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Review

TRANSCRIPTION FACTORS IN CEREBELLAR DEVELOPMENT

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ABSTRACT

The cerebellar germ arises from the rhombic lip, and it's a rostral part from the mesencephalon. The following cellular processes take place in the developing cerebellum: proliferation, migration, differentiation, synapse formation and cell death. An important step is the transformation of the Purkinje cell layer from a multilayer composition into a monolayer. This structural reorganization is followed by the foliation process. At first, the smooth surface is divided into five major lobes by four grooves in the vermis. In the next phase major lobes are subdivided into sublobes and lobules and they grow in size. The development of granular neurons in the cerebellum takes place in two phases. The first is in the rhombic lip and the second is in the external granular layer (EGL). The development is directed by some transcription factors such as sonic hedgehog (Shh), Zbtb, and Pax6.

Key words: foliation, neurogenesis, granule cells, Purkinje cells, rhombic lip

INTRODUCTION

The cerebellum has three main functions: maintenance of the balance and muscle tone, and coordination of the voluntary body movements. Two lateral parts, named hemispheres could be distinguished on the cerebellar surface. In the middle (between the hemispheres) is situated the phylogenetically oldest part, named vermis. The cerebellar surface is grooved by deep fissures, which divide the cerebellar folia. Some of the fissures are deeper than the others and separate lobes. We aimed at making a literature review on cerebellar development in mice, and the role of different factors, which orchestrate it.

Embryonal development

Phylogenetically and functionally the cerebellum is divided into three parts: ancient – archicerebellum, old – paleocerebellum and new – neocerebellum. The archicerebellum is represented by the vermis. Vermis is functionally related to the vestibular system and plays a role in equilibrium maintenance. The medial parts of both hemispheres form paleocerebellum. It receives sensory fibers from the muscle spindles and Golgi tendon organ through the spinocerebellar tract, and takes part in the regulation of the muscle tone. The lateral parts of the hemispheres are neocerebellum. It receives signals from the neocortex via corticopontocerebellar tract and coordinates the voluntary movements. Cerebellum is composed of the cortex and centrally located white matter. Among the white matter are situated four pairs of cerebellar nuclei: nucleus fastigii, globosus, emboliformis, and dentatus. Each of them has a reciprocal connection with specific areas of the cerebellar cortex (1).

The central nervous system (CNS) arises from the neuroepithelium of the neural tube. Its germ is formed by the embryonic ectoderm in the third week of development. During the closure of the neural tube is formed the neural crest. It divides longitudinally, forming left and right ganglion stripes. At the 20th day in the rostral part are formed some vesicles: prosencephalon (the germ of the forebrain), mesencephalon (the germ of the midbrain) and rhombencephalon (the germ of the rhombic brain). More caudally, in the posterior part of the neural groove is formed the germ of the spinal cord. The rhombic vesicle divides into two parts: rostral – hindbrain (metencephalon) and caudal –

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myelencephalon. In this way, the metencephalon composes the pons, which is situated ventrally. Dorsally from it lies the cerebellum. The wall of the neural tube is composed of several rows of high prismatic neuroepithelial cells, divided into four zones: ventricular, subventricular, intermediate and marginal. These are transitional structures in the development of CNS.

