The International Journal of Biochemistry & Cell Biology 41 (2009) 435–445

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Cells in focus

Cerebellar granule cells: Insights into proliferation, differentiation, and role in medulloblastoma pathogenesis

Hourinaz Behesti, Silvia Marino*

Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, E1 2AT London, United Kingdom

ARTICLE INFO

Article history:
Received 20 February 2008
Received in revised form 30 May 2008
Accepted 9 June 2008
Available online 5 August 2008

Keywords:
Granule
Cell
Proliferation
Differentiation
Medulloblastoma

ABSTRACT

Cerebellar granule cells originate from precursors located in the dorsal region of rhombomere one within the hindbrain of developing embryos. They undergo proliferation for an extensive period well into postnatal stages of development to form the major cell type of the cerebellum, the most populous structure within the mammalian brain. Granule cell development is highly dependent upon the cerebellar environment and contact with neighbouring cells. In recent years, the molecular basis of these interactions has started to be unravelled. Granule cell precursors and the molecular mechanisms involved in controlling their proliferation have been shown to be involved in the pathogenesis of medulloblastoma, the most common malignant pediatric brain tumour. Here, we review the control of granule cell generation with emphasis on the molecular regulators of cell proliferation and differentiation during normal and malignant development.

1. Introduction

Granule cells are the main neuronal cell type of the cerebellum, a well defined anatomical structure of the brain regulating coordinated movements, muscle tone, and balance by integrating data from the brain stem, the spinal cord, and the cerebral cortex with sensory input from muscles and other areas. The cerebellum is located in the posterior cerebral fossa of the skull above the brainstem and contains a central region called the vermis flanked by the cerebellar hemispheres. The anatomical structure of the cerebellum is distinctive with multiple fissures dividing the cerebellar cortex into lobes and folia. The cerebellar cortex is divided into three layers, the molecular layer (ML), the Purkinje cell layer (PCL) and the internal granule layer (IGL), which contain stellate and basket neurons (the ML), Purkinje cells and Bergman glia (the PCL), and granule neurons, Golgi cells and astrocytes (the IGL) respectively. White matter, containing fibre tracts, as well as astrocytes, oligodendrocytes, and deep cerebellar nuclei complete the structure of the cerebellum (Altman and Bayer, 1997).

Cerebellar development relies on molecular mechanisms regulating the formation and establishment of neuronal circuits through proliferation, differentiation and migration of neural and glial cell progenitors. The formation of the cerebellum spans embryonic and postnatal development, initiating at 9 days post coitus (dpc) in the mouse. Granule neurons originate from a structure in the metencephalon, known as the rhombic lip, located at the cranial edge of the roof of the fourth ventricle. The isthmic organiser, a signalling centre at the mid-hindbrain junction, is crucial for regulating early cerebellar development and for specifying the distinct cerebellar cell types (recently reviewed in Sillitoe and Joyner, 2007). Granule cell progenitors (GCPs) proliferate and migrate from the rhombic lip along the outer surface of the cerebellar anlage to form the external granule layer (EGL), a transient secondary germinal zone. Here they undergo clonal expansion during early postnatal life (within the outer EGL) and after exiting the cell cycle, start their differentiation process (within the inner EGL). Upon migration along a grid of glial cell processes, the granule cells eventually settle in the IGL. All other cerebellar neurons (Purkinje neurons, Golgi neurons and molecular layer neurons) as well as glial cells originate at different time-points from the primary germinal zone, the ventricular zone neuroepithelium (Altman and Bayer, 1997). This review focuses on the molecular control of granule cell development with emphasis on the control of their proliferation and differentiation as well as their involvement in brain tumourigenesis.

Abbreviations: ML, molecular layer; PCL, Purkinje cell layer; IGL, internal granule layer; dpc, days post coitus; EGL, external granule layer; GCP, granule cell precursor; P, postnatal day; A-P, anterio-posterior; M-L, medio-lateral; CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; MB, medulloblastoma.

