A Role for Sox2 in the Adult Cerebellum

Nikolaos Panagiotis Mandalos1,2, Ioannis Karampelas3, Marannia Saridaki2, Ronald D G McKay4, Mark L Cohen4 and Eumorphia Remboutsika1,2,5*

1National and Kapodistrian University of Athens, School of Medicine, Department of Pediatrics, Athens, Greece
2Stem Cell Biology Laboratory, Biomedical Sciences Research Centre "Alexander Fleming", Vari-Athina, Greece
3Department of Neurosurgery, University Hospitals Case Medical Center, Cleveland, OH, USA
4Department of Pathology, University Hospitals of Cleveland, Case Western Reserve School of Medicine, Cleveland, OH, USA
5The Lieber Institute for Brain Development, Basic Sciences Division, Johns Hopkins Medical Campus, Baltimore, USA

Abstract

The cerebellum, a derivative of the hindbrain, plays a crucial role in balance and posture as well as in higher cognitive and locomotive processes. Cerebellar development is initiated during the segmental phase of hindbrain formation. Here, we describe the phenotype, of a single surviving adult conditional mouse mutant mouse, in which Sox2 function is ablated in embryonic radial glial cells by means of hGFAP-CRE. The single Sox2RGINV/mosaic adult mutant mouse displays motor disability, microsomia, reduced Central Nervous System (CNS) size and cerebellar defects associated with human genetically related congenital abnormalities.

Keywords: Neural stem cells; Neural progenitor cells; Neurogenesis; Development; Differentiation; Neurodegeneration; Sox genes

Introduction

In adult mouse the cerebellum is located dorsally to the brainstem. It plays a fundamental role in sensory-motor processing, exemplifying a well-defined and distinctive neurophysiological structure [1]. The cerebellar cortex is constituted of three layers, spatially designated as the innermost layer- a) the granule cell layer, b) the middle Purkinje cell layer and c) the outmost molecular layer. The outer molecular layer is mainly composed of the axons of granule cells and dendrites of Purkinje cells. The Purkinje single cell layer sets the border between the granule and molecular layers, while the inhibitory Purkinje cells are located between excitatory granule cells and the subpial molecular layer [2]. The complex structure of the cerebellum is well preserved among mammals and birds, budding from the neural tube at early stages of development. The morphogen Fibroblast Growth Factor Eight (Fgf8) signalling pathway is believed to play a crucial role for the setting of the axial boundaries of the cerebellar anlage [3]. In a similar mode, Otx2 has a fundamental role in determining its forebrain and midbrain boundaries [4,5], while Hoxa2 establishes the caudal limits of the cerebellum during embryonic differentiation [6,7].

The pluripotency transcription factor Sox2 governs the neural lineage commitment during cerebral development, since it controls the proliferation and differentiation of Neural Progenitor Cells (NPCs) [8]. Interestingly, Sox2 spatially and functionally defines stem cell niches of the mammalian adult cerebrum [9,10]. It has also been reported to be expressed in a variety of differentiated cerebellar glia cells in mouse embryogenesis, such as Bergmann glia cells, a radial glia subtype that plays a crucial role in the migration of the cerebellar Purkinje cells and granule cells [11]. Along with Sox1 and Sox9 [12], Sox2 has a consistent expression in the cerebellar Purkinje cell layer in adulthood [13].

We have previously used a Sox2COIN conditional mutant mouse to understand how Sox2 governs neural stem and progenitor cell fate during embryogenesis [7,10,14,15]. Expression of Sox2 along with Sox1 and Sox9 was detected at the mRNA level in both foetal and adult mouse cerebellar samples, suggesting that the maintenance of these markers in adult tissue is also observed in the human cerebellum. These markers were further confirmed at the protein level on human tissue sections, as Sox1, Sox2 and Sox9 expression was detected in the Purkinje cell layer of the adult cerebellum. Here we report the behavioural and pathoanatomical defects of a single case conditional Sox2 adult mutant mouse in radial glia cells. Sox2RGINV/mosaic adult mouse shows microsomia, motor defects, impaired CNS development and malformations of cerebellar granular and molecular cell layers.

Case Study

In an effort to understand the role of Sox2 in neural stem and progenitor cells, we have conditionally ablated the function of Sox2 in radial glial cells via Sox2COIN/COIN [7], to transgenic Tg(hGFAP-CRE) mouse intercrosses [7,14,16]. Tg(hGFAP-CRE) mice express CRE recombinase at embryonic day E13.5 in radial glial cells at dorsal and middle regions of the telencephalon, while at an early birth stage, hGFAP is ubiquitously expressed throughout the CNS, in all neural cell types derived from radial glial cells. In adult mice, hGFAP expression is mainly restricted in astrocytes of the brain, and also on neuronal niches, such as the external granule cell layer of the cerebellum, theolfactory bulb, the hippocampal area and the subventricular zone [17]. After performing Sox2COIN/COIN to Sox2RGINV/+; Tg(hGFAP-CRE) intercrosses, we observed that adult heterozygote Sox2RGINV/+ Tg(hGFAP-CRE) offspring appear normal and fertile compared with wild type mice. However, Sox2RGINV/mosaic Tg(hGFAP-CRE) mutants, hereafter referred to as Sox2RGINV/mosaic, die around E15 (data not shown).

Interestingly though, a single Sox2RGINV/mosaic mutant escaped embryonic lethality and it developed severe microsomia phenotype evident at the age of 28 postpartum days (Figure 1 A). Microsomia was accompanied by severe neurological disorders, as repetitive and stereotyped circular movements, hyperactivity, impaired motor skills.

