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TELOCYTES IN THE HUMAN BRAIN TUMOR. INFRASTRUCTURE AND RELATIONSHIPS OF THE TELOCYTES INSIDE OF THE BRAIN TUMOR

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Accepted July 4, 2022

In this paper we investigated by Transmission Electron Microscopy telocytes inside of the human brain tumor. We identified/detected telocytes and described their polymorphic infrastructural aspects as well as the homo- and heterocellular junctions established by this very peculiar cell phenotype in the human brain affected by different types of tumor. Moreover, we report about the ability of the telocytes to generate and to deliver extracellular vesicles as exosomes considered being involved in cell signaling. Our Transmission Electron Microscopic investigations clearly demonstrate that by their homo- and heterocellular communications performing a kind of 3-Dimensional network, and moreover by their ability to deliver extracellular vesicles, telocytes offer a genuine cell-to-cell communication system inside the human brain. In spite of the fact that extensive brain areas are severely altered by tumor growth and hemorrhagic aspects are visible, inside of the tumor stroma a plenty of telocytes still keeping their homocellular junctions between adjacent telopodes aspect suggests to offer to some extent a kind of mechanically protection against aggressive development of the tumor brain. We emphasize a very paradoxically aspect concerning presence of the glial cells like Schwann cells embracing the brain axons but exhibiting a solid basement membrane, knowing that inside of the central nervous tissue the only glial cell type habillited to ensure neurons' insulation by myelin enwrap is the oligodendrocyte never able/described to generate a proper basal lamina. All together data resulted from our results strength the conclusion that telocytes is a very peculiar cell phenotype requiring our attention and worth to be investigated as multifaceted cell type, for better understanding their putative roles in the human brain.

Keywords: telocytes, tumor human brain, homo- and heterocellular junctions, exosomes, microvasculature.

INTRODUCTION

Brain is the most delicate organ of human body. Like any other body tissue, nervous tissue can be affected by congenital malformations, traumatic injuries, progressive degenerative diseases, benign or malignant tumors. There are few diseases like encephalitis, multiple sclerosis, neurological disorders, stroke and tumors able to induce severe deterioration of brain function. Brain tumors encompass two major classes: primary brain tumors that start in the brain and secondary brain tumors that are generated by the cancer cells that migrated from tumors developed in the other parts of the body. Brain tumors may grow from the brain tissue itself (glioma) as well as from nerves (neuroma), dura (meningioma), or pituitary gland (craniopharyngioma or pituitary adenoma)[1].

Whether a brain tumor is benign, malignant, or metastatic, all are potentially life-threatening, especially because the rigid bony skull does not allow any room for the developing tumor mass inside of the brain so that tumor compress and displaces normal brain tissue and consequently, possible to cause a blockage of cerebrospinal fluid and increase intracranial pressure as well as extensive areas of edema.

In spite of the fact that in the Central Nervous System (CNS) glial cells as number is approx. 10:1 neurons, nerve cells as volume including their dendrites and axons represent the most of the cellular volume of the brain.

Drug treatment of the brain tumors remains a great challenging task because the presence of

blood-brain-barrier (BBB). The degree of BBB disruption differs from the malignancy of the brain tumor so that chemotherapy of low-grade brain tumors is very difficult, because of the presence of almost intact BBB[1].

Now is generally accepted that together with any other more or less sophisticated light microscopy and molecular modalities to study and establishment of precise diagnostic criteria for a large variety of benign and malignant tumors, Transmission Electron Microscopy (TEM), especially when is used as immune electron microscopy (IEM) alone or combined with in situ hybridization at the ultrastructural level (ISH-US) remains a powerful tool to gain valuable knowledge about infrastructural aspects of tumor initiation and growth, especially about subtle relationships between tumor cells and peritumoral stroma[2-4].

Recently published papers emphasized roles of the tumor microenvironment in the cancer initiation, tumor development and progression[5-12]. How to define accurately the tumor microenvironment is difficult but generally accepted terms include (1) resident or transitory stromal/interstitial cells as well as recently discovered telocytes (TCs), and (2) extracellular matrix represented mostly by acellular stroma (collagen fibrils etc.), a plenty of extracellular molecules as growth factors, cytokines/chemokines, including extracellular vesicles as cargos of bioactive molecules involved in cell signaling. Cellular component of the tumor microenvironment contains non-neoplastic cells in large amount represented in different proportions by endothelial cells and associated pericytes (PCs), fibroblasts, immune cells, stem-like cells. Telocytes (Tcs) as an important component of the tumor microenvironment become in focus for many laboratories so that there are some reports about their putative roles in tumor behavior[5,7,13-17].

When discuss about malignant cells-tumor microenvironment cross-talk, direct contacts of some stromal cells, including telocytes, with neoplastic cells is of a great interest and should be explored, as well as special attention must be paid to the soluble messengers[5,18].

To our best knowledge, there is a paucity of studies concerning the telocyte (Tc) as stromal/ interstitial cell population in different brain alterations, including brain tumors. In this context, here we report about our preliminary results concerning peculiar ultrastructural aspects in some human brain tumor types with focus on the telocytes detection, especially about their abilities to develop homo- and heterocellular junctions as well as to deliver extracellular vesicles, suggesting their putative roles in brain pathology.

TELOCYTES. DEFINITION AND IDENTIFICATION IN ALMOST TISSUE TYPES

Telocyte is a distinctive phenotype of interstitial cells exhibiting a peculiar infrastructural aspect identified in almost tissues and organs, which have a wide range of biological functions[7,9-11,13,19-21].

TCs occupying a strategic position were detected in close relation mostly to blood vessels, nerve endings, stem cell niches, smooth muscle cells and participate in inter-cellular signaling, tissue remodeling, renewal and regeneration[7,22-24].

Very important data in biomedicine research emphasize the major role played by the stromal/interstitial cells in the parenchymal cells destiny: cytodifferentiation, histogenesis, tissue physiology, apoptosis, ageing, degenerative diseases, regeneration, malignant cell transformation etc. Stromal cells are identified as a heterogeneous population of resident or transiently cells mostly of mesenchymal origin represented by fibroblasts/fibrocytes, pericytes, telocytes usually associated with tissue microvasculature[6,7,9-11,13,25].

Almost all published paper about telocyte concerning their 3-D morphology showed that Tcs undergo phenotypic changes related to the temporal-spatial and functional needs of different tissues and organs. Moreover, by their abilities to send as well as to receive cell-signals from altered tissue, Tcs, especially those located in close proximity or even inside of a stem cell niche are involved in tissue regeneration[7,14,26,27].

Telocytes are stromal cell population detected in almost normal or altered tissues represented by variable phenotypes depending on the location and temporal-vectorial requirements of that tissue. Because to date is still difficult to characterize telocytes in terms of immunophenotype, Transmission Electron Microscopy (TEM) remains the gold standard method to identify the real telocytic phenotype. Telocytes have a small cell body with an ovoidal euchromatic nucleus (clusters of heterochromatin are attached to the inner membrane of nuclear envelope) and scarce cytoplasm around nucleus filled with some profiles of endoplasmic rough reticulum and Golgi apparatus that abruptly gives rise to a variable number of extremely long and slender moniliform cytoplasmic prolongations called telopodes. Moniliform appearance of telopodes is conferred by the irregular alternation of slender segments, termed podomers, and small cistern-like dilations, referred to as podoms, which accommodate few organelles such as mitochondria, endoplasmic reticulum cisternae and caveolae ($Ca2^{+}$ uptake/ release units).

By their telopodes, telocytes establish homo- and hetrocellular junctions forming a three-dimensional (3-D) network playing putative roles especially concerning cell signaling mainly when telocytes become in direct contact with tissue stem cell niches. Moreover, in this line, mention must be made that by their ability to form and deliver extracellular vesicles cargo telocytes may transfer different bioactive molecules between different distant cells inside of a tissue suggesting important roles in tissue engineering and regenerative medicine[7,18,22,28].

WHAT ABOUT THE TELOCYTE PHENOTYPE PRESENCE INSIDE OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM?

The central nervous system (CNS) is represented by the brain and the spinal cord. The peripheral nervous system (PNS) consists of the nervous ganglia and peripheral nerves.

Presence of telocytes as stromal cell type inside of the central and peripheral nervous systems generates a great interest so that some laboratories published their observations but we are still far to know especially telocytes' putative roles.

Beginning with the year 2000, Dănăilă[29,30] identified by TEM a morphologically elongated interstitial cell type termed cordocytes present inside of the human cerebrovascular tissue, later assimilated to telocyte phenotype[30-34].

Telocytes were detected inside of meninges and choroid plexus, both structures involved in modulation of brain function, described in direct contact with blood capillaries and putative stem cells suggested that telocytes might have a role during neurogenesis stages[31,35-37]. The choroid plexus is mainly involved in producing cerebrospinal fluid. Being the siege of different niches of cells as pluripotent hematopoietic cells, neuronal progenitors cells as well as for telocytes, the choroid plexus is a very special tissue on the CNS[38].

Using a large panel of immunohistochemical antibodies and TEM analysis to study the so called periadventitial cells (PAC) enveloping the arteries within the CNS Dănăilă and Arsene[34] concluded that PAC have a complex phenotype being assimilated to the fibroblasts, pericytes or even telocytes.

So far, there is a reduced number of studies concerning the telocytes as stromal cells inside of the human tumor brain. Mitrofanova *et al.*[14], reported the presence of telocytes inside of the glioblastoma, mostly in relationships with pericytes associated to the blood capillary, Tcs and Pcs being involved in glioblastoma neovascularization.

Using an immunohistochemistry combined with electron microscopy study concerning the human trigeminal ganglion, Rusu *et al.*[39], identified a perineuronal cell population with long and moniliform processes intermingled with satellite glial cells and reported to be considered as telocytes.

In the central nervous system oligodendrocytes are the glial cells wrap themselves around neurons and habilitated to generate the myelin sheath surrounding axons[40-42].

Concerning telocytes' detection inside the peripheral nerves, mention must be made that Mirancea *et al.*[5], Diaz-Flores *et al.*[43], Păiș and Păiș[32], Mirancea[7], Diaz-Flores *et al.*[44,45], reported about presence and presumptive roles of the telocytes in the normal and pathological peripheral nervous system.

