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From Cell Biology to Tissue Engineering

Spontaneous intracerebral hemorrhage as presentation of atypical central neurocytoma: the role of angiogenesis through the characterization of tumor endothelial cells

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Summary, A 36-year-old white man presented with sudden-onset headache and rapid deterioration of consciousness. Computer tomography revealed a right capsular intra-parenchimal hemorrhage with an intraventricular component; therefore, emergency surgery was performed. Once the hematoma was evacuated, the cause of the hemorrhage was identified as a tumor mass and it was resected. Histopathological and immunohistochemical examinations of the surgical specimen disclosed a diagnosis of atypical central neurocytoma.

By using a protocol recently set up in our laboratory, we succeeded in isolating and propagating, for the first time, human endothelial cells from central neurocytoma (CN-ECs). Different analyses revealed that isolated CN-ECs consist of a pure endothelial cell population, with the expression of endothelial markers (CD31, CD309/VEGFR2, CD105, eNOS) and with angiogenic properties, such as the uptake of LDL. Moreover, CN-ECs spontaneously organize in a vascular-like structure.
The goal of this case report is to stress the need for further studies focused on understanding the causes of the onset of an intra-parenchimal hemorrhage in the presence of an atypical central neurocytoma in order to tailor treatments to each single patient and achieve the best clinical outcome.

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Introduction

Central neurocytomas (CNs) are uncommon benign tumors of the central nervous system that can have both neuronal and glial origin (Mozes et al., 2014). Most CNs are benign and are well-differentiated tumors. Furthermore, CNs have an indolent course after their clinical presentation that includes symptoms related to increased intracranial pressure secondary to obstructive hydrocephalus, such as headache and visual disturbances (Schmidt et al., 2004). A subpopulation of these tumors displays more aggressive clinical behaviours and are defined as "atypical central neurocytomas" (Rades et al., 2004). Although intracranial hemorrage is a well-recognized complication in numerous neuroepithelial tumors such as glioblastoma, astrocytoma, oligodendroglioma, and medulloblastoma (Piepmeier and Baehring, 2004), tumoral hemorrage in CNs is not generally acknowledged and many aspects such as the histogenesis of CN remain unknown because of its rarity. In this regard, Vaquero and colleagues developed an experimental model of neurocytoma to study neurocarcinogenesis in brain tumors (Vaquero et al., 1993).

Here, we report on a case of atypical CN that presented as a spontaneous intracerebral haemorrhage (ICH). The added value of our study is the fact that, for the first time, we isolated the endothelial microvascular component, which showed the typical morphology and

phenotypes of tumor endothelial cells, and the functional ability to perform cord formation and uptake of LDL. In our case, CN displays an uncommon modality for this type of tumor and illustrates the difficult task of making the differential diagnosis of hemorrhagic lesions, especially in emergency cases.

Materials and methods

Tissue samples

All procedures involving human participants were in accordance with the ethical standards of the institution and with the declaration of Helsinki. Tissue was obtained from patient underwent tumor resection, with informed consent and institutional review board approval. Histological diagnosis of CN in the original specimen was established ultrastructurally and immunohistochemically. The diagnosis was made by a neuropathologist, according to the WHO classification (Louis et al., 2007). Tumor sample was collected at the time of surgery, washed in D-PBS (Euroclone, Milan, Italy) and suspended in DMEM/F12 (Gibco, Grand Island, NY, USA) containing 1% penicillin/streptomycin (Gibco) at 4°C.

Cell culture preparations

Dissociation of the tumor tissue was carried out according to the following method (Navone et al., 2013a). Briefly, the specimen was minced into pieces approximately 1 to 2 mm in size with two sterile scalpel blades and incubated at 37°C in an atmosphere of 5% CO_2 and 95% air with Liberase Blendzyme 2 (Roche, Mannheim, Germany) in D-PBS at a concentration of 0.625 WU/ml at 37°C on a rotator for 1 hour. After enzymatic digestion, the cell suspension was washed with D-PBS, centrifuged at 276 g/10 min and filtered by a 70 μm mesh (BD Bioscience, San Jose, CA, USA), to obtain a monocellular suspension. The sample was centrifuged, the supernatant discarded and the cell pellet was plated in Endothelial Proliferation Medium (EndoPM), a medium specifically developed by our laboratory for the isolation and expansion of the tumoral endothelial component (Navone et al., 2013b; Di Vito et al., 2017). Cells were maintained at 37°C, 5% CO_2 in bovine collagen type I-coated culture 25 cm^2-flask in fresh EndoPM medium. The medium was changed every 7 days, the cultured cells were passaged at a split ratio of 1:4 every 14 days and detached by TrypLE Select (Gibco). Cultured central neurocytoma endothelial cells (CN-ECs) at passages P3-P5 were used as indicated for all experiments. Experiments were performed in triplicate. Cells were routinely observed with an inverted phase-contrast microscope (Leica Microsystems, Wetzlar, Germany), and images were acquired with a digital camera (Carl Zeiss Microscopy GmbH, München Germany).

