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PERIADVENTITIAL CELLS OF MENINGEAL AND INTRAPARENCHYMAL ARTERIES AND ARTERIOLES, CHARACTERIZED BY IMMUNOHISTOCHEMISTRY AND ULTRASTRUCTURAL EXAMINATION OF HUMAN FRESH AND POSTMORTEM TISSUE

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The small arterial branches of the brain surface and penetrating the nervous parenchyma display some peculiar peripheral cells coating their surface. These cells are not entirely identified regarding their structure or function. We intended to study them thoroughly using a large panel of immunohistochemical antibodies and transmission electron microscopy on both postmortem and fresh human tissues. In the arteries located within the subarachnoid space, the periadventitial cells showed strongest positivity for vimentin, α-smooth muscle actin, collagen type IV, PDGFR-β and PDGFR-α. Lower positivity was found for TE-7 fibroblast marker, S100 protein, CD68, CD63, CD133, and caveolin-1. For the arteries located within the brain parenchyma, the strongest reaction retained was only for vimentin and collagen type IV. In both locations, these periadventitial cells were negative for NOTCH-1, CD13, DDR1, FLK1, CD45, nestin, laminin, desmin, or DDR2. Our results suggest that these cells are myofibroblasts with limited stem cell characteristics and most probably involved in angiogenesis. Furthermore, we found that the arteries within the subarachnoid space were not enveloped by pia mater, unlike those penetrating the brain parenchyma.

Key words: human brain, arteries, adventitia, myofibroblasts, pericytes.

INTRODUCTION

The cells enveloping the arteries within the central nervous system are rarely characterized in depth. These cells, which we named periadventitial cells (PACs), are located at the outer limit of the vessel, either positioned on a thin collagen layer, or embedded in this one (Fig. 1). PACs could have several origins, functions and structure. We intended to unravel the fundamental structural details of PACs at molecular level. The peripheral portion of cerebral vessels seems to be involved in many processes, the perivascular drainage of deleterious compounds (i.e. beta-amyloid) being an important one[1,2,3,4]. This is in close relationship with the lymphatic clearance of the brain[5,6], subsequently stated as being involved in some neurodegenerative conditions[7,8]. Since they are located in this peripheral position, PACs, apparently originating in the pia mater, are described as being a structural part of the pial-glial basement membrane[4]. These PACs are, however, insufficiently characterized in humans and until now immunohistochemistry played only a modest role in this characterization process. Therefore, a more detailed approach to their structure and functions seemed appropriate.

MATERIALS AND METHODS

Since PACs could be simple fibroblasts, we tried to verify this hypothesis first. TE7 is an antibody especially designed to react with human fibroblasts, while not cross reacting with other connective tissue cells[9]. Also, the antibody seems to be more specific than other ones conceived for the same purpose, 1B10 and 5B5[10]. To test the inclusion of these cells in the fibroblast category, DDR1, DDR2, PDGFRα, PDGFRβ and collagen IV staining were performed. DDR1 (*discoidin domain receptor 1*) is a receptor

interacting with all types of collagen (from type I to type V)[11]. It is involved in tissue homeostasis, cell proliferation, differentiation, adhesion, migration and invasion[12]. DDR2 (*discoidin domain receptor 2*) is also involved in cancer development and metastasis. DDR1 is expressed by epithelial cells, whereas DDR2 appears in mesenchymal cells[14,15]. DDR2, as well as PDGFRα, are known as fibroblasts cell membrane components, at least in the cardiac ones[16]. Both PDGFRα and PDGFRβ have increased expressions on fibroblasts membrane, related to their proliferation potential and transformation into myofibroblasts[17]. Collagen IV, in conjunction with laminin, is a fundamental component of tissue specific basement membranes, including the vascular ones[18]. It is also known as being involved in myofibroblasts development and migration[19].

On the other hand, PACs could also be considered as having progenitor cell potential, as already described[20]. To this purpose, we performed immunostainings for CD34, c-kit (CD117), Flk-1 (VEGFR-2) and CD133, molecules known as being expressed by progenitor cells. Some studies also define globally the meninges as a stem cell niche[21], so we tested this hypothesis on PACs using CD133, CD45 and NOTCH-1 staining[22], in conjunction with the rest of the panel of antibodies already mentioned (Flk-1, CD34, or c-kit). Nestin is also mentioned as being present in the meninges, which were considered as being a source of stem cells[23], therefore we also stained the tissue for this marker.

For the potential inclusion of PACs in the pericyte category, we used markers currently described as being expressed by this cell type[24], as α-smooth muscle actin, nestin, vimentin, and platelet derived growth factor receptor-beta (PDGFR-β), respectively. All these are currently used to identify pericytes[25]. Also, desmin and alanyl-aminopeptidase (CD13) are both mentioned as useful pericyte markers[26].

