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MEDICINE
Research article

CORDOCYTES IN THE HUMAN CHOROID PLEXUS: ANTIHEMORRHAGIC ROLE IN A CASE WITH INTRAVENTRICULAR EPENDYMOMA

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Accepted October 12, 2014

The authors examined the relations of special leptomeningeal cells from the stroma of the human choroid plexus with erythrocytes found in the hemorrhage areas in a case of intraventricular ependymoma. Specialized stromal cells were morphologically similar to those identified and named by us "cordocytes" in many locations and pathologies of the human brain, and demonstrated as multifunctional cells, primarily against erythrocyte invasion. These choroidal cordocytes were found in degenerated and hemorrhagic tissular areas containing numerous and extremely dilated blood vessels, and extravasated erythrocytes, closely surrounded by cordocytes. Other cells with cordocytic phenotype are directed specifically toward regions with erythrocytes accumulations. In addition, to impede new deleterious hemorrhages, some cordocytes seem to cooperate with precursor/stem cells to form new functional cells on cordocytic line, also very good defenders antihemorrhage. Our hypothesis merit attention in biomedical research, because the choroid plexus, like pia mater, contains numerous vessels, surrounded by these special interstitial cells with supervising and protective roles, stopping any abnormal movement of cells and particuled material, without entering into ventricular cavities or brain parenchyma.

Key words: choroid plexus, ependymoma, cordocytes, antihemorrhage, stem cells, cooperation.

INTRODUCTION

Since 1956, it was suggested that the relation of pial cells to the ependymal epithelium and blood vessels is the most interesting feature of the choroid plexus, because pial cells tend to interpose (by means of cytoplasmic sheets) between epithelium and endothelium[1]. Meninges enter the central nervous system (CNS) by projecting between structures in the stroma of chororid plexus, modulating most of the physiological and pathological events of CNS throught the life, playing multiple roles, from protective membrane to stem cell niche[2].

The choroid plexus is an important part of CNS that can be primary or secondary location for many congenital abnormalities or pathological conditions as neoplasms, infections, inflammatory processes, cysts and vascular malformations[3]. Recent data indicate that besides its secretory and barrier functions, the choroid plexus has a novel role in attenuating the differentiation of adjacent neural progenitors[4] and can works as non-neuronal source, like the meninges, for ambient GABA which can modulate the properties of neural progenitors during neocortical development[5].

In the light of our recent achievements regarding morphological implications of cells named cordocytes

which populate many anatomical locations in CNS, including pia mater[6-10], we performed this histological analysis to better document the protective role of cordocytes and potential roles of precursor/stem cells on cordocytic line from the choroidal stroma, in a clinical case with intraventricular ependy-moma. This work is for the first time reported on this topic in literature.

MATERIAL AND METHOD

We obtained a choroid plexus fragment by surgery from a female (aged 63 years), operated on for intraventricular ependymoma developed into the third ventricle, in accordance with ethical guidelines. Conventional light microscopy was used to identify and characterize structures under light microscopy. Samples to be observed under the light microscope were fixed with 10% buffered formaldehyde; after fixation, cuts of 4-6 μm in thickness were made with a microtome. The sections were mounted on glass slides and stained under standard conditions for hematoxylin-eosin technique. The specimens were then observed under a Leica SCN400 Slide Scanner.

RESULTS

In the areas with spliting of the stroma become visible very long and thin prolongations of the specialized leptomeningeal cells, *i.e.*, the cordocytes in our opinion. These cells are overlapping running in parallel towards hemorrhagic places (Fig. 1). The distribution of these cells into areas with ectactic vessels is suggestive for an anti-hemorrhage protective role because they surround entirely both the vessels and hemorrhagic zones, and only exceptionally one can see a few erythrocytes beyond their barriers, in the ventricular space. Additionally, these protective cells are capable to form numerous filopodia or lateral thin ramifications which catch up erythrocytes. These short branches which fix erythrocytes on their cell membrane are developed only in that zones what contain an increased number of erythrocytes, but a new short branch is growing very fast if in the proximity is encountered a single red blood cell (Fig. 2). At another sectioning level, we observed the same characteristic aspect, namely, all erythrocytes are adherent on the cordocytic membrane and only a few vascular cells are escaped into the ventricular space (Fig. 3). In areas with massive hemorrhage, the cordocytes either surrounded hematic masses or they enwrapped concentrically the capillaries, retaining numerous erythrocytes in their network. Aditionally, new differentiated cells of cordocytic phenotype can appear from precursor/stem cells in these activated and perturbated areas what contain psammoma bodies as well (Fig. 4). There are many protective cells distributed between degenerated choroidal epithelium and subjacent hematic mass (Fig. 5). At higher magnification, one can see in this area how these protective cells form multiple layers as physical barriers around the small vessels and surrounding hematic masses like a dense trapping network. In the same time become apparent a tendency to form new cordocytes from mesenchymal precursor/stem cells localized either perivascularly or at margin of hematic mass (Fig. 6).

A particular aspect was observed in some areas with epithelium disintegrated, where a material similar to the plasmatic material is clearly surrounded by the cellular barrier and not diffusible into the environmental milieu (Fig. 7). In fact, in that area do exist a complex pattern of cellular events indicating a perturbed dynamics and including an extensive cell proliferation in the thickness of vascular wall with disapeearance of the choroidal epithelium, but plasmatic material remaining firmly atached to the vascular wall, despite the desaggregated epithelial tissue around it (Fig. 8). Another perturbed cell dynamics is related of psammoma bodies formation, when it can be seen both cellular proliferations or differentiations and different morphological stages of these bodies.