Flow cytometry analysis

Flow cytometry (FC) was performed on CN-ECs to evaluate stemness and endothelial markers using phycoerythrin (PE)-conjugated CD133 (Miltenyi, Miltenyi Biotec S.r.l. Bologna, Italy), CD309/VEGFR2 (BD Pharmingen, Becton Dickinson, Franklyn Lakes, NJ, USA), and fluorescein isothiocyanate (FITC)-conjugated CD105 (AbDSerotec, Raleigh, NC, USA). Briefly, 5×10^4 cells/tube were stained with anti-human monoclonal antibodies (mAbs) and incubated for 15 minutes at room temperature (RT) in the dark. Samples were centrifuged at 300g/5 minutes and washed twice with D-PBS. For data acquisition, a FACS Canto II with FACSDIVA software (both from BD) was used. The controls were isotype-matched mouse IgG. Non-viable cells were excluded by 7-AAD staining.

Immunofluorescence analysis

To evaluate the expression of endothelial markers by immunofluorescence, permanox chamber slides (Nunc, Naperville, IL, USA) were coated with bovine collagen type I for 1 hour and left to air dry. Subsequently, CN-ECs were plated at the density of $2x10^4$ cell/well and after 48 hours were fixed with Paraformaldehyde 4% (15 min, RT). To avoid quenching, CN-ECs were treated with 0.1M glycine (10 min, RT). Subsequently, the samples were permeabilized with 0.1% Triton X-100 for 10 min. Cells were incubated overnight with anti-endothelial nitric oxide synthase (eNOS) primary antibody (1:200, purchased from ABR Affinity Bioreagent, Golden, CO, USA) and then, with Alexa564 conjugated secondary antibody (1:1,000; Life Technologies, Milan, Italy) at RT for 45min, washed, and the nuclei were counterstained with DAPI (Life Technologies). The sample was mounted with ProLong® Gold Antifade Mountant (Life Technologies) and images were captured using a Leica TCS SP5 confocal microscope (Leica). The staining was performed in triplicate.

CN-ECs cord formation on Matrigel

50 µL of Matrigel (12.5 mg/mL, BD Bioscience) at 4°C were transferred to pre-chilled 96-well culture plates. After gentle agitation to ensure complete coating, plates were incubated for 30 minutes at 37°C to allow the solidification of Matrigel. CN-ECs were then seeded at a concentration of 1×10^4/well in EndoPM (Navone et al., 2013a). Cord formation was detectable after 12 hours of incubation in ten fields randomly photographed.

Functional assays for CN-ECs

To determine the uptake of Dil-labeled acetylated low-density lipoprotein (LDL), CN-ECs were incubated with 10 mg/mL Dil-Ac-LDL (Molecular Probes,

Invitrogen) at 37°C for 4 hours. The cells were washed with D-PBS and mounted with ProLong® Gold Antifade Mountant (Life Technologies). The slides were analyzed using a Leica TCS SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany).

Results

A 36-year-old white male was admitted to the emergency unit of our centre presenting with sudden-onset headache and a rapid deterioration of consciousness. The patient became unresponsive to external stimulation (GCS 3) during his transport to the computer tomography (CT) room. Blood pressure was 170/100, blood analysis were normal. CT scan of the brain (Fig. 1A) showed right capsular intraparenchimal haemorrhage with an intraventricular component, necessitating emergency surgery. No relationship with the circle of Willis and no vascular malformation were seen on CT angiographic sequences; there was no pathological enhancement after injection of iodine contrast. First, the hemorrhage was evacuated through a large fronto-temporo-parietal decompressive craniotomy to treat the brain swelling (Fig. 1B). Intraoperative specimens of hemorrhagic lesions were not diagnostic for a tumor. The patient was initially in intensive care and then in a rehabilitation program. The bone flap was replaced and the patient recovered neurologically although a slight left sided hemiparesis remained. At a 10-month follow-up visit, brain magnetic resonance imaging (MRI) revealed a residual iso-ipointense, a heterogeneously enhancing lesion with areas of pseudocystic degeneration in the right lateral ventricle (Fig. 1C,D). Whole spine MRI did not show drop metastases. The patient underwent resection of the mass by a transcortical transventricular approach. At a 7-month follow-up visit after the second surgery, a brain MRI showed a small area of enhancing contrast. The patient refused to undergo an additional surgical operation to remove the residual mass and he decided against any adjuvant treatment (no chemotherapy or radiotherapy). At the 36 months follow-up visit, clinical and radiological conditions of the patient were stable (Fig. 1E-G).

