA-seq data demonstrated that *Enpp6* mRNA transcript displays a bi-phasic expression pattern along the lineage progression: one peaks in newly formed OLs and the other in mature OLs (Figure 2). Furthermore, histological detection of *Enpp6* mRNA by in situ hybridization requires stringent tissue processing to protect RNA degradation and is time-consuming compared to immunohistochemical staining. The protein product ENPP6 is mainly detected at the processes/myelin sheath and absent from the cell bodies of OLs (Morita et al., 2016), thus making it difficult for precise histological quantification. Paradoxically, the expression of ENPP6 protein (evaluated by Western blot assay) is maintained at a high level in the adult brain (Morita et al., 2016) in which the population of newly formed OLs is substantially reduced. Of note, the high level of ENPP6 protein in the adult brain is consistent with the second peak of the mRNA transcript in mature OLs (Figure 2). More recently, Fard and colleagues proposed BCAS1 as a specific marker for newly generated premyelinating OLs (Fard et al., 2017). Like ENPP6, BCAS1 is mainly detected at the plasma membrane and the processes of OLs (Fard et al., 2017), making it less accurate than nuclear markers in quantifying OL population. Furthermore, BCAS1 is also expressed in OPCs; an approximate of 17% of BCAS1$^+$ cells are NG2-expressing OPCs in the corpus callosum of the early adult brain at P40 (Fard et al., 2017). There is an imminent need to identify nuclear markers that can be detected by routine immunohistochemical staining for measuring newly generated OLs. In contrast, *Tcf7l2* mRNA transcript shows a more restricted expression in newly formed OLs and is minimal in OPCs and mature myelinating OLs (Figure 2). In this article, we propose that TCF7l2 is a specific nuclear protein that can be used by routine immunohistochemistry (IHC) to label and quantify newly generated premyelinating OLs.

3 | NOMENCLATURE CLARIFICATION OF TCF7L2 AND TCF4

Transcription factor 7-like 2 (TCF7l2, mouse gene symbol *Tcf7l2*, located in chromosome 19) is a high mobility group (HMG) box-containing transcription factor. It is one of the four members of the mammalian T cell factor/lymphoid enhancer factor (TCF/LEF) family (TCF7, TCF7l1, TCF7l2, and LEF1) which are the downstream transcriptional effectors of canonical Wnt/β-catenin signaling (Hatzis et al., 2008). In the previous literatures, TCF7l2 is also called transcript factor 4 (TCF4). It should be cautious that TCF4 (mouse gene symbol *Tcf4*, located in chromosome 18) is a basic helix–loop–helix (bHLH) transcription factor that plays a crucial role in psychiatric disorders (Amiel et al., 2007). To avoid confusion, we use the official gene symbol *Tcf7l2* and the protein product TCF7l2 throughout the review. Researchers in the field are recommended to avoid using the nomenclature TCF4 when studying the HMG-box transcriptional factor and the Wnt effector TCF7l2.

4 | TCF7L2, A USEFUL NUCLEAR MARKER FOR PREMYELINATING OLS

4.1 | TCF7l2-expressing cells belong to oligodendroglial lineage in the postnatal CNS

It has been well documented that developmental myelination occurs in a caudal (spinal cord)-to-rostral (brain) direction and follows different temporal trajectories in various anatomic regions of the brain and the spinal cord. In the murine spinal cord, oligodendrocyte differentiation, an essential step for subsequent myelination, initiates by the perinatal ages,

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peaks by the second postnatal week, and completes by the first postnatal month. Double fluorescent IHC data have shown that TCF7l2$^+$ cells are not co-labeled with GFAP, an astrocytes marker, or NeuN, a neuronal marker (Ye et al., 2009) and that TCF7l2 is exclusively co-labeled with panoligodendroglial lineage markers Sox10 (Olig2 or Olig1) (Figure 1) in the CNS white matter of during normal developmental myelination (Fancy et al., 2009; Fu et al., 2009; Hammond et al., 2015; Ye et al., 2009). TCF7l2$^+$/Sox10$^+$ cell density peaks around the second postnatal week and is progressively reduced prior to the completion of developmental myelination in the spinal cord (Hammond et al., 2015). In the adult CNS where oligodendrocyte differentiation is substantial reduced despite of abundant mature oligodendrocytes, the number of TCF7l2$^+$Sox10$^+$ cells is scarce; usually less than 10 TCF7l2$^+$Sox10$^+$ cells are observed in the white matter tract of one spinal cord transverse section or one forebrain coronal section (Figure 3) (Hammond et al., 2015). The current literatures have reached a consensus that (1) TCF7l2-expressing cells belong to oligodendroglial lineage and (2) the number of TCF7l2-expressing cells is high in the developing CNS in which oligodendrocyte differentiation is actively ongoing and become barely detectable in the adult mature CNS in which oligodendrocyte differentiation is substantially reduced (Table 1). Of note, the temporal correlation betweenTCF7l2-expressing cell numbers and the rate of oligodendroglial differentiation suggests that TCF7l2-expressing cells may be newly generated premyelinating OLs, a transient population along oligodendroglial lineage progression.

It should be noted that TCF7l2 is also highly expressed in thalamic neurons in the forebrain (Ye et al., 2009) (Figure 4). The thalamic neuronal expression seems independent of the developmental ages (Figure 4). TCF7l2$^+$ thalamic neurons can be easily distinguished from TCF7l2$^+$ oligodendroglial lineage cells based on the cell size and anatomic locations. The role of TCF7l2 in thalamic neurons remains incompletely understood (Lipiec et al., 2020).

4.2 | TCF7l2 is expressed selectively in premyelinating OLs

Previous studies employed TCF7l2 antibodies from different sources to dissect oligodendroglial stage specificity. The most cited antibody is a mouse monoclonal one (clone 6H5–3) by which different conclusions had been drawn by different laboratories in 2009, from exclusive expression in OPCs (Fancy et al., 2009) to low in OPCs and high in OLs (Ye et al., 2009) to exclusive expression in post-mitotic OLs (Fu et al., 2009) (Table 1). These conflicting observations may reflect inconsistent working conditions of the antibody during immunohistochemical staining. Subsequently, we found that TCF7l2 was expressed in a transient APC$^+$CC1$^+$ oligodendrocyte population (Lang et al., 2013). However, the working conditions of the clone 6H5–3 antibody on immunohistochemical staining were inconsistent in our experiments; it depends on the tissue fixation during tissue processing and antigen retrieval during immunostaining. Tissue over-fixation and/or immunostaining without antigen retrieval yield very high non-specific background signals. By leveraging TCF7l2 genetic knockout models, we screened a most reliable TCF7l2 antibody (rabbit monoclonal #C48H11) that gives the least non-specific background signals on both frozen- and paraffin-embedded sections with flexible tissue fixation duration (2–24 h we tried) and without antigen retrieval (Hammond et al., 2015). This monoclonal antibody has been used

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to label TCF7l2^{+} cells and quantify the rate of oligodendrocyte differentiation (Wang et al., 2021; Zhang, Rasai, et al., 2018a; Zhang, Zhu, et al., 2018b).