In the developing cerebellum, as well as in the rest of the CNS, the following cellular processes take place: proliferation, migration, differentiation, synapse formation and cell death. The proliferation of neuroepithelial cells leads to the formation of neuroblasts. Initially, this process takes place in the ventricular zone, and later in the subventricular zone. After the new cells are generated they migrate to the place of their definite position and differentiate. The cerebellar germ arises from the rhombic lips, and according to new studies, its rostral part is formed by the caudal part of the midbrain vesicle (2). These two specialized germinative zones give the origin of the neurons and glia. From the rhombic lip originate granular neurons (GCs), deep cerebellar neurons and unipolar brush cells, which are glutamatergic neurons. A distinctive feature in the development is the anterior-posterior lobulation. Another moment is the formation of cerebellar fissures and folia. In this process could be distinguished into two main stages: first, the formation of the caudal lobes; and second - the formation of lobes and lobules, which also continues postnatally (3). The cerebellar development in mice could be divided into four stages: formation of the cerebellar ventricular zone between the seventh and tenth embryonic day (E7-E10); formation of Purkinje cells in the ventricular area and their specification (E10-13); migration of Purkinje cells and their reorganization into groups (E14-E17) and proliferation of these groups (E18-P20) (4). From the ventricular zone, located on the dorsal surface of the fourth ventricle originate Purkinje cells, most cerebellar interneurons, cells of the deep cerebellar nuclei and Bergman's glia. These neurons are GABAergic, and interneurons also express the transcription factor Pax2. In mice, glutamatergic projection neurons originate E10.5 and E12.5. GABAergic interneuronal precursors develop from progenitor cells in the ventricular zone during E13.5-E16.5. Glutamatergic and some cholinergic neurons are generated in the rhombic lip, while GABAergic neurons originate in the ventricular zone. After research on different expression models of transcription factors, it was found that neurons in the cerebellar nuclei migrate from the rhombic lip to the nuclear transitory zone through the subpial migratory stream, sequentially expressing the transcription factors Pax6, Tbr2, Tbr1 (5).

An important step in the development of the cerebellum is the transformation of Purkinje cell layer from a multilayer composition into a monolayer. This structural reorganization is followed by the foliation process: a genetically determined series of cellular movements and morphological changes that lead to the formation of cerebellar lobes (3). The cerebellar vermis in many types of mice consists of eight lobes and many sublobes **(Figure 1).** Rat's cerebellum is composed of ten lobes and more sublobes, than mice. Some hybrid mice and rats have one lobe more and some sublobes. In the human cerebellum could be identified ten lobes and each of them is subdivided into many lobules (6). The process of formation of functionally active neurons from neural precursors takes place mostly during the embryonal development. It can last a lifetime, but only in limited, predicated areas of the mammalian brain (7). An important process during the embryonic development of the CNS is the migration of neurons and the formation of neuronal chains. Under the influence of controlled processes, newly generated neurons migrate from their germinal area to all parts of the CNS. The migration process occurs in radial and tangential directions. Migrating neurons are polarized structures and could be distinguished as a leading process and a subsequent process. The formation of these processes is controlled by precise cellular and molecular mechanisms. Some proteins such as doublecortin (DCX), dynein, fibrillar actin, GABA are involved in this process. In GABA-deficient mice, neuronal migration is limited (8).

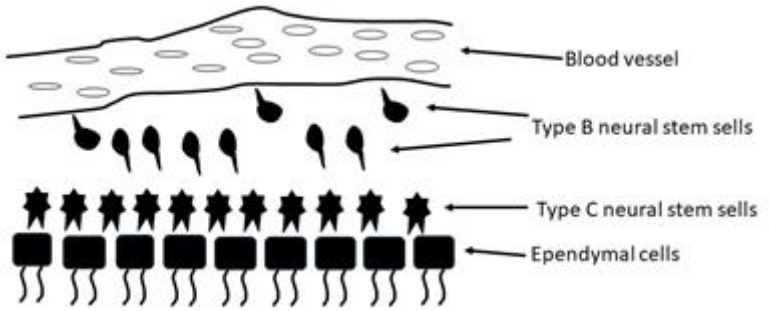

Figure 1. Cerebellar foliation arises during embryonal development and continues postnatally. In the mouse cerebellum at P8 are identified eight cerebellar folia in the vermis (labeled in roman numerals).