The cell proliferation and differentiation is related to cordocytic phenotypes when clusters of mesenchymal stem cells undergo divisions and changes of shape and size of their nuclei. At the beginning, the cell nuclei of mesenchymal stem cells have a similar morphology with a round form, and a visible nucleolus, then the nuclei become ovoid and the cell prolongations become visible surrounding collagen whorls which become calcified. Finally, entire this formation called psammoma body become very dense, but isolated cordocytes could be still identified inside the calcified formation (Fig. 9). In other areas from the modified choroid plexus, these cellular proliferations are not associated with psammoma bodies formation, but they could be identified to explain a line of differentiation of the functional protective cordocytes against massive hemorrhage. This line of differentiation is ranging from

polygonal uncommitted epithelioid mesenchymal cells, to spindle cells with less proeminent prolongations or cells well-differentiated with ovoid nucleus and characteristic long and thin prolongations. Thus, one can see proeminent proliferation of the precursor/stem cells on cordocytic exclussive lineage, either clearly demarkated from hematic mass, or as cells-collagenous matrix routes inside the hematic mass.

All these cellular complexes are very well surrounded by well-differentiated protective cordocytes. Around them is visible a normal choroidal epithelium with its characteristic stroma (Fig. 10). This highly vascularized connective tissue, is under a continuous monitoring of cordocytes which have many relations with vessels in the central nervous system, they representing the best example among the multifunctional protective cells.

DISCUSSIONS

The recent discovery of interstitial cells of Cajal-like cells (ICC-LC) in many anatomical locations in the CNS and their multifunctional morphological involvements changed our traditional concepts.

There is growing evidence that suggests a continuum of cytological changes from one phenotype to another accurately described, but the mechanisms underlying these changes only now begins to be elucidated. Thus, at least much is clear that pia mater is a cordocytic-vascular tissue and this architectural complex characterizes the stroma of the choroid plexus. The cordocyte-blood vessel cell proximity is essential in the CNS due to the antihemorrhage role played by cordocytes, perhaps their specific role in health and disease in adult life.

The main step is to start our visionary change concerning the physiological signification of these special interstitial cells throughout the CNS, irrespective of their terminology. Recently, in meninges and choroid plexus interstitial cells characterized by a small body and extremely long, moniliform, cell processes have been described and named telocytes. These cells, characterized by a specific phenotype, establish close contacts with blood capillaries, nerve fibers and stem cells, modulating the neural stem cell fate and being involved in adult neurogenesis[11]. In fact, increasing evidence indicates that neural stem/progenitor cells reside in many regions of the CNS including the subventricular zone of the lateral ventricle, subgranular

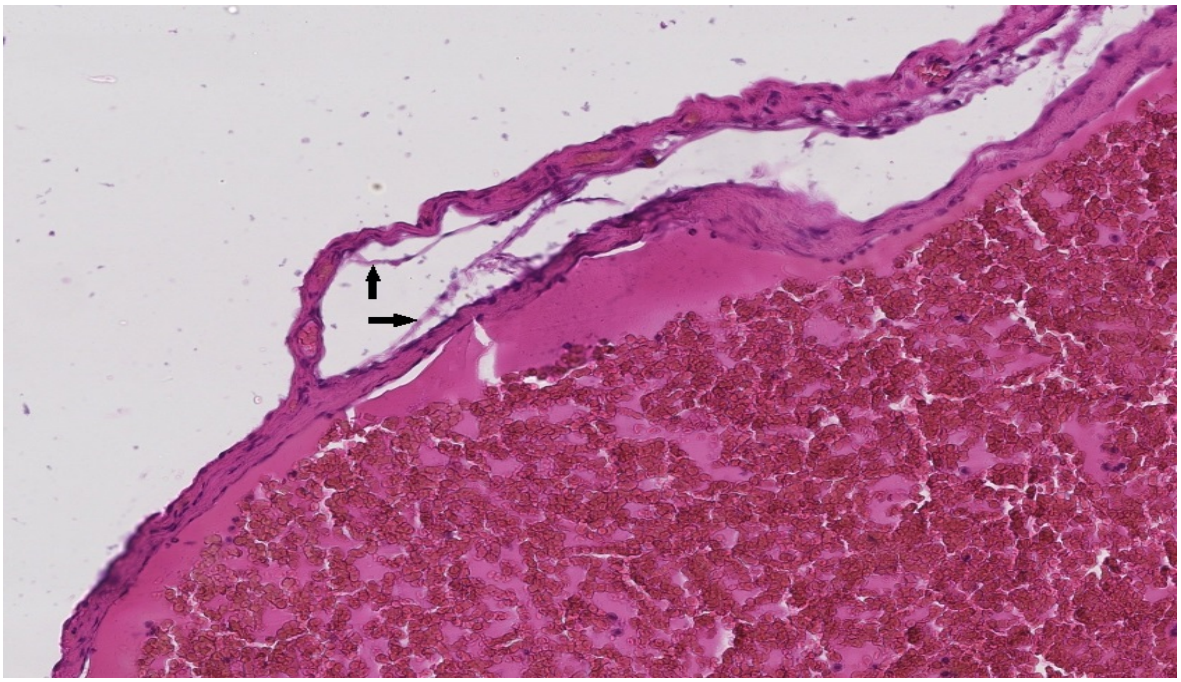

Figure 1. This image shows the stroma splitting, with evidence of cordocytes (arrows). Significantly, the cordocytic network is arborescent and contains many thin cell processes only in the proximity of erythrocytes ×200.