TCF7l2 meets the following criteria that define a histological marker as premyelinating oligodendrocyte-specific: (1) its expression is restricted to postmitotic oligodendroglial cells, (2) its expression is minimal or absent in OPCs, (3) its expression is upregulated in myelin gene-expressing OLs morphologically characteristic of premyelinating OLs, and (4) its expression is downregulated in fully matured myelinating OLs in the adult CNS.

1. TCF7l2 expression is restricted to postmitotic oligodendroglial cells. OPCs are the major cell population that is highly proliferative during postnatal CNS development. Previous data from Fu et al, and Hammond et al., demonstrated that immunoreactive signals of TCF7l2 and Ki67, an endogenous marker for cell cycle, were exclusive from each other (Fu et al., 2009; Hammond et al., 2015). EdU pulse labeling (2 h) also showed non-colocalization of EdU and TCF7l2 immunoreactive signals (Hammond et al., 2015). Notably, neither TCF7l2-low nor TCF7l2-high cells were co-labeled with Ki67 or EdU both in vitro and in vivo (Fu et al., 2009; Hammond et al., 2015). Collectively, these data support the concept that TCF7l2 labels a population of oligodendroglial lineage cells that already exit cell cycle and become post-mitotic.

2. TCF7l2 expression is absent from OPCs and restricted to OLs. Primary OPCs maintained in PDGF-containing growth medium are highly proliferative and rapidly expand their population numbers in the dish; they exit cell cycle and differentiate into myelin protein-expressing OLs when cultured in T3-containing differentiation medium. Previous Western blot assay (Hammond et al., 2015) shows that TCF7l2 is absent from primary OPCs and upregulated in differentiating OLs in vitro (Hammond et al., 2015). Oligodendroglial differentiation initiates around the second postnatal week in the murine forebrain during which TCF7l2^{+} cells become apparent in the corpus callosum. Double or triple fluorescent IHC showed that TCF7l2 is absent from in vivo OPCs which are identified by PDGFRα or NG2 (Hammond et al., 2015). This is consistent with recent scRNA-seq data (mRNA isolated from the mouse brain) demonstrating that cells expressing *Tcf7l2* mRNA transcript is developmentally distinct from OPCs which are characterized by the mRNA expression of *Pdgfra* and *Cspg4* (NG2) (Marques et al., 2016). All TCF7l2^{+} cells are CC1 positive, yet only a subpopulation of CC1^{+} cells are TCF7l2 positive in the CNS at various developmental stages. Importantly, the proportion of CC1^{+} OLs that express TCF7l2 is gradually decreased during the progression of developmental myelination; in the adult CNS, a neglectable proportion (<5%) of CC1^{+} OLs that are TCF7l2 positive. Given that OL differentiation is substantially reduced after completion of developmental myelination, these data suggest that TCF7l2 marks newly generated OLs.

3. TCF7l2^{+} cells are myelin gene-expressing OLs displaying premyelinating OL morphology. RNA-seq data show that *Tcf7l2* mRNA^{+} cells are developmentally distinct from cells expressing major myelin gene transcripts (termed as myelin

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forming OLs, MFOLs, Figure 2) (Marques et al., 2016). We tested whether TCF7l2 protein$^+$ cells expressed major myelin gene transcripts and myelin proteins. Dual IHC and mRNA in situ hybridization (dual IHC/ISH) showed that TCF7l2 protein was present in *Plp* and *Mbp* mRNA-expressing cells (Figure 5a,b). Double IHC also demonstrate that TCF7l2$^+$ cells were MBP and CNP-expressing OLs in vivo (Figure 5c–g) and in vitro (Fu et al., 2009). These data suggest that TCF7l2 protein marks a population of oligodendroglial cells that expressing the transcripts and proteins of major myelin genes.

The major myelin sheath structure proteins, such as MBP, CNP, and PLP, are absent from OPCs, present in newly formed premyelinating OLs, and further upregulated in fully matured myelinating OLs. The subcellular distribution of these myelin sheath structure proteins is different in immature premyelinating OLs from that in mature myelinating OLs; they are primarily located at the cell bodies and proximal processes of premyelinating OLs and subsequently incorporated into the distal processes and myelin sheath of myelinating OLs. In the murine spinal cord of perinatal ages when premyelinating OLs start to appear, all TCF7l2$^+$ cells display the characteristic subcellular distribution of MBP and CNP in premyelinating OLs (Figure 5c–e, arrowheads). Similarly, in the corpus callosum at P6 when premyelinating OLs start to appear, TCF7l2 is expressed in all cells that exhibit MBP immunoreactivity in the cell bodies and proximal processes despite the number of TCF7l2$^+$MBP$^+$ cells is very low (Figure 5f). In the corpus callosum at P8, some MBP$^+$ cells are TCF7l2 positive, however, others exhibiting parallel myelin sheath along callosal axonal bundles start to downregulate TCF7l2 expression (Figure 5g). The observation suggests that TCF7l2 may be transiently expressed in premyelinating OLs and downregulated in myelinating OLs.

4.3 | TCF7l2 is co-labeled with adenomatous polyposis coli which is transiently upregulated in newly generated OLs

Adenomatous polyposis coli (APC) is one of the components of the destruction complex responsible for proteosome-mediated degradation of the key Wnt signaling molecule β-catenin (Guo et al., 2015) and its mutations result in hyperactivity of Wnt/β-catenin signaling in various types of cancer cells (Rowan et al., 2000). APC is expressed in radial glia (Yokota et al., 2009), neurons (Ruane et al., 2016), and astrocytes (Leroy et al., 2001). In the oligodendroglial research field, the pan-oligodendrocyte marker CC1 (an antigen recognized by the antibody clone CC1) had been mistakenly used to designate as APC. The apparently different cellular specificity between *Apc* mRNA (Bhat et al., 1994) and antigen recognized by clone CC1 (referred to as CC1) (Bhat et al., 1996) suggests that clone CC1 antibody recognizes an OL-enriched protein different from APC, which is subsequently identified as the RNA-binding protein Quaking 7 (QKI7) (Bin et al., 2016). Previous data reported that, within the oligodendroglial lineage cells, APC is transiently expressed in newly differentiated OLs during developmental myelination and in newly regenerated OLs during remyelination after myelin damage (Lang et al., 2013).

