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Faulty position of cerebellar cortical neurons as a sequel of disturbed neuronal migration

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Abstract

The object of our report is the presentation of the morphological picture of cerebellar cortex malformation as a sequel of disturbed neuronal migration. In the disarranged tissue, cavities with a network of meningeal tissue and embedded pathological vessels were noted. The external granule cells did not form a proper external granule layer, but moved deeper, forming irregular aggregates. Abnormally aggregated external granular cells invaded the cerebellar tissue. Abnormal Purkinje cell positioning and a disarranged molecular layer were observed. The normal layered pattern of the cerebellar cortex was disorganized. The presented cases represent a spectrum of morphological changes which are the consequence of aberrant migration. Against a background of vascular pathology affecting the meningoglial network the migration pathways were disrupted. The defective movement of neurons and their faulty maturation resulted in disturbances of cortical layering, and defects of cerebellar folia formation.

Key words: *cerebellum, development, cerebellar cortex, cerebellar malformation*

Introduction

During normal development of the cerebellum neurons migrate from the place of origin to the place of residence in the correct laminar position within the cerebellar cortex. The glial and neuronal cells that compose the cerebellum migrate to their final locations in the cerebellar hemispheres through two general pathways [2]. The neurons that will form the deep cerebellar nuclei and the Purkinje cell layer (PCL) of the cerebellar cortex migrate radially outward from the germinal matrix in the wall of the fourth ventricle. Another set of neuroepithelial cells migrate along the pial surface to form a secondary germinal matrix, the external germinal (granular) layer. The neuronal precursors migrate tangentially from the germinal zone in the lateral portion of the rhombic lips, using the pial basal lamina as a guiding structure. The cells in the external granular layer (EGL) retain the capacity to divide and many of the daughter cells are destined to form the internal granular layer. These cells migrate inwards from the proliferative zone in the EGL, just below the pial surface membrane. Final division of granule cells occurs in the upper zone of the molecular layer, then cells become bipolar by extending horizontal processes [7,16]. The granule cell attaches to the Bergmann glial fibre and then neurons pass through

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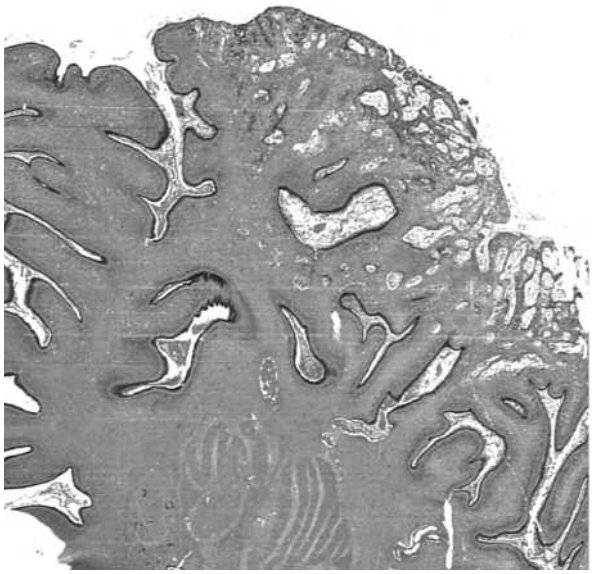

Fig. 1. Cerebellum with disorganized folial structure. The malformed folia coupled together and cavities. HE. Glass magnification

the molecular layer, by Purkinje cells, and beneath the PCL form the mature internal granular layer (IGL).

The migration of the Purkinje cells depends on the expression of genes *En1*, *En2*, *wnt3*, *disabled-1 gene* [11,14]. Granule cell viability and migration are supported by several genes, the most important being *Pax6*, *Zic1* and *Math1*, whereas the Bergmann glia are preserved by the expression of *Pax3* [9,12].

The objective of our report is to present the morphological picture of cerebellar cortical malformation with reference to neuronal migration. Very similar dysplastic changes of the cerebellar cortex are described in two cases.

Case I: A 1350 g boy was stillborn at 26 weeks of gestation (gw). Age according to biometry measurements was 27 gw. Cerebral sonography showed abnormally enlarged lateral ventricles. Sonography revealed also abnormality of chest, enlarged hyperechogenic lung on the right side, ascites. The postmortem finding on autopsy was congenital emphysema of the right lung.

