Elucidating the role of a mitochondrial protein – Mmd2 in neuron-glial fate choice determination in the dorsal root ganglia

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Abstract: Metabolic syndromes often result in congenital birth defects in the formation of neural crest (NC) derived tissues, indicating that metabolism plays a crucial role in NC development. The hierarchical transcriptional control on how NC cells (NCCs) give rise to different cell types have been extensively studied, but how metabolism regulates NC lineage determination remains unknown. Early migratory NCCs are bipotent neuron-glial (N/G) progenitors, which can further differentiate into sensory neurons and glial cells in the dorsal root ganglia (DRG), forming the somatosensory component of the peripheral nervous system (PNS). In this project, we aim to study the role of a mitochondrial metabolic regulator –Monocyte to Macrophage Differentiation Associated 2 (Mmd2) in determining neuron-glial fate choice in the DRG in chick embryo using gain and loss of function studies. It is well-known that Sox10, a high mobility group (HMG) transcription factor is responsible for specifying N/G progenitors into glial cells instead of sensory neurons. We found that Mmd2 expression was initiated in bipotent N/G progenitors and later retained in glial lineage in the DRG, suggesting a role for Mmd2 in determining neuron-glial lineage choice. Indeed, overexpression (OE) of Mmd2 led to an increase in N/G progenitors and glial cells in the DRG, whereas Mmd2 knockdown (KD) did not appear to affect the formation of N/G progenitors, glial cells and sensory neurons. In addition, we further revealed that Sox10 OE induced ectopic Mmd2 expression, suggesting that Sox10 functions as an upstream regulator of Mmd2. Together, these results suggest that Mmd2 functions downstream of Sox10 as a mitochondrial metabolic factor in regulating glial lineage specification. How Mmd2 promotes glial specification remains to be elucidated. This study provides insights into how metabolic regulation influences NC lineage determination.

Introduction

NC development and the formation of PNS

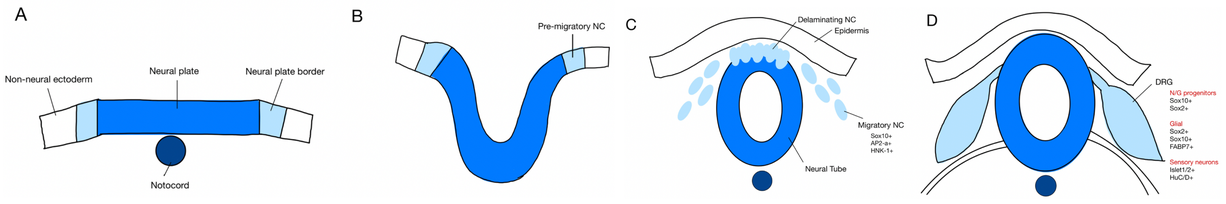


Figure 1. **The development of NC and the PNS**. Neural plate border is specified between non-neural ectoderm and neural plate border in the gastrula stage of chick embryos. (B) Neural plate closes to form a neural tube together with the specification of pre-migratory NC in the neural plate border region. (C) Upon neural tube closure, prospective NCCs delaminate from the dorsal neural tube and undergo epithelial to mesenchymal transition (EMT) to form a multipotent migratory population. (D) A subpopulation of migratory NCs migrate to the DRG where they differentiate into sensory neuron and glial cells, forming the PNS.

In the central nervous system (CNS):

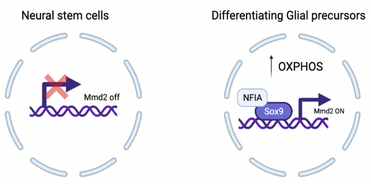


Figure 2. Sox9 and its co-factor NFIA activate a mitochondrial protein –Mmd2 expression which promotes oxidative phosphorylation (OXPHOS), leading to glial specification in the CNS.

In the peripheral nervous system (PNS):

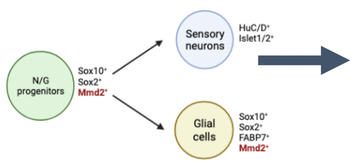


Figure 3. Mmd2 expresses in migratory NCCs and later retain in N/G progenitors and glial precursors in the PNS

Hypothesis:

Mmd2 plays a metabolic role in glial specification from N/G progenitors in the PNS

Sox10 (glial lineage transcription factor) acts upstream of Mmd2 to promote glial specification

Significance of studying Mmd2 in neuron-glial fate determination

By studying a metabolic regulator that promotes OXPHOS –Mmd2, neuron-glial fate determination in the PNS, this allow us to unravel a functional importance of mitochondrial metabolic regulation in NCC fate determination and a deeper understanding of the etiology of defects in NC-derived tissues that are caused by metabolic disruption.