TCF7l2 and APC were expressed in the same population of CC1$^+$ OLs in the murine spinal cord of early postnatal ages (Lang et al., 2013). To extend this concept to other CNS regions, we observed that the nuclear TCF7l2 and cytoplasmic APC were expressed

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in the same populations of cells in the white matter tracts of the cerebellum, the forebrain, and the spinal cord at the onset of oligodendrocyte differentiation (Figure 6a–c). Nearly 100% of nuclear TCF7l2$^+$ cells express cytoplasmic APC in the CNS white matter tracts regardless of the developmental ages. Even in the adult CNS where newly differentiated OLs are sparse, all TCF7l2$^+$ cells exhibit cytoplasmic APC expression (Figure 6d). These data provide further support that TCF7l2, when used alone or together with APC, is an effective marker that specifically labels immature premyelinating OLs. Since APC plays a crucial role in downregulating Wnt/β-catenin signaling activity, the overlapped expression of TCF7l2 and APC suggests a Wnt-independent role of TCF7l2 in regulating oligodendrocyte differentiation (Zhang et al., 2021) (Discussed in Section 5).

4.4 | TCF7l2 is a nuclear marker labeling newly regenerated premyelinating OLs in multiple sclerosis and in animal models of demyelination

Oligodendrocyte regeneration and remyelination after myelin damage usually recapitulate the process of oligodendroglial lineage progression and maturation during developmental myelination. It is widely appreciated that TCF7l2-expressing cells are scarce in the healthy adult CNS of mice (Fu et al., 2009; Hammond et al., 2015; Lurbke et al., 2013) and humans (Fancy et al., 2009; Lurbke et al., 2013). In the brain of multiple sclerosis (MS), a chronic inflammatory demyelinating disorder, TCF7l2$^+$ cells are observed in the early lesions where spontaneous remyelination occurs but minimal or absent from the chronic lesions where little remyelination happens (Fancy et al., 2009; Lurbke et al., 2013). Interestingly, in the early MS patients, TCF7l2$^+$ cell number is increased in periplaque white matter (PPWM) and remyelination (RM) lesions and few TCF7l2$^+$ cells are observed in actively demyelinating (AD) lesions and demyelinated (DM) lesions (Lurbke et al., 2013). More interestingly, most TCF7l2$^+$ cells in PPWM and RM lesions are identified as cells that express Nogo-A (Lurbke et al., 2013), a pan-oligodendrocyte marker (Kuhlmann et al., 2007) particularly upregulated in newly formed OLs (gene symbol *Rtn4*) (Marques et al., 2016; Zhang et al., 2014). Conversely, only a small proportion of Nogo-A$^+$ oligodendrocytes (less than 10%) express TCF7l2 (Lurbke et al., 2013). These data suggest that TCF7l2 may only mark newly regenerated premyelinating OLs among the total Nogo-A$^+$ oligodendrocyte population. These observations (Lurbke et al., 2013) seem different from an earlier study in which TCF7l2$^+$ cells in early active lesions were identified as OPCs based on the expression of the transcript factor Olig2 (Fancy et al., 2009). It should be noted that Olig2 is a pan-oligodendroglial lineage marker expressed not only in OPCs but also in differentiated OLs regardless of their maturation stages (Figure 1).

Cuprizone-induced demyelination and experimental autoimmune encephalomyelitis (EAE)-induced demyelination in mice are two commonly used demyelination models for MS (Wang et al., 2022). In the cuprizone demyelination model in which complete remyelination is achieved, the number of TCF7l2$^+$ cells is increased in the corpus callosum at the time of myelin repair and progressively decreased to a barely detectable level at the time of remyelination completion (Hammond et al., 2015; Lurbke et al., 2013). Notably, nearly all TCF7l2$^+$ cells in the remyelinating corpus callosum are positive for APC which is transiently upregulated in newly regenerated OLs after myelin damage (Hammond et al., 2015; Lang et al., 2013). Previous fate mapping data suggest that new OLs are generated in

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the spinal cord of EAE-induced demyelination mice (Guo et al., 2011; Tripathi et al., 2010) despite newly regenerated OLs are much fewer in EAE-induced demyelination than those in cuprizone-induced demyelination. Double fluorescent IHC results show that the TCF7l2-expressing cells are Sox10 positive and that number of TCF7l2$^+$Sox10$^+$ cells is increased in EAE spinal cord compared to that in the spinal cord of EAE controls and healthy adults (Hammond et al., 2015). Taken together, these data suggest that TCF7l2 is a histological nuclear marker that can also be used for labeling newly regenerated premyelinating OLs in MS and animal models.

4.5 | Comparison of endogenous TCF7l2 and the lineage tracing approaches in identifying newly formed premyelinating OLs

Traditionally, lineage tracing approaches by exogenous mitotic tracer such as BrdU/EdU (BrdU birth-dating) and Cre-loxP-based transgenic lineage tracing (genetic fate-mapping) have been applied to identify newly generated cells. In BrdU birth-dating approach, thymidine analog BrdU (or EdU) is initially incorporated into proliferating OPCs and subsequently passed down to their progeny cells. By immunodetecting BrdU signals together with differentiated oligodendrocyte markers (such as CC1), one could identify BrdU$^+$CC1$^+$ cells as newly generated OLs. Genetic fate-mapping approach takes advantage of OPC-specific Cre transgenic lines (such as *NG2-CreER^{T2}*) and *loxP* reporter lines in which a STOP codon that is placed before the reporter gene coding sequences. The *loxP-STOP-loxP*-reporter transgenes are inserted into either ubiquitous Rosa26 promoter (for instance *Rosa26$^{loxP\text{-}STOP\text{-}loxP\text{-}EGFP}$* reporter line) or Tau promoter (for instance *Tau$^{loxP\text{-}STOP\text{-}loxP\text{-}mGFP}$* reporter line). In Cre-loxP hybrids of *NG2-CreER^{T2}:Rosa26$^{loxP\text{-}STOP\text{-}loxP\text{-}EGFP}$*, the reporter gene is initially expressed in OPCs and persists in differentiated OLs (Zhu et al., 2011) due to the ubiquitous Rosa26 promoter activity in both OPCs and OLs, whereas in *NG2-CreER^{T2}:Tau$^{loxP\text{-}STOP\text{-}loxP\text{-}mGFP}$* hybrids, deletion of the STOP codon occurs in NG2$^+$ OPCs but the reporter gene expression starts in the progeny cells differentiated from STOP-deleted OPCs because activity of the Tau promoter is only in differentiated OLs but not in OPCs (Chen et al., 2021; Guo et al., 2021). By immunodetecting reporter signals together with differentiated oligodendrocyte markers (such as CC1), one could identify reporter$^+$CC1$^+$ cells as newly generated OLs in the genetic fate-mapping approach.

