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# WNT signaling suppresses oligodendrogenesis via Ngn2-dependent direct inhibition of Olig2 expression

Min Jiang, Dan Yu, Binghua Xie, Hao Huang, Wenwen Lu, Mengsheng Qiu* and Zhong-Min Dai*

## Abstract

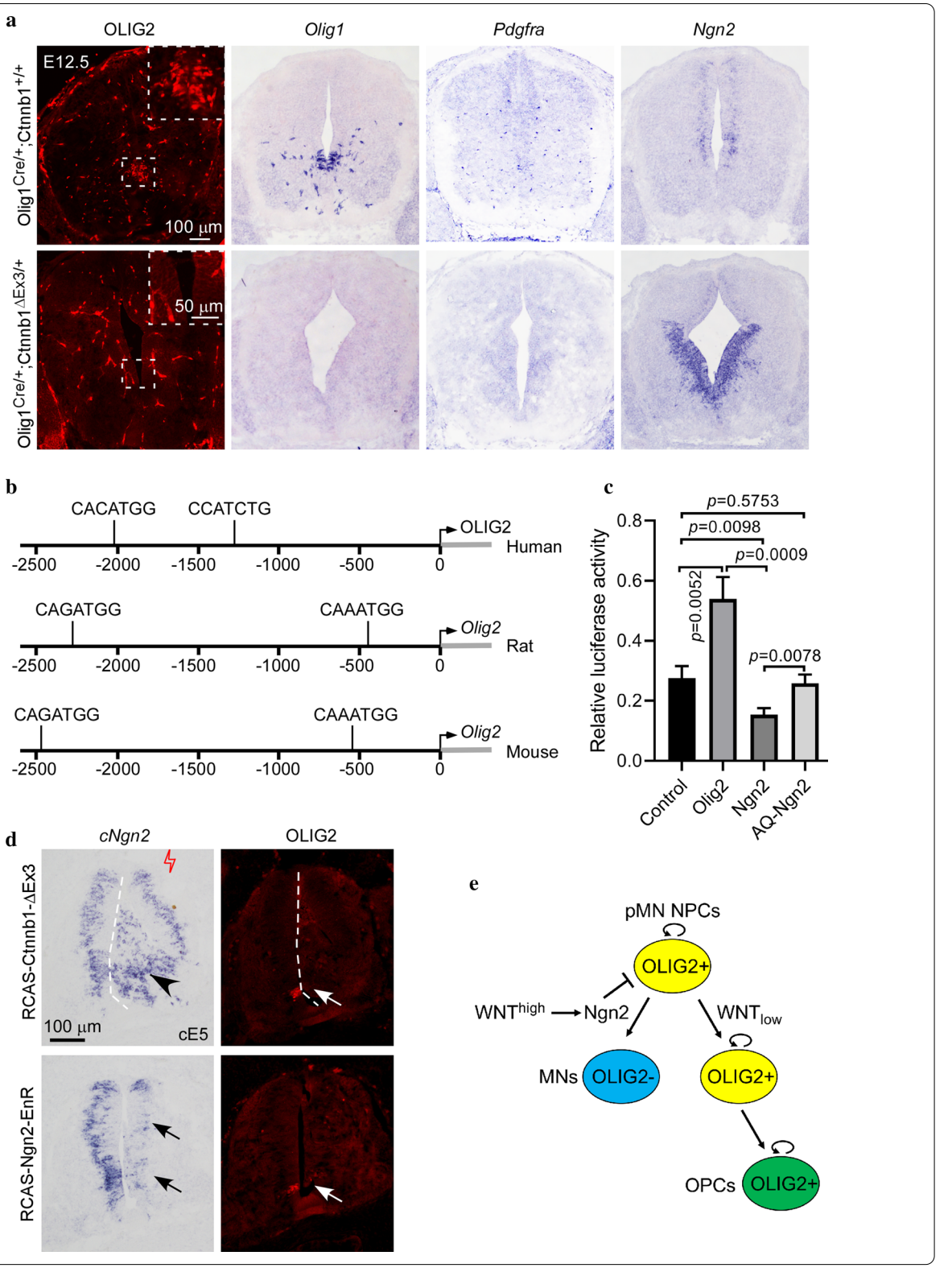
*Olig2* transcription factor is essential for the maintenance of neural progenitor cells (NPCs) in the pMN domain and their sequential specification into motor neurons (MNs) and oligodendrocyte precursor cells (OPCs). The expression of *Olig2* rapidly declines in newly generated MNs. However, *Olig2* expression persists in later-born OPCs and antagonizes the expression of MN-related genes. The mechanism underlying the differential expression of *Olig2* in MNs and oligodendrocytes remains unknown. Here, we report that activation of WNT/β-catenin signaling in pMN lineage cells abolished *Olig2* expression coupled with a dramatic increase of *Ngn2* expression. Luciferase reporter assay showed that *Ngn2* inhibited *Olig2* promoter activity. Overexpression of Ngn2-EnR transcription repressor blocked the expression of *Olig2* in ovo. Our results suggest that down-regulation of WNT-*Ngn2* signaling contributes to oligodendrogenesis from the pMN domain and the persistent *Olig2* expression in OPCs.