Since PACs appear as exceedingly elongated cells, their possible relationship with the so-called telocytes[27-35] could not be ignored. To test this hypothesis, positive staining for c-kit protein (CD117), CD34, PDGFR-α, CD63, smooth muscle actin and vimentin would have been considered as significant, even though some of them are also expressed by pericytes. In this case, the cell shape and localization as well as its ultrastructural details were taken into account.

CD34 is a transmembrane protein expressed mostly on hematopoietic progenitor cells but also on several primitive pluripotential stem cells[36]. Since it labels the majority of gastrointestinal stromal tumors (GIST)[37] which seem to originate in telocytes, we also used it as a marker in our series. CD117, also known as c-kit, is a receptor tyrosin kinase protein encoded by KIT gene, expressed on multipotent progenitor cells and hematopoietic stem cells. It is mentioned as being useful for identifying vascular stem cells[22], while also identifying reliably the mast cells[38]. Both CD34 and CD117 are also considered as markers for telocytes[29]. Flk-1 (VEGFR-2) is a receptor tyrosin kinase expressed on progenitor cells, with potential role in vasculogenesis and neurogenesis[39]. CD133, on the other hand, is currently used for identification of stem cells in both normal and tumor tissue[40]. CD45, also known as common leukocyte antigen (CLA), is a membrane glycoprotein expressed mostly on leukocytes, as its name suggests, but also in circulating cancer stem cells[41] and in the vessel wall stem cells[22]. Nestin, an intermediate filament, is expressed in various cell types, including mostly multipotent neural stem cells[42] but also in endothelial stem cells, even though it seems not to be present in mature vasculature[43]. Notch-1 is a transmembrane receptor mentioned as marker for vascular stem cells[22], being involved in stem cell processes as survival, differentiation or proliferation[44].

CD63 is a tetraspanin protein related to exosomes[45]. Caveolin-1, also known to be present in telocytes[46], was tested in our panel of antibodies.

Since some studies included these vascular coating cells (probably PAC) in the category of pial cells, without supplementary specifications[47], we also decided to compare the immunoprofiles of the two categories (PAC and pial cells) in order to see if identity is found.

PATIENTS

We analyzed the structure of cerebral vessels in a series of 20 patients, 13 men (65%) and 7 women (35%), ranging from 44 to 85 years old (mean 65.35) deceased in the neurology and neurosurgery departments of our institute. Informed consent was obtained in each case, from the patients or their relatives. The work was approved by the Ethic Committee of the institute according to international requirements. Complete autopsies were performed in each deceased patient. The interval between death and tissue sampling varied from 6 hours to 36 hours. The major inclusion criterion was the

macroscopic integrity of the brain surface and leptomeninges at macroscopic examination. Cases were excluded when presenting cerebral pathological conditions which could significantly change the structure of the brain and leptomeninges, as infarcts or hemorrhages with superficial involvement, tumors or neurodegenerative diseases.

After a few weeks fixation in 10% buffered formalin, the brain was sectioned in each subject in 1 cm wide slices. Previously, in each case we harvested the whole leptomeningeal coating one hemisphere and embedded it in paraffin as a single block. We worked on archival paraffin tissue blocks prepared in this manner and retrieved from the archive of our institute. Both the arachnoid blocks as well as those with brain fragments taken randomly from cortical areas of the frontal, parietal, and occipital lobes were sectioned and stained with Haematoxylin and Eosin for microscopic examination. All available clinical and pathological data (resulting from autopsy specimen examination) were retrospectively examined. Separately, in two patients undergoing surgical procedures for meningioma ablation (females, aged 50 and 65 respectively), small fragments of vessels and adjacent nervous parenchyma were sampled for electron microscopy.

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

All samples processed for histopathology were analyzed for the expression of several immunohistochemical markers. Two micrometer-thick sections were obtained from the previously histologically examined paraffin blocks. Each section was deparaffinized and hydrated in graded ethanol concentrations. Heat induced antigen retrieval was obtained with the buffer indicated by each antibody supplier for 30'. The slides were then treated with 3% hydrogen peroxide for 20' at room temperature to block endogenous peroxidase activity. Tissue slides were incubated with primary antibodies solution overnight at room temperature, followed by two PBS (phosphate buffered saline) changes. The list of primary antibodies used is shown below (see the table).