Histopathological and immunohistochemical examination of the surgical specimen revealed a diagnosis of atypical CN. Photomicrographs after hematoxylin and eosin stain showed a monotonous population of tumor cells with round nuclei and clear cytoplasm (Fig. 2A-C). A CD34 immunostaining revealed the presence of numerous thin-walled capillaries (Fig. 2D). Tumor cells showed immunoreactivity for synaptophysin (Fig. 2E) and a relatively high mitotic rate (MIB-1 index 6%, Fig. 2F). Endothelial cell population, isolated and expanded from tumoral tissue, seen by phase-contrast microscopy, formed a monolayer with the typical cobblestone-like morphology (Fig. 3A). CN-ECs were characterized by flow cytometry analysis (Fig. 3B). Results showed high expression for CD31 (94.3±3.2%), CD309/VEGFR2 (77.7±2.5%), and CD133 (83.7±2.8%). Moreover a moderate expression was revealed for CD105 (9.1±0.8%), the endoglin, a specific and sensitive marker for evaluation of neoplastic angiogenesis, particularly expressed in glioblastomas (Afshar Moghaddam et al., 2015). To determine whether EndoPM cultured CN-ECs were able to show angiogenic function, 1×10^4 cells were plated on Matrigel and the cultures were examined for capillary tube-like structure formation. CN-ECs exhibited an angiogenic response within 12 h and migrated to form net-like, capillary tube-like structures (Fig. 3C). Using immunofluorescence staining, *in vitro* endothelial functionality was demonstrated by evaluating the uptake of Dil-Ac-LDL (Fig. 3D) and the expression for eNOS (Fig. 3E).

