

Developmental study of the cerebellar granule cell precursors in the *weaver* condition

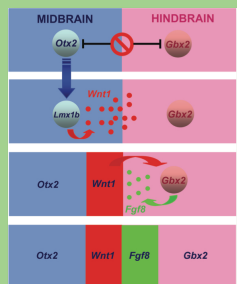
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1. Introduction

Mice carrying the *weaver* (*wv*) mutation are characterized by a wide range of severe symptoms. Although it is known that the responsible of its abnormalities is the death of several cell types mainly in the CNS, the details of the link between the genotype and the phenotype remain unclear. One of the most affected cell types are the granule cells of the cerebellar cortex, thus being an interesting model to study the effects of the *wv* mutation. Poor research has been done in the context of their prenatal life, so studying the development of the granule cell precursors may unveil unknown mechanisms that drive the mutant genotype to its corresponding phenotype.

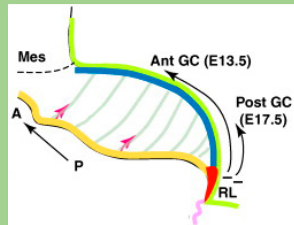
3. Key steps in the prenatal development of the cerebellar granule cell precursors



1. Establishment of the isthmus organizer (IsO)
(Carletti B, Rossi F; 2008)

Key factors:
- OTX2
- GBX2
- FGF8

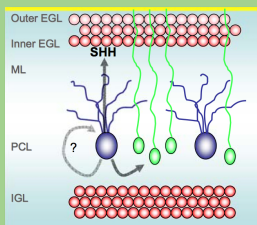
Age: E7.5



GCPs markers:
- MATH1
- ZIC1

Age: E10-E15

2. Proliferation of Granule cell precursors (GCPs) in the Rhombic lip and migration to form the external granular layer (EGL)
(Chédotal A; 2010)



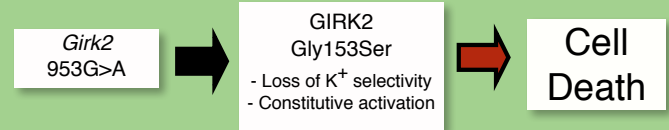
Key factors:

- SHH
- GLI1
- GLI3
- Cyclins A2, B1, D1, D2
- FOXM1
- N-MYC
- PTC

Age: E13.5-P15

3. Proliferation and maturation of the GCPs in the EGL
(Vaillant C, Monard D; 2009)

2. The *weaver* mutant

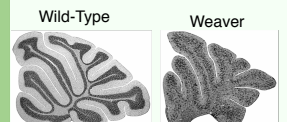


- Small size
- Instability of gait
- Weakness
- Hypotonia
- Male sterility

Weaver Mouse
(www.und.nodak.edu)

Cerebellar abnormalities:

- Structural deficiencies.
- Depletion of granule cells (most affected), Purkinje cells and deep cerebellar nuclei neurons.



Sagittal sections of Wild-Type and Weaver cerebellums (Martí J. et al, 2007)

4. Working hypothesis

An incorrect prenatal development of the cerebellar GCPs leads to the phenotype of the *wv* mutants.

5. Objectives

General objectives:

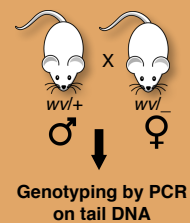
- Study the prenatal development of the GCPs in the *wv* condition

Specific objectives:

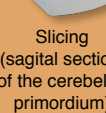
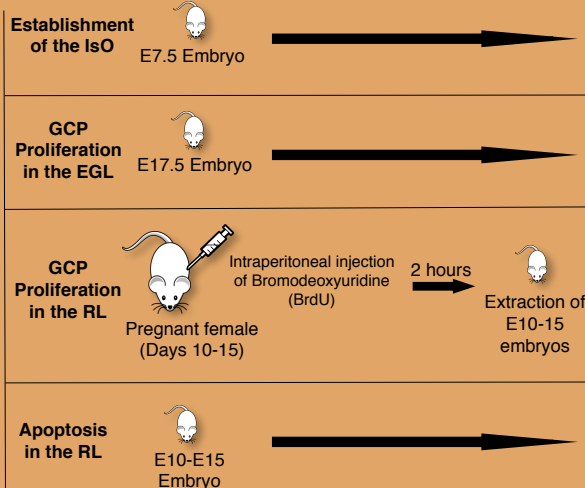
- Check the correct expression of the key factors involved in the establishment of the IsO.
- Check if the *wv* mutation decreases the proliferation rate of the GCPs in the stage of RL.
- Check if the *wv* mutation increases the apoptosis rate of the GCPs in the stage of RL.
- Check the correct expression of the key factors involved in the GCP proliferation in the EGL.

6. Methodology

Mutant stock maintenance
(First heterozygous mutants from Jackson Laboratory, ME, U.S.A.)



Experimental or procreational use of descendants



Immunostaining

Targets of immunostaining

OTX2 GBX2
FGF8

SHH Cyclins N-MYC
GLI1 GLI3
FOXM1 PTC

BrdU
PCNA (Proliferating cell nuclear antigen)
MATH1
ZIC1

Caspase-3 / TUNEL
MATH1
ZIC1

FLUORESCENT-MICROSCOPY

Semiquantitative analysis
+/+ vs *wv/wv*

Quantitative analysis
+/+ vs *wv/wv*

7. Expected Results

- Significant differences in the expression of *Otx2*, *Gbx2* or *Fgf8* between *weaver* and wild-type mice at the IsO level.
- Significant differences in the expression of the key factors involved in the Shh signalling pathway between *weaver* and wild-type mice at the EGL level.
- Decreased number of proliferating (BrdU positive) cells in *weaver* mice at the RL level.
- Increased number of apoptotic GCPs (Caspase-3 or TUNNEL positives) in *weaver* mice at the RL level

8. References

- Martí, J. et al., Purkinje cell age-distribution in fissures and in foliar crowns: a comparative study in the weaver cerebellum. *Brain struct. Funct.* **212**, 347-57 (2007)
- Harrison, S. M. & Roffler-Tarlov, S. K. Cell death during development of testis and cerebellum in the mutant mouse *weaver*. *Dev Biol.* **195**, 174-86 (1998)
- Carletti, B. & Rossi, F. Neurogenesis in the cerebellum. *Neuroscientist* **14**, 91-100 (2008)
- Chédotal, A. Should I stay or should I go? Becoming a granule cell. *Trends Neurosci.* **33**, 163-172 (2010)
- Vaillant, C. & Monard, D. SHH pathway and cerebellar development. *Cerebellum* **8**, 291-301 (2009)