Neurogenesis in the developing cerebellum

During the embryonal development could be distinguished two main areas of neurogenesis-rhombic lip and the subventricular zone **(Figure 2).** The glutamatergic neurons of the deep cerebellar nuclei and unipolar brush cells (UBCs) originate from the rhombic lip. They differentiate after mitosis. Subsequently, migrate over the cerebellar rudiment to form the nuclear transition zone (9). The other progenitor zone in the developing cerebellum is the ventricular zone. From the ventricular zone come the astrocytes of the cerebellum, a subpopulation of oligodendrocytes and Bergman's glia. A complex research of neurogenesis in adult individuals began after the introduction of bromodeoxyuridine (Brdu), a nucleotide analogue that labels mitotic neurons (10). This way was found, that it lasts during a lifetime in almost all mammals. This process is limited in two brain areas: the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone of the lateral ventricle. Granular neurons are generated in the SGZ. In the subventricular zone the neurons migrate through the rostral migration stream (RMS) to the olfactory bulb to become interneurons (11). This process is dynamic and is modulated by various physiological, pathological and pharmacological factors. Neurogenesis in other areas of the CNS in normal conditions is limited, but can be stimulated after some damage (12). The development of granular neurons in the cerebellum takes place in two phases. The first takes place in the rhombic lip, and the second in a temporarily existing outer (external) granular layer (EGL) of proliferating cells. It is formed by the tangential migration of granular precursors (13). This proliferation occurs under the influence of the sonic hedgehog protein (Shh) and Jag1 signaling molecules (14). The precursors in this layer express the Atoh1 (Math1) gene. When this gene is mutated, EGL is not formed (15). The proliferation of the precursors in EGL is characterized by symmetrically occurring mitoses (16). This process is supported by the signaling protein SDF1a, which is secreted by the developing meninges. It regulates cell adhesion (17). Recently it was found, that these precursors form exclusively granular neurons (16). This is a contradiction with earlier assumptions (18) regarding EGL proliferation. The precursors of granular neurons in EGL are stimulated by Shh, secreted by Purkinje cells (19). Shh signaling determines the EGL size as well as cerebellar foliation (20). The proliferation of precursors in EGL is important for increasing in size of the postnatal cerebellum. As the size of the posterior cranial fossa does not increase significantly, the cerebellum begins to fold along the anteroposterior axis (21). In this way begins the process of cerebellar foliation. The basic shape of foliation remains similar in all vertebrates in the process of evolution. It is initiated by the formation of multiple "attachment centers" in stereotypical areas of the cerebellar primordium. This will form each cerebellar fissure, separating two adjacent folia (22). In the mouse cerebellar vermis four attachment centers form the five main lobes. After birth, fast cell proliferation transforms EGL from a monolayer to a multilayer structure. EGL consists of two sublayers - a proliferating outer layer and a differentiating inner layer. Proliferating precursors of granular neurons are actively spreaded, and in mice, this occurs between the first and fourteenth postnatal days (P1-P14) (23). After mitosis begins the cell differentiation in EGL. This process is accompanied by radial migration to the underlying part of the cerebellar cortex during the second postnatal week. As a result, EGL disappears. In this way is formed the inner granular layer located below the Purkinje cell layer (24). The temporary stimulation by the EGL is of great clinical importance. It is supposed, that a significant proportion of medulloblastomas arise as a result of mutations in Shh signaling in this period (25). This affects the proliferation of

granular cells (26). The granular neurons in EGL spread and cover the developing Purkinje cell layer. This phenomenon suggests an interaction between Purkinje cells and granular neurons. Accordingly, the Purkinje cell layer does not develop normally in the absence of granular neurons (27).

In mice, Purkinje cells leave the ventricular zone at E10.5. They stop their proliferation and begin migration to the EGL to form a multilayered structure called a Purkinje cell plate. Early generated Purkinje cells migrate first around E14.5. This occurs mainly in the posterior ventricular zone. These cells move tangentially, similar to the cells derived from the rhombic lip (28). Later generated Purkinje cells, as well as early-generated cells in the anterior ventricular zone, migrate farther than their precursors. A marker for the molecular heterogeneity of Purkinje cells is zebrin II (ZII). Early generated Purkinje cells are ZII + and late generated cells remain ZII- (29). By E14.5, the cerebellar plate begins reorganization, forming over than 50 different clusters up to E18.5. They are composed of ZII + or ZII- Purkinje cells (30). The clusters transform into sagittal stripes as the cerebellum grows along the anteroposterior axis. This increase is due to the proliferation of precursors of granular neurons and their migration inward. In this way, Purkinje cells are transformed into a monolayer around the fourth-fifth postnatal day (P4-P5) (31). At this stage, each Purkinje cell begins to form dendrites, and subsequently a long axon (32). Purkinje cells are generated in a relatively short time interval (33), and gene expression in the ventricular zone is constant (34).