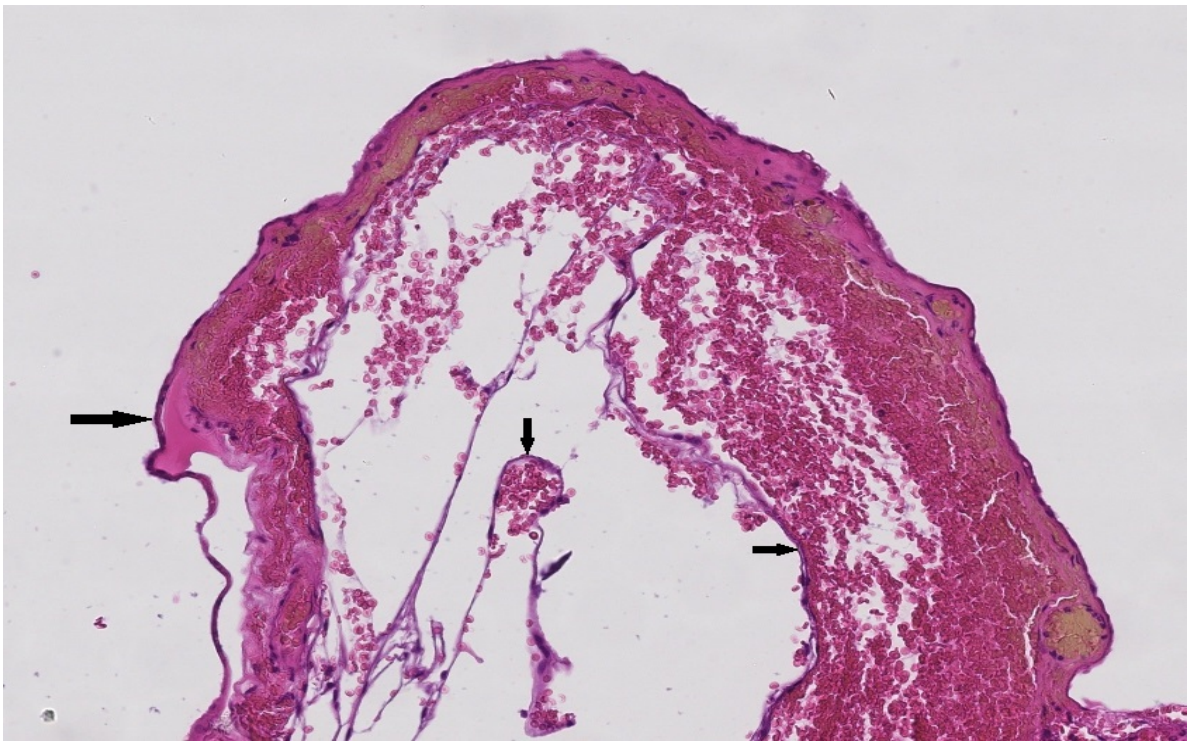

Figure 2. Long interconnected and overlapped cordocytes surrounding the connective tissue (long arrow) and retaining numerous erythrocytes in their network (short arrows) ×400.