While these lineage tracing approaches have strengths in identifying newly generated oligodendrocytes, the following crucial caveats may limit their usage compared to TCF7l2. First, unlike the endogenous TCF7l2 expression, lineage tracing approaches require time-consuming generation of *Cre* and *loxP* double transgenic mice. Second, the identification of BrdU$^+$ or reporter$^+$ newly generated OLs is critically dependent on the timing of immunohistochemical assay because the lineage tracers (BrdU or the reporter genes) is not only present in initially labeled premyelinating OLs but also persists in fully matured myelinating OLs. Rather, detecting the endogenous TCF7l2 expression represents a "real-time" approach for identifying newly generated premyelinating OLs regardless of the timing of immunohistochemical assay and developmental ages. Thirdly, BrdU dilution during multiple rounds of OPCs proliferation prior to differentiation would result into underestimation of newly generated premyelinating OLs. Fourth, specificity concerns

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of promoter-driven Cre expression would also create uncertainties in identifying newly generated premeylinating OLs by lineage tracing approach. Therefore, IHC-based detection of endogenous TCF7l2 circumvents these caveats and is superior to exogenous lineage tracing approaches in identifying newly generated OLs.

5 | TCF7L2 PROMOTES OLIGODENDROCYTE DIFFERENTIATION AND MATURATION

The role of TCF7l2 in oligodendrocyte differentiation and maturation had been under debating. Given that TCF7l2 is the major Wnt effector expressed in the oligodendroglial lineage cells and that hyperactivation of Wnt/β-catenin signaling inhibits the differentiation of OPCs into immature OLs (Shimizu et al., 2005), TCF7l2 was originally proposed as an crucial inhibitor for oligodendroglial differentiation through transcriptionally activating Wnt/β-catenin signaling (Fancy et al., 2009). TCF7l2 expression was also used as an indication of inhibited differentiation of OPCs into OLs (He et al., 2007; Pedre et al., 2011). Conditional disruption of the Wnt negative regulator APC results in hyperactivation of Wnt/β-catenin signaling in APC-deficient oligodendrocytes (Fancy et al., 2014; Lang et al., 2013). Interestingly, the transcription factor TCF7l2 is downregulated whereas LEF1, another Wnt effector which is otherwise undetectable in oligodendroglial lineage cells, is upregulated in APC-deficient oligodendrocytes (Hammond et al., 2015). These contrasting observations suggest that TCF7l2 may play a dispensable role in transcriptionally activating Wnt/β-catenin signaling and that Wnt/β-catenin signaling through LEF1 may play a major role in inhibiting oligodendrocyte differentiation. Oligodendroglial-specific conditional knockout (cKO) of APC and LEF1 (or TCF7l2) is needed to test these hypotheses.

Earlier experimental data derived from TCF7l2 genetic knockout mutants (*Tcf7l2*$^{-/-}$) indicate that TCF7l2 is required for oligodendrocyte differentiation from OPCs, as the number of OLs is reduced in *Tcf7l2*$^{-/-}$ mutants at E18.5 (mutants die within hours after birth) (Fu et al., 2009; Ye et al., 2009). Subsequent experiments leveraging *Cre-loxP*-mediated TCF7l2 cKO convincingly demonstrate that TCF7l2 is an intrinsic positive regulator for postnatal oligodendrocyte differentiation and maturation. Our group employed inducible TCF7l2 cKO (*Olig2-CreER*^{T2}*:Tcf7l2*$^{fl/fl}$) and constitutive TCF7l2 cKO (*Cnp-Cre: Tcf7l2*$^{fl/fl}$) system and concluded that TCF7l2 is required for the transition of OPCs into OLs and/or subsequent myelination (Hammond et al., 2015). Zhao and colleague used a different Cre-driven constitutive TCF7l2 cKO system (*Olig1-Cre:Tcf7l2*$^{fl/fl}$) and reached the same conclusion (Zhao et al., 2016), which altogether weakens previous hypothesis that TCF7l2 inhibits oligodendrocyte differentiation.

The mechanism underlying TCF7l2-regulated OL differentiation and maturation seems multi-modal and depends on TCF7l2's interacting protein partners (Figure 7). TCF7l2 is a transcription factor that can activate or repress its downstream gene expression by recruiting potential co-activators or co-repressors in a context-dependent manner. The binding of the co-activator β-catenin to TCF7l2 displaces the co-repressors Groucho/TLE family members from TCF7l2 and activates Wnt/β-catenin signaling target genes (Clevers, 2006). Under physiological conditions, TCF7l2 seems to preferentially interact with the

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co-repressors Groucho/TLE, HDAC1/2 (Zhang et al., 2021), and Kaiso/Zbtb33 (Zhao et al., 2016) over the co-activator β-catenin, suggesting that TCF7l2's transcriptional repression function may play a major role in driving the transition from OPCs into OLs. Consistent with this hypothesis, previous data demonstrate that TCF7l2 promotes OL differentiation through transcriptionally inhibiting BMP4-mediated signaling and/or Wnt/β-catenin signaling (Zhang et al., 2021; Zhao et al., 2016); conditional disruption of TCF7l2 resulted in upregulation of BMP and Wnt signaling target genes, which is opposite to previously proposed Wnt signaling activation role of TCF7l2 during OL differentiation. Importantly, simultaneous BMP4 deletion rescues the inhibitory extent of OL differentiation elicited by TCF7l2 cKO in the CNS (Zhang et al., 2021), suggesting that TCF7l2 promotes OL differentiation by dampening autocrine BMP4-mediated signaling in vivo (Figure 7b). Interestingly, during oligodendrocyte maturation, TCF7l2 seems to switch its binding partner from co-repressors to co-activators, such as Sox10, a potent myelin gene activator during OL maturation (Figure 7a). TCF7l2 recruits and cooperates with Sox10 to activate myelin genes and cholesterol synthesis genes and promote myelination (Zhao et al., 2016). Taken together, the role of TCF7l2 in oligodendroglial lineage differentiation and maturation depends on its potential binding partners. Whether TCF7l2 expression in premyelinating OLs regulates myelination remains elusive. Since any defects in OL differentiation and maturation invariable result in abnormalities in subsequent myelination, the constitutive TCF7l2 cKO systems used in previous studies (Zhang et al., 2021; Zhao et al., 2016) could not definitively dissect the observed myelination defects from inhibited OL differentiation. In this regard, time conditional TCF7l2 disruption specifically in differentiated OLs is needed to test the role of TCF7l2 in CNS myelination.

6 | CONCLUSION AND PERSPECTIVES

The production of premyelinating OLs is an essential step for developmental myelination and experience-dependent adaptive myelination in the adult CNS in response to various external stimuli (Chen et al., 2021; Osso et al., 2021; Xiao et al., 2016; Xin & Chan, 2020). Identifying histological markers that are specifically expressed in premyelinating OLs provides a sensitive readout for perturbed oligodendrocyte differentiation. The identification of premyelinating OL markers is particularly important for quantifying adult oligodendrocyte differentiation during adaptive myelination (Xiao et al., 2016) given that the extent of oligodendrocyte differentiation in the adult CNS is much smaller and subtler than that in the developing CNS (Tripathi et al., 2017).