Materials & Methods

In ovo electroporation

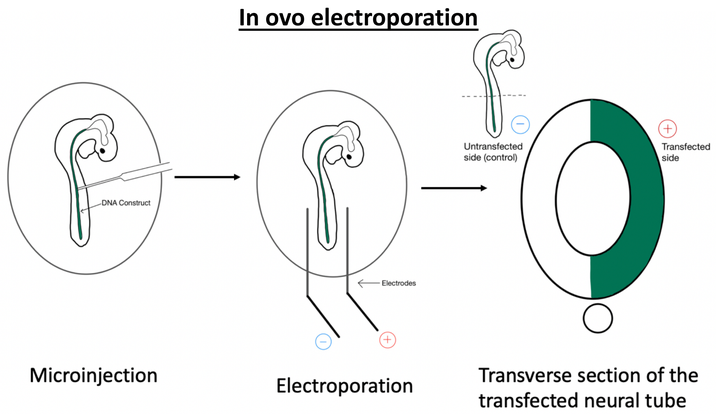


Figure 4. Diagram illustrating how in ovo electroporation was conducted at Hamburger and Hamilton (HH) stage 14 chick embryos. DNA constructs were injected into the neural tube through a micro glass pipette followed by electroporation. Negatively charged DNA constructs were transfected to the right side of the neural tube where positively charged electrode is placed next to it. The left side of the neural tube served as the untransfected control.

Mmd2 Morpholino (MO) design & expression vectors

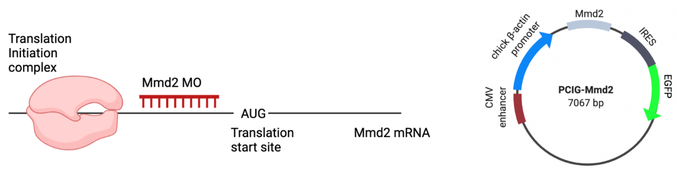
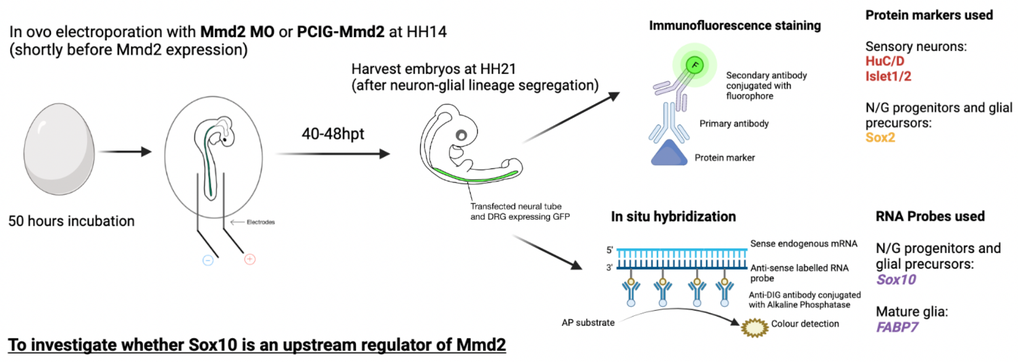
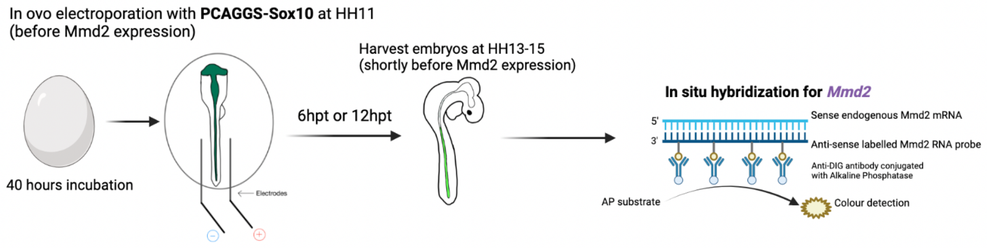


Figure 5. Mmd2 MO design and PCIG-expression vector. Mmd2 MO are synthetic antisense oligonucleotides composed of 22 nucleotides that are designed to block and bind the translation start site of *Mmd2* mRNA. Full length chick *Mmd2* cDNA was inserted upstream of an internal ribosomal entry site (IRES) and a nuclear localization sequence (nls)-tagged EGFP in a pCIG expression vector driven by both CMV promoter and chick beta-actin enhancer.

