NEOPLASMA accepted, ahead of print manuscript
Cite article as https://doi.org/10.4149/neo_2026_250623N278

Running title: Microvilli expression in glial and glioma cell cultures

Cell surface topography differs in the human "glia-like" and glioma cultures

Ivana Sivakova[1], Maria Lorencova[2], Anna Perzelova[1], Paulina Galfiova[2], Stefan Polak[1,2]

[1]Department of Anatomy, Faculty of Medicine Comenius University in Bratislava, Bratislava, Slovakia
[2]Center of Electron-microscopic Laboratory Methods, Institute of Histology and Embryology, Faculty of Medicine Comenius University in Bratislava, Bratislava, Slovakia

*Correspondence: galfiova1@uniba.sk

Received June 23, 2025 / Accepted January 19, 2026

Glioblastoma multiforme is the most malignant and incurable primary brain tumor. Infiltrative growth of gliomas into surrounding brain tissue may cause the presence of normal cells in glioma cultures. The aim of this study is to develop a simple, rapid method for detecting normal cells in short-term glioma cultures, to be applied primarily to personalized glioma treatment.
Cell lines with permanent cell growth consist solely of cancer cells. Here, we examined two glioblastoma cell lines (8-MG-BA and 170-MG-BA), one brain metastatic carcinoma cell line (135-BCA), five short-term glioblastomas, and five human "glia-like" cultures using scanning electron microscopy (SEM), standard phase contrast microscopy, and GFAP immunofluorescence.
All cells in glioblastoma and carcinoma cell lines were covered with microvilli of varying density, 4/5 of short-term glioblastoma cultures contained 1-3% cells with sparse microvilli, and one culture (139-GBM) showed microvilli in 15-20% of the cells and a higher percentage of GFAP-positive cells. A rare occurrence (less than 1%) of cells bearing microvilli was observed in all "glia-like" cultures. Using SEM, we observed similar cells with microvilli in both glioblastoma cell lines, but in the 135-BCA line, the microvilli were significantly shorter. Microvilli rarely occurred on normal "glia-like" cells. Based on this observation, we conclude that our 4/5 of short-term glioblastoma cultures contain predominantly normal "glia-like" cells.
SEM could be a valuable method for distinguishing normal and tumor cells in short-term glioblastoma cultures, which have similar morphologies at light microscopy and immunophenotypes. We conclude that microvilli are characteristic of a specific tumor cell surface topography compared to "glia-like" cells.

Key words: glioblastoma; "glia-like" cells; SEM; microvilli

Gliomas are primary CNS tumors arising from glial cells. *Glioblastoma multiforme* is the most malignant and invasive glioma with a lethal prognosis. Glioma resistance to treatment is attributed to inter- and intra-tumoral heterogeneity [1, 2]. The heterogeneity of gliomas in the expression of intermediate filaments which are cell-types specific cytoskeletal proteins, is notable. Glial fibrillary acidic protein (GFAP) is considered to be specific marker for cells of astroglial origin [3]. Most permanent glioblastoma cell lines are negatively stained for GFAP or lose GFAP expression after serial passages [4, 5]. Human cultured "glia-like" cells do not express GFAP [6-8]. Cytokeratins (CK), specific for normal and neoplastic epithelial cells were found in glioma tissue [9]. Recently, we described a subpopulation of CK-positive cells in glioma cell lines as well as in human "glia-like" cells [5, 10, 11]. Nestin is a protein of intermediate filament originally identified as a marker for neuroepithelial precursor cells [12]. However, nestin is also expressed in a variety of neoplasms, including brain tumors [13]. Previously, we detected nestin-positive subpopulation in "glia-like" cultures as well as in glioblastoma cell lines [5, 14]. Initially, vimentin intermediate filament was described as specific to mesenchymal cells. However, vimentin was also present in GFAP-positive astrocytes, in all "glia-like" cells and glioblastoma cell lines [5, 8, 14]. Although intermediate filaments are often used as the markers, they have limited use in identifying cell types under culture conditions.

We established and characterized four glioblastoma cell lines: 8-MG-BA, 42-MG-BA, 170-MG-BA, and 538-MG-BA [5, 15], and one metastatic brain cell line from large lung carcinoma 135-BCA [16]. Glioblastoma cell lines showed intertumoral and intratumoral heterogeneity. Subpopulation of GFAP-positive cells was present in 8-MG-BA and 42-MG-BA, while in 170-MG-BA, and 538-MG-BA was not observed [5, 15]. However, the 135-BCA carcinoma cell line maintained specific CK expression throughout whole culturing.