**Keywords:** WNT, β-catenin, Oligodendrocyte, *Ngn2*, *Olig2*

*Olig2* is the key transcription factor that not only maintains the neural progenitor cells (NPCs) of pMN domain, but also regulates the sequential specification of NPCs into motor neurons (MNs) and OPCs [1–5]. Since persistent expression of *Olig2* is inhibitory to post-mitotic MN genes [6], the expression of *Olig2* rapidly declines in newly generated MNs, but remains high in later-born cells of oligodendrocyte lineage [2–6]. The mechanism of down-regulation of *Olig2* expression in MNs remains elusive. WNT signaling is known to regulate the balance between the proliferation and differentiation of NPCs during neurogenesis [7]. It is interesting that endogenous WNT/β-catenin signaling is activated in newly generated MNs [8]. Activation of WNT/β-catenin signaling has been reported to inhibit the specification of OPCs and astrocytes from NPCs during early stages of gliogenesis [9–11]. However, the mechanism underlying the inhibition of OPC specification from pMN NPCs by WNT/β-catenin signaling remains to be determined.

Here, we utilized the $Olig1^{Cre/+}$;$Ctnnb1^{\Delta Ex3/+}$ transgenic mice to activate WNT signaling in the pMN domain. At embryonic day 12.5 (E12.5) when oligodendrogenesis commences, expression of *Olig1* and *Olig2* remains high in the pMN neural progenitor cells from which *Pdgfra*+ OPCs arise (Fig. 1a). Strikingly, in $Olig1^{Cre/+}$;$Ctnnb1^{\Delta Ex3/+}$ transgenic mice, activation of WNT signaling totally abolished the expression of *Olig1*, *Olig2* and *Pdgfra* (Fig. 1a), indicating a complete inhibition of oligodendrogenesis. By contrast, the number of ISL1-positive MNs was only decreased slightly in $Olig1^{Cre/+}$;$Ctnnb1^{\Delta Ex3/+}$ mice (Additional file 1: Fig. S1), consistent with the previous finding that *Olig1* is intermittently expressed in pMN NPCs and only weakly expressed during neurogenesis stage [12]. Although $Olig1^{Cre}$ was also transcribed in P3 domain at early stages

*Correspondence: m0qiu001@yahoo.com; zhongmindai@hznu.edu.cn
Institute of Life Sciences, Key Laboratory of Organ Development and Regeneration of Zhejiang Province, College of Life Sciences, Hangzhou Normal University, Hangzhou 310029, People's Republic of China


a
OLIG2
Olig1
Pdgfra
Ngn2
E12.5
Olig1Cre/+;Ctnnb1+/+
100 μm
Olig1Cre/+;Ctnnb1ΔEx3/+
50 μm
b
CACATGG
CCATCTG
OLIG2
Human
-2500
-2000
-1500
-1000
-500
0
CAGATGG
CAAATGG
Olig2
Rat
CAGATGG
CAAATGG
Olig2
Mouse
c
p=0.5753
p=0.0098
p=0.0009
p=0.0052
p=0.0078
Relative luciferase activity
0.8
0.6
0.4
0.2
0.0
Control
Olig2
Ngn2
AQ-Ngn2
d
cNgn2
OLIG2
RCAS-Ctnnb1-ΔEx3
100 μm
cE5
RCAS-Ngn2-EnR
e
pMN NPCs
OLIG2+
WNThigh
Ngn2
WNTlow
MNs
OLIG2-
OLIG2+
OPCs
OLIG2+