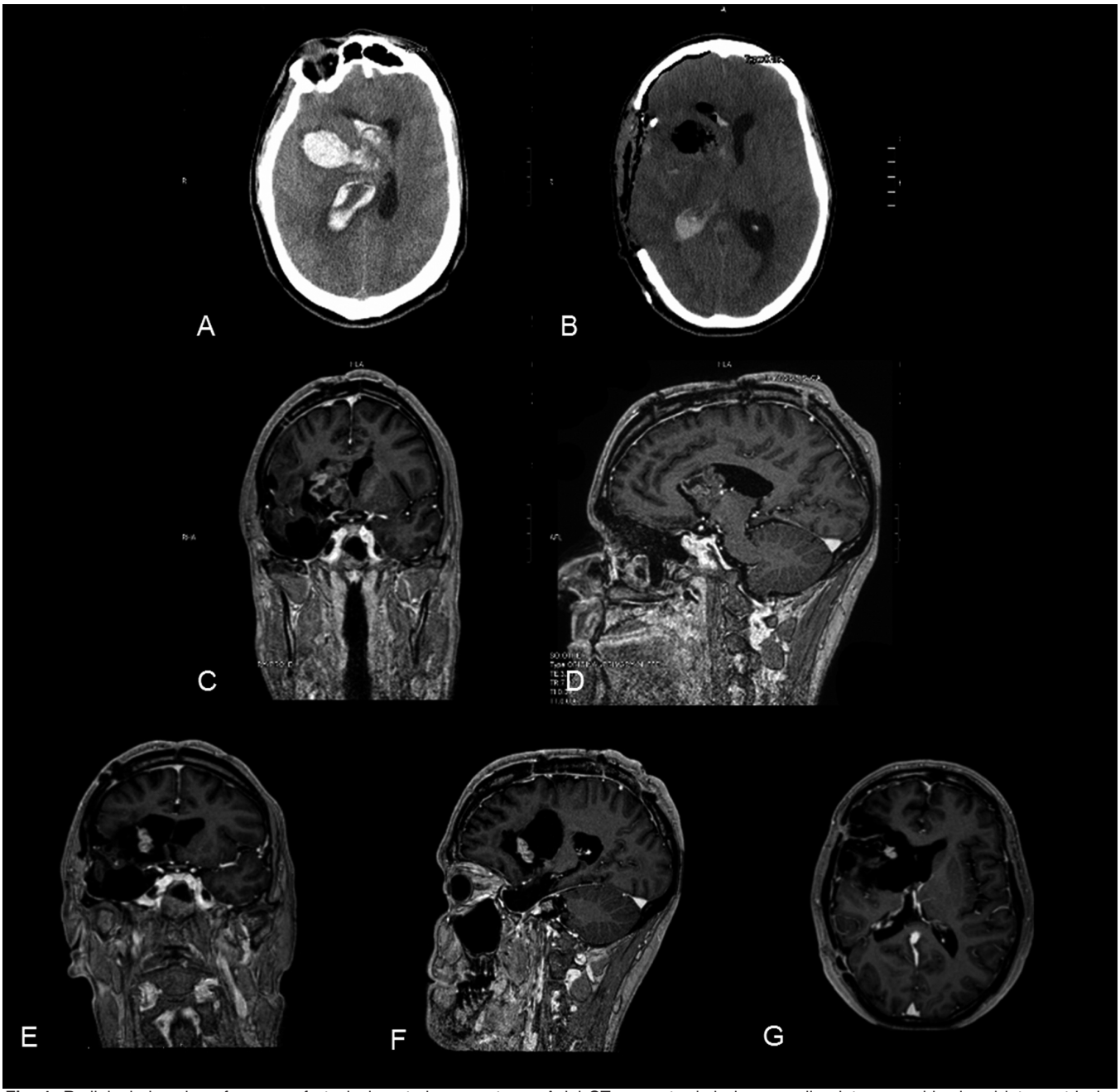
Discussion

CN are rare tumors of the central nervous system, accounting for about 0.25-0.5% of all intracranial tumors, and are classified as a grade II brain tumor according to the WHO classification (Louis et al., 2016). They are considered benign lesions and are generally associated with a favourable outcome. CN occurs predominantly in young adults around the third and fourth decade of life and usually arises within the supratentorial ventricolar system in proximity to deep midline structures, causing visual changes, altered consciousness, headaches, and other symptoms related to non-communicant hydrocephalus and increased intracranial pressure (Schmidt et al., 2004). A subpopulation of CNs exhibits more aggressive clinical behaviours and are termed atypical CNs. This subgroup is characterized by a higher mitotic activity (e.g. MIB-1 index >2%) compared to normal CNs or atypical histological features, such as focal necrosis, cellular pleomorphism, and endothelial and vascular proliferation (Mackenzie, 1999; Moreno et al., 2001; Rades et al., 2004; Mozes et al., 2014). Atypical CNs tend to disseminate in the central nervous system both intracranially and intraspinally, causing drop metastases, and to recur locally after surgical resection (Mozes et al., 2014). Furthermore, several studies have shown a significant correlation between mitotic rate and a poor outcome (Mackenzie, 1999).

Spontaneous intracranial hemorrhage (ICH) in young adults may represent a difficult challenge in the differential diagnosis among different hemorrhagic lesions. Intracerebral brain hemorrhage is more frequently due to arterial hypertension and non-tumoral lesions, such as cavernous angioma and vascular malformations. In addition, tumoral lesions presenting ICH normally include mainly high grade glial tumors, metastatic tumors, cerebral lymphoma and other malignancies (Wakai et al., 1982; Kase, 1986). CN is not commonly viewed as a hemorrhage-producing tumor and few hemorrhagic CNs have been reported in the literature (Smoker et al., 1991; Okamura et al., 1995;

Balko and Schultz, 1999; Jamshidi et al., 2001; Smets et al., 2005; Nishibayashi et al., 2006; Terakawa et al., 2010). Moreover, although histological hypervascularity in these cases were described, the factors predisposing to this feature and to hemorrhage remain to be determined (Jamshidi et al., 2001; Nishibayashi et al., 2006).

In this paper we report on a case of ICH caused by an atypical CN, which is an uncommon presentation for this kind of tumor. In our case, radiological examination of the patient by means of CT allowed us to diagnose the



Fig, 1. Radiologic imaging of a case of atypical central neurocytoma. Axial CT scan at admission revealing intraparenchimal and intraventricular hemorrhage **(A)**. Immediately postoperative CT scan showing a decompressive craniotomy and evacuation of the intraparenchimal hematoma **(B)**. Coronal **(C)** and sagittal **(D)** MRI T1-weighted images with contrast at 10 months after hemorrhage showing a solid mass with heterogeneous enhancement and cystic portions inside lateral ventricle and third ventricle. Coronal **(E)**, sagittal **(F)**, axial **(G)** MRI T1-weighted images after Gadolinium administration at 36 months after second surgery showing stable small area of enhancing contrast.

