Math1 is essential for genesis of cerebellar granule neurons

Nissim Ben-Arie‡*, Hugo J. Bellen†‡, Dawna L. Armstrong§, Alanna E. McCall¶, Polina R. Gordadze†, Qixia Guo∥, Martin M. Matzuk‡§ and Huda Y. Zoghbi†‡

Departments of *Molecular and Human Genetics, ‡Pediatrics, §Cell Biology, ¶Pathology, and †Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030, USA

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The cerebellum is essential for fine motor control of movement and posture, and its dysfunction disrupts balance and impairs control of speech, limb and eye movements. The developing cerebellum consists mainly of three types of neuronal cells: granule cells in the external germinall layer, Purkinje cells, and neurons of the deep nuclei. The molecular mechanisms that underlie the specific determination and the differentiation of each of these neuronal subtypes are unknown. Math1 (refs 2, 3), the mouse homologue of the Drosophila gene atonal4, encodes a basic helix-loop-helix transcription factor that is specifically expressed in the precursors of the external germinall layer and derivatives of these cells. Here we report that mice lacking Math1 fail to form granule cells and are born with a cerebellum that is devoid of an external germinall layer. To our knowledge, Math1 is the first gene to be shown to be required in vivo for the genesis of granule cells, and hence the predominant neuronal population in the cerebellum.

The mammalian cerebellum consists of deep centrally located neurons, referred to as the deep nuclei, and a peripheral cortex. The cortex contains two principal neuronal subtypes, the Purkinje cells and the cells of the internal granular layer (IGL). The IGL neurons, which eventually constitute most of the neurons in the cerebellum, are derived from peripherally located cells in the external germinal layer ( EGL) which migrate postnatally along radial glia5,6. The mouse cerebellar anlage is specified at embryonic-day 9 (E9) just anterior to the area where closure of the neural tube is incomplete. The neuroepithelium of the ventricular zone in the metencephalon gives rise to the precursors of the neurons of the deep nuclei and Purkinje cells. The precursors of the EGL are derived at E13–E15 from a structure named the rhombic lip (also known as the germinal trigone), which is located at the posterior edge of the cerebellar anlage (Fig. 1). The proliferating cells of the rhombic lip, which are committed to become EGL neurons, disperse rostrally over the surface of the cerebellar anlage, where they establish the EGL. Proliferation of these granule cell precursors continues in the EGL until postnatal day 15 (P15). The EGL cells migrate inwardly to give rise to the IGL, starting at birth until day P20, when maturation of the cerebellum is complete.1,3,7

The Drosophila atonal gene is essential for the generation of the chordotonal organs, the proprioceptors, and the R8 photoreceptor precursors in the eye. Adult flies that lack atonal function do not have photoreceptors, are uncoordinated and tend not to fly8. Expression of Math1, the mouse atonal homologue, is highly restricted to a few neurons in the hindbrain and dorsal neural tube1,3. Math1 is expressed in the precursors of the EGL in the rhombic lip, and it is restricted to a few neurons in the hindbrain and dorsal neural tube1,3. Math1 expression ceases during the inward migration phase of the granule cells to the IGL. To investigate the role of Math1 in cerebellar development, a targeted deletion of the Math1 gene (Math1 KO) was generated by using embryonic stem (ES) cell technology (Fig. 2a). Heterozygous mice were intercrossed to obtain mice homozygous for the Math1 deletion (Fig. 2b). Absence of a hybridization signal with DNA from homozygous mutant mice, when probed with an internal probe spanning the basic-helix–
loop–helix (bHLH) domain, confirmed that the mutation is a null allele (data not shown).

Mice heterozygous for the Math1 deletion are viable, fertile, and appear normal. Genotyping of newborn mice from heterozygous intercrosses demonstrated that the targeted allele did not result in embryonic lethality. The observed ratio of genotypes fitted that expected for a fully penetrant, mendelian recessive inheritance pattern, with 28 (25.2%) wild types, 55 heterozygote (49.5%) and 28 (25.2%) homozygote (null) mice. Math1-null mice are born alive, but fail to breathe, become cyanotic, and die a few minutes after birth. We observed no morphological abnormalities in the lungs (data not shown). Furthermore, the lack of expression of Math1 in the lungs, combined with the expression pattern in the brainstem1, suggest a central mechanism for this respiratory failure.