MATERIALS AND METHODS

From a total of 17 patients with different tumors affecting human central nervous system investigated by TEM, here we reported about three patients with frontal brain tumor, one patient with a temporal brain tumor and a patient with meningioma developed in the ponto-cerebelar angle/flexure.

Biological materials represented by tumor and peritumoral nervous tissue were surgically prelevated from pacients affected by tumor brain with patients' agreement in accordance with ethical guidelines. Specimenes were processed for electron microscopy analysis following TEM protocols published elsewhere[3,46]. Briefly, in order to perform TEM investigations, small fragments of tissue (2-4 mm^3) were pre-fixed overnight in 4% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4 at 4°C. After post-fixation with 4% OsO_4 in 0.1 M cacodylate buffer for 2h at room temperature, and dehydration in a graded series of ethanol and infiltration with propylene oxide, samples were embedded with glycid ether and polymerized at 60° C for 48 hours.

After the sectioning was performed on the III LKB ultramicrotome with a glass knife, the ultrathin sections (70–90 nm thick) were double counterstained with UA-Zero non-radioactive EM Stain (Agar Scientific) for 20` and subsequently lead citrate (15`). All the prepared grid samples were analyzed using a JEM-1400 TEM (JEOL, Japan) operated at 80 kV accelerating voltage, and visualized with Quemesa CCD camera (Olympus Soft Imaging Solutions). In order to highlight the presence of TCs, several electron microscopic images were digitally colored.

RESULTS

Electron microscopic investigations showed different degrees of alterations concerning microanatomy of nervous tissue, mostly depending of the cell type origin involved in the brain tumor development. Indeed, more or less extensive areas of nervous tissue exhibiting nervous tissue displacement are hemorrhagic and/or edematous, sometimes fibrotic.

Inside of the brain tumor stroma numerous telocytes can be counted. When the plane of tissue cutting in order to obtain ultrathin sections for TEM analysis crosses the nucleus, then the whole telocyte with nucleus and its telopodes is visible (Figs. 1–3).

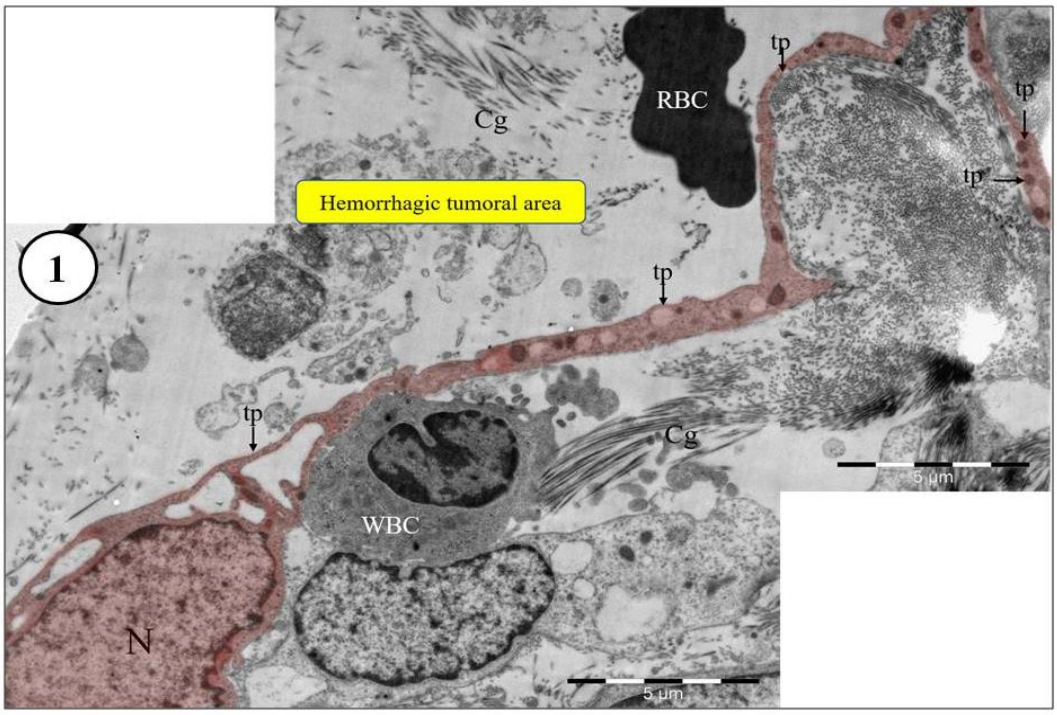


Figure 1. Inside of the brain hemorrhagic tumor stroma a nucleated telocyte (Tc) with a very long telopode (tp) is detected. Extravasated red (RBC) and white (WBC) blood cells are visible. N = nucleus. Cg = collagen. Scale bar = 5 µm. Temporal brain tumor.

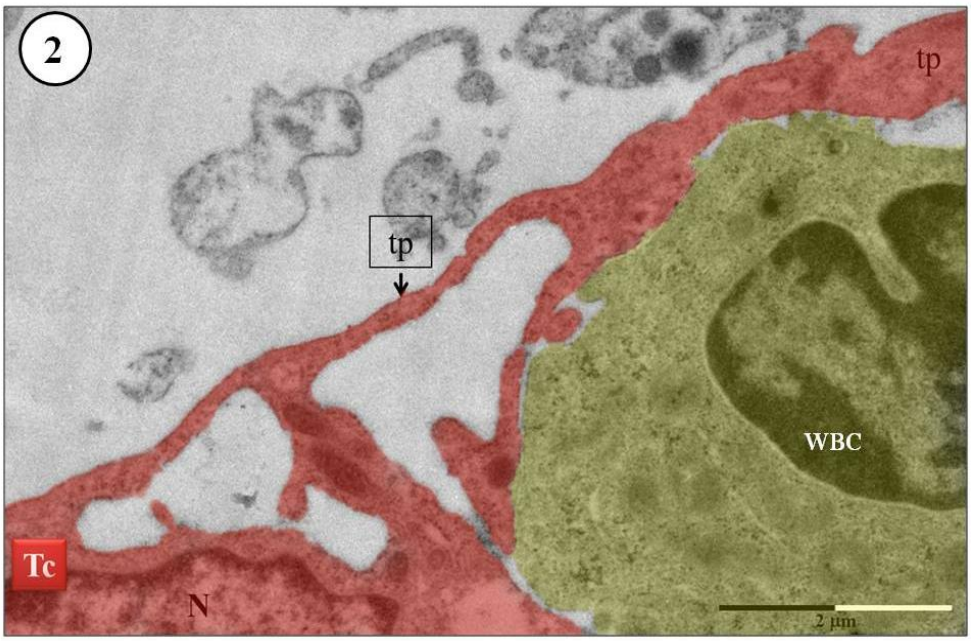


Figure 2. A detail from Fig. 1 shows the telocyte (Tc) in close contact with an extravasated white blood cell (WBC). N = nucleus. Tp = telopode. Scale bar = 2 µm.

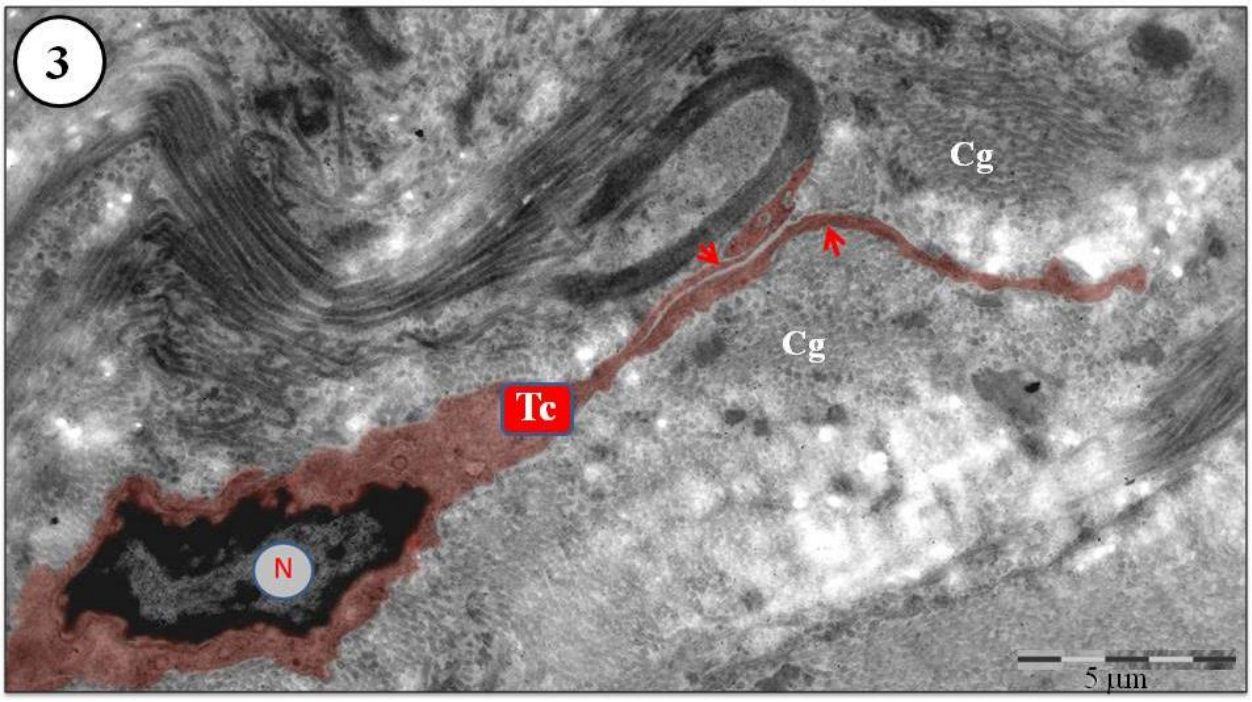


Figure 3. A nucleated telocyte (Tc) exhibits a long and bifurcated telopode (red arrows). N = nucleus. Cg = collagen. Scale bar = 5 µm. Meningioma.