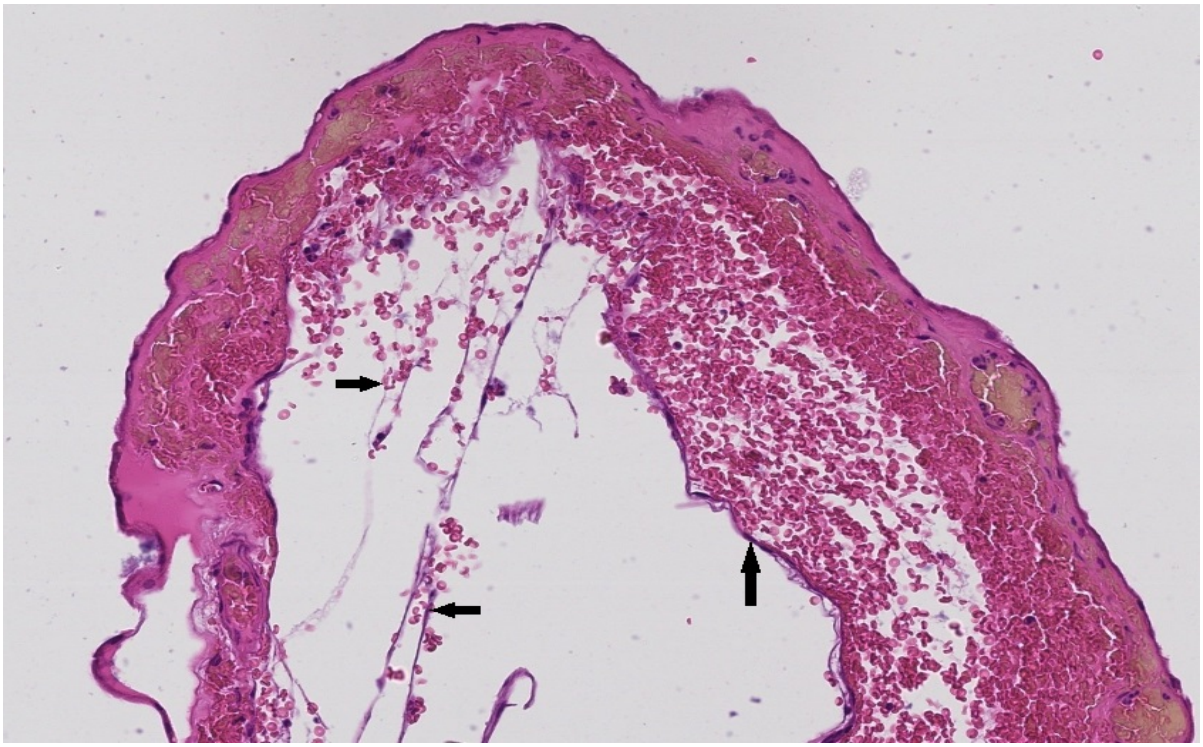

Figure 3. Another sectioning level from the same area illustrated above showing strong adhesion of the erythrocytes to the cordocytic network (short arrows) as well as stratified arrangement of the cells which prevent erythrocyte spreading (long arrow) ×400.

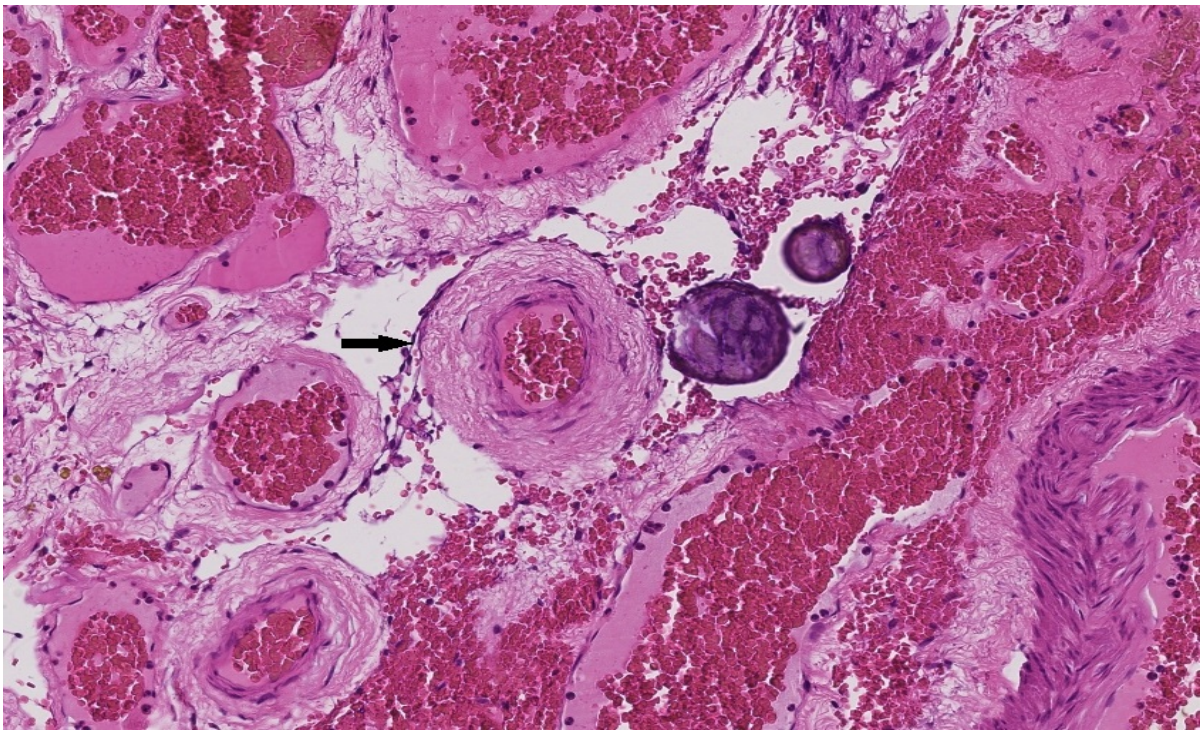

Figure 4. Extensive hemorrhage and numerous ectactic vessels containing cordocytes concentrically distributed which can pass from one vessel to another (arrow). Significantly, there is a cytogenetic zone where new cordocytes appear ×200.