The structure and histology of the cerebellum of Math1-null mice is abnormal compared to their littermates. The most peripheral cell layer in the cerebellum, the EGL, is normally about eight cells thick at E18.5. These rapidly proliferating cells are the granule cell precursors and express Math1 abundantly2. Math1-null mice completely lack the EGL, as shown by haematoxylin-and-eosin staining (Fig. 3a, b), and cresyl violet staining (data not shown), and have a cerebellum that is reduced in size. To analyse the changes in the cytoarchitecture of the cerebellum, we stained the two principal neuronal populations in the cerebellum, the Purkinje cells (Fig. 3c, d) and the granule cells (Fig. 3e, f). RNA in situ hybridization using RU49, a zinc-finger transcription factor that labels the EGL, the migrating granule cells and the IGL, demonstrates the absence of the EGL and migrating granule cells in the cerebella of Math1-null mice (Fig. 3c, f). In contrast to the lack of granule neurons, the Purkinje cells and the neurons of the deep nuclei are still present in mutant mice, as demonstrated by staining with anti-calbindin antibody (Fig. 3c, d) and Nissl staining (data not shown). However, because of the absence of the EGL, the Purkinje cells, which are normally located beneath the EGL, are now localized at the periphery of the cerebellum and do not form a distinct and organized layer. In addition, a significant subpopulation of Purkinje cells fails to migrate from the central area of the cerebellum into the periphery (Fig. 3c, d). Staining with glial fibrillary acidic protein (GFAP) and nestin11, which both label glial cells, demonstrates that there is not excessive gliosis, and that the radial glia are localized properly (data not shown). These results show that Math1 is essential for the formation of the EGL but not the Purkinje cells, the neurons of the deep nuclei, or the glial cells. In Math1-null mice, the absence of the EGL also leads to the lack of foliation of the cerebellum typically observed in normal embryos starting from E18 (Fig. 3a, b).

Possible causes for the missing EGL in mutant mice include failure in fate determination of granule precursors at the rhombic lip, aberrant migration of precursors to form the EGL, or degeneration of the neurons already localized at the EGL. The granule neuron precursors originate at E13–E15 in the proliferation zone of the rhombic lip1,7. At E14, the EGL precursors initiate migration from the rhombic lip over the surface of the cerebellar primordia. At this stage, Math1 is expressed in the granule precursor cells localized at the rhombic lip and in the emerging EGL2. As shown in Fig. 4a–d, Math1-deficient mice contain fewer cells in the rhombic lip than do control embryos. In addition, there is no migration of cells out of the rhombic lip to form the EGL. Bromodeoxyuridine (BrdU) labelling demonstrates the absence of proliferating cells in both sites that normally expand the pool of granule cells: the rhombic lip and the cells that have already migrated to the EGL (Fig. 4e, f). Hence, the lack of the cerebellar EGL in Math1-null mice is evident early on and is caused either by the absence of the granule cell precursors or by the inability of these cells to proliferate at the rhombic lip. Thus, the agenesis of granule cell precursors in Math1-null mice is a much earlier event than the postnatal apoptotic degeneration of the EGL seen in weaver mice12,13.
It has been suggested that inferior olive neurons are derived from the rhombic lip, which consists of an upper and a lower component (Fig. 1) and that it is the lower lip that gives rise to the neurons of the inferior olive. Math1 is only expressed in the germinial trigone that is part of the upper lip and not the lower lip (Fig. 1). The inferior olive neurons are present in Math1-null mice and appear normal (Fig. 5), consistent with Math1 expression in the upper rhombic lip. This finding supports the histological and functional division of the rhombic lip into an upper and a lower lip, each contributing to the generation of different types of neurons that migrate to separate destinations.

As Math1-null mice die shortly after birth from respiratory failure and Math1 is expressed in the hindbrain, we wanted to establish whether there is selective loss of neurons implicated in the control of respiration, including those of the nucleus tractus solitarius, nucleus ambiguus, dorsal vagus nucleus and trigeminal ganglion. These brainstem nuclei were identified and analyzed for neuronal density and morphology. No differences between Math1-null mice and littermate controls were noted (Fig. 5). The lack of morphological defects suggests that Math1 has different roles in the brainstem and cerebellum, or that Math1 is required in other unidentified neuronal cells involved in respiratory control.