Usually, one or more than 2-3 long profiles of telopodes can be detected inside of the fibrotic area of the tumor (Figs. 4–12).

Alternation of podomes and podomers is a general characteristic of the slender telopodes (Fig. 7) but some variability concerning this general rule is recorded as we described here. Sometimes, one telopode reoriented abruptly their growth axes and may perform a kind of loop running back parallel very closely with their long axes (Fig. 8).

Telocytes perform (1) homo- and (2) heterotypic junction formation. Two or more telopodes, for a short distance from their lengths become in very close contacts each other to perform so called homocellular junctions (Figs. 9–17).

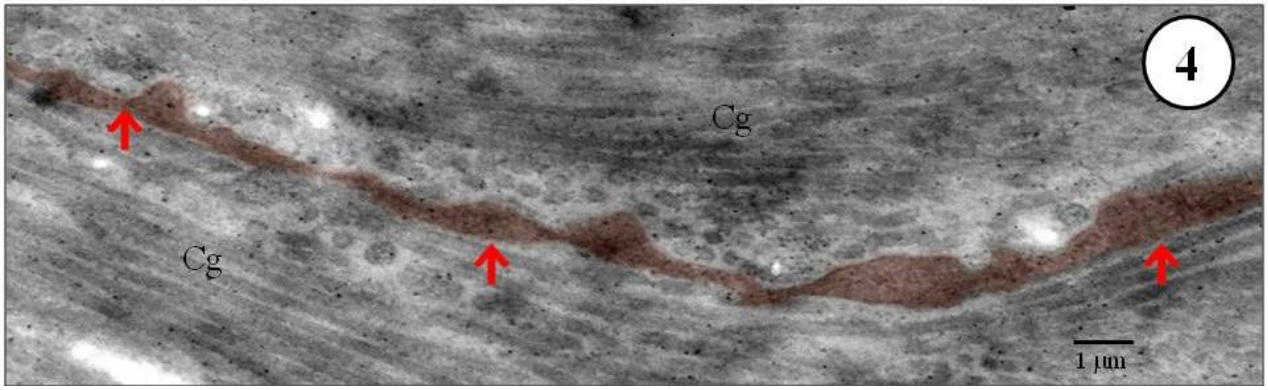
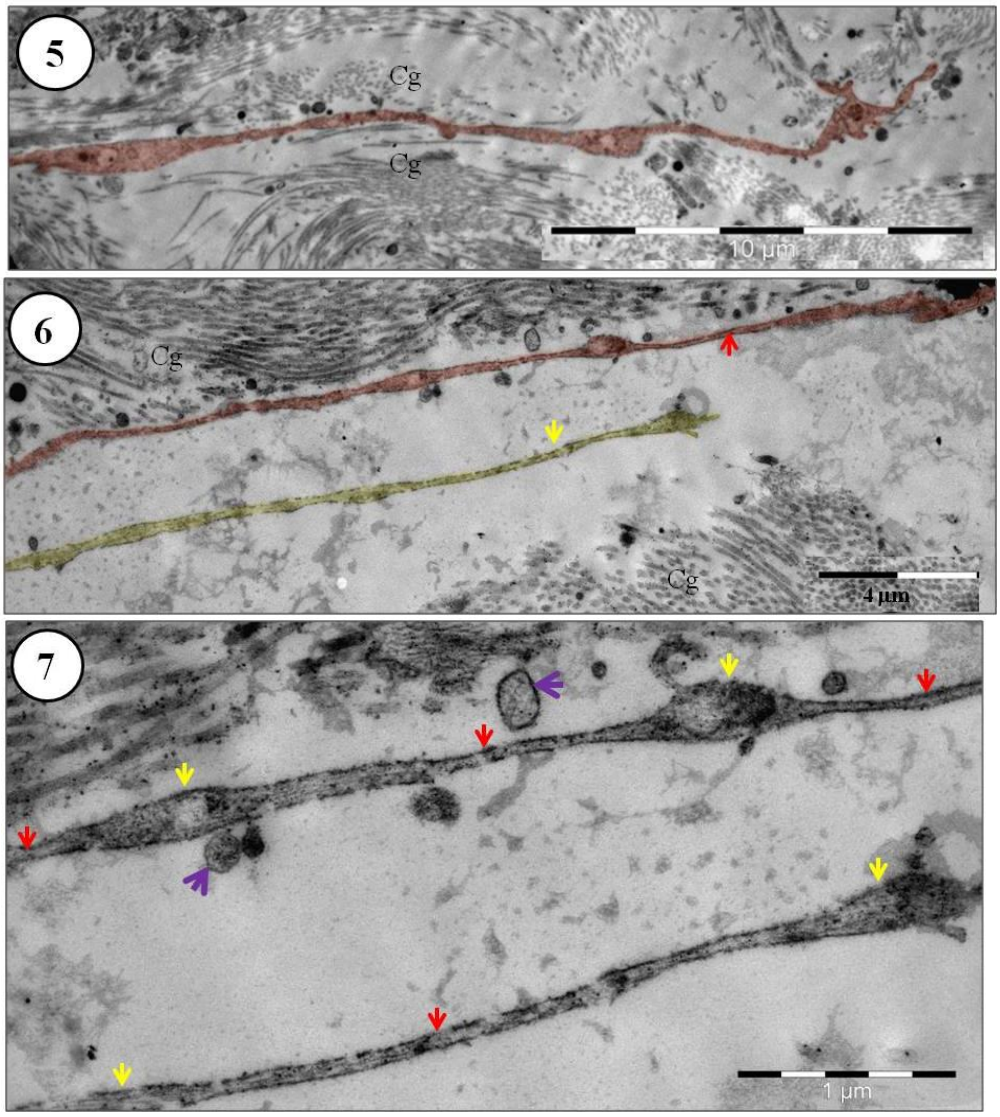


Figure 4. In a very fibrotic area, a long telopode (red arrows) with moniliform aspect is visible. Cg = collagen. Scale bars = 1 µm Meningioma.



Figures 5–7. Few slender telopodes inside of a fibrotic stroma exhibiting an alternation of podoms (yellow head arrows) and podomers (red head arrows) as is detailed in Fig. 7. Pink head arrows mark exosomes presumably delivered by a telopod. Cg = collagen. Scale bars: 10 µm in Fig. 6; 4 µm in Fig. 7; 1 µm in Fig. 8. Temporal brain tumor.

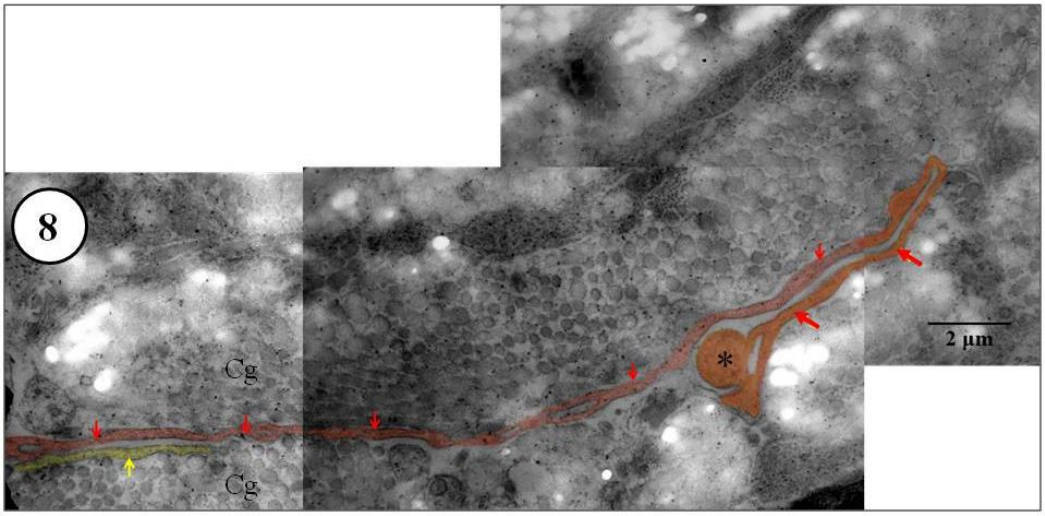


Figure 8.Two telopodes (red and yellow small arrows) run parallel each other for a distance. The longest one (red arrows makes an abruptly flexure, like an anse and endet with a sferic button (asterisck). Cg = collagen. Scale bar = 2 µm. Meningioma.

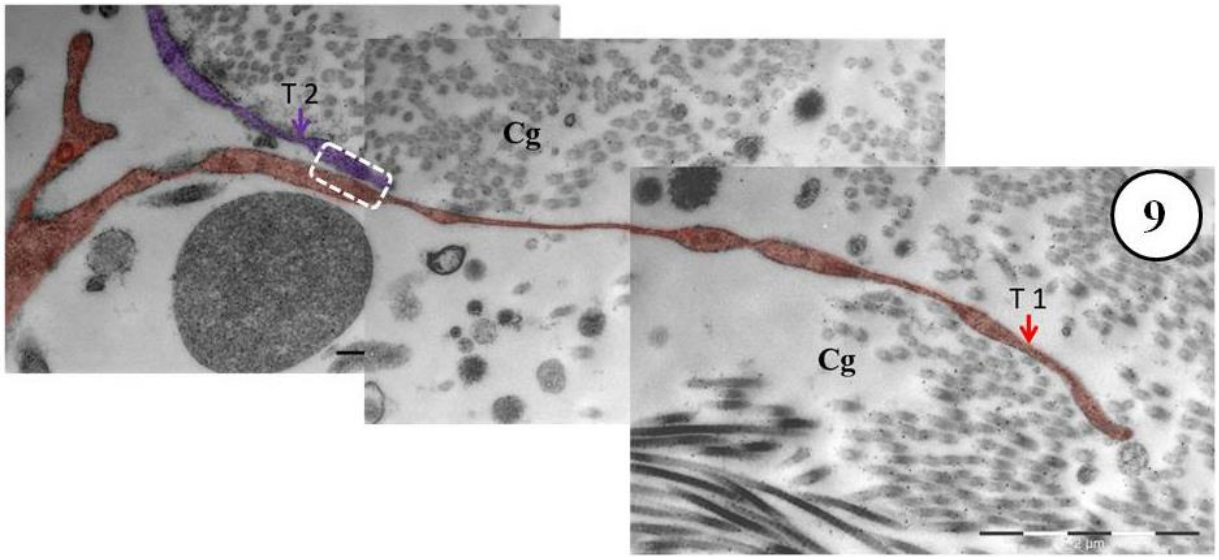


Figure 9. Telopodial extensions of two telocytes (T1 and T2) become in close contact each other (see delimited area by interrupted white lines). Cg = collagen. Scale bar = 2 µm. Right frontal brain tumor.