The transcript factor TCF7l2 is specifically expressed in oligodendroglial lineage cells; it is absent from OPCs, the major proliferative cells in the CNS, and upregulated in newly formed premyelinating OLs. Current literatures have reached a consensus that TCF7l2 functions as a positive regulator for oligodendroglial progression and maturation. We conclude that TCF7l2 is a nuclear marker for identifying premyelinating OLs. Given its nuclear localization, TCF7l2, as a potential premyelinating OL marker, is superior to recently identified markers (BCAS1 and ENPP6) (Fard et al., 2017; Xiao et al., 2016) in histological quantification. TCF7l2 can be used alone, or in combination with other oligodendroglial linage markers (such as Sox10, Olig1/2, and APC) to quantify the rate of oligodendrocyte differentiation during developmental myelination (Zhang, Rasai, et al.,

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2018a; Zhang, Zhu, et al., 2018b) and to measure the extent of oligodendrocyte regeneration during remyelination (Wang et al., 2021; Zhang, Zhu, et al., 2018b). Future studies are needed to investigate if TCF7l2 can be used to reliably identify remyelination lesions (or shadow plaques in medical term) in clinical MS patients and if TCF7l2 or TCF7l2-regulated signaling pathways, could be a therapeutic target for promoting remyelination in MS and animal models.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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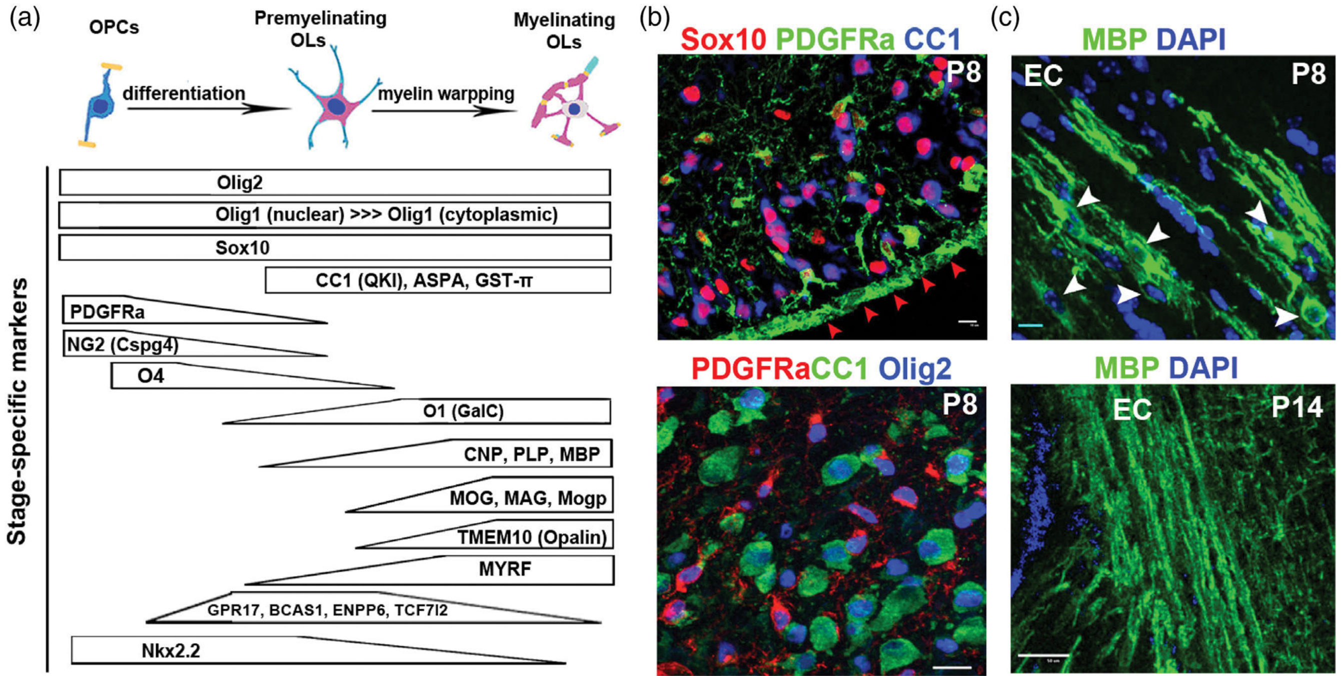

FIGURE 1.

Commonly used stage-specific markers of oligodendroglial lineage cells. (a) Schematic diagram depicting the expression dynamics of commonly used protein markers. (b) Triple immunostaining of Sox10 (or Olig2), PDGFRα, and CC1 identifying OPCs (Sox10^{+}PDGFRα^{+} or Olig2^{+}PDGFRα^{+}) and OLs (Sox10^{+}CC1^{+} or Olig2^{+}CC1^{+}) in the same sections of the murine spinal cord at postnatal day 8 (P8). Red arrows point to meningeal layers. (c) Immunostaining of myelin basic protein (MBP) in the external capsule (EC) of the murine brain at P8 and P14. Note that MBP is located in cell bodies (arrowheads) and proximal processed of newly generated premyelinating OLs

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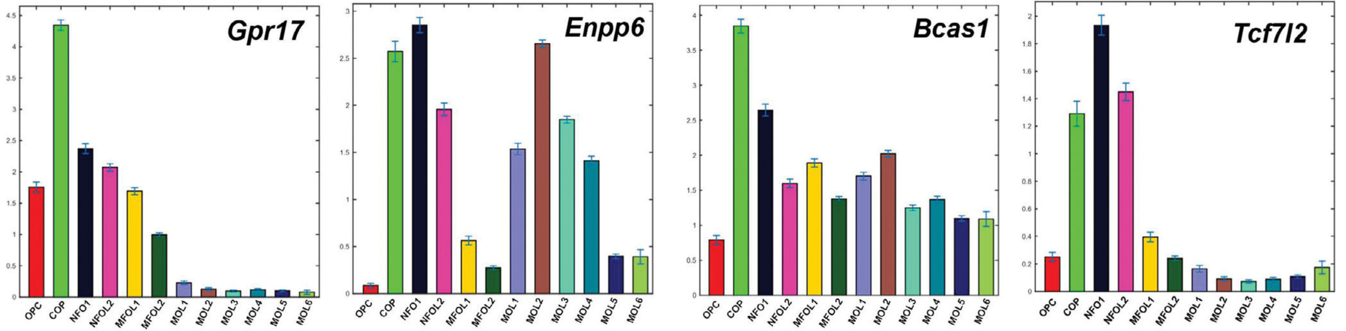

FIGURE 2.