To investigate whether Mmd2 plays a role in glial specification



To investigate whether Sox10 is an upstream regulator of Mmd2



Results

Mmd2 is expressed in migratory NCCs containing N/G progenitors and in glial cells in the DRG

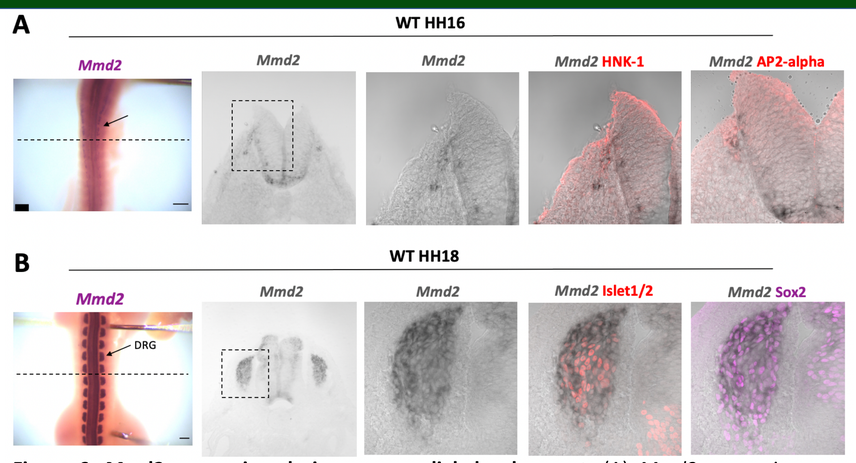


Figure 6. Mmd2 expression during neuron-glial development. (A) *Mmd2* expression was initiated in migratory-NC at HH16 as revealed by co-expression of migratory markers HNK-1 and AP2-alpha. (B) *Mmd2* expression retained in N/G progenitors and glial cells but not sensory neurons in the DRG at HH18 as revealed by co-expression with Sox2 and Islet1/2. Abbreviations: WT: Wild-type, HH: Hamburger and Hamilton stages.

Mmd2 KD did not affect N/G progenitors, glial cells and sensory neurons formation

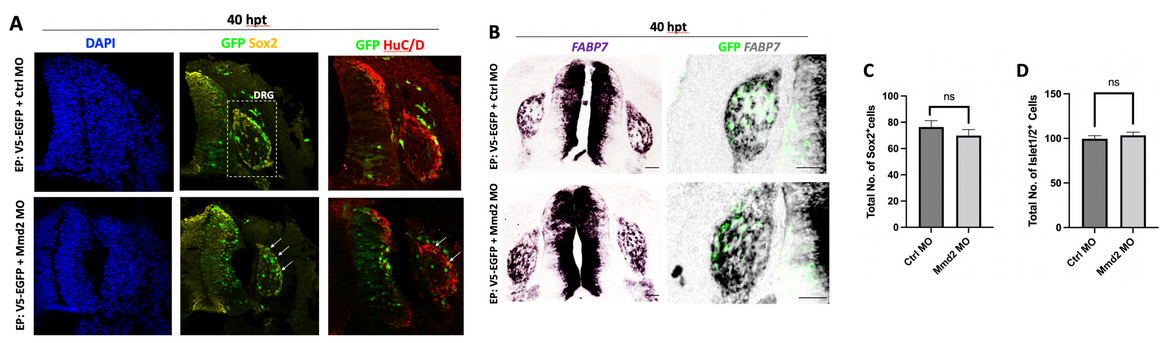


Figure 7. Mmd2 KD did not affect N/G progenitors, glial cells and sensory neurons formation. (A) GFP+ cells in the DRG overlap with Sox2 and HuC/D expression, indicating that N/G progenitors, glial cells and sensory neurons formation are not affected upon Mmd2 KD. (B) GFP transfected cells in the DRG did not affect glial marker *FABP7* expression, further confirming that glial formation was not affected by Mmd2 KD. (C) & (D) $Sox2^+$ and $Islet1/2^+$ cells number did not change upon Mmd2 KD. Abbreviations: Ctrl: control, hpt: hour post-transfection, EP: electroporation, ns: non-significant