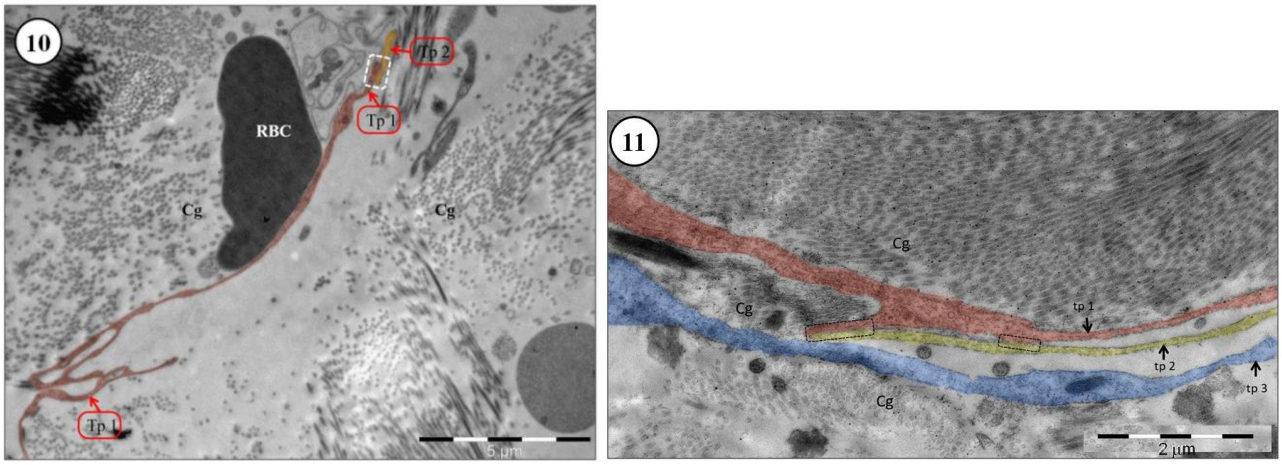


Figure 10. A long and tortuous telopode (Tp 1) become in close contact with another telopode (Tp 2) (see delimited area by interrupted white lines). An extravasated red blood cell (RBC) is barred by the telopd Tp 1. Cg = collagen. Scale bar = 5 µm. Right frontal brain tumor.

Figure 11. Three telopodes (tp 1, tp 2 and tp 3) run parallel into a fibrotic stroma. To some extensions tp 1 and tp 2 become in close contacts (delimited areas by black interrupted lines). Cg = collagen. Scale bar = 2 µm. Temporal brain tumor.

The lengths of affronted two Tcs' telopodes involved in a homocellular junction may vary from 50 nm to few microns. Sometimes, more or long profiles of basal lamina accompany to some extent the telopodes (Fig. 12).

The homocellular junction can be realized also as end-to-end of two adjacent telopodes. Mention must be made an amorpho-fibrillar substance (a kind of electron dense plaque) can be detected at this type of junction (Fig. 15).

Interestingly, homotype junction as *a plug and socket synapse* between two telopodes can be detected (Fig. 16 and Fig. 17).

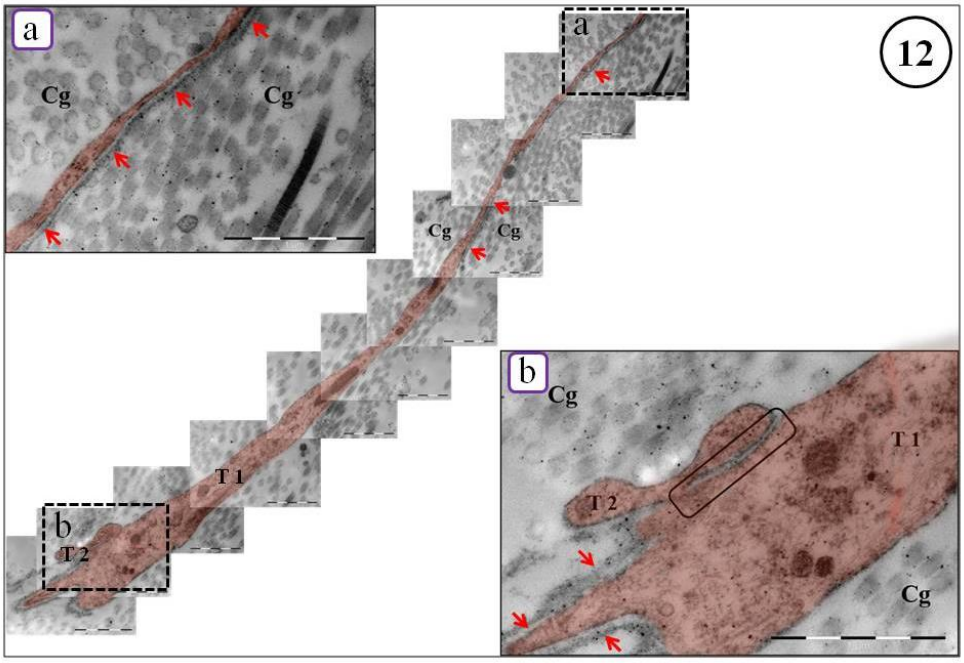


Figure 12. A long and slender profile of a telopode (T 1) detected inside of a fibrotic stroma exhibits to some extents short segments of basal lamina (red arrows) as is detailed in inset **a**, and have a synaptic junction with another telopode T 2 detalied in inset **b**, and also short segments of basal lamina can be seen. Cg = collagen. Scale bar = 1 μm. Right frontal brain tumor.

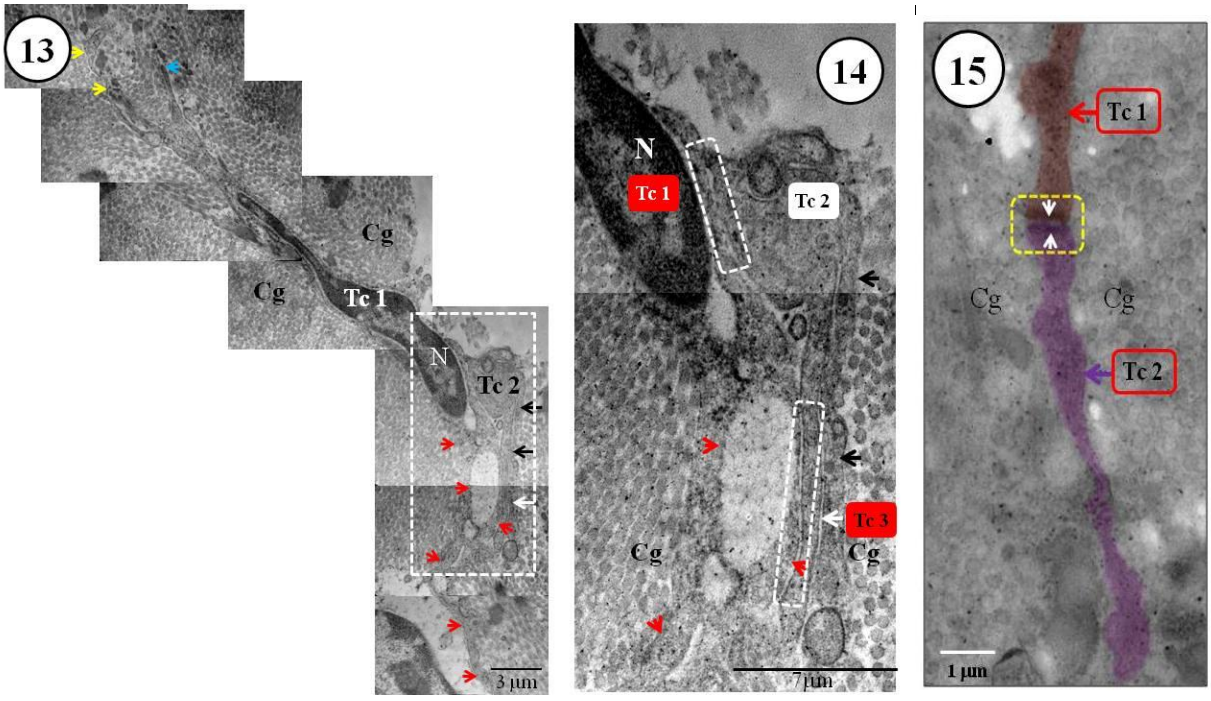


Figure 13. A nucleated telocyte (Tc 1) with a bifurcated telopode (yellow and blue small arrows) becomes in close contacts with other two telocytes (Tc 2 and Tc 3, here represented by short segments of telopodes marked by black and white small arrows) by his second telopode (red small arrows) (rectangular areas delimited by white interrupted lines) and detailed in the Fig. 16. N = nucleus. Cg = collagen. Scale bar = 3 μm. Meningioma.

Figure 14. Nucleated telocyte 1 (Tc 1) performed synaptic junctions with other two telocytes (Tc 2 and Tc 3) here represented by their telopodes (see the two rectangular areas delimited by white interrupted lines). N = nucleus. Cg = collagen. Scale bar = 7 μm. Meningioma.

Figure 15. Two telocytes (Tc 1 and Tc 2) represented by their telopodes performed an end-to-end homocellular communication. At the junctional interface, where the two telopodes are affronted, two dense electronomicroscopic materials are detected (see the white arrows in the yellow interrupted lines rectangular area). Cg = collagen. Scale bar = 1 μm. Meningioma.