*Corresponding author: Eumorphia Remboutsika, National and Kapodistrian University of Athens, School of Medicine, Department of Pediatrics, Athens, Greece, Tel: 00306974706252; E-mail: remboutsika@gmail.com / eremboutsika@med.uoa.gr / erembou1@jhu.edu

Received June 30, 2018; Accepted July 24, 2018; Published July 27, 2018


Copyright: © 2018 Mandalos NP, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
(Figure 2), tremors and lack of appetite, grabbing defects, uncontrolled movements indicating intense stress, phenotypes that are related to brain abnormalities in mouse models with Autistic Spectrum Disorder (ASD), and that have been associated to cerebellar disruptions during development [18].

After observing this pathological phenotype, we opted to focus on the pathoanatomical traits of the cerebellum of this mouse Sox2\textsuperscript{RGINV/ mosaic} mutant, while littermates used as control mice in this study are referred as Sox2\textsuperscript{RGINV/+}. PCR genotyping was performed for both case study Sox2\textsuperscript{RGINV/mosaic} and its Sox2\textsuperscript{RGINV/+} littermates (Figure 1 (B)). Performing dissection of the CNS of the Sox2\textsuperscript{RGINV/mosaic} mouse, we observed substantially smaller size of the CNS, comparing with its Sox2\textsuperscript{RGINV/+} littermate. A substantial number of brain structures such as the olfactory bulb, the cerebellar cortex and the cerebellum were

![Figure 1](image_url)
Figure 2: Impaired motor skills in Sox2RGINV/mosaic mutant.

significantly reduced, especially the cerebellar vermis (Figure 1 (C)).
On the assumption that the role of the cerebellum is to memorize
information relating to neuromuscular actions we further studied the
pathoanatomical structure of the cerebellar vermis, by performing
tangential cryosectioning of the vermis in its midline (Figure 1 (C)), and
stained with Hematoxylin and Eosin (H&E). \cite{10.4172/2157-7633.1000433}

Discussion

The cerebellar cortex is divided in the granule cell layer (innermost
layer), the Purkinjje cell layer (middle layer) and the molecular layer
(granule cells' axons and Purkinjie cells' dendrites). Cerebellar granule
cells are small densely packed neurons, representing the highest
number of neurons in the cerebellum and account for more than half
of the neurons of the entire brain \cite{10.4172/2157-7633.1000433}. Sox2 gene has recently been
reported outside of established Neural Stem Cells (NSCs) niches, such
as the Purkinjje cell layer of the adult cerebellum \cite{10.4172/2157-7633.1000433}.

Neural development and neurogenesis is governed by the
temporal patterning and differentiation of early embryonic precursors
of neuroectoderm. Segmentation of the hindbrain that is initiated
around E8.5 of mouse embryonic development sets the boundaries
of cerebellar formation. The rhombic lip that appears later at E10.5,
plays a crucial role for the separation of GABAergic and glutamatergic
neuronal progenitors, both of which underlie the formation of Purkinje
and granule cells.

The present case study reveals for the first time that the prolonged
cerebellar tangential, followed by radial, migration of the referred
neuronal cell types, whose allocation continues even in the early
postnatal stage, depends on Sox2 \cite{10.4172/2157-7633.1000433}. These results suggest that Sox2
loss of function in radial glial cells can generate neurological defects
in cognitive behaviour, as a result of overproduction of immature/non
committed granule cell neuron progenitor cells in the adult cerebellum.

Interestingly, our pathoanatomical analysis experiments could
suggest that Sox2 is associated with the gene regulatory network that
is responsible for the correct spatiotemporal allocation of granule cell
precursors. This regulatory network includes a significant number of
kinases and neurotrophins and theirs receptors with the most important
to include Brain Derived Neurotrophic Factor (BDNF), Tyrosine
Receptor Kinase B (TrkB), Calcium Dependent Secretion Activator 2
(CAP52/CADPS2), neurotrophin 3, cAMP response element-binding
(creb) protein, Calcium/Calmodulin-dependent protein kinase
phosphatase 2 (CAP2), Calcium/Calmodulin Dependent Protein Kinase
2, C-X-C Motif Chemokine Ligand 12, semaphoring 6a, Plexin A2
genes as reviewed by \cite{10.4172/2157-7633.1000433}. Further molecular analysis on Sox2
embryonic cerebellum would reveal the genetic regulatory partnerships
that could potentially regulate the spatiotemporal tangential and radial
migration of granule cell neuron progenitor cells, a process that extends
from early embryogenesis till early adulthood.

Materials and Methods

Experimental animals

Mice were described elsewhere \cite{10.4172/2157-7633.1000433}. All animals were handled
in strict accordance with good animal practice as defined by the
Animals Act 160/03.05.1991 applicable in Greece, revised according
to the 86/609/EEC/24.11.1986 EU directive regarding the proper
care and use of laboratory animals and in accordance to the Hellenic

Genotyping

Tail, yolk sack or embryonic tissues were isolated and processed according to previously described methodology. PCR amplification conditions and primers used are described elsewhere [7,14,16].

Embryo processing and histological analysis

The mice were perfused intracardially with 4% PFA fixative in 0.1 M Phosphate Buffer (PB, pH 7.4) using a 27 gauge needle under anaesthesia (Avertin/Tribromoethanol injection at the peritoneal cavity). For histological analysis, embryos were fixed with 10% Formalin for 24 hours at room temperature and then washed several times with PBS, placed in embedding cassettes and sectioned in a Leica RM2125RT microtome. Paraffin sections (10 μm) were stained with Hematoxylin and Eosin and mounted with xylene based mounting medium, according to standard procedures [7].

References