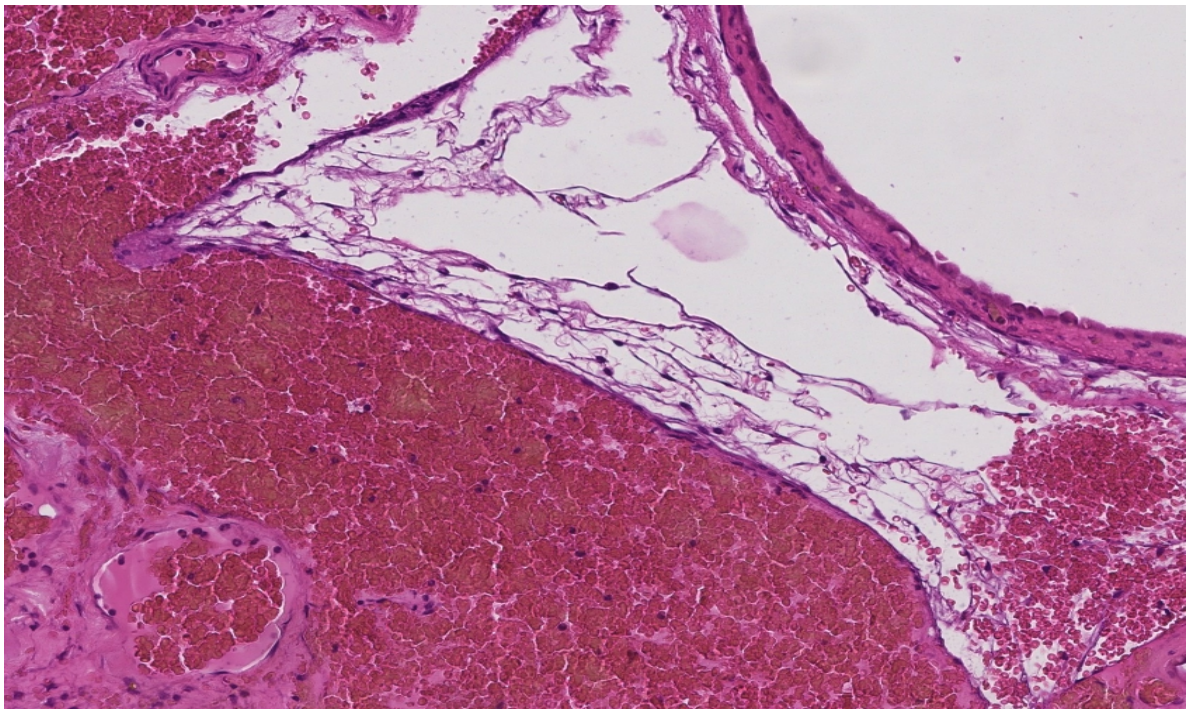

Figure 5. Fragment of the choroid plexus showing flattened epithelium above leptomeningeal cordocytes stratification on the hematic mass ×200.

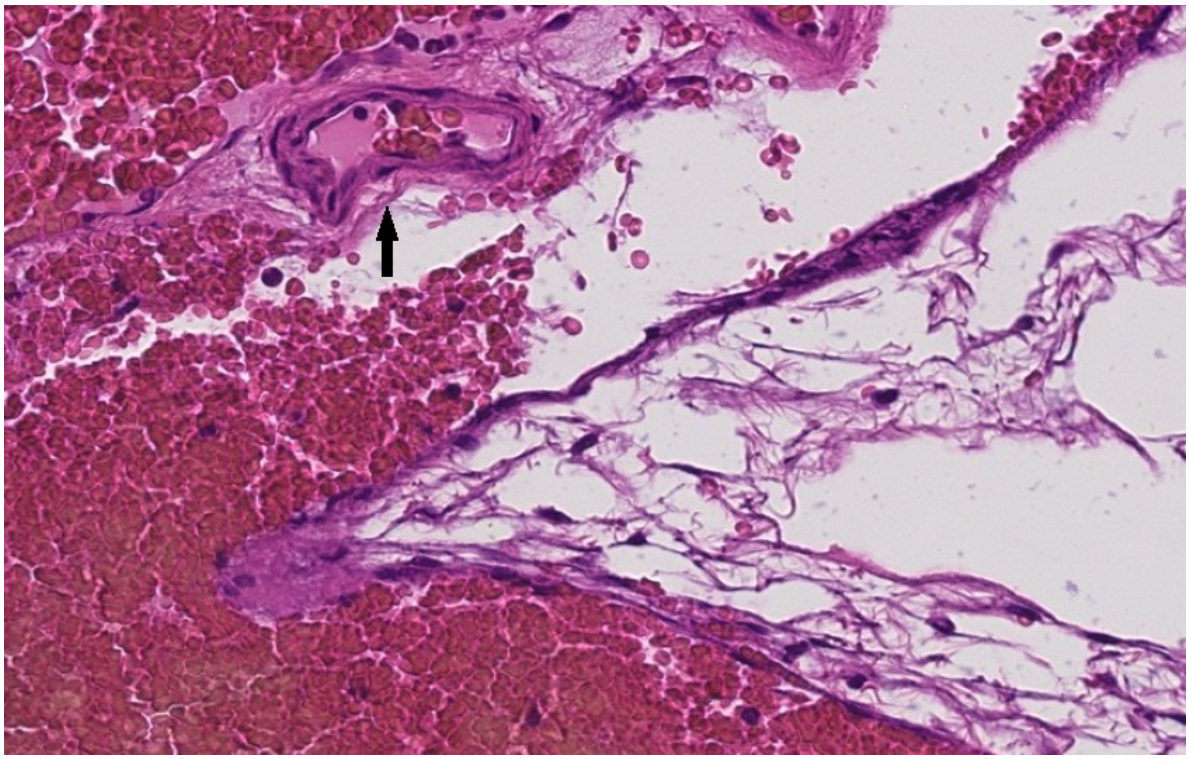

Figure 6. Detail from Fig. 5 showing stratified arrangement of cordocytes around the small vessels (arrow), acting against hematic spreading ×400.

