

NAME: ODIWAMKPA UCHENNA
MATRIC NUMBER: 17/ MHS01/228
COURSE: NEUROANATOMY
DEPARTMENT: MEDICINE AND SURGERY

QUESTION

- 1) Write a concise review on the developmental genetics of cerebellum and highlight the genetic bases of known cerebellar disorders

ABSTRACT

The study of the links and interactions between development and motor learning has noticeable implications for the understanding and management of neurodevelopmental disorders. This is particularly relevant for the cerebellum which is critical for sensorimotor learning. Advances in genetics have led to major improvements in our appraisal of the genes involved in cerebellar development, especially studies in mutant mice. Cerebellar neurogenesis is compartmentalized in relationship with neurotransmitter fate. The Engrailed-2 gene is a major actor of the specification of cerebellar cell types and late embryonic morphogenesis. Math1, expressed by the rhombic lip, is required for the genesis of glutamatergic neurons. Mutants deficient for the transcription factor Ptf1a display a lack of Purkinje cells and GABAergic interneurons. Rora gene contributes to the developmental signaling between granule cells and Purkinje neurons. The expression profile of sonic hedgehog in postnatal stages determines the final size/shape of the cerebellum. Genes affecting the development impact upon the physiological properties of the cerebellar circuits. For instance, receptors are developmentally regulated and their action interferes directly with developmental processes. Another field of research which is expanding relates to very preterm neonates. They are at risk for cerebellar lesions, which may themselves impair the developmental events. Very preterm neonates often show sensori-motor deficits, highlighting another major link between impaired developments and learning deficiencies. Pathways playing a critical role in cerebellar development are likely to become therapeutic targets for several neurodevelopmental disorders.

INTRODUCTION

Cerebellum plays critical roles in learning sensorimotor tasks (Manto, 2010). For instance, it is widely accepted that the olivocerebellar tract is one of the key pathways contributing to learning of new motor skills (Ito, 2006). Although the involvement of cerebellar circuits in motor learning is known to be critical, their precise role in the acquisition and storage of

new motor abilities or rather in the performance of the acquired motor skills is still a matter of debate. Several authors have pointed that attention should be paid to functional states (as opposed to neural sites) able to generate motor learning in mammals (Delgado-García and Gruart, 2002). Permanent or temporary disconnection of a given nodal center in a neural circuit will not determine the whole functional state of the involved circuit and the transformation of neural signals occurring at the different neural centers included in the circuit (Harvey et al., 1993; Delgado-García and Gruart, 2002).

There is currently a growing awareness that neurodevelopmental disorders are associated with cerebellar deficits and learning impairments. Still, the molecular mechanisms of the cerebellar defects remain poorly understood in many cases. Cerebellum is likely to become a major platform to investigate how development and learning interact in mammals. Indeed, cerebellar circuits are modular and stereotyped from the morphological standpoint and provide thus a structure of choice to investigate the relationships between regional developmental defects and learning, especially from the anatomical/functional point of view. Moreover, marked morphological changes still occur after birth, allowing the detailed assessment of developmental abnormalities with various techniques and their phenotypical impact after the pregnancy. In addition, developmental studies have revealed that the cerebellum evolves in successive waves of progenitors proliferation/migration throughout the embryonic and postnatal phases. The possibility to act selectively on these waves opens new therapeutic doors.

This review covers recent advances in the understanding of the gene networks contributing to cerebellar development. Potentially clinically relevant discoveries are highlighted.

THE KEY FEATURES OF CEREBELLUM DEVELOPMENT

Because the circuits of the cerebellum are unique in their morphology, the mechanisms of cerebellar neurogenesis are a subject of intense investigation (Carletti and Rossi, 2008). Neuronal/glial migrations as well as dendritogenesis are fundamental processes leading to functional cerebellar microcircuits being effective for plasticity and learning.