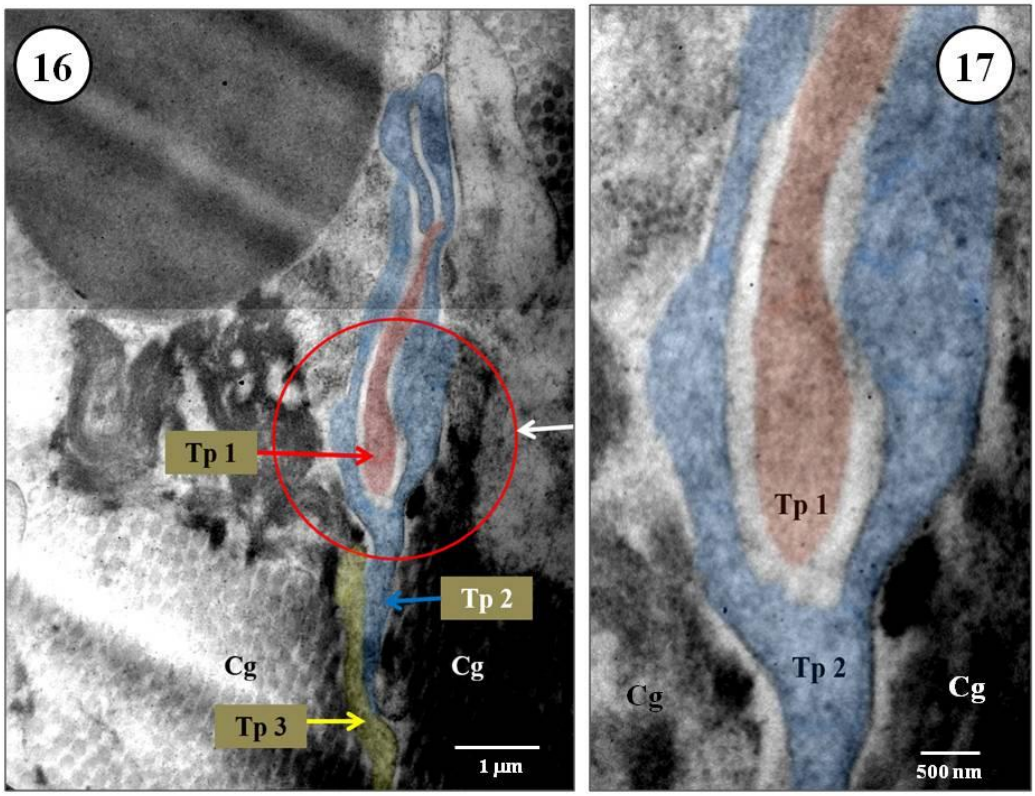


Figure 16. Three telocytes represented by their telopodes (Tp 1, Tp 2 and Tp 3) performed homocellular junctions as follows: a plug and socket synapse between telopode Tp 1 and telopode Tp 2 is detected (encircled area, detailed in Fig. 19) while telopode Tp 2 and telopode Tp 3 realized also a clear synapse. Cg = collagen. Scale bar = 1 µm. Meningioma.

Figure 17. Detail for the plug and socket synapse between telopode Tp 1 and telopode Tp 2. Cg = collagen. Scale bar = 500 nm. Meningioma.

Inside of the nervous tissue involved in an advanced state of degradation (myelinic envrappment of axons is severely destructured) and only short profiles of telopodes can be seen in that area of the brain (not shown).

Telocytes inside of the tumor brain performed also so called hetero-cellular junctions. Tcs' telopodes become in contact with different resident or transitory cell present in the brain parenchymal tissue (for ex. with extravasated white blood cells as is depicted in Fig. 1 and detailed in Fig. 2), or with amorphous/acellular peritumoral stroma components (for ex. with collagen fibrils (Figs. 3–17).

We emphasize that telocytes by their telopodes become in very close vicinity to nerves and associated glial cells (Fig. 18 and Fig. 19).

Nerves cross sectioned represented by non-mielinated axons enwrapped by a glial cell exhibiting a basal lamina can be detected (Figs. 20–22).

In Fig. 23 is depicted a nucleated glial cell which captured some non-mielinated axons and a continuous and strong basal lamina follow the whole glial plasma membrane.

Like in case of many other types of stroma tissues often, telocytes appear associated with blood capillary (Fig. 24). Here, interestingly, is that telopodial extension of a telocyte appears interposed between two basal laminas which seem to be secreted one by the pericyte and another by a presumably glial cell which enwraps two apparently degraded non mielinated axons.

Moreover, very often telocytes are involved in exosome deliverance inside of the extracellular matrix (Fig. 7 and Figs. 25–28). Numerous exosomes appear as microvesicles system delivered by telopodes.

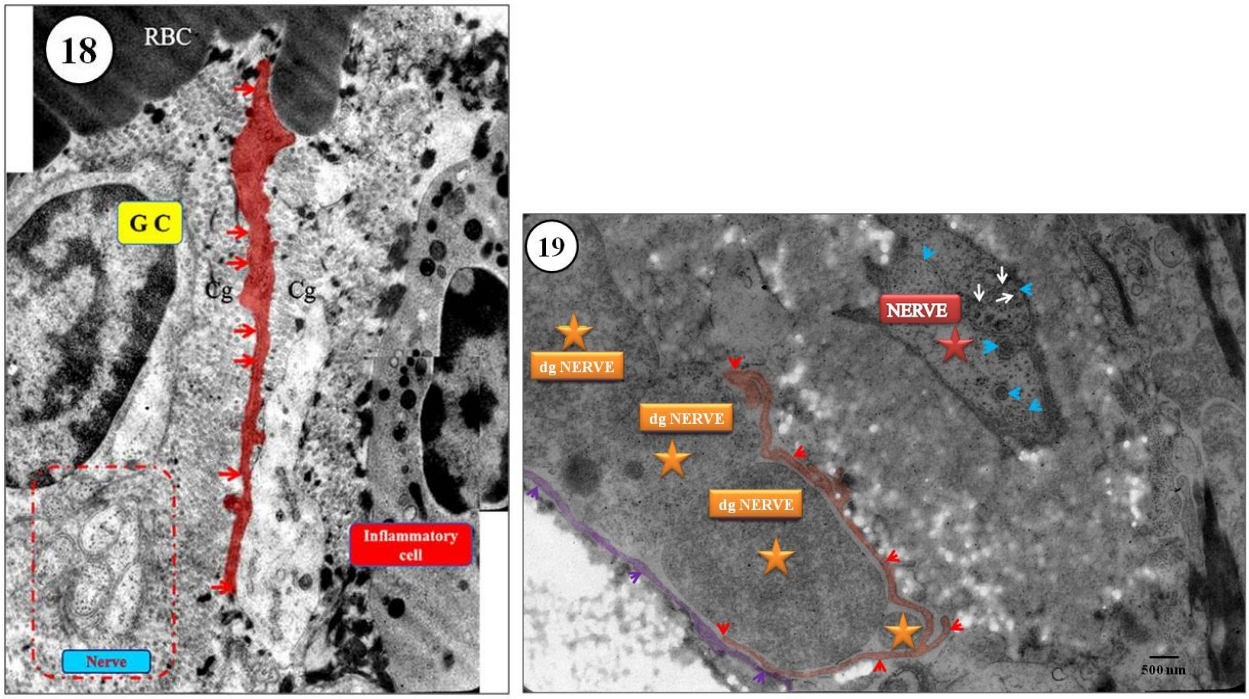


Figure 18. In a hemorhhagic area (see the inflammatory cell and red blood cells) of the right ponto-cerebelous angle meningioma an short segment of a telopode can be seen not far from a glial cell (GC) which envrapp a nerve (red rectanular area). Cg = collagen. Meningioma.

Figure 19. On the right side of the picture a cross sectioned nerve represented by a glial cell (red star) envrapping few axons, while on the left side presumably degraded nerves/glial cells (orange stars) appear in very close contact with two telopodes. Blue arrows indicate non-myelinized axons, while white arrows indicate axonal neurotubules. Scale bar = 500 nm. Meningioma.

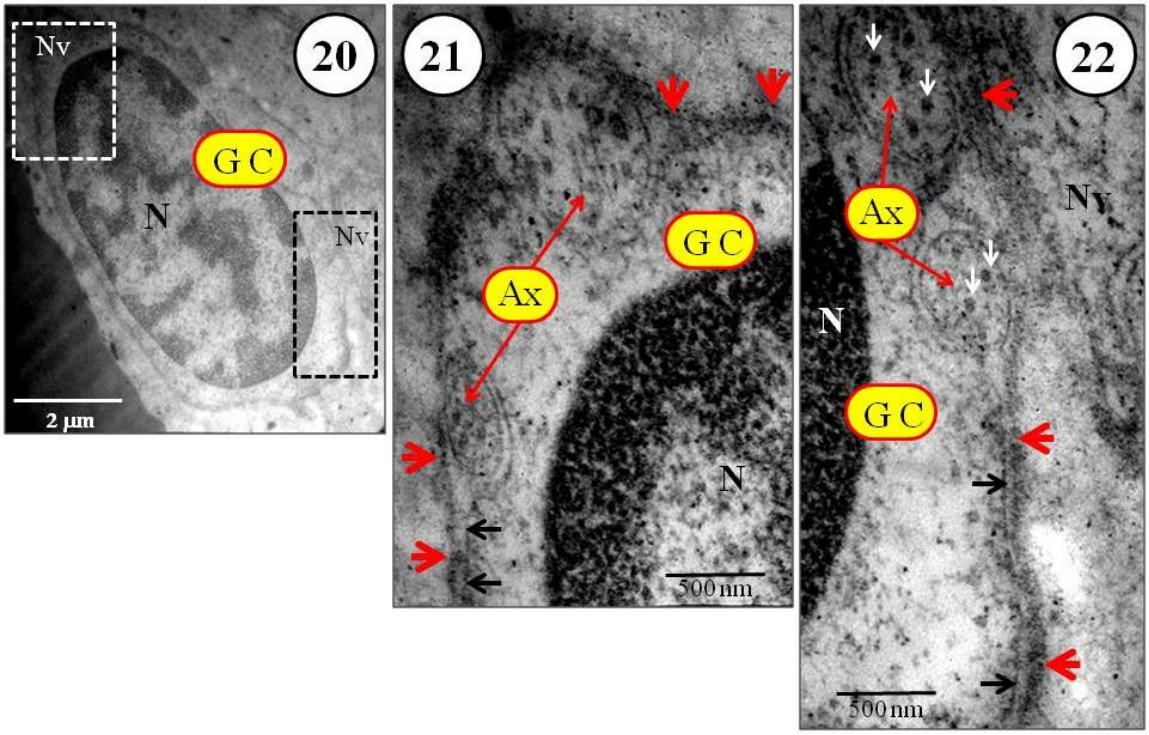


Figure 20. A nucleated glial cell enwrapps two nerves (Nv) detailed as follows: white rectangular area in Fig. 22 and black frame area in Fig. 23. N = nucleus. Scale bar = 2 µm. Meningioma.