Relative expression of the mRNA transcripts of premyelinating OL-enriched genes along the 12 maturation stages of oligodendroglial lineage cells clustered by scRNA-seq (Marques et al., 2016). COP, differentiation-committed OPC; MFOL, myelin forming oligodendrocytes; MOL, mature oligodendrocytes; NFOL, newly formed oligodendrocytes; OPC, oligodendrocyte progenitor cells. The figure panels are retrieved from the single cell RNA-seq (www.linnarssonlab.org/oligodendrocytes) (Marques et al., 2016). Note that *Tcf7l2* mRNA transcript shows a more transient and restricted expression pattern along oligodendroglial lineage progression and is absent from OPCs and mature myelinating OLs. *Enpp6* mRNA transcript shows a bi-phasic expression: One peak in newly formed OLs and the other in mature OLs. *Gpr17* is also expressed in OPCs

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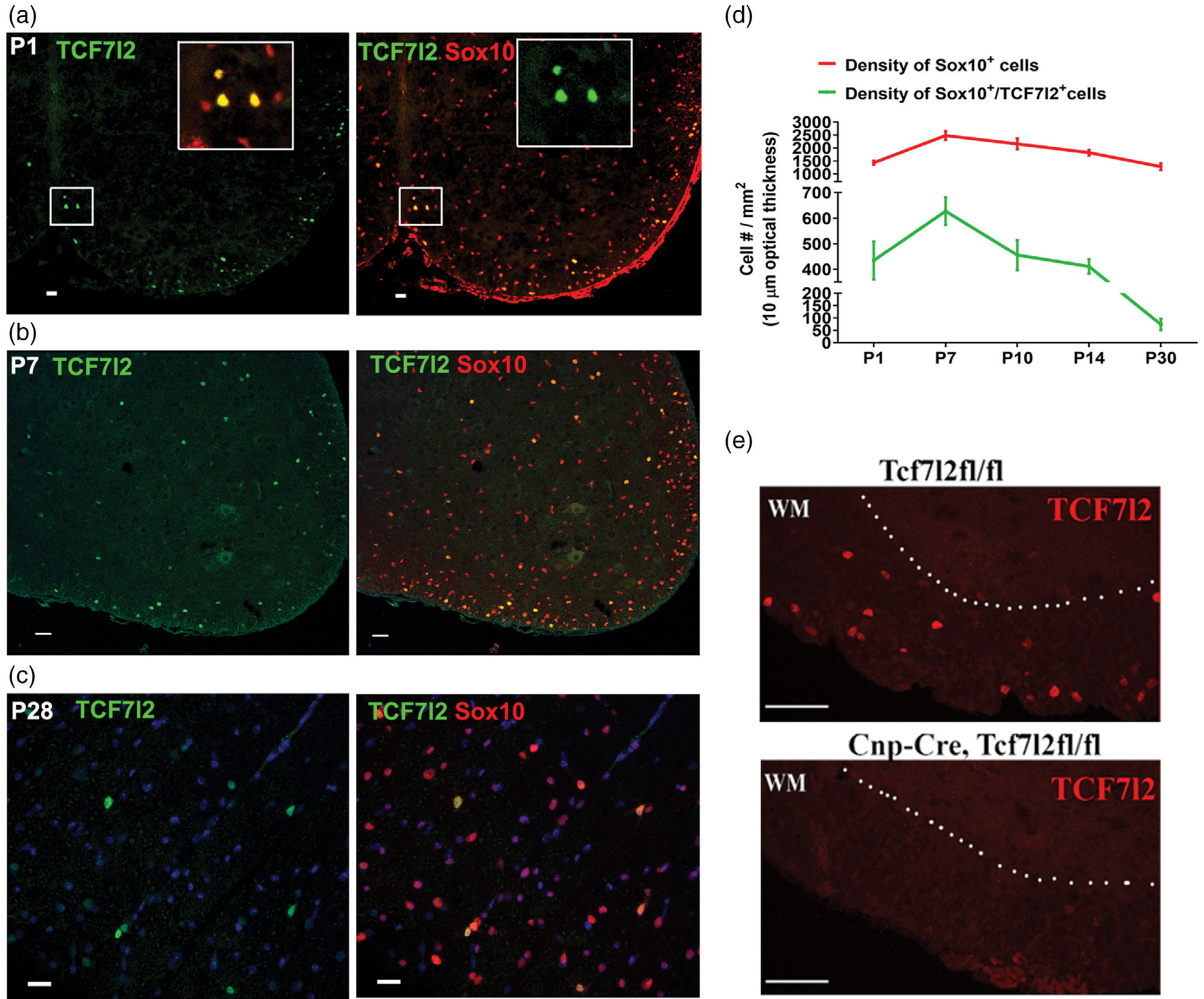

FIGURE 3.

TCF7l2-expressing cells belong to oligodendroglial lineage in the murine CNS, a notion illustrated by pan-oligodendroglial lineage marker Sox10. (a–c) Double fluorescence immunohistochemistry of TCF7l2 and Sox10 in the murine spinal cord at P1, P7, and P28. (d) Quantification of $Sox10^+$ and $Sox10^+TCF7l2^+$ cells in the white matter of the spinal cord. (e) The antibody specificity of TCF7l2 immunoreactive signals was validated by Cre-loxP-mediated TCF7l2 knockout in *Cnp-Cre:Tcf7l2*$^{fl/fl}$ double transgenic mice; P5 spinal cord was used for IHC. (d, e) Adapted from a previous paper (Hammond et al., 2015). TCF7l2 antibody was rabbit monoclonal antibody purchased from Cell Signaling Technology (clone # C48H11, catalog #2569)

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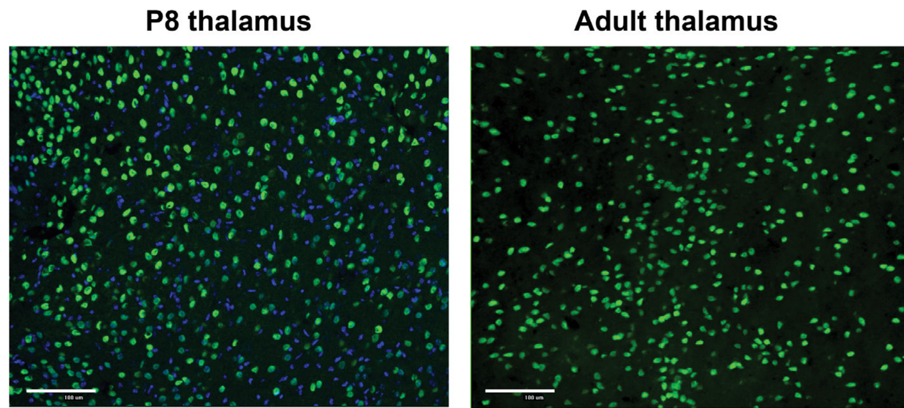

FIGURE 4.