Case II: A girl weighing 1500 g, length 39 cm, stillborn at 33 gw, the first pregnancy, unrelated parents. The clinical background during pregnancy revealed anhydramnion. The postmortem autopsy study showed foetal congenital malformations of the urinary system (hypoplastic kidneys, abnormal bladder, lack of urethra), digestive system (hypoplastic stomach) and of skeletal system (horseshoe-shaped foot).

Neuropathological data

Macroscopically in case I the meninges were thin and brownish coloured. On the frontal sections a wide left lateral ventricle was observed. The cerebellum was hypoplastic. Grossly in case II the brain did not show any obvious abnormality. The cerebellum disclosed vermian hypoplasia.

Microscopically, the most notable lesions were located in the medial upper part of the anterior lobe of the cerebellar hemispheres (Fig. 1). The lateral part was recovered with a normal layered pattern developmentally appropriate for gestational age. In the malformed part folia were coupled together, and the folial cerebellar pattern was obliterated.

In the disarranged tissue, cavities with a network of meningeal tissue and embedded pathological vessels were observed. The vessels within cavities and meninges were abnormal, cavernous, thin--walled. Vasculomeningeal proliferation fused with the underlying external granular layer. The external granule cells did not form proper EGL, but pushed in deeper, forming irregular aggregates (Fig. 2). The aggregation of granule cells invaded cerebellar tissue, clustering in abnormal rings of granule cells around cavities. The numerous cavities, various in size, were surrounded by widely scattered granule cells (Fig. 3). The congested granule cells formed around cavities the EGL pattern, with a mostly obliterated molecular layer. Large clusters and abnormal pericavital aggregations of the external granule cells disturbed normal cortical lamination. In some places nodules of chaotic appearance of granule cells formed a structure resembling polymicrogyric cortical invaginations (Fig. 4).

Disorientation of Purkinje cells was observed. The alignment of Purkinje cells was disturbed. Calbindin-immunoreactive Purkinje cells formed fragments of monolayer among granule cells (Fig. 5). They were embedded in the granule neurons mass. Abnormal orientation of the Purkinje cells was related to the pericavital external granule cells. The narrow molecular layer was occasionally visible, but mostly it disappeared. In large parts of cerebellar tissue Purkinje cells were not observed; they did not migrate to the place of residence. Purkinje neurons arrested in their course of migration were found in

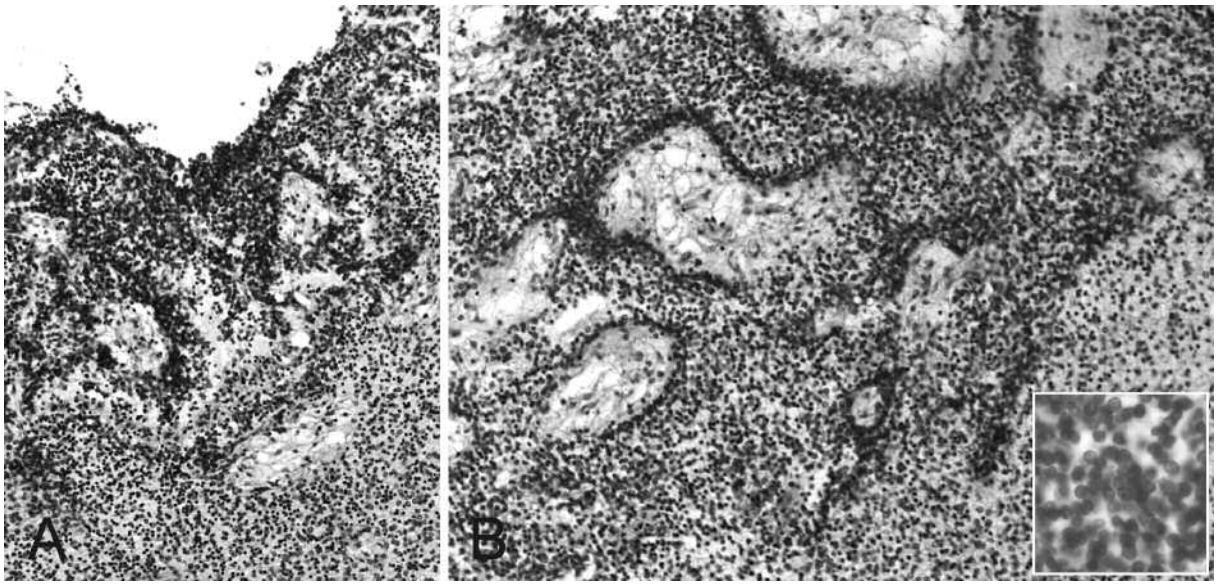

Fig. 2. Aggregations of granule cells invading the cerebellar tissue. A. chaotic appearance of granule cells in the superficial area. B. wide scattered cells and abnormal ring clusters around cavities. Insert: granule cells. Cresyl violet. Orig. magn. A,B x 200; insert x 400