Figure 21. A sector of glial cell (GC) enwrapps two non-myelinated axons (Ax). N = nucleus. Small black arrows indicate plasma membrane of the glial cell while red head arrows mark a strong basal lamina of the glial cell. N = nucleus. Scale bar = 500 nm. Meningioma.

Figure 22. Two non-myelinated axons (Ax) are enwrapped by a glyal cell (GC). N = nucleus. Small white arrows indicate axonal neurotubules. Small black arrows = plasma membrane of the glial cell. Red head arrows = basal lamina of the glial cell. N = nucleus. Scale bar = 500 nm. Meningioma.

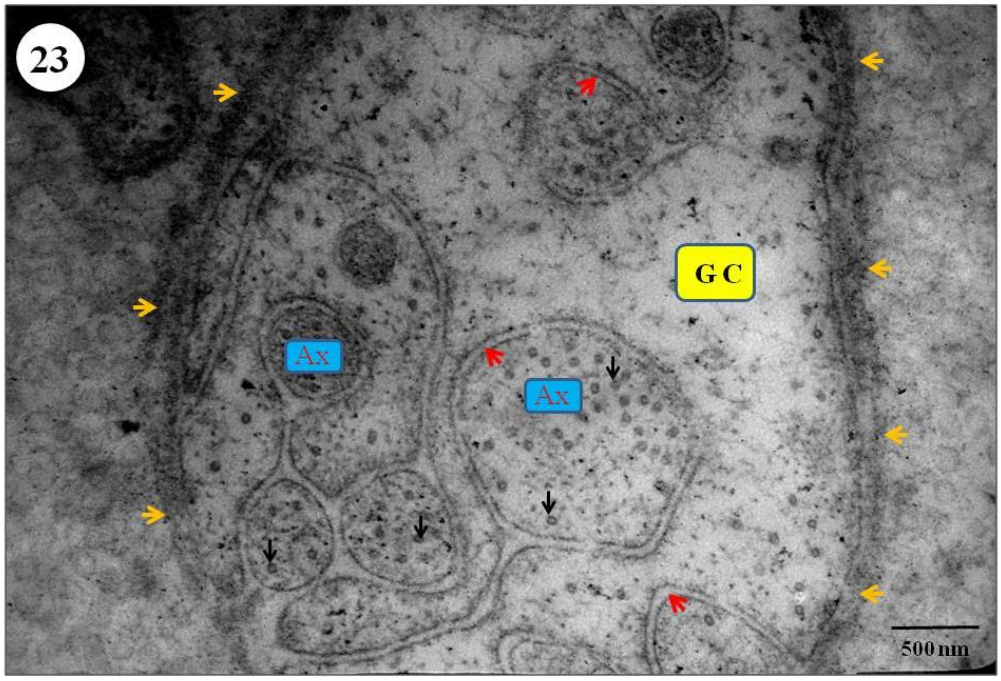


Figure 23. A cross sectioned nerve; non-myelinizated axons have a distinct axolema (blue head arrows) envrapped by glial cell extensions. Red arrows inicate axonal neurotubules. A basement membrane (small yellow arrows) follows the external face of plasmamembrane of the glial cell (indigo arrows). Chlatrin coated vesicles = white rectangular area. Cg = collagen. Scale bar = 500 nm. Meningioma.

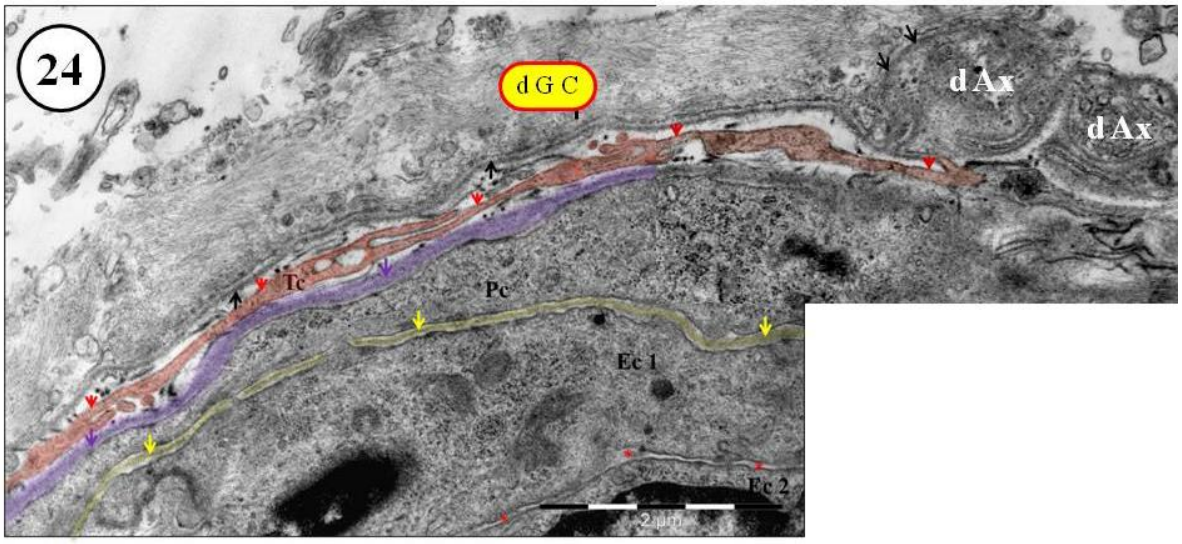


Figure 24. A sector of sanguine capillary with collapsed lumen (red asterisks) where two endocytes (Ec 1 and Ec 2) covered by a pericyte (Pc) are visible. Between endocytes and pericyte a continuous basal lamina (yellow arrows) is detectable while a strong basal lamina accompanied alluminal face of the pericyte interposed between pericyte and a telopodial extension (indigo small arrows) of a telocyte (Tc). Black head arrows indicate delivered exosomes by the telocyte. A presumable degraded glial cell (dGC) with a continuous basal lamina (small black arrows) becomes in very close vicinity with the telocyte's telopode. Scale bar = 2 µm. Left frontal brain tumor.

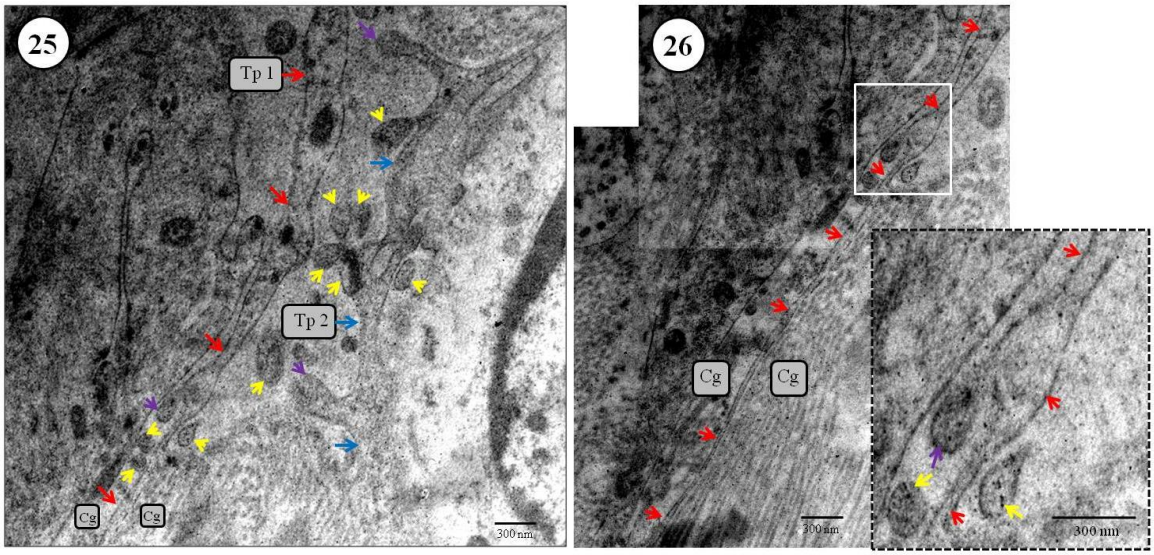


Figure 25. Two telopodes (Tp 1 = red arrows, and Tp 2 = blue arrows) involved in exosomes deliverance: indigo head arrows indicate exosomes stil attached to the telopode while yellow head arrows indicate just delivered exosomes into extracellular microenvironment of tumor stroma. See the continuity of the telopode Tp 1 in Fig. 32. Cg = collagen. Scale bar = 300 nm. Temporal brain tumor.

Figure 26. The telopode Tp1 from the Fig. 31 is continued as extremely slender telocytic prolongation (red arrows). In inset: delimited area in white rectangular lines shows exosomes deliverance. Indigo head arrow indicates exosomes still attached to the telopode while yellow head arrows indicate just delivered exosomes into extracellular microenvironment of tumor stroma. Cg = collagen. Scale bar = 300 nm. Temporal brain tumor.