TCF7l2 is highly expressed in thalamic neurons in the forebrain. Fluorescent immunostaining showing abundant TCF7l2 expression in thalamic neurons at early postnatal and adult ages. TCF7l2 antibody, Cell Signaling #2569 (rabbit clone C48H11). Scale bar = 100 μm

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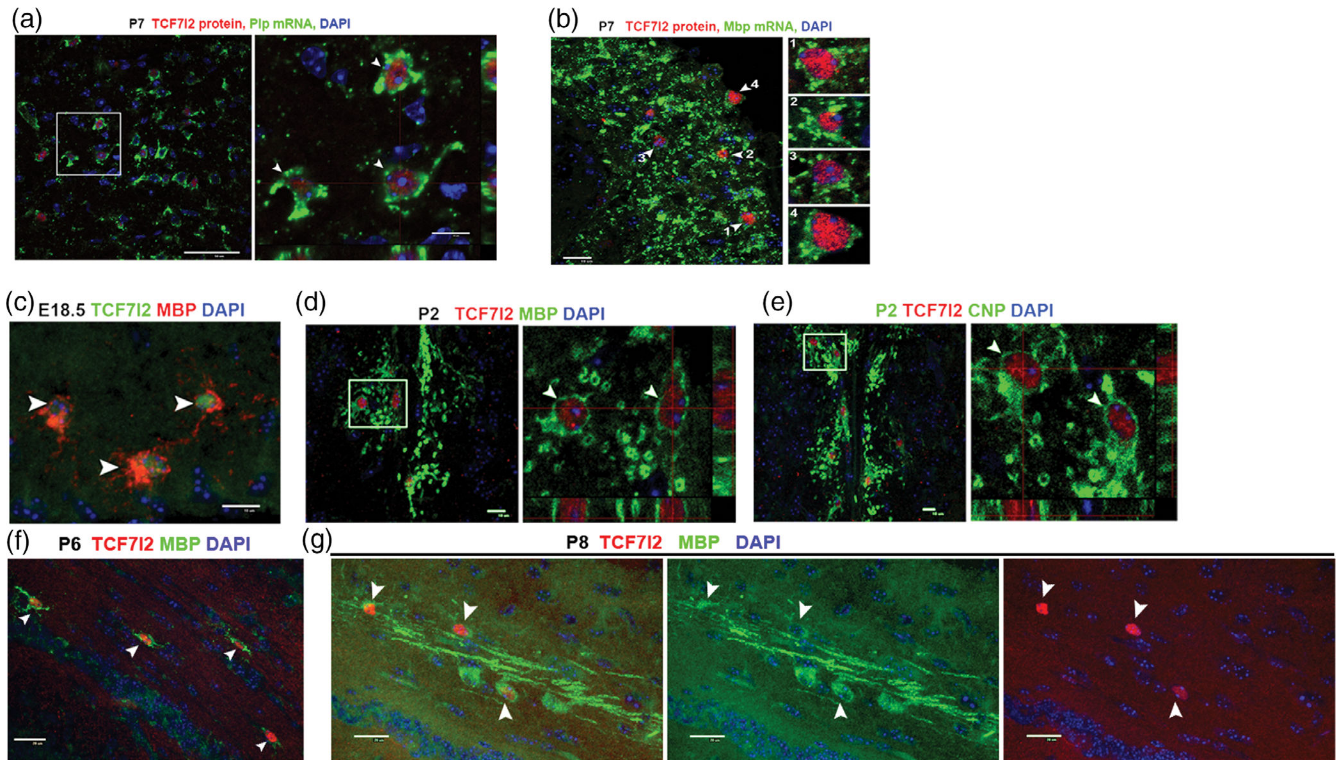

FIGURE 5.

TCF7l2^{+} OLs are myelin protein-expressing cells that are characterized by premyelinating morphology. (a) IHC of TCF7l2 and *Plp* mRNA in situ hybridization (IHC/ISH) on the sections of P7 spinal cord. Arrowheads, double positive cells. (b) IHC of TCF7l2 and *Mbp* mRNA in situ hybridization (IHC/ISH) on the sections of P7 spinal cord. Arrowheads, double positive cells. (c) Double fluorescent IHC of TCF7l2 and MBP in the spinal cord of mouse embryonic 18.5 days. (d–e) Double fluorescent IHC of TCF7l2 and MBP (d) or CNP (e) in the dorsal column of P2 mouse spinal cord. The boxed areas in (d) and (e) were shown as orthogonal views of confocal single optic slice at the right. (f–g) Double fluorescent IHC of TCF7l2 and MBP in the corpus callosum of P6 (f) and P8 (g) mice. The data of panel (a–e) and the concept underlying panels (f–g) are from previous paper (Hammond et al., 2015). Arrowheads in all panels point to double positive OLs. Blue, DAPI nuclear counterstaining. Scale bars in (f–g), 20 μm. TCF7l2 antibody, Cell Signaling #2569 (rabbit clone C48H11)

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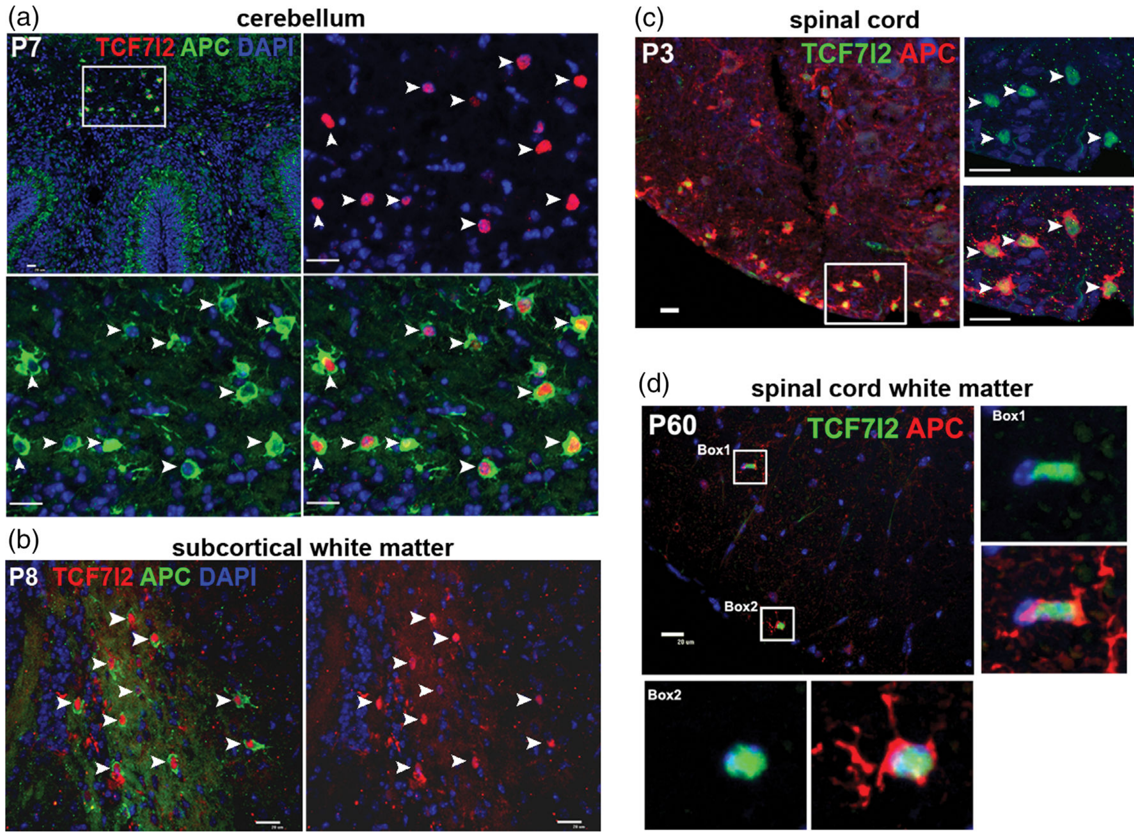

FIGURE 6.