Epitope	Source	Dilution
Vimentin	Dako, Glostrup, Denmark	1:50
Desmin	Dako, Glostrup, Denmark	1:50
TE7	Merck, Millipore, Billerica, CA, USA	1:50
CD13	Leica Biosystems, Newcastle upon Tyne, UK	1/100
PDGFRα	NeoMarkers, Fremont, CA, USA	1:40
Laminin	Sigma-Aldrich, St Louis, MI, USA	1/1000
Nestin	Santa Cruz Biotechnology, CA, USA	1:50
DDR1	Santa Cruz Biotechnology, CA, USA	1:50
DDR2	Santa Cruz Biotechnology, CA, USA	1:50
αSMA	Dako, Glostrup, Denmark	1/100
S100 protein	Dako, Glostrup, Denmark	1/500
CD63	Leica Biosystems, Newcastle upon Tyne, UK	1:50
CD117	Dako, Glostrup, Denmark	1/400
Caveolin	Santa Cruz Biotechnology, CA, USA	1:50
CD45	Leica Biosystems, Newcastle upon Tyne, UK	1/100
NOTCH-1	Santa Cruz Biotechnology, CA, USA	1/100
CD133	MyBioSource, San Diego, CA, USA	1/500
Collagen type IV	Leica Biosystems, Newcastle upon Tyne, UK	1/100

The reaction was visualized using Poly-HRP-GAM/R/R IgG detection kit for 30' (Immunologic, Duiven, The Netherlands) using 3,3'-diamino-benzidine, followed by counterstaining with hematoxylin. Negative control was obtained by omitting the primary antibodies and replacing them with PBS (phosphate buffered saline). Positive control was also available for each antibody according to manufacturer's instructions.

Images were taken using a system with an Olympus B51 light microscope with attached Olympus SP-350 camera, while being further acquired, measured and annotated using the QuickPhoto MICRO 2.2 software (PROMICRA, Prague, Czech Republic). We examined all category of vessels – arteries, arterioles, veins, venules, capillaries – in the subarachnoid space, on their trajectory entering the brain and within the nervous parenchyma. Focus was set on arterial and arteriolar vessels, since veins, venules and

capillaries possess a vascular wall too thin to enable a correct identification of a distinct adventitia or its structural components. Cells located at the vascular periphery, at the outer limit of adventitia, were our target, regarding their shape, distribution, and immunophenotype (Fig. 1). We described these cells as periadventitial cells (PACs).

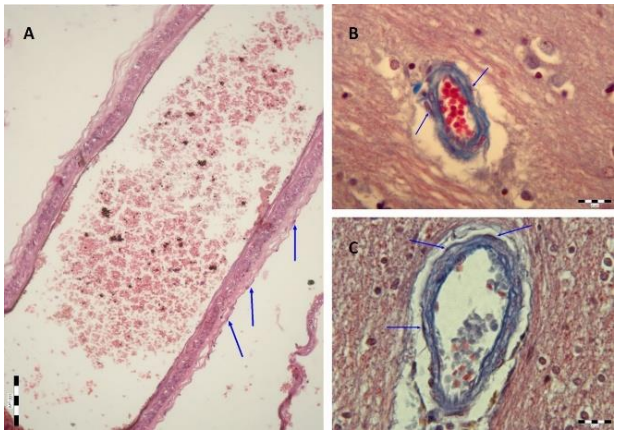

Figure 1. In (A), a large artery (440 µm in diameter) within the subarachnoid space is cut longitudinally. Several elongated cells, whose nuclei are highlighted by arrows, are coating the vascular surface. (B) and (C). High power view of two intraparenchymal arteries, at cortical-subcortical limit (B) and within the white matter (C) show the same elongated cells at their surface; arrows point their nuclei. (Hematoxylin and Eosin – (A). Original magnification 200×; Mallory trichrome – (B), (C). Original magnification 1000×).

We graded the immunostaining extension and intensity as following: 0 for no reaction; 1 for isolated cells around the vessel circumference showing strong positivity or alternatively, a weak positivity on most of the same section area; and 2 for strong, diffuse staining of all or almost all these cells. Since all antibodies were supposed to be expressed in a cytoplasmic or membranous manner (no nuclear ones were used), these were the expressions we considered in our examination. Separate examination was performed for subarachnoid arteries/arterioles, intraparenchymal arteries/arterioles and pia cells.

ELECTRON MICROSCOPY

In the two cases of patients undergoing surgery, the fresh tissue composed of small fragments of nervous parenchyma and small vessels adjacent to the lesion were harvested and immediately immersed in glutaraldehyde. Transmission electron microscopy was performed on small (1 mm^3) tissue fragments processed according to a routine Epon embedding procedure. Thin sections (50–60 nm) were examined with a Morgagni 286 transmission electron microscope (FEI Company, Eindhoven, The Netherlands) at 80 kV.

RESULTS

The histological findings were uniform across the whole series, in all regions of brain and within the entire leptomeningeal tissue, regardless of the patient's age or subjacent pathology.