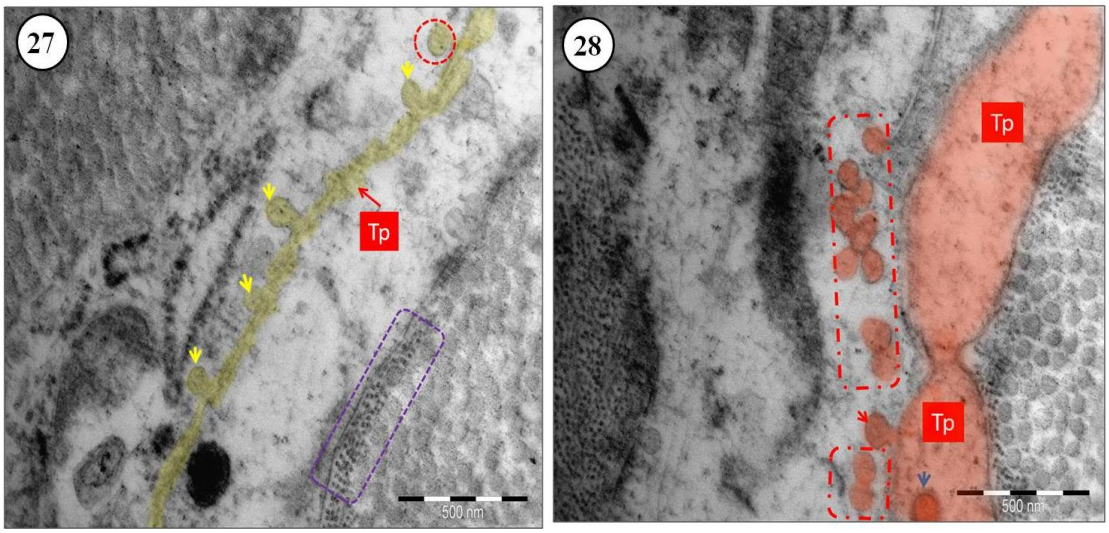


Figure 27. A short segment of a telopode (Tp) involved in exosomes deliverance. Many exosomes still attached to the telopode while in encircled area indicate an exosome just delivered exosome into extracellular microenvironment of tumor stroma. Scale bar = 500 nm. Right frontal brain tumor.

Figure 28. A short segment of a telopode (Tp) involved in exosomes deliverance. Red short arrow indicates an exosome still attached to the telopode while rectangular delimited area indicates just delivered exosomes into extracellular microenvironment of tumor stroma. Blue head arrow indicates a chlatrin coated vesicle. Scale bar = 500 nm. Right frontal brain tumor.

Concerning the microanatomy/histo-architecture and the real nervous cells status inside of the tumor brain mention must be made that there is a great variability related mostly to the tumor brain type and the degree of the damage provoked by tissue displacement or the degree of the local hemorrhagic area. Indeed, inside of the tumor nervous tissue, extensive areas in advanced degraded stage of the nervous tissue (neurons, glial cells) can be microscopically recorded (Figs. 29–32). Severe ultrastructural alterations observed suggest that affected nervous areas are functional totally compromised. Neurons and glial body cells are almost dissolute, axons as well as dendrites and myelin sheaths are swollen, often destructured. When still present, microvasculature also is

affected so that endothelial cells become to be swollen (Fig. 30). Perivascular edema could induce a severe impairment of the blood-brain-barrier. Interestingly, even in advanced state of the neuropil, in an extensive edematous area, apparently "free" swollen axonal synaptic boutons with totally dispersed synaptic vesicles are visible (Fig. 31). Inside of the more or less altered nervous tissue axo-dendritic synapses can be still well preserved to some extent. Indeed, the two types of Gray's synapses detectable inside of the normal central nervous system as Gray's type I asymmetrical synapse with post-synaptic membrane thickening (usually considered excitatory) (Fig. 32) and Gray's type II symmetrical synapse usually considered inhibitory (not shown here) were also recorded by TEM analysis. Swollen axonal synaptic boutons exhibited a high or totally dispersion of synaptic vesicles (Fig. 32).

Concerning 3-D network Tcs' status of deeply altered parenchyma of brain tumor, remnants of short profiles of Tcs' telopodes can be detected only.

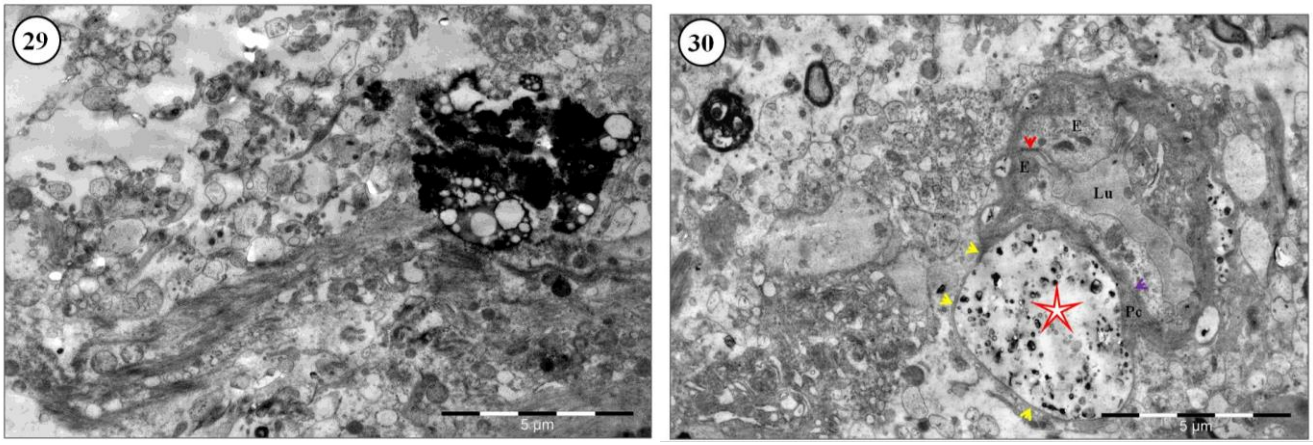


Figure 29. Advanced altered tumor nervous tissue. A large conglomerate of degraded amorphous material is visible. Scale bar = 5 µm. Left frontal brain tumor.

Figure 30. In an advanced altered tumor nervous tissue, a blood capillary with still preserved tight junctions (red head arrow) between endothelial cells (E) and a basal lamina (indigo head arrow) as well as an attached pericyte (Pc). An advanced degraded cell (star) filled with electron dense amorphous material but still preserved basal lamina (yellow head arrows) makes difficult to identify cell type attached to the capillary. Lu = capillary lumen. Scale bar = 5 µm. Left frontal brain tumor.

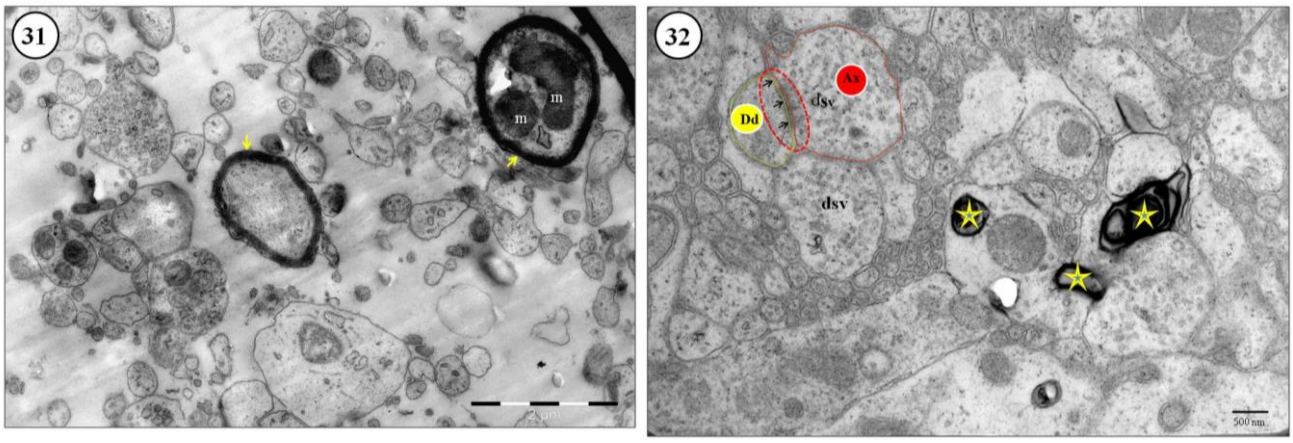


Figure 31. A lot of polymorphic vesicles are visible in an area of degraded tumor nervous tissue. Two myelin sheaths enwrapped severe altered axons. m = giant mitochondria. Scale bar = 2 µm. Left frontal brain tumor.

Figure 32. In a meningioma tumor area among severe altered axons with collapsed myelin sheaths (stars) there is still detectable apparently normal axo-dendritic synapse (elliptic area) with post-synaptic thickening (arrows) but exhibiting highly or totally dispersed synaptic vesicles (dsv) and. Ax = axon. D = dendrite. Scale bar = 500 nm. Meningioma.

DISCUSSION

Here we report about our results concerning detection, localization and detailed infrastructure of telocytes inside of diverse tumor brain with focus on special relationships aspects of telocytes themselves and/or other cell types present in the human brain.

So far, there are two major theories concerning cancer neogenesis: the somatic mutations theory (SMT) as a consequence of gradually accumulation of one or more mutations of oncogenes and tumor suppressor genes or chromosomal alterations and tissue organization field theory (TOFT)[47].

Generally, once initiated tumor cells compete with the normal microenvironment. Initially, there is a battle between peritumoral stroma and malignant transformed cells but cancer cells by their altered behavior as neighbors will "try" to make the normal cells as their friends[48]. On the other hand, using different ways, normal microenvironment exerts anti-tumorigenic pressures[49,50]. *In situ/in vivo*, different normal cell types trigger appropriately mechanisms to overcome aggressive behavior of tumor cells. Among the fighters, immune surveillance cells habilitated to patrol and to perform a synergistic attack against abnormal cells are the first responding. Moreover, even apparently inert resident stromal cells as fibroblasts will participate to limit/stop aggressive growth of tumor mass, finally by producing a fibrotic tissue. Moreover, it seems that telocytes are involved in this battle. Many published papers underline beneficial actions of this peculiar cell phenotype, namely telocytes, not only in some aspects of early embryo development, in maintaining tissue/organ homeostasis but also in tissue regeneration consequently to some pathological events[51,52].