TCF7l2 is co-expressed with adenomatous polyposis coli. Double fluorescent IHC of TCF7l2 and APC in P4 cerebellum (a), P8 external capsule of the forebrain (b), and P3 spinal cord (c). Note TCF7l2 and APC were overlapped in same cell populations. (d) Sparse TCF7l2^{+} cells were observed and co-labeled with APC in the white matter of P60 spinal cord. Scale bar = 20 μm. The concept underlying the figure panels are published in the previous papers (Lang et al., 2013). APC antibody, Santa Cruz Biotechnology (catalog # sc-896); TCF7l2 antibody, Cell Signaling #2569 (rabbit clone C48H11).

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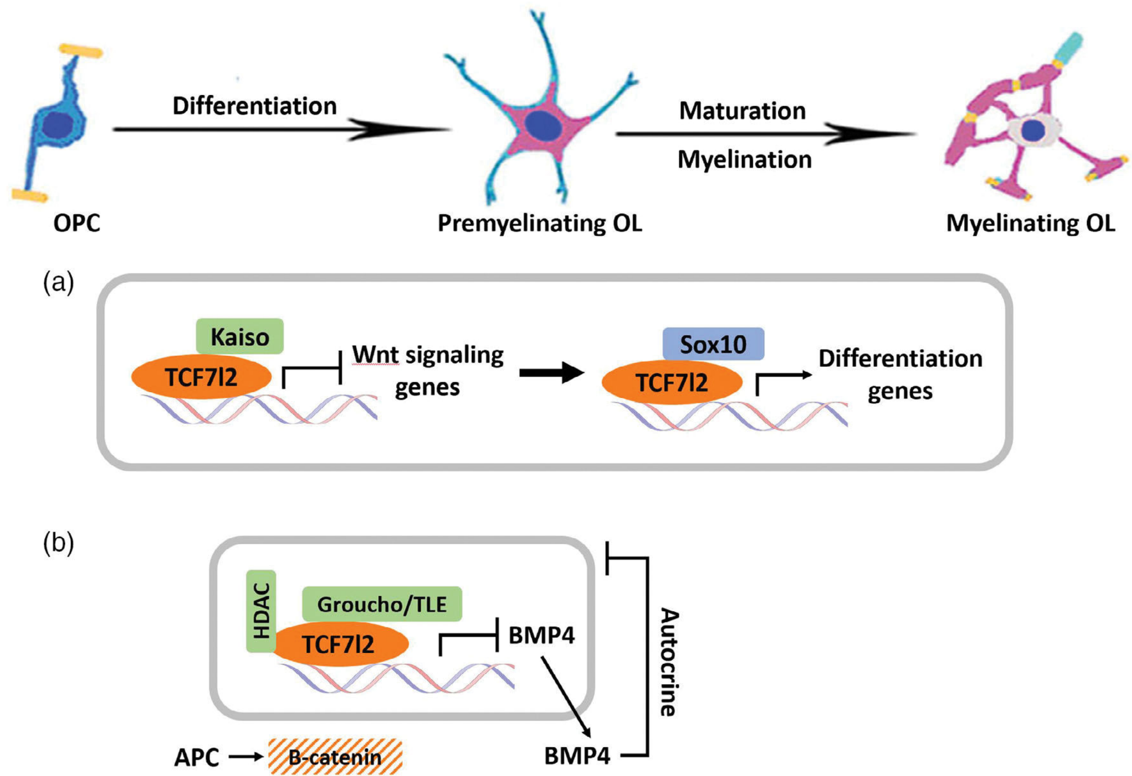

FIGURE 7.

Proposed molecular mechanism of TCF7l2-regulated oligodendroglial differentiation and maturation. (a) TCF7l2 recruits the Wnt inhibitor Kaiso to dampen Wnt/β-catenin signaling activity during early differentiation and Sox10 to enhance myelin gene expression during late maturation (Zhao et al., 2016). (b) TCF7l2 is co-expressed in the same population of OLs that are positive for APC (Lang et al., 2013) (also see Figure 6), a crucial regulator for β-catenin degradation. TCF7l2 represses autocrine BMP4-mediated signaling to promote early OL differentiation (Zhang et al., 2021)

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TABLE 1

Source of TCF7l2 antibodies and major expression conclusions from previous studies

References	TCF7l2 antibodies for immunostaining	Reported expression patterns and specificity
Fancy et al., 2009	• Upstate #05-511 (mouse, clone 6H5-3) • Cell signaling #2565 (Rabbit, Clone C9B9) • Abnova #H00006934-M04 (mouse, clone 1A9)	• TCF7l2 was expressed in OPCs but not in OLs. • TCF7l2 expression was high during developmental myelination and barely detectable in the adult CNS
Ye et al., 2009	• Upstate #05-511 (mouse, clone 6H5-3)	• TCF7l2 protein was low in PDGFRα$^+$ OPCs and high in CC1$^+$ OLs
Fu et al., 2009	• Upstate #05-511 (mouse, clone 6H5-3)	• TCF7l2 was absent from OPCs and present in postmitotic (Ki67$^+$) myelin gene-expressing premyelinating OLs. • TCF7l2 expression was high during developmental myelination and barely detectable in the adult CNS
Lurbke et al., 2013	• Catalog # (or clone #) not specified • Cell signaling rabbit anti-TCF7L2 • Abnova mouse anti-TCF7L2	• TCF7l2 was expressed in NogoA$^+$ OLs • TCF7l2 expression was high during developmental myelination and barely detectable in the adult CNS
Lang et al., 2013	• Upstate #05-511 (mouse, clone 6H5-3)	• TCF7l2 is expressed in APC$^+$/CC1$^+$ OLs.
Hammond et al., 2015	• Cell signaling #2569 (rabbit, clone C48H11) (*the most reliable antibody*) • Cell signaling #2565 (rabbit, clone C9B9) • Santa Cruz #sc-8632 (goat polyclonal) • Upstate #05-511 (mouse, clone 6H5-3)	• TCF7l2 is expressed in oligodendroglial cells that are post-mitotic, PDGFRα/NG2 negative, CC1 positive, MBP/CNP/PLP positive in the cell bodies. • TCF7l2 expression was high during developmental myelination and barely detectable in the adult CNS