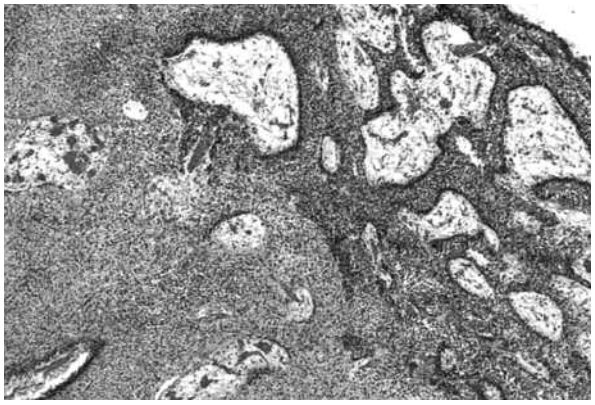

Fig. 3. Various sized cavities embedded in the widely scattered granule neurons. Cresyl violet. Orig. magn. x 60

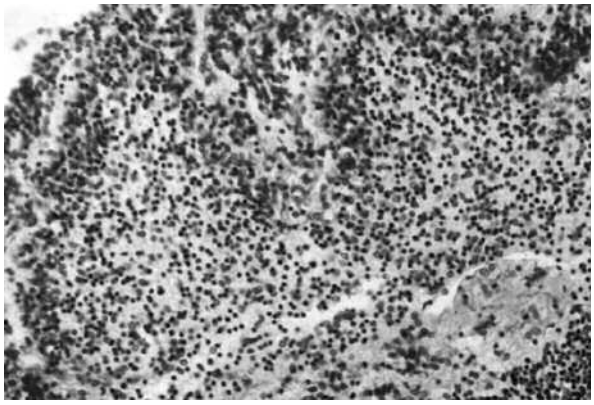

Fig. 4. Dysplastic cortex resembling polymicrogyric cortical invaginations. Cresyl violet. Orig. magn. x 200

the disintegrated tissue. The arrested neurons formed small or large improperly arranged clusters. The Purkinje cell dendrites were degenerated; the dendritic tree lost its orientation (Fig. 5).

The irregular structure of EGL and PCL corresponded to paucity of inner granular cells. The conglomerates of cells forming bands of rounded or spindle external granule cells spread in the deepest parts mimicked the internal granule layer only (Fig. 6).

There were no marked lesions in the cerebral hemispheres.

Discussion

The presented cases represent a spectrum of morphological changes which are the consequence of aberrant migration. The understanding of cerebellar malformation should be considered in relation to normal migration and normal interactions between glial and neuronal cortical components. Normal migration of neuronal progenitors relies on accurate intrinsic cellular programs as well as on extrinsic informative cues provided by extracellular