On the other hand, mention must be made that, in contrast with the very beneficial effects reported about telocytes roles in normal tissue homeostasis, tissue regeneration, protecting against cancer, there are few published papers about the dark face of telocytes. In an experimental study, Xu *et al.*[53], showed that telocytes (TCs) participate in tumorigenic, invasive, and migratory processes by secreting functional proteins and transmitting cell-to-cell information in hepatocellular carcinoma. In a very recently published paper, Diaz-Flores *et al.*[54], showed that resident CD34+Scs/Telocytes participate as an important source of cancer-associated fibroblasts in invasive lobular carcinoma of the breast.

Our observations from this study emphasize that in spite of the fact that extensive brain areas are severely altered by tumor growth and hemorrhagic aspects are visible, inside of the tumor stroma a plenty of telocytes still keep unaltered their homocellular junctions between adjacent telopodes as well as their heterocellular junctions. This aspect suggests that telocytes by their ability to realize a 3-D construct, to some extent, offer a kind of mechanically protection against aggressive development of the tumor brain. In the context of telocytes' participation to the halting cancer growth, most hidden to our knowledge remains the multitude of putative roles of cargo molecules delivered by telocytes into tumor microenvironment.

Either isolated but mostly as organized cells into specialized tissues, by their ability to receive, process and respond to the microenvironment signals as putative information is essential for all biological processes: cell division, normal histo-architecture maintenance, as well as during development of different pathological processes. There are different modalities involved in cell signaling. Direct contact as homo- or heterocellular cell-to-cell junction (eventually with plasma-membrane recombination) as well as *via* extracellular vesicles as putative molecular cargo are already well documented as reliable ways for cell communication processes[3,5,18,26,55].

Recently, a new type of cell-to-cell communication was reported: membranous F-actin nanotubes which can mediate plasma-membrane continuity between adjacent cells and consequently can mediate intercellular transport of various cellular components[56].

In the context of this fundamental process of cell-signaling, telocytes as stromal/interstitial cells described in almost all human body tissues appears as a provocative temptation to search for more knowledge/challenge especially about their putative role in the normal or affected brain.

Almost all published paper about telocyte concerning their 3-D morphology showed that Tcs undergo phenotypic changes related to the temporal-spatial and functional needs of different tissues. Moreover, by their abilities to send as well as to receive cell-signals from altered tissue, Tcs, especially those located in close proximity or even inside of a stem cell niche are involved in tissue regeneration[7,27,57,58]. TCs can transfer information to neighbor or distant located cells ensuring communication, by the release a wide variety of

extracellular vesicles: exosomes, ectosomes, and multivesicular bodies[7,19]. Telocytes exert their effect on cells either by establishing homo- and/or heterocellular contacts or in a paracrine mode *via* delivered extracellular vesicles as cargo of different bioactive molecules as proteins, RNAs, microRNA, etc. involved in cell-to-cell communication[5,7,18,22,39,59].

Telopodes aid TCs in forming homo- or heterocellular contacts; thus, assembling three-dimensional networks that organizes the stromal and the parenchymal components of the organs[19].

Our observations concerning telocytes relationships in the tumor brain were focused on different aspects. Concerning direct homocellular junctions which telocytes performed, we described two aspects: (1) the most frequent, two or more telopodes run parallel each other and become in direct contact for a different length (300-500 nm, even few micrometers) and (2) two adjacent telopodes realize an end-to-end contact. In this case, as a rule, an electronodense material is detected as a plaque between the two telopodial ends. Here some molecules involved in this end-to-end junction infrastructure must be of great interest to identify by an appropriately immune reaction the nature of that molecular species.

TEM investigations of normal and brain tumor microvasculature offer very interesting data. Within the normal brain, blood vessels participate in blood and brain tissue exchange via the blood–brain barrier (BBB). Mention must be made that inside of the brain tumor vasculature two different types of vessels are found: preexisting vessels which may be co-opted by tumor cells and vessels formed by angiogenesis known as neoangiogenic vessels[60]. Calabrese *et al.*[61], reported that endothelial cells interact closely with self-renewing brain tumor cells and secrete factors that maintain these cells in a stem cell-like state. In this context, of a great interest would be to know the putative roles of telocytes associated to the tumor brain microvasculature, as we detected and described here.

One of the very important cell components of the microvasculature is represented by pericyte, a multifunctional mural cell type, located at the abluminal side[62]. In the brain, pericytes are associated to the precapillary arterioles, capillaries, and postcapillary venules sharing a common basement membrane with endothelial cells[14]. It is well established that, in the brain close juxtapositions and physical contact between endothelial cells, pericytes, astrocytes and neurons form the neurovascular unit[63,64].

Mitrofanova *et al.*[14], postulated that pericytes and telocytes as perivascular interstitial cells may be involved in human glioma/glioblastoma neovascularisation.

Recently published papers reported about the telocytes association to the microvasculature in different organs[7,65,66]. In the present study, we also detected Tcs' telopodes accompanying brain capillaries. This aspect suggests that telocytes may participate also to the brain capillary stability, especially during/against aggressive tumor growth.

In our opinion, the well represented telocytic 3-D construct inside of the brain tumor may offer a mechanistic protection against invasive tumor growth, while multiple exosomes delivered by telocytes in our investigated case of cerebellopontine angle meningioma by their molecular cargos, presumptively may also act as antitumorigenic pressures.

Dănăilă[67] and Dănăilă and Păiş[68] suggest that telocytes may offer protection against different internal and external aggressions leading to different cerebrovascular abnormalities.

Mention must be made that inside of the nervous tissue involved in an advanced state of degradation with extensive hemorrhagic and edematous area, 3-D network of telocytes is destroyed and only short profiles of telopodes can be seen in that area of the brain**,** so that Tcs' putative protective role against aggressive tumor growth is lost/abolished.

Severe ultrastructural alterations induced by brain tumor development as we reported here clearly compromised the functionality of that affected region. Just to mention here: high or totally dispersion of presynaptic vesicles, *versus* normal polarized distribution (synaptic vesicles are concentrated close to the synaptic cleft[70]), diverse grades of infrastructural alterations of oligodendrocytes, severe altered axons and myelin sheaths. Swollen axonal synaptic boutons exhibiting a high dispersion of synaptic vesicles were reported in the rat brain experimental streptozotocin-induced diabetes considered contribute to cognitive impairments[71].

As a primary brain tumor arising from the neoplastic transformation of meningothelial cells, meningioma is the most common primary intracranial tumor. Meningiomas are heterogeneous tumors, so that up to date thirteen histologic variants of meningioma have been described and recognized in the WHO Classification of Tumors of the CNS[12,72]. Xu and Lu[36] identified and described infrastructure of telocytes in meninge's

dura. The meningiomas of the cerebellopontine angle may arise from any area of the meninge's dura and is very vascularized lesions[73].

Decimo *et al*.[37], reported that following disease, meninges increase their thickness and stromal cells, including telocytes will increase in their number. Indeed, in our investigated case of cerebellopontine angle meningioma, we observed that a plenty of nucleated telocytes are detectable and by their homocellular contacts often for more long distances (running from 500 nm to few microns) performed a strength 3-D system and moreover are very active producing large amounts of delivered exosomes into the peritumoral space.

Different from the glial Schwann cells able to mielinate each one axon from the peripheral nervous system, inside of the CNS individual oligodendrocytes each mielinate multiple, as many as 50+ axons. Another remarkable particularity is that while each Schwann cell has a prominent basal lamina, oligodendroglia does not secrete and assemble a basal lamina[74]. In fact, inside of the CNS so called basal lamina is quantitatively very reduced almost limited to blood vessels from the brain parenchyma and the pial basal lamina found on the brain surface[75]. Concerning the presence of glial cells with a continuous basal lamina enwrapping axons inside of the brain/central nervous system as we described here in a case of meningioma of the cerebellopontine angle (cerebellopontine angle meningioma), this is a big matter of debate. We may speculate only that glial cells surrounded by a continuous basal lamina can be imported *via* blood vessels which penetrated from the subarchnoidal space into the cortex. Some arteriole penetrated the depth of the cortex, other vessel become capillaries[76]. Presumptively, this kind of glial cells will migrate inside of the tumor nervous tissue.

CONCLUSIONS

1. Telocytes express their ability to perform (1) homocellular nano contacts (including *plug and socket synapse*) and (2) heterocellular junctions, especially with pericytes accompanying brain microvasculature as well as with nervous and glial cells of the tumor brain.

2. Another very interesting observation of interest is related to the possible involvement of telocytes in cell signaling and intercellular communication *via* exosomes genesis and their deliverance as a product of the microvesicular system of the brain telocytes.

3. The inner 3-D network of brain telocytes by their homo- and heterocellular communications, and moreover by their ability to deliver extracellular vesicles appears as a genuine cell-to-cell communication system inside of CNS.

4. During a brain tumor development or consequently to a secondary tumor development inside the brain, concomitantly with the brain (nervous tissue) destruction, telocytes lost their juxta-cerebral zones (meninges and choroidal plexus with neurogen potential etc.) and migrate to occupy ectopic areas of nervous tissue. In this line we detected telocytes in close vicinity of the nerves and their associated glial cells.

5. We emphasize about particular observation concerning presence of glial cells enwrapping axons inside of the brain/central nervous system and exhibiting a clear continuous basal lamina.

6. In spite of the fact that extensive brain areas are severely altered by tumor growth and hemorrhagic aspects are visible, inside of the tumor stroma a plenty of telocytes still keeping their homocellular junctions between adjacent telopodes aspect suggests to offer to some extent a kind of mechanically protection against aggressive development of the tumor brain.

Conflict of interests. The authors declare no conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by an Agreement Cooperation between Institute of Biology Bucharest of Romanian Academy and National Institute of Neurology and Neurovascular Disease. The authors acknowledge for financing support done to perform and to write the paper coming from: project No. RO1567-IBB06/2020 from the Institute of Biology Bucharest of Romanian Academy. Electron microscopic investigations were performed by a JEOL JEM 1400 transmission electron microscope, supported by DIBIOCLIM Project. The author thanks A. Brînzan and Viorica Stan for technical assistance.

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