The periadventitial cells (PACs) within the subarachnoid space were strongly positive for vimentin and collagen IV, α-smooth muscle actin, PDGFRα, and PDGFRβ. Vimentin showed strong positivity in the cytoplasm of almost all PACs coating arteries of all calibers within the subarachnoid space, as well as the muscular layer (Figs. 2 and 3).

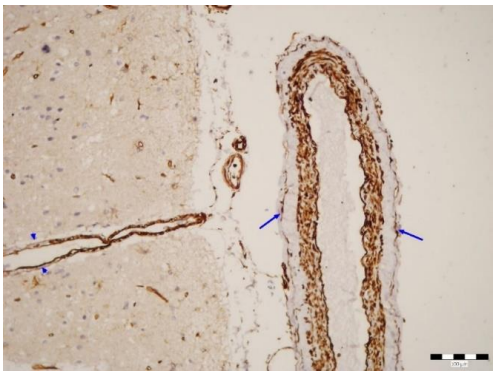

Figure 2. Vimentin is expressed almost in a continuous manner in PACs of a subarachnoid large artery. PACs are located in peripheral position or embedded in the adventitial collagen layer (arrows). A perforating artery also have a separate coating layer with positive staining for vimentin (short arrows). (Vimentin immunostaining, 200× original magnification).

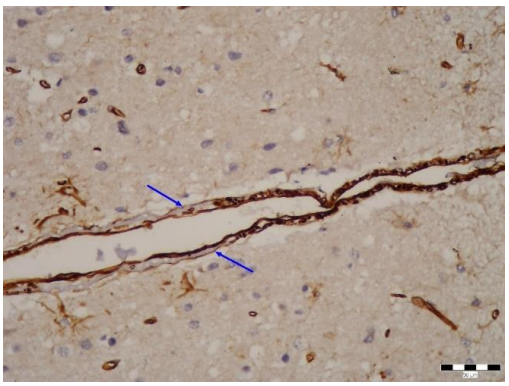

Figure 3. Details of an intraparenchymal artery sectioned longitudinally. PACs are obvious at the external border of the vessel (arrows). (Vimentin immunostaining, 400× original magnification).

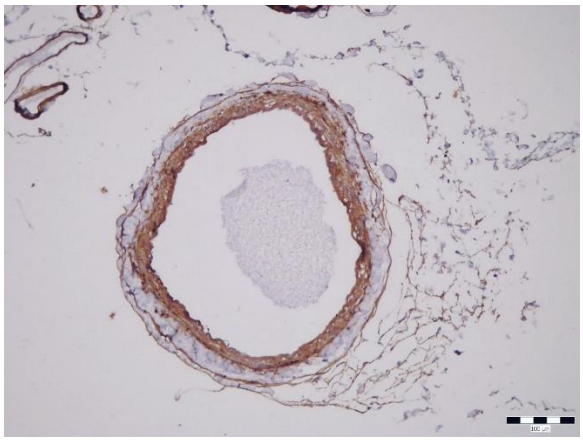

Figure 4. Collagen type IV is also expressed in all PACs around a large artery from the subarachnoid space, in a single or double layer at this point of section (Collagen IV immunostaining, 200× original magnification).

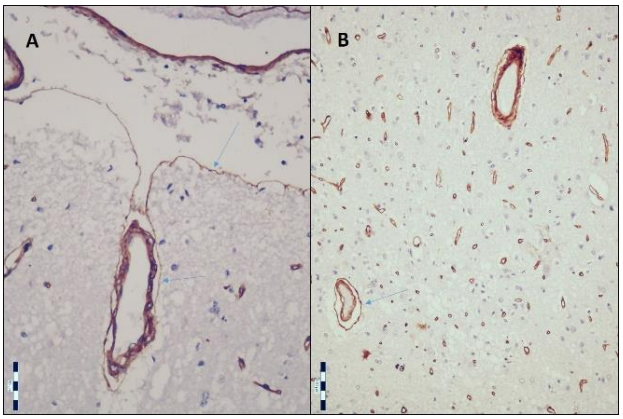

Figure 5. Collagen IV is also expressed at intraparenchymal level, but seems to represent a pial coating. (**A**) – pia mater (arrows) is following a penetrating artery. **(B)** – two sections of arterial profiles show the same coating positive for collagen IV (arrow). (Collagen IV immunostaining, 400× original magnification – (**A**); 200× original magnification – (**B**).