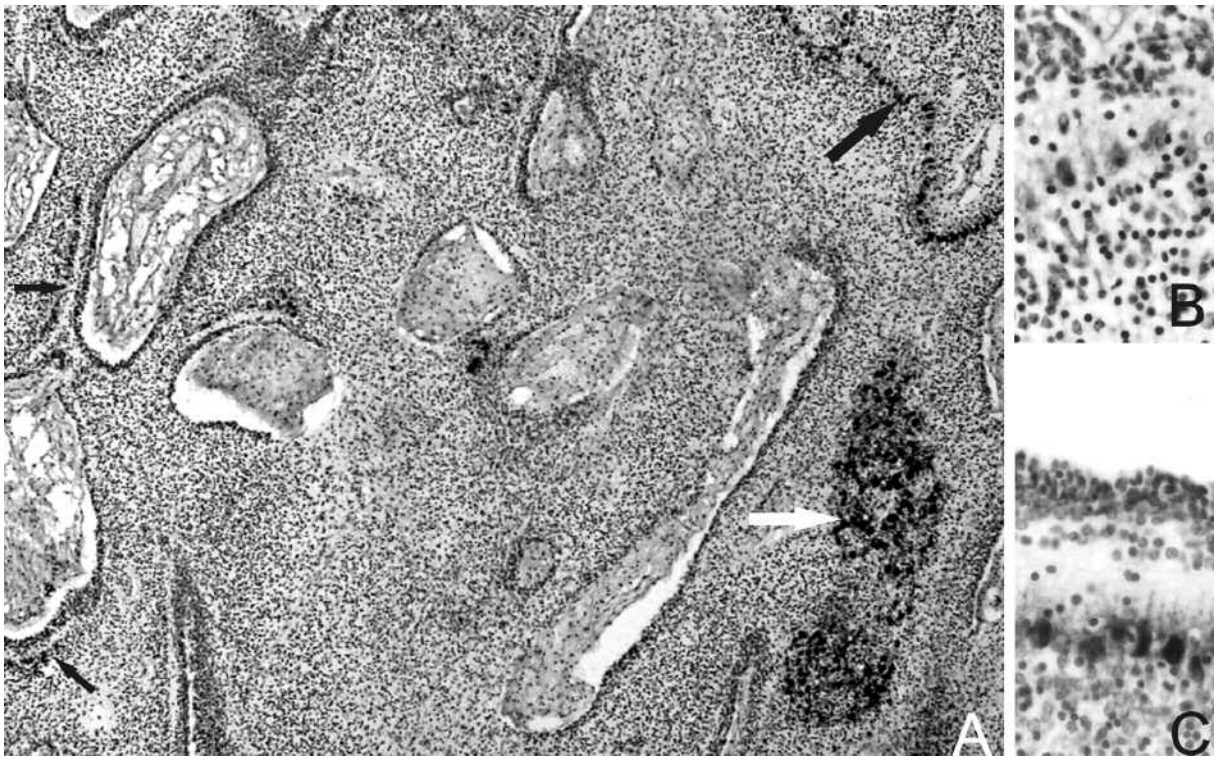

Fig. 5. A. Fragments of Purkinje cell monolayer among granule cells (black arrows). Nests of Purkinje neurons arrested in their migration (white arrow). **B.** Degenerating Purkinje neurons. **C.** Normal Purkinje cells with dendrites in the unaffected part of the cerebellum. Calbindin counterstained with cresyl violet. Orig. magn. A x 100; B,C x 400

molecules. Extracellular matrix of the developing cerebellum is composed of molecules that support neuronal migration – laminin, fibronectin, vitronectin; molecules that function as barriers to migration – chondroitin sulphate proteoglycans; and molecules that orient the migrating neurons to reach their contact locations – reelin, netrins, tenascins [10,14]. Some of these molecules, such as laminin,

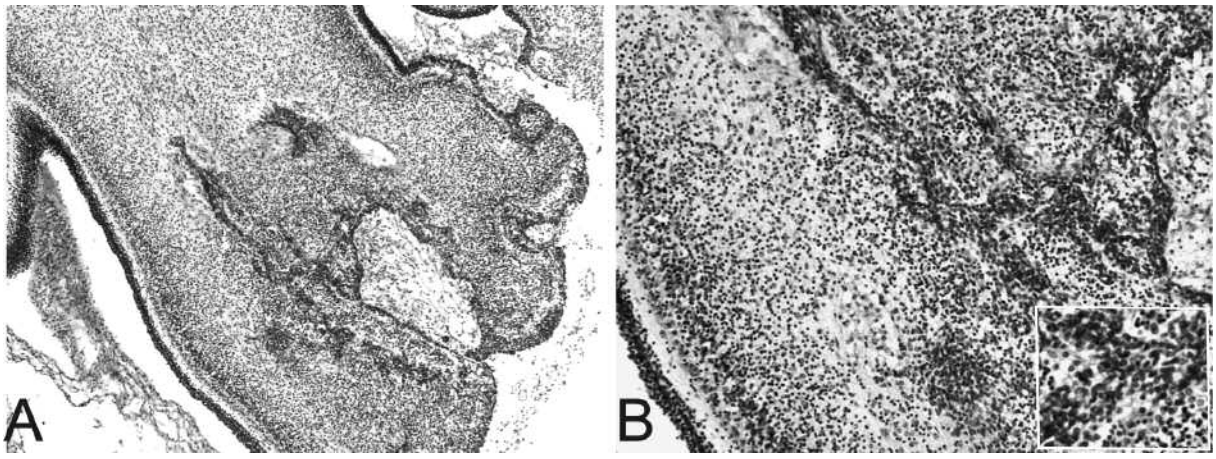

Fig. 6. A, B. Clusters of cells mimicking internal granule layer inside the folia. In the same gyrus the cortex presenting normal layered pattern. Insert: spindle cells. Cresyl violet. Orig. magn. A x 60; B x 100; insert x 200

fibronectin and vitronectin, are compounds of the vascular basal membrane [4].