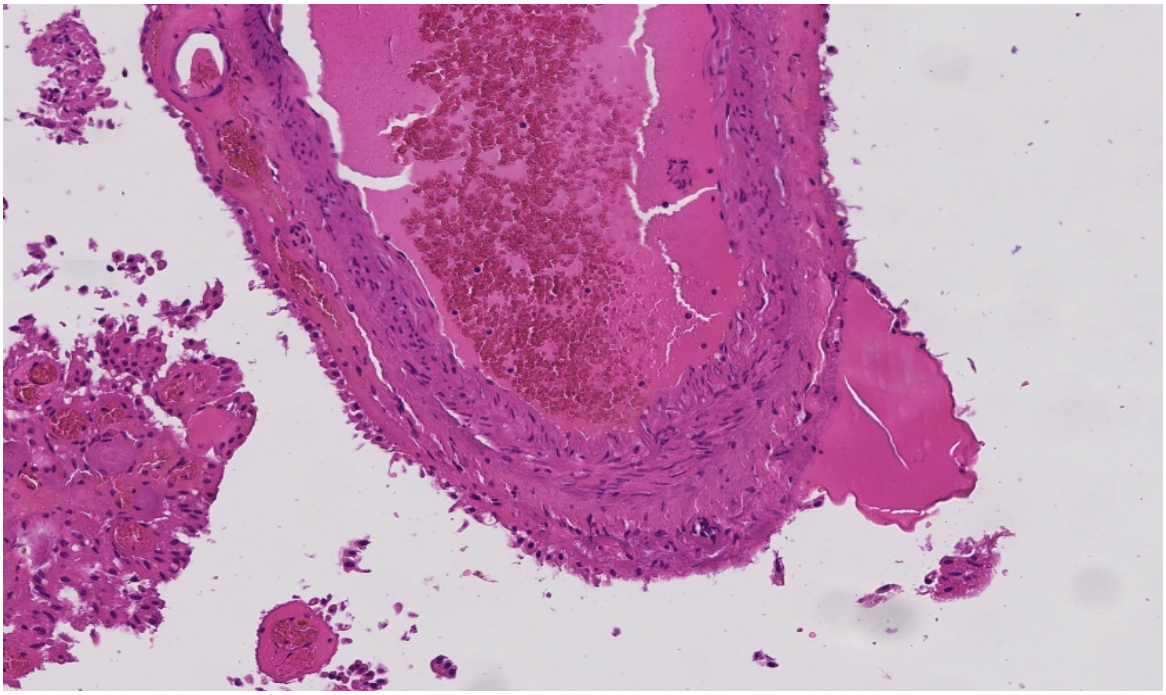

Figure 7. Cordocytes which surround a perivascular area resembling plasma material and numerous cells proliferated in the vascular wall ×200.

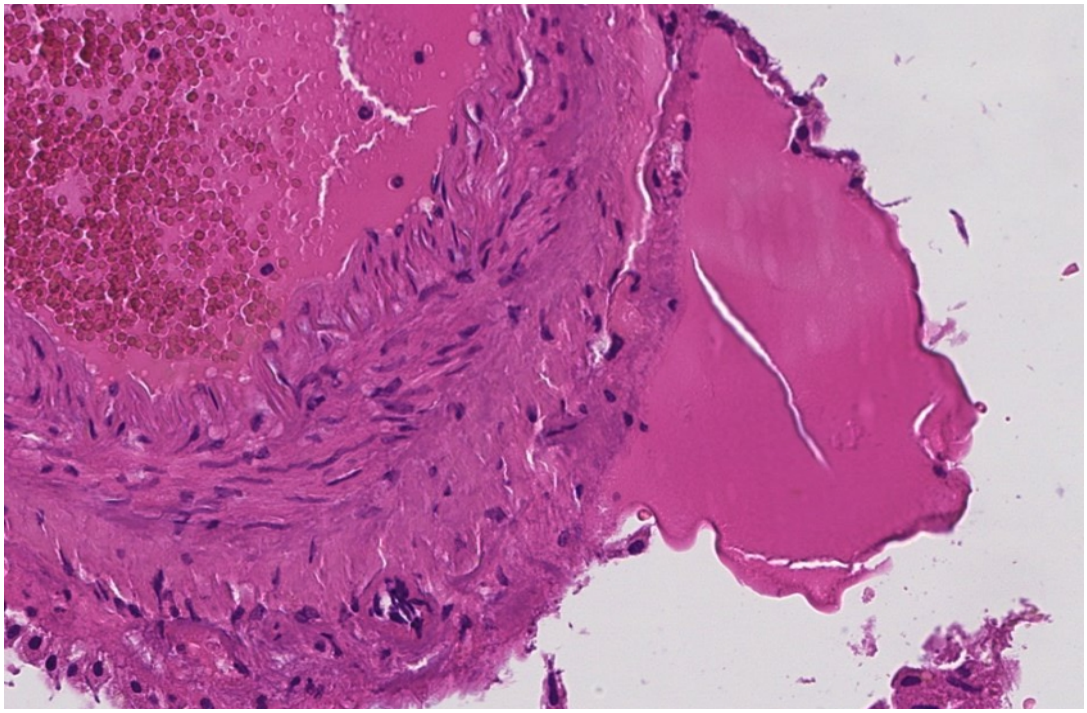

Figure 8. Detail from Fig. 7 showing marked proliferation of spindle cells in the vascular wall as well as epithelial degeneration with cellular remnants in the extracellular space, close to the presumable plasma material ×400.