Interestingly, the anatomy of the cerebellum with a midline vermis and two hemispheres located laterally is highly conserved from rodents to human, suggesting that the analysis of the development in rodents should provide direct relevant informations in human, including for cerebellar malformations.

The major features of cerebellar development can be briefly summarized as follows. Neuronal populations are generated in a sequential manner. The inhibitory interneurons emerge from the ventricular zone and the

glutamatergic neurons are generated by the rhombic lip (Carletti and Rossi, 2008). In mouse, the glutamatergic and gabaergic neurons in nuclei are produced first, followed by Purkinje neurons. It is established that gabaergic interneurons of the cerebellar cortex originate from a ventricular zone progenitor (Leto and Rossi, 2011). After generation of cerebellar nuclei, the external granular layer is formed from precursors of granule cells originating from the rhombic lip. Granule cells will migrate to form the internal granular layer. It is interesting to note that these events occur at the third trimester of development in human (see also below the impact of very premature birth upon cerebellar development). Survival and maintenance of Purkinje neurons and granule cells is dependent on the antiapoptotic protein Lifeguard, which is highly expressed in the cerebellum and is strongly upregulated during postnatal brain development (Hurtado de Mendoza et al., 2011). Lifeguard antagonizes the FAS pathway. FAS receptors tune neuronal survival following trophic factors deprivation (Raoul et al., 2000). Lifeguard affects cerebellar size, internal granular layer thickness, and Purkinje cell development, suggesting that lifeguard could participate in the pathogenesis of various human cerebellar disorders characterized by cerebellar atrophy. Glutamatergic unipolar brush cells migrate to the internal granular layer. Whereas the ventricular zone will lose its progenitors at late embryogenic stages, the rhombic lip remains active until postnatal period.

CEREBELLUM, HORMONES AND NEUROSTERIODGENESIS

The relationship between circulating hormones and cerebellar development is well demonstrated.

THYROID HORMONE

Thyroid hormone plays a critical role in brain development (Koibuchi, 2008). The thyroid hormone receptor is a ligand-regulated transcription factor binding to a specific DNA sequence called thyroid-hormone-responsive element. The receptor recruits various coregulators such as coactivator and corepressor in a ligand-dependent manner to modulate the transcription of target genes (Koibuchi, 2008). It may also interact with other nuclear receptors such as Rora (retinoic-acid-related orphan receptor alpha; see below) whose expression is regulated by the thyroid hormone during the first postnatal two weeks.

THE ENGRAILED-2 GENE

The engrailed (En) homeobox transcription factor family is critical for the patterning of cerebellar lobules and for Purkinje cells protein stripes (Kuemerle et al., 1997). The En1/2 regulates the targeting of mossy fiber systems to subsets of cerebellar lobules, showing a main role for the afferent topography in the cerebellar circuitry (Sillitoe et al., 2010). Initially, the En1/2 mRNA/protein are expressed in the ventricular zone. During early post-natal cerebellogenesis, En1/2 are expressed in spatially restricted patterns in most cell types. It is plausible that En1/2 are implicated in neurodevelopmental disorders such as autism spectrum disorder (see also below). Indeed, mutant mice EN2-/- show neurobehavioral and neurochemical deficits suggestive of autism spectrum disorder (Cheh et al., 2006).

MATH1

The specification and differentiation of glutamatergic lineages is dependent upon Math1, a transcription factor of the bHLH class. Math1 is critical for the proper development of the granular layer of the cerebellum. Mice deficient in Math1 show a loss of glutamatergic neurons in cerebellar nuclei, a loss of external granular layer and unipolar brush cells. In addition, Math1 null embryos lack interneurons giving rise to the spinocerebellar and cuneocerebellar tracts (Bermingham et al., 2001).