Noteworthy is 170-MG-BA, which shows amplification of the epidermal growth factor receptor (EGFR) gene, the most common genetic alteration in gliomas, which is the target of current therapeutic strategies [17, 18]. EGFR amplification is unstable in common glioma cell lines. However, 170-MG-BA is apparently the first conventional glioma cell line with stable high level of EGFR expression. This was confirmed by combined cytogenetic genomic and transcriptional analyses [5].

Poor treatment outcomes in glioblastoma require a deeper understanding of glioma biology. New therapeutic approaches are often investigated in glioma cell lines that have critical disadvantages: 1) cell lines cross-contamination (CLCC) [19-22]; 2) cryptic contamination with mycoplasma with the influence on cell growth [23]; 3) glioblastoma short-term cultures which may contain normal human "glia-like" cells [24]; 4) poor knowledge of glioma cells growing under different cultures conditions.

Optimization of the available cell models requires more comparative studies on glial and glioma cells. Surface morphology obtained from scanning electron microscopy (SEM) exceeds many times the structural outcome of the light microscope, standardly used for cell culture evaluation. The identification of cell surface structures opens up several morphologic options: microvilli, lamellipodia, filopodia, micro-spikes, plasma membrane blebs or ruffles [25, 26]. Cancer cells may exhibit increased numbers of all of the above cellular structures that play a key role in cancer cell migration, invasion and metastasis [27-29]. "Microvillus" is a unique term for the cytoplasmic protrusion on the apical surface of most epithelial cells. Microvilli are supported by actin filaments that are arranged into bundles. The average microvillus is about 1 μm long and 0.1 μm wide [30]. The small intestine is the primary site of microvilli. They could also be found in the respiratory, reproductive and other systems. In the brain they are found on ependymal cells and on the dendrites of sensory neurons. Microvilli appear on the surface of various tumor cells [31], including human glioma cell lines [32, 33].

The surface structures of glioma cells can be targeted for innovative therapeutic approaches. In this study, we observed detailed imaging of cell surface structures in the glioblastoma cell lines (8-MG-BA, 42-MG-BA and 170-MG-BA), short-term cultures from glioblastoma and normal adult brain tissue.

Materials and methods

Biopsy samples. Different brain biopsy samples were kindly provided by the Department of Neurosurgery, Derer's Hospital, Bratislava. They were collected between 1993 to 2011. Experiments with human brain biopsies were conducted in accordance with Slovak laws 272/1994, 76/2004 and were approved by the Ethical Committee of UNB Bratislava. For this study we selected brain biopsies from patients undergoing neurosurgical intervention for *glioblastoma multiforme* (n=5) and non-tumoral diagnoses (n=5). The samples were obtained from the frontal, temporal or occipital lobes. Clinical data of patients are summarized in Tables 1 and 2.

Tissue cultures. Tissue cultures were prepared by an explant method**.** Biopsy brain samples were cut into small pieces and seeded in uncoated plastic dishes (25 cm^2). Culture medium consisted of MEM (M0643, Sigma) Minimum Essential Medium Eagle with Earle s salts, L-glutamine, nonessential amino acids and 10% fetal calf serum (Sigma). Cells in early passages were cryopreserved in liquid nitrogen. For this study, we recultured glioblastoma 8-MG-BA and 170-MG-BA cell lines, metastatic brain carcinoma 135-BCA cell line, 5 glioblastoma tissue cultures, and 5 normal adult human brain cultures. Passaging was performed using 0.2% EDTA and 0.25% trypsin. All cell lines as well as short term cultures were cultured and passaged under the same culture conditions.

Scanning electron microscopy (SEM). Cells used for SEM were grown under the same conditions on uncoated glass coverslips (diameter 12.5 mm). Cell cultures adhered on coverslips in culture medium were gently washed by 3% glutaraldehyde buffered solution fixative for 30 min at the room temperature. Afterwards, samples were rinsed three times in phosphate buffer solution and postfixed in osmium tetroxide 1% solution for 1 hour at the 4 °C temperature. After the rinse in demineralized water, samples were dehydrated through a graded ethanol series to 100% ethanol, followed by critical point drying of liquid CO_2. Finally, they were mounted on aluminium specimen stubs with carbon adhesive tapes, sputter coated with 5 nm layer of gold/palladium and evaluated with a scanning electron microscope ZEISS type EVO LS 15 in the Center of electron-microscopic laboratory methods, at the Institute of Histology and Embryology, Medical Faculty, Comenius University in Bratislava.