Developmental events in the superficial part of the cerebellum appear to be critical for the formation of gyri [2]. Meningeal cells constitute part of the superficial glia limitans and produce components of both the interstitial matrix and the basement membrane. Pia mater cells stimulate tangential neuronal migration. The external granule cells attached to the pial basal lamina can use it as a guiding structure [5]. It is known that the overlying meningeal cells influence cerebellar foliation and lamination [15]. The role of the meninges in the pathogenesis of cortical dysplasia was also described by Sievers et al. [13]. Mercier and Hatton [8] describe a meningeoglial cellular network that courses through all layers of the meninges bordering or joining the vasculature and extends into the periventricular subependymal layer. This network consists of fibroblasts in the meninges and the walls of large blood vessels, of pericytes at the level of capillaries, of ependymocytes and a population of astrocytes in the brain parenchyma. They suggested that all the cell types of this cooperative network may communicate and control cellular proliferation, growth and differentiation. The significance of an alteration of the meningeal expansions could be important in pathological processes. The meningeal vessel pathology observed in our cases affected the meningoglial network, and led to over-migration of granule external neurons through a defective glial limiting membrane. Disturbance of proliferation of external granule cells caused in consequence abnormal development and increase of granule cells.

Granule cells are potent regulators of Purkinje cell development and enhance survival and dendritic development of these neurons. A subpial stream of rhombic lip-derived cells, the external granule neurons, express reelin – an extracellular molecule that orients the migrating Purkinje neurons to reach their contact locations [6,10] and is mainly involved in the Purkinje cells' alignment. Granule cell precursors (EGL) also play a role in the positioning of Purkinje cells through expression of the netrin-like receptor Unc5h3 [3]. The abnormal aggregation of external granule cells and unstable expression of reelin and netrin affected the misorientation of Purkinje cells, and disturbed the arrangement of Purkinje cell settlement and dendritic tree orientation. The disoriented Purkinje neurons were localized in a faulty position, or lost their orientation and were arrested in their migration to their final location.

During cerebellar development there is a reversible linkage in granule and Purkinje neuron interaction by Sonic hedgehog signalling (Shh). Shh is produced by Purkinje neurons. It is required for the control of proliferation of granule neuron precursors in EGL [1]. Processes involving Purkinje neurons influence proliferation of external granule cells. Shh also induces differentiation of Bergmann glia [1]. Bergmann glia – unipolar protoplasmic astrocytes – during cerebellar development form a close association with migrating granule cells. The directed migration of the external granule cells to their destination underneath the Purkinje cell layer is guided by and is dependent on glial processes of the Bergmann glial cells. Bergmann glial processes extend from the Purkinje cell layer to the pial surface, where their end-feet form a continuous glial boundary. Defect of the glial limiting membrane led to destruction of the well-defined glial boundary. Glia displayed abnormal end-feet, the glial processes lost their radial orientation and in consequence anomalous formation of the internal granular layer was observed.

Time of arising of this malformation can be dated to 8 postconceptional weeks, the period when the external granule layer forms.

The cascade of disturbances can be stated in the presented cases. Against a background of vascular pathology affecting the meningoglial network the migration pathways were disrupted. The defective movement of neurons and their faulty maturation resulted in disturbances of cortical layering and defects of cerebellar folia formation. The reason leading to this malformation remains to be elucidated. It should be noticed that in both cases other congenital malformations of internal organs were also observed. The lesions originating from different ontogenic periods indicate remitted or prolonged influence of teratogenic factors. Teratogens may alter gene expression, hinder differentiation of tissue, or damage the induced precursor cells. We speculate that a gene expressed in cerebellar meningeal vessel development is mutated and this mutation disrupts the glial-pial membrane and migration pathways of external granule cells and alters cerebellogenesis.

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