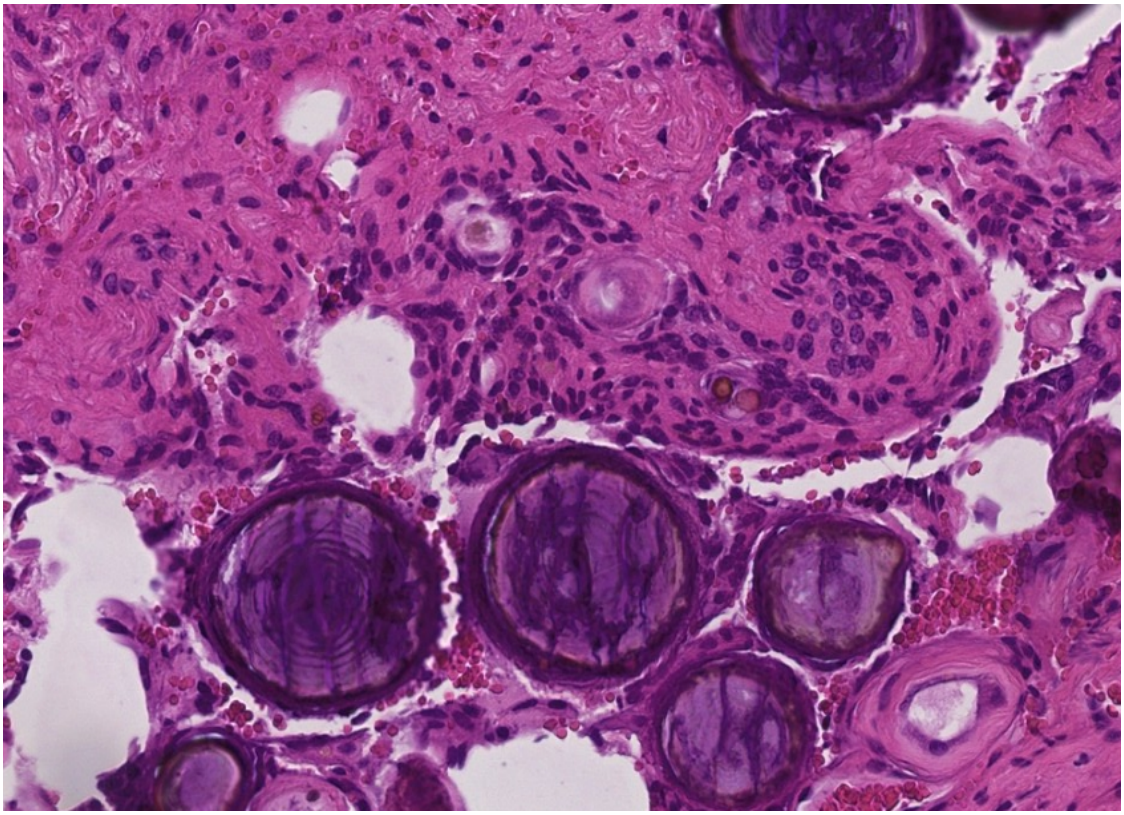

Figure 9. Choroidal stromal area containing numerous psammoma bodies and cells of the cordocytic lineage, from precursor/stem cells to well-differentiated cordocytes, some of them concentrically disposed inside psammoma bodies ×400.