Immunofluorescence staining. Cells grown on coverslips were rinsed with PBS, fixed in methanol-acetone (1:1) solution for 15 min at -15 °C, and used for indirect immunofluorescence staining. For GFAP examination we used the following antibodies: against GFAP, clone GF-01, 1:100, (Exbio, Prague), and polyclonal sera to GFAP, 1:100, (Dako). Secondary fluorescein-conjugated antibodies were purchased from Sigma and Sevapharma (Prague, Czech Republic). Cells for indirect immunofluorescence were incubated 1h with primary and 30 min. with secondary antibodies. Nuclei were stained with Hoechst 33258 fluorochrome (5 μg/ml in PBS, Sigma) for 1 min. To determine the percentage of immunoreactive cells, 30 fields were enumerated at 200× magnification, equally distributed over the coverslips fixed at different DIV and passage numbers. Fluorescence micoscopy was performed using an Olympus BX51 microscope (Olympus, Germany).

Results

Morphology at the level of phase-contrast microscopy. Morphological features of living cells were examined with inverse phase-contrast microscopy (Olympus IMT-2). Glioblastoma 170-MG-BA cell line in 110 to 120 passages consisted mainly of flat and spindle-shaped cells (Figure 1A). Glioblastoma 8-MG-BA cell line in 330 to 335 passages were predominantly flat in shape (Figure 2A). Metastatic brain carcinoma 135-BCA in passage 50 to 53 were mostly epitheloid in shape and grew in clusters (Figure 3A). Short-term cultures obtained from *glioblastoma multiforme* (Figures 4A, 4B) and cultures from normal brain tissue (Figure 5A) contained similar cell morphologies between passages 4 to 8, when we did not observe astroglial cells with long and thin processes. All cultures consisted of flat, spindle-shaped and intermediate cell morphologies. Cell surface protrusions could not be identified regarding the resolving power limits of the phase-contrast microscopes.

SEM: **Glioblastoma 170-MG-BA cell line.** Morphological properties of 170-MG-BA cell line are demonstrated at 200× magnification (Figure 1B). Cells displayed flat or spindle shape, made a confluent monolayer. Detailed observation of cellular surface is shown at higher magnifications, overcoming the light microscopy limitations. Using a SEM, considering the output, only the topography, size and density of surface protrusion could be evaluated. The majority of cells possessed numerous microvilli, which were present on flat (Figure 1C) or spindle-shaped cells (Figure 1D). Microvilli occurred at different densities on cells regardless the cell shape (Figure 1E). They were present on all cells, 10 to 15% of cells showed sparce microvilli. Microvilli in the 170 MG-BA cell line were 1-2 μm long in average and the thickness range was from 130-150 nm in diameter (Figure 1F). The size reduction had to be considered, due to the sample procedure including both, the dehydration with ethanol series and critical point drying [34, 35].

SEM: Glioblastoma 8-MG-BA cell line. Morphological properties of 8-MG-BA cell line are demonstrated at 200× magnification (Figure 2B). Cells displayed mainly flat (epitheloid) shape (Figures 2C, 2D), grew in monolayer. Detailed observation of cellular surface is shown at higher magnifications, referring that all cells possessed microvilli (Figures 2C-2E) with different size and density. The measurement of microvilli is demonstrated at 10,000× (Figure 2F). Microvilli in the 8 MG-BA cell line were 1-3.5 μm long in average and the thickness range was from 130-150 nm in diameter.

SEM: **Metastatic brain carcinoma 135-BCA cell line.** Morphological properties of 135-BCA cell line are demonstrated at 200× magnification (Figure 3B). Cells showed great variability in shape, being epitheloid, spindle-shaped, or round, growing in clusters (Figures 3B-3D). Detailed observation of the cell surface is shown at higher magnifications, referring to the fact that all cells had very short microvilli of varying density (Figures 3C-3E). The measurement of microvilli is demonstrated at 10,000× (Figure 3F). Microvilli in 135-BCA cell line had an average length 0.3-1 μm and an average thickness range was from 130-150 nm in diameter.