PTF1A and ASCL1

Cerebellar mutants have a deficit in the transcription factor Ptf1a (pancreatic transcription factor 1a). They show a lack of Purkinje cells and gabaergic interneurons. It has been demonstrated that climbing fiber neurons are derived from the Ptf1a domain (Yamada et al., 2007). In Ptf1a null mutants, immature climbing fiber neurons cannot migrate or differentiate, causing a failure in the formation of the inferior olivary nucleus. Ptf1a is also involved in the control of fate and survival of neurons during development. In human, mutations of Ptf1a are associated with cerebellar agenesis (Sellick et al., 2004).

Ascl1 directs ventricular neuroepithelium progenitors toward inhibitory interneuron fate and suppresses the astrocytic differentiation (Grimaldi et al., 2009). Mice lacking Ascl1 in the cerebellum exhibit a major decrease of cerebellar interneurons and an imbalance between oligodendrocytes and astrocytes (Sudarov et al., 2011).

RORA(RETINIOIC-ACID-RELATED-ORPHAN-RECEPTOR ALPHA) GENE

Rora is a transcription factor encoding a retinoid-like nuclear receptor which is highly expressed in the cerebellum (Hamilton et al., 1996). Rora belongs to the steroid-thyroid hormone receptor superfamily (Koibuchi, 2008). Its endogenous ligand is cholesterol which is abundantly present in each cell. Therefore, Rora acts as if it is a constitutively active nuclear receptor (Koibuchi, 2008). It was initially thought that Rora was exclusively expressed in neurons, but recent data show that it is also expressed in glial cells especially in astrocytes (Journiac et al., 2009). Rora plays a pivotal role in the development of the cerebellum, olfactory bulb, and retina (Jetten, 2009). However, its functions extend beyond development. For instance, Rora also protects neurons against oxidative stress and shows an anti-inflammatory action by inhibiting the NF-Kappa-B pathway (Delerive et al., 2001; Boukhtouche et al., 2006). The autosomal recessive staggerer mutation is associated with a severe degeneration of Purkinje neurons with a nearly total absence of granule cells at the end of the first postnatal month (Landis and Sidman, 1978). The homozygous mouse *Rorasg/Rarasg* is highly ataxic, whereas the heterozygous mouse *Rora+/Rarasg* appears phenotypically normal, showing disabilities during challenging tasks.

REELIN AND CEREBELLAR DEVELOPMENT

The external granular layer promotes Purkinje cell migration by secreting reelin (RELN), an extracellular matrix component attracting or repelling precursors and axons during development, acting as an extracellular signaling molecule. Reelin deficient mice (Reeler) show a severe cerebellar hypoplasia. They exhibit Purkinje cell migration defects and cerebellar nuclei are impaired. Foliation is absent. Reelin continues exerting activities beyond birth. It modulates long-term potentiation and is thus involved in learning (Beffert et al., 2004). In the adult brain, Reelin regulates structural and functional properties of synapses. Its overexpression may increase markedly the long-term potentiation responses and it has been proposed that Reelin controls developmental processes remaining active in the adult brain (Pujadas et al., 2010). In human, reelin might be implicated in some forms of lissencephaly (due to neuronal migration defect) and could contribute to the pathogenesis of autism.

THE CHEMOKINE RECEPTOR 4(CXCR4)-CHEMOKINE LIGAND12 (CXCL12) SYSTEM

Chemokines and their receptors are determinant in cell migration and in organogenesis (Zou et al., 1998). *CRXC4* and *CXL12* mutant mice show proliferating granule cell progenitors located in deeper location (Zou et

al., 1998). Down-regulation of CXCR4 causes an inward radial migration of granule cells precursors. The chemoattractant SDF-1 α and its receptor CXCR4 attract the cerebellar granular neuronal precursors to the outer external granular layer and promote an increase of the sonic hedgehog mitogenic effect.