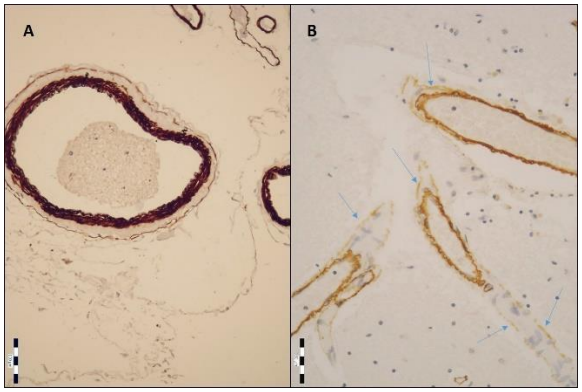

Figure 6. Smooth muscle actin is expressed in an almost continuous layer in PACs of a subarachnoid large artery (**A**). In (**B**), several arterial profiles within the brain parenchyma show actin expressed mostly with a granular pattern, either coating the vessel wall or slightly separated from it (arrows). (α-smooth muscle actin immunostaining. 200× – (**A**), and 400× – (**B**), original magnifications).

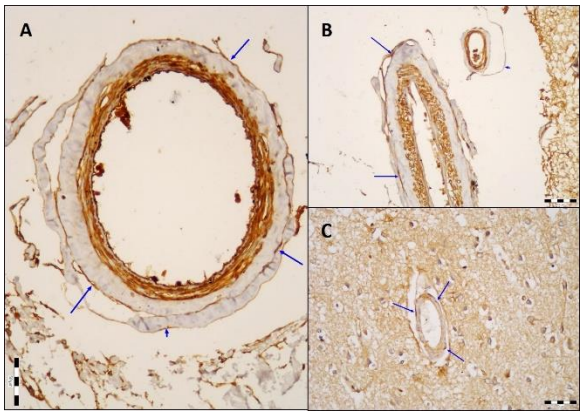

Figure 7. (**A**). PDGFRα is strongly expressed in an almost continuous layer in PACs coating a 300 µm artery of the subarachnoid space, mostly embedded in a thick collagen matrix (long arrows) but also at the outmost periphery (short arrow). (**B**) shows almost the same aspect (long arrows). However, a smaller arterial branch is negative, as is the pia adjacent to it (short arrow). (**C**) shows an intraparenchymal arterial branch with only scattered PACs showing positivity. (PDGFRα immunostaining, 400× original magnification).

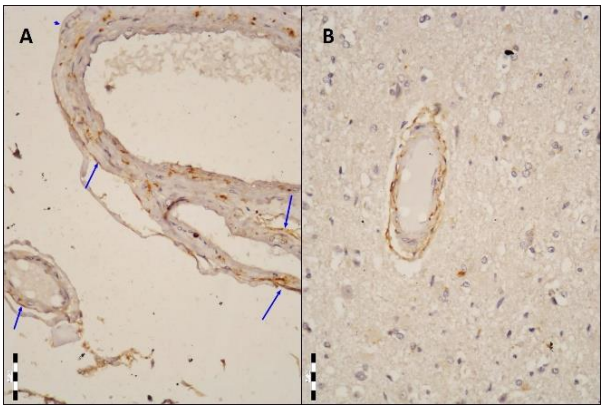

Figure 8. (**A**). CD133 is expressed in several PACs, coating larger arteries in the subarachnoid space (long arrows). However, many peripheral areas are devoid of positivity (short arrow, up left). In (**B**), CD133 shows a more granular positivity in PACs of an intraparenchymal arterial branch (CD133 immunostaining, 400× original magnification).

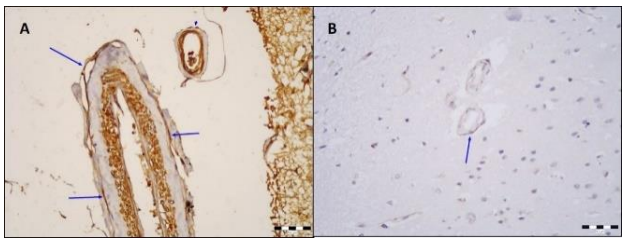

Figure 9. (**A**). PDGFRβ expression is seen almost in the same strong manner as that of PDGFRα (long arrows). Here, PDGFRβ is also expressed in PACs of the smaller artery (short arrow). Pia mater is still negative. (**B**). Two small arterial intraparenchymal profiles express PDGFRβ in a zonal manner (PDGFRβ immunostaining, 400× original magnification).

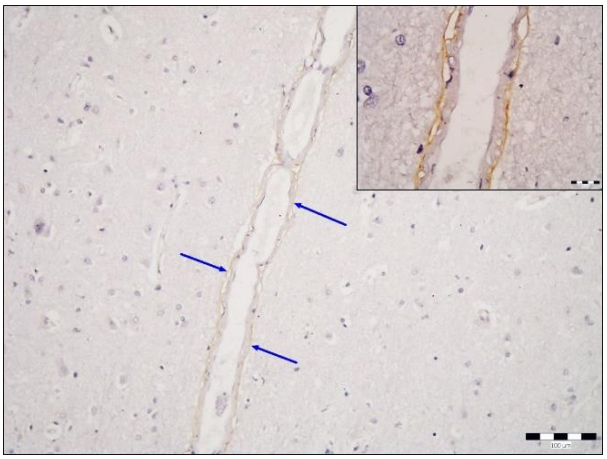

Figure 10. TE7 is expressed in an almost continuous pattern in PACs of a perforating artery (arrows). Inset: detail of positive cells coating the vessel. (TE7 immunostaining, 200× original magnification, inset – 1000× original magnification).