Figure 2. Subventricular zone is an area for neurogenesis in vertebrates. Type B are slow proliferating cells and type C are actively proliferating cells.

Unlike Purkinje cells, which originate from the ventricular zone, inhibitory interneurons are derived from proliferating precursors in the cerebellar germ (28). These cells have high mitotic activity in the first postnatal week (9). Interneuronal progenitors retain their developmental potential till the end of cerebellar development.They acquire the phenotype of mature cells through the influence of signals in the developing white matter (35). Cellular signaling, which begins at E10.5 in the cerebellar germ, is essential for astrocyte development (36). Bergman's glia originates directly from the radial glia, lining the ventricular zone. This process begins at E13.5, after the origin of Purkinje cells (22). Bergman's glia has many ascending processes that pass through the molecular layer and the outer granular layer. Granular cells migrate inward from EGL to IGL along these processes. Bergman's glia also guides the migration of interneurons into the molecular layer (37). In rodents, the number of Bergman's glia fibers increases significantly during the first two postnatal weeks, when proliferation and migration of granular neuronal precursors was observed. This means that these precursors influence the Bergman's glia formation. The differentiation of these glial cells depends on the Shh factor secreted by Purkinje cells (38).

Transcription factors during cerebellar development

The embryonal development is determined by the individual genome (the totality of all genes and the information encoded in them). The genes determine protein synthesis. In turn, proteins regulate the expression of other genes. They also play a role of signaling molecules that guide development. There are about 23,000 genes in the human genome. Due to the different levels of regulation, the number of proteins synthesized by them is significantly higher. The first hypothesis of a single-protein gene has been refused. By different mechanisms, one gene may be responsible for the synthesis of many proteins. Genes are located in the DNA strand and contain regions called exons, which can be translated into proteins, and introns, which are diffused between exons and which are not translated into proteins. The promoter is a region that binds RNA polymerase to initiate transcription. However, to bind to this place, the polymerase system requires additional proteins called transcription factors. Transcription factors have a specific DNA-binding dominant and a

transactivating domain, that activates or inhibits the transcription of the gene whose promoter is bound. In combination with other proteins, transcription factors activate gene expression. For example, the transcription factor Pax6, which is involved in the development of the pancreas, eye and neural tube, contains three separate activators. Each of them regulates its specific expression in these tissues.

Role of transcription factor Zbtb20

Zbtb, originally referred to as zinc finger BTB / POZ from dendritic cells (DPZF) belongs to a family of transcription factors with an N-terminal BTB / POZ domain and a C-terminal DNA-binding zinc finger domain. Zbtb20 (zinc finger and BTB-domain containing 20) is a member of the BTB / POZ transcription factor family and functions as a transcriptional repressor (39). It is an important regulator in the cartilage tissue development. Its expression has been found in the developing chondrocytes. Removement of the transcription factor leads to disturbances in endochondral ossification and growth retardation (40). The level of pituitary hormones was monitored in Zbtb20 knockout mouse models. It was found that from E14 to P0 the level of somatotropin was reduced. For the other pituitary hormones: luteinizing, follicle-stimulating and thyroid-stimulating hormone, no difference was found compared to control models. Zbtb20 is important for the terminal stages of prolactin-secreting cell differentiation. Removal of Zbtb20 leads to hypoplasia of the anterior pituitary and complete loss of lactotropic cells (41). According to (42) defects in the differentiation and proliferation of somatotropic and lactotropic cells are found. It is established, that Zbtb20 is expressed in the cerebral cortex of mice during the embryonal and postnatal development (43). In advanced embryonal stage of development, as well as in the postnatal period, Zbtb20 is expressed mainly in differentiating granular neurons. It's expression is also found in the pyramidal neurons of the hippocampal formation and gyrus dentatus. Zbtb20 is transiently expressed in immature post-mitotic neurons derived from the hippocampal neuroepithelium, as well as in migrating precursors of granular neurons of the dentate gyrus. In wild type mice, the two isoforms of Zbtb20 (Zbtb20S and Zbtb20L), are expressed in the projection neurons of hippocampus, gyrus dentatus, subiculum and neocortex (44). The gene expression begins at E11 and extends throughout the pallium at E15. At P4 the expression is mainly in the superficial layers of the mantle and at P12 it almost disappears. Expression is almost undetectable in the deep layers. Zbtb20 deficiency leads to reduced proliferation of late cortical progenitors (45). Zbtb20 is required for astrocytogenesis in experiments in vivo (46). It is found increased expression of Zbtb20 in pain sensory neurons during embryonal development (47). A protein synthesized by Zbtb20 has also been found in indusium griseum, in hippocampal neuroepithelial cells and fornix. Postnatally Zbtb20 expression was found in the intermediate hippocampal area, pyramidal neurons of the cornu ammonia (CA1 and CA3). And also in granular neurons of gyrus dentatus, outer and inner granular layers of the cerebral cortex, molecular cell layer and white matter of the cerebellum (39). During postnatal cerebellar development, transient expression of Zbtb in the granular layer is established. Granular neurons are generated postnatally in the outer granular layer (EGL) and then these post-mitotic precursor cells migrate inward through the molecular layer to reach the inner granular layer. In mice, most granular neurons are generated during the first 3 weeks of postnatal development. In the fourth week, the expression of Zbtb in the inner granular layer decreases. These data indicate that Zbtb is transiently expressed in the nuclei of migrating granular neuronal precursors. Expression decreases during maturation of granular neurons in the inner granular layer (43). In Zbtb20S, Zbtb20L and Zbtb20S / L transgenic mice deviations from normal cortical development were found. Sometimes even a lack of corpus callosum was found (44).