SEM: Short-term cultures from glioblastoma multiforme. The basic morphological properties of short-term glioblastoma cultures are demonstrated at 200× magnification (Figure 4C). The cells were predominantly flat in shape. We examined 5 cultures, 4 of which showed similar number of microvilli bearing cells, scanty microvilli were present in 1 to 3% of all cells (Figure 4E). The remaining cells showed a morphology without microvilli (Figures 4D, 4F). One culture (139-GBM) contained higher number of microvilli-bearing cells, approximately 15 to 20% of all cells (Figures 4G, 4H). The length of glioblastoma cell microvilli ranged from 1.5-4.5 μm, in some places. However, the average length of microvilli was approximately 2 μm. Microvilli showed a similar thickness, 130-150 nm on average, compared to microvilli of the glioblastoma cell line (Figure 4I).

SEM: Short-term cultures from normal brain tissue. The basic morphological properties of short-term normal brain cultures are demonstrated at 200× magnification (Figure 5B). Flat cells predominated over spindle-shaped ones. Higher magnifications were used for the detection of microvilli. All cultures contained predominantly cells without microvilli (Figures 5C, 5D), rarely (less than 1%), cells with sparse microvilli were found (Figure 5E), Ranging in length from 0.2-1.8 μm and about 120 nm in diameter in thickness (Figure 5F). Microvilli of normal cells from brain tissue cultures were less frequent and the shortest of all cell cultures.

Immunofluorescence. The results of indirect immunofluorescence staining for GFAP are shown in Figure 6. GFAP-positive cells in all five cultures of normal human brain were present in a low percentage (0.1%). They were mainly long and thin processes-bearing astrocytes or large flat cells (Figures 6A, 6B). All GFAP-positive cells disappeared after passage number 3. In glioblastoma short-term cultures, GFAP-positive cells occurred in 1/5 cultures. Positive cells in 139-GBM reached for about 50% of cells at passage number 4. They showed mainly flat or spindle shaped morphologies, only a small proportion of cells had thin and longer processes (Figures 6C, 6D). The percentage of GFAP-positive cells decreases markedly with passaging until they eventually disappear at passage number 15.

Infiltrative growth of gliomas. Biopsy samples may consist of varying amounts of normal brain tissue infiltrated with glioma cells. Under culture conditions, it is not possible to distinguish normal cells from glioma cells, if they have similar morphology and immunophenotype. The different ratio of normal cells and glioma cells in culture may reflect subsequent passaging of culture, in which we observed three modifications. A) normal cells overgrew the glioma cells and stopped cell growth between passages 12-15. B) GFAP-positive glioma cells made up to 50% of the cells in the early stage. These cells disappeared during passaging, and the remaining flat cells stopped growing. C) A higher number of GFAP-negative glioma cells overgrew normal cells and gave rise to a glioblastoma cell line with continuous cell growth. The numbers of cells with microvilli in cell lines and short-term cultures are shown schematically in Fig. 8. in form of graphic visualization of the cell counts.

Discussion

We have recently demonstrated a comparative analysis of adult human "glia-like" cells and glioblastoma short-term cultures. Immunostaining, growth and morphological characteristics were similar in both cultures [24]. GFAP-positive cells are rare or absent in adult human cultures, often referred to as "glia-like" cells [6, 7]. These non-passaged primary cultures contain low numbers of morphologically and immunocytochemically distinct glial cell types: 2-5% microglial cells, 0.1% GFAP-positive astrocytes, less than 0.01% oligodendrocytes, and 95-98% flat or spindle-shaped "glia-like" cells positively stained for vimentin and fibronectin [8, 14]. However, all of these glial cell types rapidly disappear with repeated passaging. Some authors consider flat GFAP-negative "glia-like" cells to be of non-glial origin [7, 36]. However, we have previously found that GFAP-negative cells at the beginning of culture become GFAP-positive during terminal passages. These findings provide evidence for a glial origin of "glia-like" cells [37, 38].