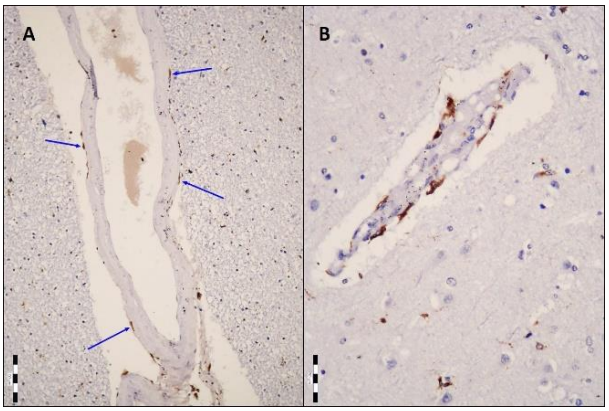

Figure 11. (**A**). The expression of CD68 is restricted to limited areas of PACs on a subarachnoid artery (arrows). (**B**). Within the parenchyma, several PACs express CD68, with a granular pattern, mostly adjacent to the nucleus (CD68 immunostaining, 200× original magnification – (**A**), 400× original magnification – (**B**).

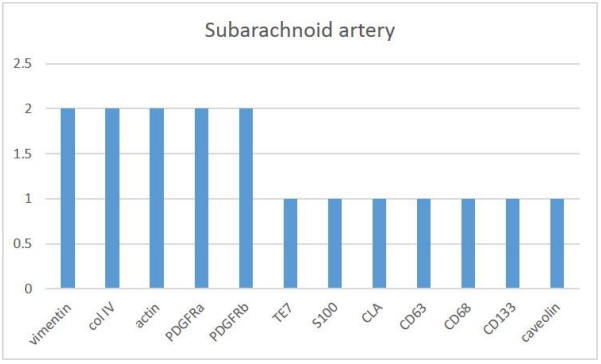

Figure 12. The overall reactivity of PACs in the subarachnoid space. Maximal staining was obtained for vimentin, collagen type IV, α-smooth muscle actin, PDGFRα, and PDGFRβ. (Abbreviation: sub=subarachnoid).

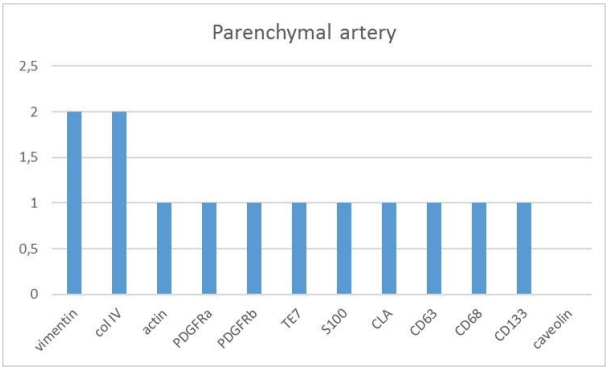

Figure 13. The reactivity of PACs in the cerebral parenchyma. Unlike the superficial vessels, the intraprenchymal ones were strongly positive only for vimentin and collagen type IV.

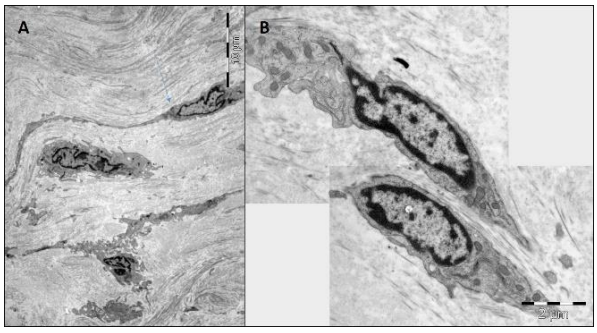

Figure 14. Ultrastructural details of PACs. In (**A**), the elongated cell is visibly embedded in a dense collagen matrix (arrow). In (**B**), the image is composed of two sections, showing the nuclear and perinuclear details of PACs, with numerous mitochondria and sparse endoplasmic reticulum. No obvious junctions are visible with the surrounding extracellular structures (Electron microscopy).