Mmd2 OE promotes the formation of N/G progenitors and glial cells

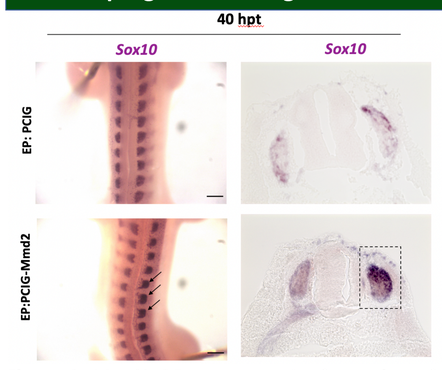


Figure 8. Mmd2 OE promotes the formation of N/G progenitors and glial cells in the DRG. The size of the DRG marked by *Sox10* in Mmd2 transfected embryos is enlarged compared with the untransfected side and embryos transfected with vector control. Transverse section of the DRG revealed that *Sox10* expression increases in the transfected DRG (right) when compared to control, meaning that the number of $Sox10^+$ N/G progenitors and glial cells increases in the DRG upon Mmd2 OE.

Sox10 is an upstream regulator of Mmd2

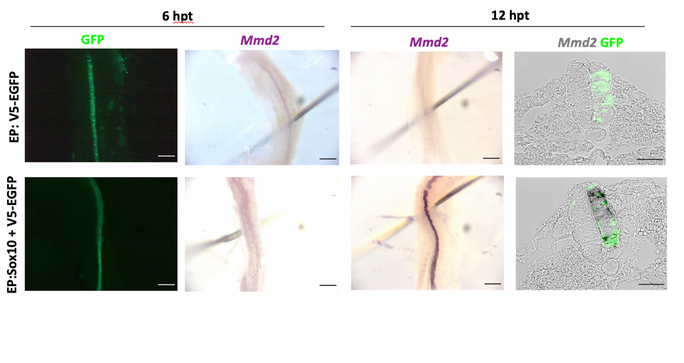
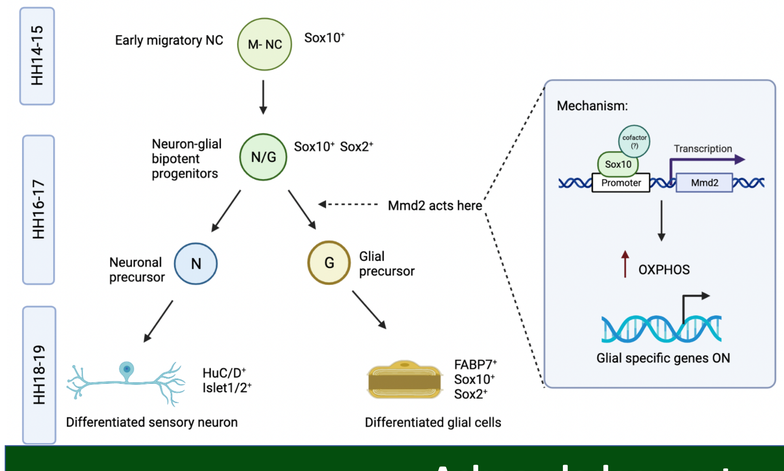


Figure 9. Sox10 OE induces ectopic *Mmd2* expression. Ectopic *Mmd2* expression is observed in the transfected neural tube (right) of Sox10 OE embryos at 12hpt but not 6hpt. Transverse sections of 12hpt Sox10 OE embryos revealed that ectopic *Mmd2* expression overlaps with GFP expression, indicating that the effect is cell autonomous – only Sox10 overexpressing cells induce ectopic *Mmd2* expression. These results suggest that Sox10 is an upstream regulator of Mmd2 in promoting glial specification.

Discussion



-Mmd2 KD did not affect the formation of N/G progenitors, glial and sensory neurons possibly due to the low KD efficiency of Mmd2 MO or functional compensation of closely related members of Mmd2 such as Mmd.

-Mmd2 may promote glial specification through increase in OXPHOS that activates expression of glial determinants.

-Sox10 with or without co-factor promotes glial specification through activating of Mmd2, via an indirect mechanism or directly binding to its promoter

Acknowledgement

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