In this study, we attempted to distinguish between normal and tumor cells in short-term cultures using the SEM method. Cultures prepared from glioblastoma multiforme biopsy specimens and brain tissue biopsies from patients with a non-tumor diagnosis were examined over 4 to 8 passages. For comparative study we used three cancer cell lines: two established from glioblastoma (8-MG-BA and 170-MG-BA) and one brain metastatic carcinoma 135-BCA. Unexpectedly, our SEM observations revealed microvilli in all cells in all cancer cell lines. In contrast, a rare occurrence of microvilli-bearing cells was found in all normal "glia-like" and in 4/5 short-term glioblastoma cultures. Only one culture (139-GBM) showed a higher percentage of cells covered with microvilli.

Our immunofluorescence staining with anti-GFAP antibodies revealed a higher percentage of GFAP-positive cells in only one short-term glioblastoma culture (139-GBM). All other normal and glioblastoma cultures were negatively stained for GFAP. Remarkably, the short-term glioblastoma culture with a higher percentage of microvilli-bearing cells contained an even higher percentage of cells positively stained for GFAP. Similarly, we recently published that in the 170-MG-BA glioblastoma cell line, GFAP-positive cells comprised 50-70% in the early passages, but their number gradually decreased and completely disappeared after the 20^{th} passage [5]. GFAP immunostaining only indicate the heterogeneity of gliomas. High percentage of GFAP-positive cells in culture is only one sign of malignancy, but it is not useful to distinguish "glia-like" cells in glioblastoma cultures.

Other studies on the surface topography investigated on human and rat glioma cell lines showed numerous microvilli and filopodia. As long filopodia of glioma cells they consider microvilli at the periphery of the cells at the time of air drying. They concluded that microvilli are composed of microfilaments due to depolymerisation of microfilaments using cytochalasin B. After this treatment microvilli collapsed and the surface of these cells smoothened out [33].

In conclusion, the presence of normal cells in glioblastoma cultures may be due to infiltrative growth of glioma into surrounding brain tissue; short-term cultures of glioblastoma may contain normal “glial-like” cells. In this study, we observed a predominant localization of microvilli on cancer cells, but normal “glia-like” cells were free of microvilli. Short-term cultures from gliomas are used to screen anticancer drugs for potential clinical efficacy. However, these cultures with a low percentage of microvilli-bearing cells can be considered as “glia-like” cells. These findings offer a simple method to distinghuish normal and glioblastoma cells in short-term cultures based on the presence of different cell surface topographies.

Acknowlegements: The study was financed under the provisions of the institutional budget.

References

[1] BECKER AP, CELLS BE, HAQUE SJ, CHAKRAVARTI A. Tumor heterogeneity in glioblastomas: From light microscopy to molecular pathology. Cancers 2021; 13: 761. https://doi.org/10.3390/cancers13040761

[2] LAUKO A, LO A, AHLUWALIA MS, LATHIA JD. Cancer cell heterogeneity and plasticity in glioblastoma and brain tumors. Semin Cancer Biol 2021; 82: 162-175. https://doi.org/10.1016/j.semcancer.2021.02.014

[3] ENG LF, GHIRNIKAR RS. GFAP and astrogliosis. Brain Pathol 1994; 4: 229-237. https://doi.org/10.1111/j.1750-3639.1994.tb00838.x

[4] RESTREPO A, SMITH CA, AGNIHOTRI S, SHEKARFOROUSCH M, KONGKHAM PN et al. Epigenetic regulation of glial fibrillary acidic protein by DNA methylation in human malignant gliomas. Neuro Oncol 2011; 13: 42-50. https://doi.org/10.1093/neuonc/noq145

[5] MACLEOD RAF, SCHNEIDER B, SIVAKOVA I, NAGEL S, DIRKS WG et al. High level EGFR amplification in a newly established glioblastoma cell line: 170-MG-BA. Neoplasma 2019; 66: 109-117. https://doi.org/10.4149/neo_2018_180427N278

[6] PONTEN J, WESTERMARK B. Properties of human malignant glioma cells in vitro. Med Biol 1978; 56: 184-193.

[7] RUTKA JT, KLEPPE-HOIFORD H, EMMA DA, GIBLIN JR, DOUGHERTY DV et al. Characterization of normal human brain cultures. Lab Invest 1986; 55: 71-85.