* Corresponding author. Tel.: +44 207 882 2201.
E-mail addresses: h.behesti@qmul.ac.uk (H. Behesti), s.marino@qmul.ac.uk (S. Marino).

1357-2725/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2008.06.017

2. Granule cell proliferation

GCPs proliferate during embryogenesis to form the EGL. However, the bulk of proliferation of the progenitor pool occurs postnatally, leading to growth of the EGL from one to eight cells in thickness (Hatten et al., 1997). The proliferative period peaks between postnatal days (P) 4 and 8, and is completed by P15 in the mouse. Pulse-labelling studies of GCPs with tritiated thymidine (^{3}H-thymidine) in rats have revealed regional differences in proliferation within the EGL. Analyses carried out just after the postnatal peak of GCP proliferation (P11–P16) have shown differences along the anterio-posterior (A-P) axis with the anterior-most and posterior-most lobes containing fewer labelled cells than the central lobes (Altman and Bayer, 1997). Along the medio-lateral (M-L) axis, the hemispheres show a higher percentage of ^{3}H-thymidine labelling in comparison to the vermis. As there is currently no evidence for a differential cycling rate during GCP proliferation in different regions of the cerebellum, areas with higher levels of ^{3}H-thymidine labelling are thought to develop later (Altman and Bayer, 1997).

At the molecular level, regional differences in gene expression have been identified along the A-P axis. In particular, activity of Sonic Hedgehog (Shh), a powerful mitogen for GCP proliferation, is highest in the anterior and posterior lobes up to P5 and equally distributed thereafter in the mouse (Corrales et al., 2004). Genetic studies have provided further evidence for the existence of regional differences in EGL proliferation. For example, targeted deletion of the retinoblastoma (*Rb*) tumour suppressor gene in the cerebellar vermis causes a more profound delay in cell cycle exit in the EGL of central lobes compared to the anterior-most and posterior-most lobes (Marino et al., 2003). Manipulations of Shh signalling have resulted in alterations in cerebellar foliation (Corrales et al., 2006). It would be interesting to investigate whether the underlying mechanism is a quantitative regional difference in the rate of proliferation, a mechanism recently proposed as the driving force for the morphogenesis of the developing heart (Cai et al., 2005).

3. The role of cell cycle genes in the developing rodent cerebellum and the regulation of GCP proliferation

Central to regulation of cell proliferation are the molecular regulators of the cell cycle, which in eukaryotic cells has been divided into two major phases with gaps in between according to the events of DNA replication and partition. These phases are controlled by proteins belonging to families of cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (CDKIs) (Nurse, 2000). At the heart of cell cycle regulation, is the phosphorylation status of the Rb protein, carried out by CDK4/Cyclin D or CDK2/Cyclin E complexes. When hyperphosphorylated, Rb dissociates from the E2F transcription factors, which promote entry into the DNA synthesis phase (S-phase) of the cell cycle by transcriptional regulation of necessary genes.

Rb is expressed in the developing and adult brain (Lee et al., 1987; Bernards et al., 1989; Jiang et al., 1997). Conditional inactivation of *Rb* driven by *Engrailed(En)2-Cre*, which bypasses the embryonic lethality of the null allele (Lee et al., 1992; Clarke et al., 1992; Jacks et al., 1992), has revealed an essential role for Rb in the proliferation to differentiation/migration switch in GCPs, and the complete differentiation and survival of granule cells. These mice display an abnormal persistence of the EGL at P20, with cells expressing markers of early differentiation but also proliferation. Granule cell loss by apoptosis is detected from P15 (Marino et al., 2003). Moreover, conditional deletion of *Rb* driven by *GFAP-Cre*, which has been shown to induce Cre-mediated recombination in GCPs, results in similar phenotypes as well as ectopic proliferation within the IGL and delayed complete differentiation of granule cells at P15. The ectopically proliferating IGL cells were reported to undergo apoptosis resulting in the absence of a dramatic phenotype by P30 (Shakhova et al., 2006). Targeted deletion of *p107*, another member of the Rb family, exasperates the phenotypes seen upon *Rb* inactivation, indicating a certain degree of cooperation and functional similarity between the family members (Marino et al., 2003). Loss of *p107* alone, or in combination with the third Rb family member *P130* does not result in cerebellar defects (Cobrinik et al., 1996; Lee et al., 1996).