SONIC HEDGEHOG AND CEREBELLAR DEVELOPMENT

Sonic hedgehog is highly expressed in the cerebellum (Vaillant and Monard, 2009). Sonic hedgehog is a morphogenetic factor which is a masterplayer in cerebellar patterning and foliation (Vaillant and Monard, 2009). Indeed, sonic hedgehog controls the proliferation of progenitors in the cerebellum (Figure 3). Sonic hedgehog pathway involves the GLI family of transcription factors. The binding of sonic hedgehog to the transmembrane receptor Patched 1 triggers a cascade of events tuning cAMP production (DeCamp et al., 2000). A link between cholesterol metabolism, sonic hedgehog and cerebellar development has been established. Indeed, cholesterol deficiencies are associated with defects in the sonic hedgehog signaling (cholesterol is an activator of sonic hedgehog) and cause cerebellar malformations (Lanoue et al., 1997). Sonic hedgehog is implicated in the formation of medulloblastoma, an aggressive tumor of the cerebellum. Mutations of the PTC receptor lead to an overactivation of sonic hedgehog (Vaillant and Monard, 2009). The overexpression of sonic hedgehog in neural progenitors of the cerebellum causes a medulloblastoma (Weiner et al., 2002). GLI1 expression is markedly increased in a subgroup of patients developing a medulloblastoma (Ferretti et al., 2008). Development of sonic hedgehog antagonists might be considered to manage this tumor.

THE PURKINJE CELL DEGENERATION MODEL

Purkinje cell degeneration (pcd), an autosomal recessive mutation in the mouse, causes the postnatal death of nearly all cerebellar Purkinje cells during the third and fourth postnatal week (Landis and Mullen, 1978; Sotelo and Alvarado-Mallart, 1986). This strain has undergone an extensive investigation (Wang and Morgan, 2007). Several independent phenotypic alleles have been identified with mutations in the *Nna1* gene. The model is characterized by a moderate ataxia developing between three and four weeks of age. The degeneration of Purkinje neurons begins around 18 days and progresses quickly over two weeks. At about four months, most of the Purkinje neurons have degenerated. Before Purkinje cells start degenerating, they appear to receive all their synaptic contacts (Landis and Mullen, 1978). The mice show impaired eyeblink conditioning and abnormal spatial navigation learning.

MUTATIONS OF HERC GENE

Proteins with HECT domains act as ubiquitin ligases. Recently, it has been shown that mutations in the highly conserved N-terminal RCC1-like domain of the HERC1 protein cause a progressive Purkinje cell loss leading to severe ataxia with reduced growth and lifespan in homozygous mice aged over two months (tambaleante mutant mice; Mashimo et al., 2009). Activities of the proteins encoded by the HERC gene family are critical in a number of important cellular processes such as cell cycle, cell signaling, and membrane trafficking. It is now established that they play a key contribution in the physiology of Purkinje neurons.

NEURODEVELOPMENTAL DISORDERS

Thanks to novel perinatal neuroimaging techniques, cerebellar malformations are increasingly recognized in the fetal period (Bolduc et al., 2011).

JOUBERT SYNDROME

The disorder presents with developmental delay, hypotonia, impaired respiration, abnormal eye movements, and ataxia (Joubert et al., 1969). Motor learning is strongly impaired. The “Molar tooth sign” (deep interpeduncular fossa, enlarged superior cerebellar peduncles which are more horizontally oriented and hypoplastic cerebellar vermis) is very suggestive. Joubert syndrome is associated with mutations of genes encoding components of the primary cilia. Interestingly, primary cilia are determinant for sonic hedgehog signal transduction (Vaillant and Monard, 2009). Disruption of primary cilia formation blocks the proliferation of neural progenitors of granule cells mediated by sonic hedgehog (Spassky et al., 2008).