[8] MACIKOVA I, PERZELOVA A, MRAZ P, BIZIK I, STENO J. GFAP-positive astrocytes are rare or absent in primary adult human brain tissue cultures. Biologia 2009; 64: 833-839. https://doi.org/10.2478/s11756-009-0136-1

[9] HIRATO J, NAKAZATO Y, OGAWA A. Expression of non-glial intermediate filament proteins in gliomas. Clin Neuropathol 1994; 13: 1-11.

[10] MACIKOVA I, PERZELOVA A, MRAZ P, STENO J, BIZIK I. Heterogeneity of keratin intermediate filaments expression in human glioma cell lines. Neoplasma 1999; 46: 390-393.

[11] SIVAKOVA I, PERZELOVA A, KUBIKOVA E, MRAZ P. Unexpected presence of cytokeratins in human “glia-like” cells. Bratisl Med J 2013; 114: 274-278.

[12] FREDERIKSEN K, MCKAY RDG. Proliferation and differentiation of rat neuroepithelial precursor cells in vivo. J Neurosci 1988; 8: 1144-1151.

[13] HIRAO A, HAYATO I, KEN-ICHI S, SUMIHITO N, JUNKO H. Nestin expression in brain tumors: its utility for pathological diagnosis and correlation with the prognosis of high-grade gliomas. Brain Tumor Pathol 2012; 29: 160-167. https://doi.org/10.1007/s10014-012-0081-5

[14] PERZELOVA A, MACIKOVA I, TARDY M, MRAZ P, BIZIK I. Subpopulation of nestin positive glial precursor cells occur in primary adult human brain cultures. Biologia 2007; 62: 633-640. https://doi.org/10.2478/s11756-007-0123-3

[15] PERZELOVA A, MACIKOVA I, MRAZ P, BIZIK I, STENO J. Characterization of two new permanent glioma cell lines 8-MG-BA and 42-MG-BA. Neoplasma 1998; 45: 25-29.

[16] MACIKOVA I, PERZELOVA A, MRAZ P, STENO J, BIZIK I. Establishment, morphological, growth and cytoskeletal properties of 135-BCA carcinoma cell line derived from lung brain metastasis. Neoplasma 2001; 48: 479-482.

[17] HATANPAA KJ, BURNS S, ZHAO D, HABIB AA. Epidermal growth factor receptor in glioma: Signal transduction, neuropathology, imaging and resistance. Neoplasia 2010; 12: 675-684. https://doi.org/10.1593/neo.10688

[18] WESTPHAL M, MAIRE CL, LAMSZUS K. EGFR as a target for glioblastoma treatment: An unfulfilled promise. CNS Drugs 2017; 31: 723-735. https://doi.org/10.1007/s40263-017-0456-6

[19] MACLEOD RAF, DIRKS WG, MATSUO Y, KAUFMAN M, MILCH H et al. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. Int J Cancer 1999; 83: 555-556. https://doi.org/10.1002/(sici)1097-0215(19991112)83:4<555::aid-ijc19>3.0.co;2-2

[20] ROJAS A, GONZALES I, FIGUEROA H. Cell line cross-contamination in biomedical research: a call to prevent unawareness. Acta Pharmacol Sin 2008; 29: 877-880. https://doi.org/10.1111/j.1745-7254.2008.00809.x

[21] CAPES-DAVIS A, THEODOSOPOULOS G, ATKIN I, DREXLER HG, KOHARA A et al. Check your cultures! A list of cross-contaminated or misidentified cell lines. Int J Cancer 2010; 127: 1-8. https://doi.org/10.1002/ijc.25242

[22] ROUTRAY I, MAHMOOD A, NGWA NE, TASLEEM M, SAHIN K et al. Cell line cross-contamination and accidental co-culture. J Stem Cell Res Ther 2016; 1: 179-185. https://doi.org/10.15406/jsrt.2016.01.00031

[23] DREXLER HG, UPHOFF CU. Mycoplasma contamination of cell culture: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology 2002; 39: 75-90. https://doi.org/10.1023/A:1022913015916

[24] SIVAKOVA I, MACLEOD R, MRAZ P, KUBIKOVA E, PERZELOVA A. Short-term glioblastoma cultures may contain normal “glia-like” cells. Bratisl Med J 2019; 120: 625-629. https://doi.org/10.4149/BLL_2019_103

[25] ROTTER K, SCHAKS M. Assembling actin filaments for protrusion. Curr Opin Cell Biol 2019; 56: 53-63. https://doi.org/10.1016/j.ceb.2018.09.004

[26] LEE SY, FUJIOKA T, OSUGA M et al. Lamellipodia and filopodia. in Plasma membrane shaping. Elsevier 2023, pp. 245-263.