There are two families of CDKIs in mammalian cells: the CIP/KIP (p21, p27, and p57) and the INK4 (p15, p16, p18, p19) families. These proteins act to prevent entry into S-phase by inhibiting CDK activity. At P7, p27 protein is expressed in the inner EGL and in the IGL as well as in cells in the molecular layer (van Lookeren Campagne and Gill, 1998; Miyazawa et al., 2000). Mice with a targeted deletion of *p27* display larger cerebella and delayed cell cycle exit within the EGL (Miyazawa et al., 2000). Although delayed, cell cycle exit and GCP differentiation do occur in these mice, and specifically in *P27*$^{-/-}$ cells as recently shown in mosaic mice (Muzumdar et al., 2007), suggesting the involvement of additional cell intrinsic regulators in this process. Targeted deletion of *p21* or *p57* however, does not cause cerebellar defects (Deng et al., 1995; Yan et al., 1997; Zhang et al., 1997), excluding a major role for the other CIP/KIP family members in GCP cell cycle exit.

The expression of members of the INK4 family in the nervous system is dynamic with only *p18* and *p19* being detected during embryogenesis. Expression of p19 protein is first detected at 14.5 dpc and unlike p18, continues into adulthood in the rat brain. The expression of *p15* and *p16* is first detected postnatally (Zindy et al., 1997; Shambaugh et al., 2000). No cerebellar phenotypes have been reported upon targeted mutagenesis of single or multiple members of the INK4 family (Serrano et al., 1996; Kamijo et al., 1997; Latres et al., 2000; Krimpenfort et al., 2007), confirming that the CIP/KIP family member p27 is the major regulator of cell cycle exit in GCPs during normal cerebellar development. However, this does not exclude a role for the INK4 family as back-up or enforcement in the absence of normal expression of the CIP/KIP family genes or other cell cycle regulators. In support of this view, it has been reported that *p18* is upregulated in the ventricular zone neuroepithelium during embryogenesis in mice with a conditional inactivation of the proto-oncogene *Nmyc*, driven by *Nestin-Cre* (Knoepfler et al., 2002).

Cyclin D1, which promotes progression through the G1 phase of the cell cycle, is detected in an anterior-high, posterior-low gradient in the EGL at 16.5 dpc and later by P6 uniformly in the outer EGL (Pogoriler et al., 2006). Mice homozygous for a targeted deletion of *Cyclin D1* display reduced cellularity of the EGL and IGL due to reduced cell proliferation as assayed by BrdU incorporation at P0 (Pogoriler et al., 2006). *Cyclin D2* is also expressed in the outer EGL at P6 and unlike *Cyclin D1*, continues to be expressed at P12. Notably, no expression of Cyclin D2 is seen in the early EGL during embryogenesis (Ross et al., 1996; Huard et al., 1999; Pogoriler et al., 2006). In agreement with the temporal difference in expression pattern, mice homozygous for a targeted deletion of *Cyclin D2* display cerebellar hypoplasia only postnatally; a reduction in the size of the EGL due to reduced proliferation and increased cell death is first apparent at P3 (Huard et al., 1999). These data support the idea that proliferation of GCPs is differentially regulated during embryonic and postnatal development with Cyclin D1 being the main embryonic cyclin regulator of GCP proliferation with Cyclin D2 contributing postnatally (Fig. 1). Cyclin G1 has also been reported to be expressed in the EGL as well as in the IGL as soon as it forms (van Lookeren Campagne