(See figure on previous page.)
**Fig. 1** WNT signaling inhibit *Olig2* expression through upregulation of *Ngn2* expression. **a** Transverse sections of spinal cord at E12.5 from control and WNT signaling activated (*Olig1*$^{Cre/+}$;*Ctnnb1*$^{\Delta Ex3/+}$) mice were subjected to IF with anti-OLIG2 antibody or ISH with *Olig1*, *Pdgfra* and *Ngn2* riboprobes. The cells positive for OLIG2, *Olig1* and *Pdgfra* are absent in the spinal cord from *Olig1*$^{Cre/+}$;*Ctnnb1*$^{\Delta Ex3/+}$ mice, whereas *Ngn2* is upregulated. Inset highlights the expression of OLIG2 in pMN domain, note that vascular development was abnormal in the spinal cord of *Olig1*$^{Cre/+}$;*Ctnnb1*$^{\Delta Ex3/+}$ mice. **b** There are putative *Ngn2* binding sequences in the promoter regions of *Ngn2* from human, rat and mouse. **c** Luciferase report assay revealed that *Ngn2* but not its DNA binding deficient mutant AQ-*Ngn2* inhibit the promoter activity of mouse *Olig2*. *$p < 0.05$, t-test. **d** Over-expression of *Ngn2*-EnR mimics the phenotype caused by over-expression of *Ctnnb1*-ΔEx3. Both expression of *Ctnnb1*-ΔEx3 and *Ngn2*-EnR suppressed the expression of OLIG2 *in ovo*. Arrowhead indicates induced expression of chick *Ngn2* (cNgn2). Arrows represent reduced expression of endogenous genes. **e** OLIG2 maintains proliferation of pMN domain neural progenitor cells. High level of WNT signaling upregulates *Ngn2* expression, NGN2 in turn coordinate with OLIG2 to promote motor neurons specification and suppress *Olig2* expression in newly generated motor neurons. OPCs were specified OLIG2+ cells when WNT signaling is declined at the gliogenesis stage

[13, 14], expression of P3 domain marker NKX2-2 was not suppressed in *Olig1*$^{Cre/+}$;*Ctnnb1*$^{\Delta Ex3/+}$ mice (Additional file 1: Fig. S1). However, the number of *Ngn2*-positive cells was dramatically increased within the ventral ventricular region in *Olig1*$^{Cre/+}$;*Ctnnb1*$^{\Delta Ex3/+}$ mice (Fig. 1a), demonstrating that WNT activation promotes *Ngn2* expression. In support of this notion, overexpression of *Ctnnb1*$^{\Delta Ex3}$ in embryonic chicken spinal cord also caused an increase of *Ngn2* expression, coupled with a reduced expression of *Olig2* (Fig. 1d). At E18.5, although a few dorsally-derived [15–17] OPCs were generated from *Olig1*$^{Cre/+}$;*Ctnnb1*$^{\Delta Ex3/+}$ mice, *Plp1*-positive mature oligodendrocytes were still undetectable (Additional file 1: Fig. S2) since dorsal OPCs differentiate only after birth [14]. Together, these results strongly suggest that *Ngn2* is the candidate gene that mediates the suppression of oligodendrogenesis from pMN NPCs by WNT signaling.

In line with this concept, two NGN2 recognition sequences are identified in the upstream promoter of the *Olig2* gene in human, rat and mouse (Fig. 1b). Luciferase reporter assay revealed that *Ngn2* but not its DNA binding defective mutant AQ-*Ngn2* can inhibit the promoter activity of mouse *Olig2* (Fig. 1c), demonstrating that *Ngn2* can bind to the promoter of *Olig2* and repress its expression. To confirm that *Ngn2* mediates WNT inhibition of oligodendrogenesis, we overexpressed *Ngn2* in embryonic chicken spinal cord by in ovo electroporation and found a significant decrease of *Olig2* and *Pdgfra* expression in the electroporated side at cE7 (Additional file 1: Fig. S3). Since *Ngn2* can function either as a transcriptional activator or a repressor, we next investigated whether the inhibition of *Olig2* expression is mediated by the transcriptional repressor activity of *Ngn2*. RCAS-Ngn2-EnR (DNA binding domain of NGN2 fused with EnR transcription repressor) was employed as a repressor-only NGN2 chimeric protein. It was found that overexpression of this chimeric repressor caused a significant reduction of *Olig2* expression (Fig. 1d), mimicking the effect of full-length *Ngn2* protein. This finding demonstrated that *Ngn2* inhibits *Olig2* expression by its transcriptional repressor activity.

In conclusion, our results suggest that WNT signaling up-regulates the expression of *Ngn2*, and *Ngn2* in turn inhibits *Olig2* expression and oligodendrogenesis during MN specification (Fig. 1e).

## Supplementary information

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s13041-020-00696-0.

**Additional file 1:** Supplementary materials and results.

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**Authors' contributions**
ZMD conceived the project. MJ, DY, BX, HH, WL and ZMD performed the experiments. MJ, DY, BX, HH, MQ, WL and ZMD analyzed the data. MQ and ZMD supervised the project. MJ, MQ and ZMD wrote the paper with input from the other authors. All authors read and approved the final manuscript.

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**Availability of data and materials**
All date generated during this study are included in this article.

**Ethics approval and consent to participate**
The use of animals was approved by the Committee on Laboratory Animals, Hangzhou Normal University.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no conflict of interest.

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