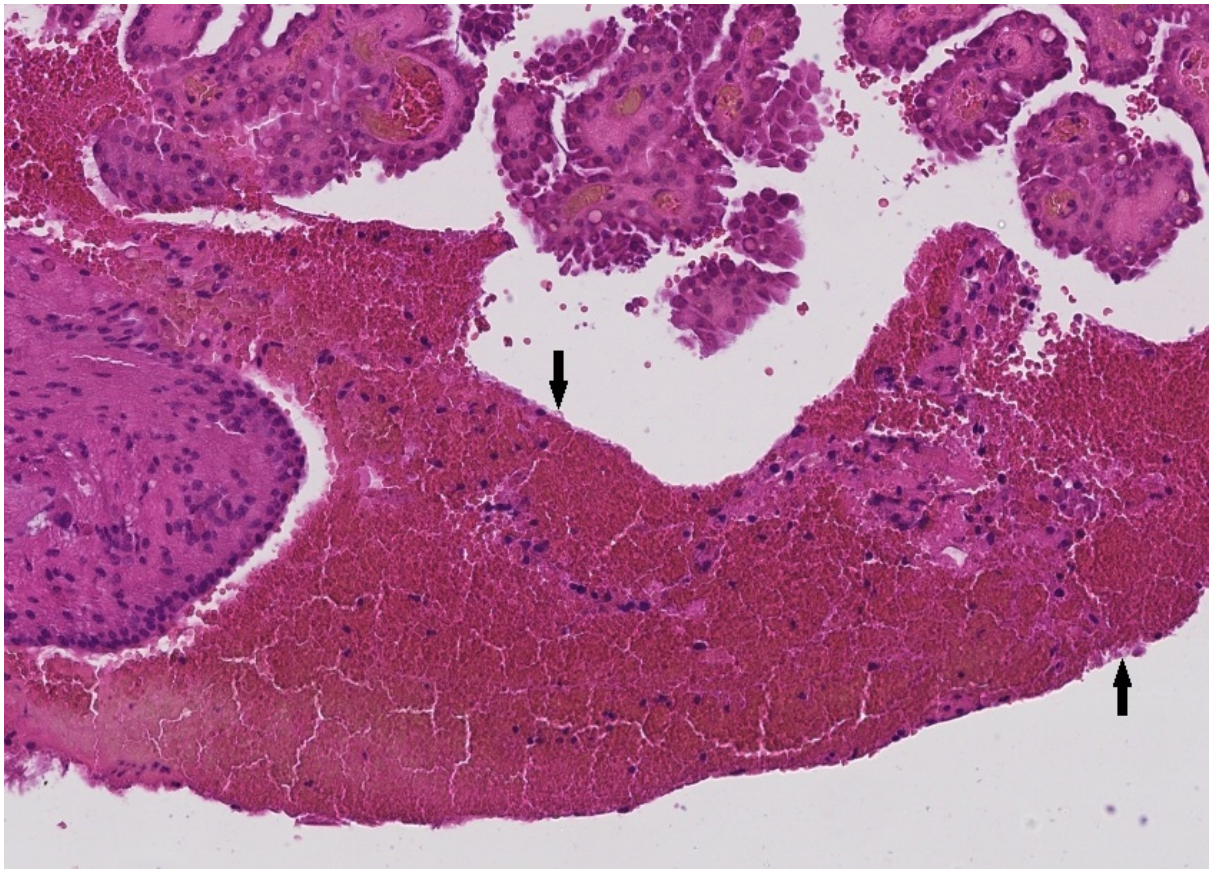

Figure 10. Large hematic mass surrounded by well-differentiated cordocytes (arrows) containing a cytogenetic area with proliferating/differentiating cells of cordocytic lineage, and spreading within the hematic mass ×200.

zone of the hippocampal dentale gyrus, cortex striatum, pia mater which covers the entire cortex[12,13]. Blood vessel walls harbor a reserve of progenitor cells that may be intergral to the origin of the elusive mesenchymal stem cells (MSCs) and other related adult stem cells[14]. On the other hand, the choroid plexus is a multifunctional organ that sits at the interface between the blood and cerebrospinal fluid (CSF), providing continuous immune surveillance by CD4+ T cells, macrophages and dendritic cells, regulating the selection of cells that transmigrate by the epithelial cells and which play prominent roles in controlling the development of immune responses within CNS[15]. The CSF may contain cells (*e.g.*, lymphocytes, monocytes) and proteins that include cytokines or chemokines that stimulate leukocyte movement and regulate the migrating of leukocytes from blood to tissues. Cultured leptomeningeal cells secrete a plethora of CSF proteins including β-trace, cystatin-c, β_2-micro-globulin, apolipo-proteiuns, ubiquitin, cyclophilin c, lysozyme and superoxide dismutase[16]. Also, a major component of extracellular fluids including CSF is albumin, with neuroprotective effects partly attributed to anti-oxidant property and modulation of intracellular signalling of neuronal or glial cells[17]. However, specialized leptomeningeal cells in the stroma of the choroid plexus form collagen whorls that become calcified with age[18], and the psammoma bodies[19], now considered "friends or foes" of the aging choroid plexus[20] were described 25 years ago by scanning electron microscopy (SEM). It has been reported an unusual example of a fourth ventricle choroid plexus papilloma with diffuse leptomeningeal seeding (the leptomeningeal dissemination normally is exceptional)[21], and a pia mater-like structure which covered the tumor surface in two cases with meningiomas[22]. Because the leptomeningeal tissue of the choroid plexuses contains multilayered cordocytes with multifunctional behaviours, it is very attractive to know rapidly the specific molecules which are expressed on their cellular membrane, some

functionally implicated in communication or adhesion and others in metabolic pathways strictly related to specific cordocyte growth.

The novel pathways for cell-to-cell communication with implications in health and disease involve nanotubes, exosomes, apoptotic bodies, and nucleic acid-binding peptides[23]. It is known that stem cells release paracrine factors into the surrounding tissue that subsequently direct a number of restorative processes including protection, neovascularization, and differentiation[24]. Paracrine actions during cell cooperation have to be better documented to define the mechanisms behind the specific differentiation of cordocytic lineage. The mechanisms underlying the mobilization, target tissue integration, differentiation, and the observed therapeutic benefits of progenitor cells in peripheral blood are now elucidated[25]. Also, it is known that adult stem cells (ASCs) and transit-amplifying cells (TACs) are protected and controlled in their self-renewing capacity and differentiation in their specialized physical location named niche[26]. We take into consideration the fundamental role of the cordocytes both for different protective actions, mainly antihemorrhagic, and coordination in reparatory processes.

Because the morphofunctional characteristics of cordocytes are better understood in the light of evidence in clinical conditions, from our pioneering studies, we have to establish the concept of "cordocytic phenotypes" with their clinical and therapeutic implications in humans. From neural crest stem cells which are capable of differentiating into mesodermal and ectodermal lineages[27] to well-differentiated cordocytes, we must understand correctly the phenotypic transition, interaction, role, and control for each cellular type in its microenvironment and epigenetic disregulations at the same time. However, in the meantime, it is necessary to know the rules that govern cordocytes-stem cells cooperation, being given their benefits.

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