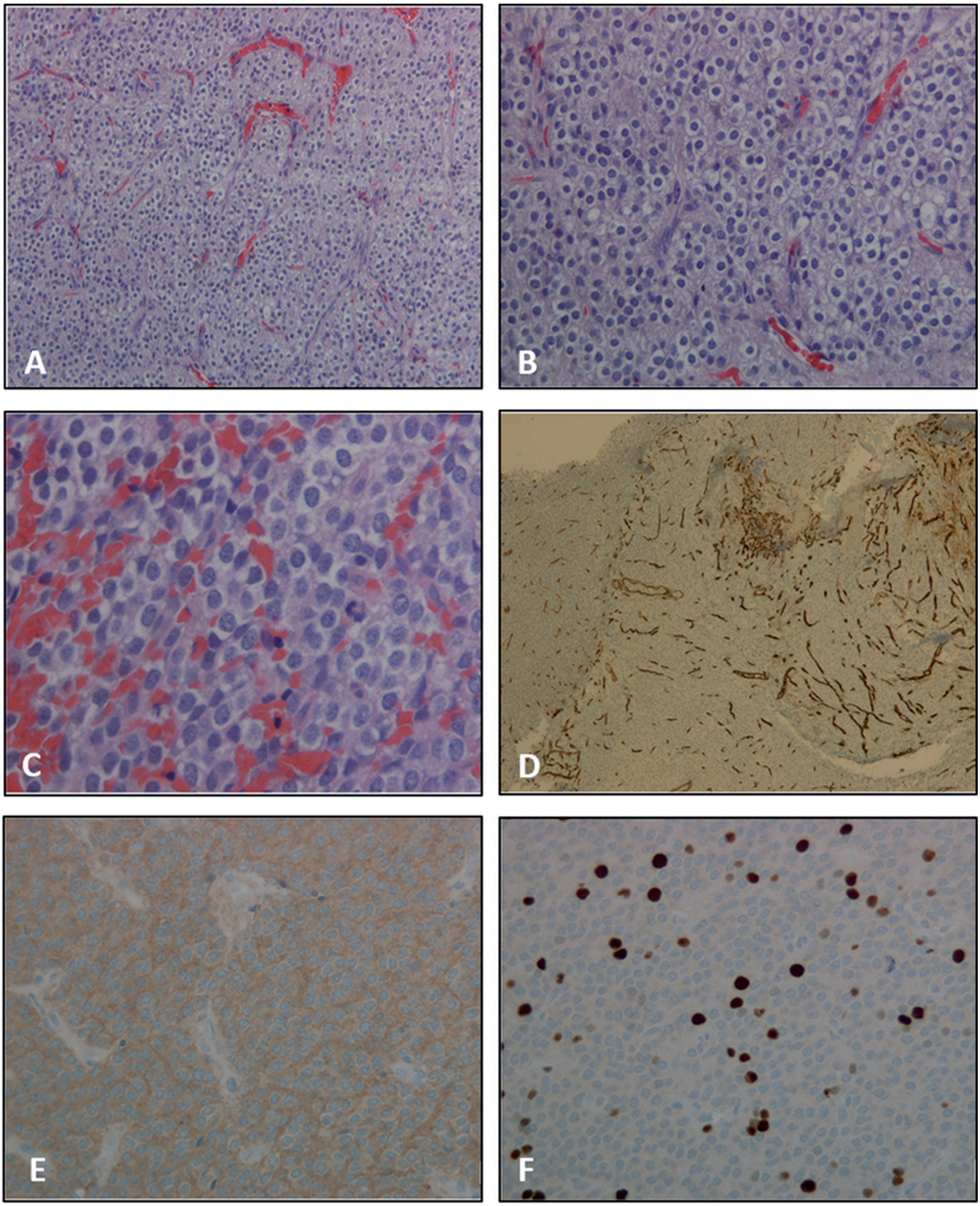


Fig. 2. Photographs of the tumour specimen. **A-C.** Monotonous population of tumor cells, with round nuclei and clear cytoplasm (HE stain). **D.** Thin walled capillary sized vessels in an arborizing pattern CD34 immunostaining. **E.** Synaptophysin immunostaining. **F.** MIB1 index. A, x 100; B, E, F, x 200; C, x 400; D, x 40.

presence of an intraparenchimal hemorrhage and to make the decision to perform an emergency surgical intervention. After the evacuation of the hematoma the hemorrhagic cause was identified as a tumor mass. Subsequent histopathological and immunohistochemical evaluations were necessary to characterize the tumor as CN with atypical features, observing similar histological and cellular features as those found in other case reports in the literature. The cause of bleeding in central neurocytoma is still unclear but several factors have been proposed as cause of hemorrhage, such as the presence of numerous thin-walled tumor vessels