RHOMBENCEPHALOSYNAPSIS

Another disorder clearly associated with learning disabilities is a malformation of the hindbrain characterized by fusion of the cerebellar hemispheres and dentate nuclei. It is assumed that the disorder is due to a failure of dorsal patterning at the midbrain-hindbrain boundary (Pasquier et al., 2009). Other cerebellar malformations which are encountered in daily practice include Dandy–Walker malformation, vermis hypoplasia, mega cisterna magna, and posterior fossa retrocerebellar cyst. Sonic hedgehog might also be involved in the pathogenesis of Dandy–Walker malformation through a contribution of

Zinc finger transcription factors which modulate the sonic hedgehog pathway (Aruga, 2004).

AUTISM SPECTRUM DISORDERS

This is characterized by difficulties in communication, social skills, and repetitive behavior. Cerebellar networks might be critically involved in the pathogenesis of autism. An immune dysfunction with local inflammation contributes to the pathogenesis of autism (Wei et al., 2011). The expression of IL-6 is increased in the cerebellum of autistic patients. IL-6 impacts upon the development of the cerebellum, impairing neural cell adhesion, migration, and causing an excessive formation of excitatory synapses (Wei et al., 2011).

Recent studies underline the a high prevalence of neurologic, developmental, and functional disabilities including motor, cognitive, language, and social-behavioral deficits in children with cerebellar malformations (Bolduc et al., 2011). The associated supratentorial anomalies, chromosomal findings, and malformations affecting the cerebellar vermis are independent predictors of neurodevelopmental disabilities. Furthermore, the associated supratentorial anomalies and chromosomal findings are predictive of cognitive impairment, gross and fine motor delay. Moreover, malformations of the vermis are predictors of gross motor delays (Bolduc et al., 2011). There is a clear need for early intervention services aiming to improve the daily care of these patients, especially when gross motor impairments and learning deficits are on the forefront of the syndrome.

THE EXAMPLE OF A COMMON DEVELOPMENTAL DISORDER: DEVELOPMENTAL DYSLEXIA.

Developmental dyslexia can be defined as “a disorder in children who, despite conventional classroom experience, fail to attain the language skills of reading, writing, and spelling commensurate with their intellectual abilities” (according to the World Federation of Neurology). Its prevalence is very high, from 3% to 10% of learning disabilities (Bishop and Snowling, 2004). Skills in children are very heterogeneous. There is evidence that dyslexia is associated with mild clumsiness and deficit in fine motor skills (Nicolson and Fawcett, 2011). Dyslexia is associated with difficulties in making skills automatic (Nicolson and Fawcett, 1990). Most patients exhibit balance difficulties. The hypothesis of a cerebellar dysfunction is straightforward given the major role played by the cerebellum in learning, automaticity of skills and its contribution to language (Nicolson et al., 1995). An ontogenetic framework has been proposed to explain how cerebellar differences at birth cause a range of

difficulties in children (Nicolson and Fawcett, 2011). The language-related regions of the cerebellum, namely lobules VI and VIIIB, would be affected in dyslexia. Procedural learning circuits involving the cerebellum would be primarily affected. An abnormal development of the brain, including the cerebellum, during the gestation has been proposed. Genetic investigations will very likely lead to the discovery of the mechanisms leading to some of these learning disabilities.

CONCLUSION

In this mini-review, we have summarized recent advances in our understanding of the molecular mechanism governing cerebellar development. We have discussed the complex interactions between cerebellar development and motor learning. The identification of several pathways which are potential targets for novel therapies in the future, such as cerebellar neurosteroidogenesis, *En1/2*, *Math1*, *Ptf1a*, *Rora*, or sonic hedgehog, is now bringing hope in a field which has often remained neglected because of a lack of understanding of the molecular events leading to the malformations. There is still a growing need to identify new targets, since neurodevelopmental disorders are heterogeneous and will impact upon the whole life of patients in most cases. Protecting the developing cerebellum—a concept which could be called cerebelloprotection—is now attracting the interest of the scientific community, especially with discoveries of the roles of the cerebellum in cognitive skills. Very preterm neonates are an example of a population of patients at risk and which could benefit from neuroprotecting actions.

REFERENCES

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