Collagen type IV was also strongly positive in the cytoplasm of PACs at this level, the cellular processes giving an almost continuous pattern, organized on one or two layers, outside and within the collagen coating of the arterial wall (Fig 4). The same profile was obtained with α-smooth muscle actin (Fig. 6 A), PDGFR-α (Fig. 7 A, B), and PDGFR-β (Fig. 9 A).

A weaker staining was seen for CD133, TE7, CD45 (CLA), CD63, caveolin and S100 protein. As an example, CD133 positivity stained only scattered PACs coating large arteries, while large areas were devoid of positivity (Fig. 8 A). CD68 was expressed only by rare PACs, coating the arteries within the subarachnoid space, in a strong manner, but limited to the cell body, adjacent to the nucleus and apparently sparing the cellular processes (Fig. 11 A).

For PACs located inside the brain parenchyma, the strongest reaction was obvious for collagen IV and vimentin, whereas PDGFRβ, TE7, PDGFRα, α-smooth muscle actin, CD45, CD63, CD133 and S100 protein were only mildly expressed. A

representative picture of vimentin expression of PACs within the parenchyma is showed (Fig. 3).

For the collagen type IV expression within the parenchyma, representative images are shown in Figure 5. However, it was obvious that pia mater is strongly positive for this marker and that the coating layer around perforating arteries at cortical level is composed of an extension of this pia material (Fig. 5 A). Arterial profiles in more profound zones of the brain keep the same structure, with a coating layer at certain distance from the arterial wall, probably determined by a certain degree of cerebral edema, present in almost all cases, due to their subsequent pathological states (Fig. 5 B). For α-smooth muscle actin, the reaction of PACs was different at this level, with an obvious coating layer apposed on the arterial wall and local separation in other areas (Fig. 6 B). Almost the same aspect was visible for CD133, with the important difference that the staining was more granular, dispersed within the cytoplasm of PACs (Fig. 8 B). PDGFRα (Fig. 7 C) and PDGFRβ (Fig. 9 B) were expressed in rare, scattered PACs around small arteries within the parenchyma. The fibroblast marker TE7 was present as a continuous layer around the intraparenchymal arteries, practically surrounding the vessels (Fig. 10). CD68 expression was more consistent at this level, with numerous PACs showing strong positivity, although in a discontinuous arrangement (Fig. 11 B). No reaction was present for CD13, CD34, CD117, FLK1, NOTCH-1, DDR1, DDR2, nestin, laminin or desmin, in either of the two vascular locations, while maintaining varying positivity in other cells, as endothelium, smooth muscle cells, scattered neurons or astrocytes (internal control). As a consequence, we ignored all these positive cells and considered only our targeted structures. Caveolin-1 showed a non-specific staining on all arterial structures in the subarachnoid space, and no positivity within the parenchymal arteries. S100 protein, in exchange, was positive in all arterial structures and pia mater, thus we decided not to take into account both reactions (not shown). The overall immunophenotype in our series is summarized in Figures 12 and 13.

The ultrastructure of PACs showed elongated shapes, with extremely long processes, cytoplasmic organelles but lacking conspicuous membrane junctions with the extracellular matrix (Fig. 14).

DISCUSSION

We tried to evaluate the peripheral adventitial cells (PACs) surrounding the brain vessels from an immunohistochemical and ultrastructural point of view. Subarachnoid PACs were only weakly positive for CD133, S100 protein, and the fibroblast marker TE7. They showed strong positivity for vimentin, collagen type IV, α-smooth muscle actin, PDGFRβ, and PDGFRα. PACs around smaller arteries within the parenchyma were weakly positive for CD133, S100 protein, smooth muscle actin, PDGFRβ, PDGFRα, and TE7, and strongly only for vimentin, and collagen type IV. On the other hand, pia cells were weakly positive for CD34 and S100 protein, while showing strong staining with antibodies against vimentin and collagen type IV.

No positive reaction of PACs was seen for nestin, CD13 or desmin. These antibodies are considered as being characteristic markers for pericytes[24-26]. However, their expression within the central nervous system is not strictly limited to a certain cell, being dynamically regulated as a consequence of various injuries and also a shift between cell populations at this level is possible[26]. As an example, fibroblast markers can be expressed by pericytes[48]. Many cell types, including fibroblasts and smooth muscle cells, express both PDGFRα and PDGFRβ. PACs expressed in both locations these two molecules, therefore a certain mix of fibroblast and smooth muscle cell immunoprofile (myofibroblast) is probable for them, furthermore reinforced by the strong reaction for α-smooth muscle actin.