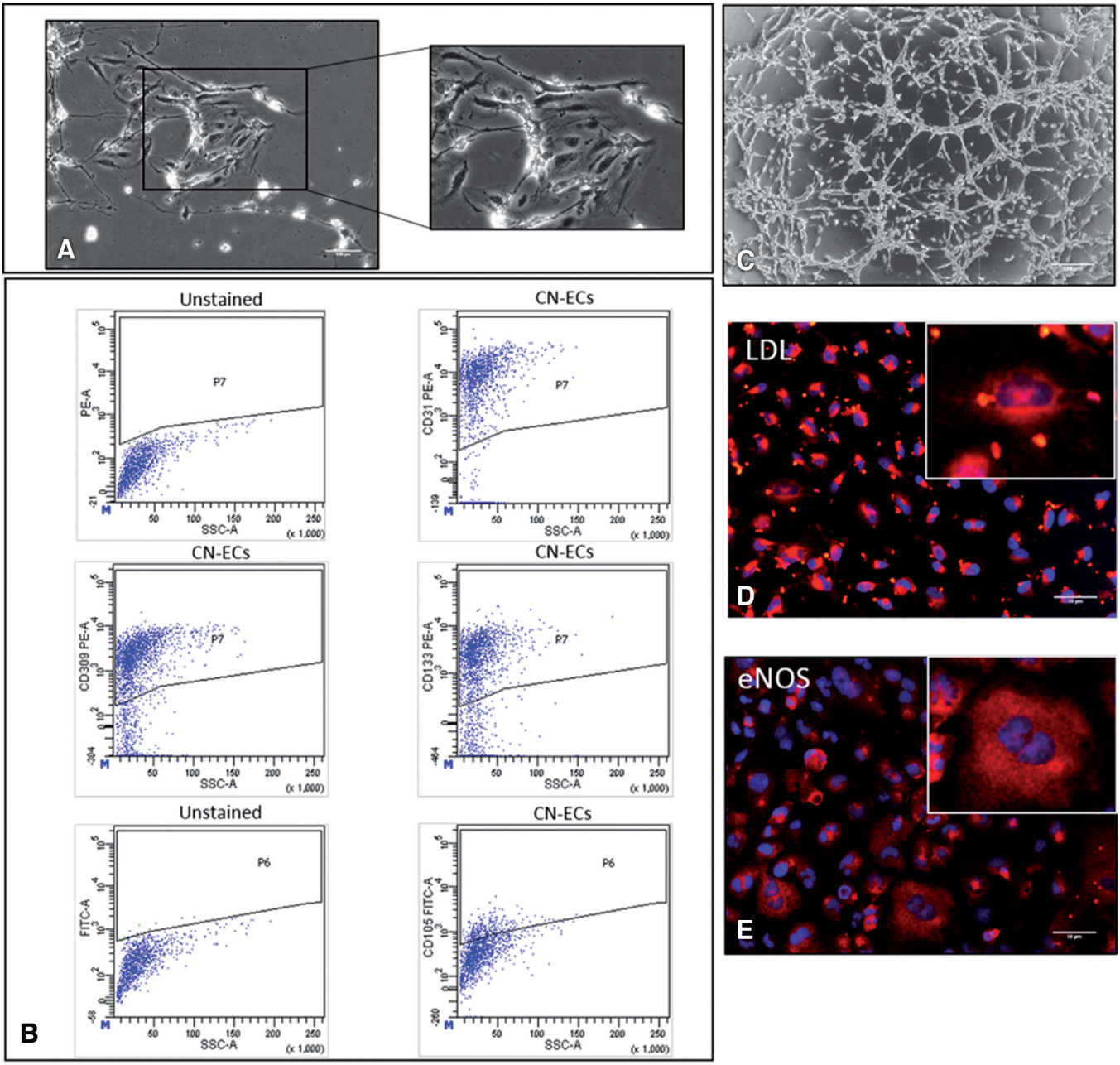


Fig. 3. Neurocytoma endothelial cells (CN-ECs) characterization. Characterization and functional features of CN-ECs. **A.** Phase contrast micrographs of confluent monolayers CN-ECs. Cells present the typical "cobblestone appearance". **B.** Flow cytometric analysis of CN-ECs. Cells resulted positive for CD31, CD309/VEGFR2, CD133 and a moderate positivity for CD105. **C.** Capillary tube-like structure produced by CN-ECs, 12h after plating onto Matrigel. **D.** LDL-uptake assay on human CN-ECs. **E.** Immunofluorescence for eNOS in CN-ECs. All nuclei were counterstained with DAPI (blue). One representative of three independent experiments performed in blind is shown for each figure. Scale bars: A, 100 µm; C, 200 µm; D, E, 50 µm.