Other transcription factors

Even Zbtb20, other transcription factors also participate in the cerebellar development. For example a signaling molecule of fibroblast growth factor 8 (Fgf8) plays a role in formation of axial boundaries (48). Same role has Otx2 in the development of prosencephalon and mesencephalon (49). The transcription factor Hoxa2 determines the caudal boundaries of the cerebellum (50). Sox2 plays a role in proliferation and differentiation of neuronal progenitor cells (NPC) (51). Expression of this factor is found in cerebral stem cells (52), glial cells. Bergmann glial cells (subtype of radial glia) express Sox2. They play a role in Purkinje and granular cells migration (53). In Sox2 knockout mice behavioral disturbances, deficit

in motor skills are found. Impaired CNS development and malformations of the granular and molecular cell layers are observed (54).Cerebellar foliation in rodents occurs in some phases (55). At first the smooth surface is divided in five major lobes by four grooves in the vermis. In the next phase major lobes are subdivided in sublobes and lobules. The foliation pattern continues two weeks after birth in mice, and three weeks in rats. It is still unknown the mechanism through which is determined the position of the fissures. Also is an enigma the transcription factors, which regulate the size and foliation of the lobes. It is unknown if there is a correlation between foliation and the number of the fissures. The cerebellum increases its size one thousand times during development (56). After birth, this is determined by growth factors and is accompanied by the process of foliation. This process is in a close connection with generation or cerebellar granule cells. If the formation of granule cells in mice and rats is embarrassed, the cerebellum is smaller and with less folia (57). The granular cell precursors (GCP) proliferation depends on Purkinje cells. In loss of Purkinje cells (caused by genetic mutation) it is observed loss of granule cells (58). This effect of Purkinje cells over granule cells is caused by the transcription factor sonic hedgedog (Shh). This factor is secreted after E17,5 in mice. It is found, that this factor increases and maintain proliferation of GCP in cell cultures (59). Shh expression from E17,5 until early postnatal stages is in the regions of the prospective fissures. After Purkinje cells become postmitotic, Shh does not affect GCP anymore (20). Inhibition of Shh by antibodies in chicken cerebellum leads to disturbance in the foliatin and spreading of GCP. Inactivation of mouse Shh during neurulation induces cerebellar malformations (59).

CONCLUSION

The cerebellar development is similar in most of the vertebrates. The main processes in the cerebellar morphogenesis and histogenesis are explored on histologic slides from experimental animals. The researchers use more often transgenic animals and wild type (as controls). Many of the cellular mechanism are visualized through labeling, directly injected in the developing embryos. Some of the molecular pathways are still unclear. Cell migration during neurulation and formation of CNS is a complicated event. Understanding migration defects can help explore CNS malignancies.

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