Globally, despite their elongated phenotype, the meningeal cells of the pia mater and those ones surrounding the brain vessels – PACs – were phenotypically various in our study. To summarize, they expressed almost equally markers for fibroblasts (TE7, collagen IV), pericytes (α-smooth muscle actin, PDGFRβ), telocytes (PDGFRα, α-smooth muscle actin) and less for progenitor cells (CD133). Vimentin was also strongly expressed, but without specificity, since it is considered as a common element for all these cell categories. The strong reaction of PACs to PDGFRα in our study could only suggest a certain relation of these cells to telocytes, which are known to express this marker[49,50]. On the other hand, CD34 and CD117, considered as the essential markers for characterizing telocytes[51,52] were negative in our study.

The strong expression of PDGFRα suggests a certain involvement of these cells in angiogenesis by recruiting other perivascular cells and proliferation if needed, through the action of PDGF[53]. We found α-smooth muscle actin to be positive in multiple PACs, whereas pia mater was completely negative for this molecule. This suggests that PACs are not an extension of the pia layer, even though former structural studies (based only on ultrastructural examination) considered the two cell populations as a continuum[54].

Also, the positivity for α-smooth muscle actin is described as being present in telocytes, a common feature shared by pericytes[51]. Telocytes are described at vascular level only in medium sized arteries, and limited to the endothelial surface of the vessel, not as adventitial cells[55]. At cerebral level, they were detected in the meninges within dura mater (at least in dogs), close to capillaries[35]. Since telocytes are still difficult to be characterized in terms of immunophenotype[56], the cells we identified as PACs could be conceivably described as telocytes (according to their ultrastructural features), but exhibiting a particular phenotype, supposedly demanded by local structural and functional conditions. PACs could also be involved, at this level, in modulation of the local homeostasis, as a rudimental second nervous network[32].

The putative scavenger role of perivascular cells was affirmed in older studies and on animal models[57]. To verify this, we performed CD68 staining, with inconclusive results. This was because the vast majority of meningeal and parenchymal PACs were devoid of CD68 reactivity, except for some very rare oval shaped cells, suggesting possibly perivascular macrophages. Some studies found that pericytes are able to transform and migrate as microglia within the nervous parenchyma[58] and that they are actively involved in the immune processes of the brain[59]. In this regard, the presence of CD68 positive cells in our series, in both the vascular periphery (only as macrophages) and within the adjacent parenchyma (as activated microglia) has not confirmed this potential association. The role of some perivascular cells in local repairing mechanisms as well as their presumed potential of motility[60] remain, however, under debate until much larger series of patients will be examined with more complex methods.

On the other hand, the structure and function of pia mater itself are still not conclusive. Early studies using cell cultures speculated the potential identity of pia and arachnoid cells, since not normal but tumor (meningioma) cells were used[61]. Ultrastructural studies on human meninges rarely supplemented by rudimental immunostaining[62] affirm that the coating of cerebral vessels is composed of pia mater[54,63,64]. The perivascular space between pia mater and the brain vessels is considered as being critical in the process of brain metastasis[65]. This perivascular compartment is also thought to be a way for lymphatic drainage of the brain[66]. PACs could play a regulatory role in this unusual transport system of brain fluids. This could occur in conjunction with the extremely complex elements composing the vascular matrisome (extracellular matrix and associated proteins)[67].

PDGF activity is conditioned by the presence of its receptors PDGFR-α and PDGFR-β, respectively, inducing angiogenesis and modulating the proliferation and recruiting of perivascular cells[53]. Since PACs in the subarachnoid space expressed both markers in our study, a certain potential for angiogenetic activity could be presumed, demanded by pathological situations as ischemic or tumoral conditions.

Our study has some shortcomings, such as a relatively narrow age category of subjects. Extreme age category as very young (*i.e.* infant cases) or near centenary ones would have been necessary to compare the overall phenotypic expression of PACs. However, no significant differences were visible among the cases included in our series, regardless their age. Another potential bias generator is the variability in the tissue processing steps. Such parameters, as postmortem delay, different fixation times, and fixative concentration, paraffin embedding materials and temperature, which are not usually standardized[68] could affect the immunohistochemical reactivity of specific cell types from a case to another[69]. However, in our series there was a remarkable uniform immune-phenotype among the same cell type within the same location (arterial coating), regardless of all these potentially confounding factors.

CONCLUSIONS

Peripheral cells of the adventitial layer possess a complex phenotype, suggesting their potential inclusion in several cellular categories: fibroblasts, pericytes and possibly telocytes. Overall, they are most probably myofibroblasts with limited stem cell potential and strong angiogenic capabilities. A remarkable uniformity of these findings was

obvious regardless the age, pathological state among subjects or region of the brain where the vessels were located. Their scavenger role was not confirmed. With certainty, the arteries and arterioles within the brain parenchyma seemed to be surrounded by an extension of pia mater, while those ones within the subarachnoid space were not, even though the shape of their coating cells was almost similar within the two locations.

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