(Smoker et al., 1991; Nishibayashi et al., 2006), hemodynamic stress caused by arteriovenous shunting (Liwnitz et al., 1987), coexistence of an aneurysm originating from a feeding artery into the tumor (Vates et al., 1982), hypertensive heart disease (Balko and Schultz, 1999), and thrombocytopenia (Smets et al., 2005). On the contrary, hypervascularity does not seem to be directly correlated to the occurrence of hemorrhage (Terakawa et al., 2010). In our case, we observed the presence within the tumor tissue of thin-walled capillaries, although without a particularly high vascularity. The formation of these fragile vessels would, hence, give rise to intracranial hemorrhage. Moreover, we found that the tumor cells from the resected tissue had a relatively high mitotic rate (MIB-1 index 6%), even if a high proliferation rate of the tumor appears not to be related with the incidence of hemorrhage in central neurocytomas (Terakawa et al., 2010). This suggests that the particular characteristics of the endothelial cells composing the blood vessels of the tumor tissue are strictly related to the occurrence of hemorrhagic events. Since in the literature there is no relevant study regarding the cause of bleeding in CN, following our protocol recently published (Navone et al., 2013a), we tried and succeeded in isolating the endothelial microvascular component from the tumor tissue biopsy in order to prove the feasibility of the procedure and suggest the possibility of further studies on the endothelial component associated to central neurocytoma. The endothelial cells were characterized by flow cytometry and immunofluorescence techniques showing typical features of the endothelial lineage with high expression of CD31 and CD309/VEGFR2. Moreover, CN-ECs expressed a moderate positivity for CD105, a typical marker of neoplastic angiogenic endothelial cells (Afshar Moghaddam et al., 2015). The angiogenic markers that we tested are commonly used during tumor endothelial cell characterization. In particular, CD31 and CD309/VEGF/R2 are usually highly expressed by endothelial cells in both vascularized neoplasm and normal tissues. CD105 (or endoglin), on the contrary, seems to have a differential positive expression among different types of brain tumors. CD105 is normally highly expressed in many different types of malignancies associated with low patient survival rates (Kong et al., 2016). In brain tumors, for example, glioblastoma cells highly express this angiogenic marker and this is often associated to the particular structural composition of its blood vessels. The hypervascularized tissues from glioblastoma contain blood vessels with endothelial cells presenting fenestration, and these characteristics are normally associated to extremely high aggressive glioblastoma (Dallas et al., 2008). On the other hand, for example, low-grade gliomas, which have a lower aggressiveness, present blood vessels with tight junctions instead of fenestrations and their endothelial cells show a lower expression of CD105 than glioblastoma cells (Kong et al., 2016). As we show, CD105 expression of CN-ECs appears to be positive, with levels closer to those measured in low-grade gliomas rather than those found in glioblastomas. Furthermore, a number of studies reported that the structure of CN blood vessels appears to be similar to that found in low-grade gliomas, i.e. ECs with tight junctions, while different from that found in glioblastomas (Miyagami and Nakamura, 1997). All this evidence suggests that there could be a tight correlation between the expression of CD105 and the blood vessel structure within brain tumors.

For this reason, it is evident the need for further studies on the endothelial cell component of brain tumors, such as CN, with the aim of disclosing the etiology of the abnormal angiogenesis and the related hemorrhage. Further studies on this subject could help scientists to eventually propose possible molecular targets for future biological treatments, specific to the type of tumor to treat, such as anti-angiogenic adjuvant therapies after the resection of the tumor mass by surgical intervention. In this context, we think that our study could be helpful to stress this need and to plan future experiments where bleeding neurocytoma-derived endothelial cells are considered.

In conclusion, the study of CN-ECs could have a positive feedback in the diagnosis, therapy and monitoring the disease in patients with CNs. However, multicentre studies are essential in order to investigate and confirm the role of the endothelial microvascular component in a larger number of cases. This may help treating physicians in the therapeutic decision-making in order to tailor treatments to each single patient and achieve the best clinical outcome.

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References

Afshar Moghaddam N., Mahsuni P. and Taheri D. (2015). Evaluation of endoglin as an angiogenesis marker in glioblastoma. Iran. J. Pathol. 10, 89-96.

Balko M.G. and Schultz D.L. (1999). Sudden death due to a central neurocytoma. Am. J. Forensic. Med. Pathol. 20, 180-183.

Dallas N.A., Samuel S., Xia L., Fan F., Gray M.J., Lim S.J. and Ellis L.M. (2008). Endoglin (CD105): a marker of tumor vasculature and potential target for therapy. Clin. Cancer Res. 14, 1931-1937.

Di Vito C., Navone S.E., Marfia G., Abdel Hadi L., Mancuso M.E., Pecci A., Crisà F.M., Berno V., Rampini P., Campanella R. and Riboni L. (2017). Platelets from glioblastoma patients promote angiogenesis of tumor endothelial cells and exhibit increased VEGF content and release. Platelets 28, 585-594.

Jamshidi J., Izumoto S., Yoshimine T. and Maruno M. (2001). Central neurocytoma presenting with intratumoral hemorrage. Neurosurg. Rev. 24, 48-52.

Kase C.S. (1986) Intracerebral hemorrage: non-hypertensive causes.

Stroke 17, 590-595.