We have shown that the Math1 gene is required for differentiation of the EGL cells and their derivatives. Although ~30 mutations in mice cause cerebellar defects and ataxia, none of these mutations causes a phenotype like that of Math1-null mice. For example, the developmental mutant roeler causes a defect in cell migration in the cerebellum, cerebral cortex and hippocampus, rather than a defect in cell specification or proliferation. In the cerebellum of roeler mutant mice, proliferation of EGL precursor cells is normal, but failure of granule cell migration from the EGL to the IGL causes degeneration of this neuronal population.

The cerebellum is derived from the posterior end of the mesencephalon and from the metencephalon. The expression of Wnt-1 at the mesencephalon–metencephalon junction stabilizes the expression of the engrailed homologues En-1 and En-2, thereby specifying the cerebellar anlage. Absence of Wnt-1 or of En-1 and En-2 causes a loss of cerebellar components, including the deep nuclei, Purkinje cells and IGL. The absence of Math1, the cerebellum develops but there is selective loss of only the EGL precursors and granule neurons. Other defects, like the ectopic localization of the Purkinje cells and the lack of foliation are most likely to be secondary to the absence of EGL. Mice lacking Mash1, the murine homologue of the Drosophila proneural gene complex achaete-scute, suffer from loss of olfactory neuronal progenitors and are arrested in the differentiation of sympathetic neuronal precursors. Therefore, Mash1 and Math1 are both essential for the generation of neurons through progression of a differentiation programme, possibly in selected progenitors. On the basis of our results, we propose that Math1 plays a role in cerebellar granule fate specification or proliferation. This role is functionally similar, although not identical, to the role of its homologue, the Drosophila atonal gene. In both mice and fruitflies, loss of function leads to the absence of specific neuronal cell types early in neurogenesis. However, Math1 is expressed in cells that are already committed to be neural tissue, whereas atonal plays a role in the decision of epidermal rather than neuronal cell fate.

Methods

Targeted deletion of Math1. The genomic locus was targeted by homologous recombination using two isogenic flanking fragments of 3.7 kb (EcoRI–EcoRV) and 3.1 kb (XbaI–ApaI), which were isolated from a mouse 129 SvEv genomic DNA library (Stratagene). A human hypoxanthine–guanine phosphoribosyltransferase (hprt) and the herpes simplex virus thymidine kinase gene were used as positive and negative selection markers, respectively. The linearized targeting construct was electroporated into hprt-negative AB2.1 embryonic stem (ES) cells and selection was achieved using HAT (hypoxanthine,
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Correspondence and requests for materials should be addressed to H.Y.Z. (e-mail: hzoghbi@bcm.tmc.edu).

Impaired mast cell-dependent natural immunity in complement C3-deficient mice

Andrey P. Prodeus, Xiaoning Zhou, Marcus Maurer, Stephen J. Galli* & Michael C. Carroll†

* Departments of Pathology, Harvard Medical School and † Beth Israel Deaconess Medical Center, Boston, Massachusetts, 02115, USA

The complement system is widely regarded as essential for normal inflammation, not least because of its ability to activate mast cells—however, recent studies have called into question the importance of complement in several examples of mast cell-dependent inflammatory responses. To investigate the role of complement in mast cell-dependent natural immunity, we examined the responses of complement-deficient mice to caecal ligation and puncture, a model of acute septic peritonitis that is dependent on mast cells and tumour necrosis factor-α (TNF-α). We found that C4- and C3-deficient mice were much more sensitive to caecal ligation and puncture than wild-type (WT) controls (100% versus 20% in 24-h mortality, respectively). C3-deficient mice also exhibited reductions in peritoneal mast cell degranulation, production of TNF-α, neutrophil infiltration and clearance of bacteria. Treating the C3-deficient mice with purified C3 protein enhanced activation of peritoneal mast cells, TNF-α production, neutrophil recruitment, opsonophagocytosis of bacteria and resistance to caecal ligation and puncture, confirming that the defects were complement-dependent. These results provide formal evidence that complement activation is essential for mast cell-dependent natural immunity in complement C3-deficient mice.


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