Kong X., Wang Y., Liu S., Xing B., Yang Y., Li Y., Ren Z., Su C., Ma W. and Wang R. (2016). CD105 over-expression is associated with higher WHO grades for gliomas. Mol. Neurobiol. 53, 3503-3512.

Louis D.N., Ohgaki H., Wiestler O.D., Cavenee W.K., Burger P.C., Jouvet A., Scheithauer B.W. and Kleihues P. (2007). The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 114, 97-109.

Louis D.N., Perry A., Reifenberger G., von Deimling A., Figarella-Branger D., Cavenee W.K., Ohgaki H., Wiestler O.D., Kleihues P. and Ellison D.W. (2016). The 2016 World Health Organization classification of tumors of the central nervous system: A summary. Acta Neuropathol. 131, 803-820.

Liwnitz B.H., Wu S.Z. and Tew J.M. (1987). The relationship between the capillary structure and hemorrhage in gliomas. J. Neurosurg. 66, 536-541.

Mackenzie I.R.A. (1999). Central neurocytoma: Histologic atypia, proliferation potential, and clinical outcome. Cancer 85, 1606-1610.

Miyagami M. and Nakamura S. (1997). Ultrastructure of microvessels and tumor cell processes on central neurocytoma. Brain Tumor Pathol. 14, 79-83.

Moreno A., de Felipe J., García Sola R., Navarro A. and Ramón y Cajal S. (2001). Neuronal and mixed neuronal glial tumors associated to epilepsy. A heterogeneous and related group of tumours. Histol. Histopathol. 16, 613-622.

Mozes P., Szanto E., Tiszlavicz L., Barzo P., Cserhati A., Fodor E. and Hideghety K. (2014). Clinical course of central neurocytoma with malignant transformation - an indication for craniospinal irradiation. Pathol. Oncol. Res. 20, 319-325.

Navone S.E., Marfia G., Invernici G., Cristini S., Nava S., Balbi S., Sangiorgi S., Ciusani E., Bosutti A., Alessandri G., Slevin M. and Parati E.A. (2013a). Isolation and expansion of human and mouse brain microvascular endothelial cells. Nat. Protoc. 8, 1680-1693.

Navone S.E., Marfia G., Nava S., Invernici G., Cristini S., Balbi S., Sangiorgi S., Ciusani E., Bosutti A., Alessandri G., Slevin M. and Parati E.A. (2013b). Human and mouse brain-derived endothelial cells require high levels of growth factors medium for their isolation, in vitro maintenance and survival. Vasc. Cell 5, 10-15.

Nishibayashi H., Uematsu Y., Terada T. and Itakura T. (2006). Neurocytoma manifesting as intraventricular hemorrage - Case report. Neurol. Med. Chir. 4,6, 41-45.

Okamura A., Goto S., Sato K. and Ushio Y. (1995). Central neurocytoma with hemorrhagic onset. Surg. Neurol. 43, 252-255.

Piepmeier J. and Baehring J.M. (2004). Surgical resection for patients with benign primary brain tumors and low grade gliomas. J. Neurooncol. 70, 371.

Rades D., Fehlauer F. and Schild S.E. (2004). Treatment of atypical neurocytomas. Cancer 100, 814-817.

Schmidt M.H., Gottfried O.N., von Koch C.S., Chang S.M. and McDermott W.M. (2004). Central neurocytoma: a review. J. Neurooncol. 66, 377-384.

Smets K., Salgado R., Simons P.J., De Clercq R., De Smedt K. and Cras P. (2005). Central neurocytoma presenting with intraventricular hemorrage: case report and review of literature. Acta. Neurol. Belg. 105, 218-225.

Smoker W.R.K., Townsend J.J. and Reichman M.V. (1991). Neurocytoma accompanied by intraventricular hemorrage: case report and literature review. Am. J. Neuroradiol. 12, 765-770.

Terakawa Y., Tsuruno T., Ishibashi K., Okada Y., Shimotake K. and Murata T. (2010). Central neurocytoma presenting with massive hemorrage leading to coma. Neurol. Med. Chir. 50, 139-143.

Vaquero J., Coca S., Zurita M., Oya S., Arias A., Morales C., Buján J. and García N. (1993). Experimental neurocytomas. Histol. Histopathol. 8, 91-96.

Vates G.E., Arthur K.A., Ojemann S.G., Williams F. and Lawton M.T. (2001). A neurocytoma and an associated lenticulostriate artery aneurysm presenting with intraventricular hemorrhage: case report. Neurosurgery 49, 721-725.

Wakai S., Yamakawa K., Manaka S. and Takakura K. (1982). Spontaneous intracranial haemorrage caused by brain tumor: its incidence and clinical significance. Neurosurgery 10, 437-444.

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