[27] DJUZENOVA CHS, FIEDLER V, MEMMEL S, KATZER A, HARTMANN S et al. Actin cytoskeleton organization, cell surface modification and invasion rate of 5 glioblastoma cell lines differing in PTEN and p53 status. Exp Cell Res 2015; 330: 346-357. https://doi.org/10.1016/j.yexcr.2014.08.013

[28] MEMMEL S, SUKHORUKOV L, HORING M, WESTERLING K, FIEDLER V et al. Cell surface and membrane folding in glioblastoma cell lines differing in PTEN and p53 status. PLoS One 2014; 9: e87052. https://doi.org/10.1371/journal.pone.0087052

[29] ALBLAZI KM, SIAR CH. Cellular protrusions-lamellipodia, filopodia, invadopodia and podosomes-and their roles in progression of orofacial tumours: current understanding. Asian Pac J Cancer Prev 2015; 16: 2187-2191. https://doi.org/10.7314/APJCP.2015.16.6.2187

[30] WOJCIECH P, ROSS MH (Eds.) Histology: A Text and Atlas: With Correlated Cell and Molecular Biology, Wolters Kluwer Health 2020; 8^{ed}, pp. 928. ISBN-13: 978-1-4963-8342-6

[31] REN J, HAMADA J, OKADA F, TAKEICHI N, MORIKAWA K et al. Correlation between the presence of microvilli and the growth or metastatic potential of tumor cells. Jpn J Cancer Res 1990; 81: 920-926. https://doi.org/10.1111/j.1349-7006.1990.tb02668.x

[32] MACHADO CML, SCHENKA A, VASSALO J, TAMASHIRO WMSC, GONCALVES EM et al. Morphological characterization of a human glioma cell line. Cancer Cell Int 2005; 5: 13. https://doi.org/10.1186/1475-2867-5-13
[33] HOA N, GE L, KUZNETSOV Y, PHERSON A, CORNFORTH A et al. Glioma cells display complex cell surface topographies that resist the actions of cytolytic effector lymphocytes. J Immunol 2010; 185: 4793-4803.
[34] GOLDSTEIN J, NEWBURY JD, LYMAN C, ECHLIN P, LIFSHIN E et al (Eds.). Scanning electron microscopy and X-ray microanalysis. Kluwer Academic Plenum Publishers 2003; New York; pp. 688. ISBN 0-306-47292-9
[35] SCHATTEN H (Eds.) Scanning electron microscopy for the life sciences. Cambridge University Press 2013; pp. 298. ISBN: 978-0-521-19599-7
[36] GIBBONS HM, HUGHES SM, VAN ROON-MOM W, GREENWOOD JM, NARAYAN PJ et al. Cellular composition of human glial cultures from adult biopsy brain tissue. J Neurosci Methods 2007; 166: 89-98. https://doi.org/10.1016/j.jneumeth.2007.07.005
[37] PERZELOVA A, MARES V. Appearance of GFAP-positive cells in adult human brain cultures spontaneously decelerated in growth. Glia 1993; 7: 237-244. https://doi.org/10.1002/glia.440070307
[38] SIVAKOVA I, MRAZ P, PERZELOVA A. Astroglial differentiation of fibronectin-positive human "glia-like" cells. Bratisl Med J 2023; 124: 437-441. https://doi.org/10.4149/BLL_2023_066

Figure Legends

Figure 1. Morphological properties of 170-MG-BA glioblastoma cell line. Phase-contrast microscopy (A), and SEM topography (B-F). Living cells at magnification ×100, scale bar 100 µm (A), and morphology of cell line at magnification ×200 (B). Cells with microvilli: flat cells (C, magnification ×1,500), spindle-shaped cell (D, magnification ×3,000), different density of microvilli (E, magnification ×3,000), microvilli lengths (F, magnification ×10,000).

Figure 2. Morphological properties of 8-MG-BA glioblastoma cell line. Phase-contrast microscopy (A) and SEM topography (B-F). Living cells at magnification ×100, scale bar 100 µm (A). Morphology of cell line at magnification ×200 (B). Cells rich in microvilli, rare occurrence of cells with sparse microvilli (C, D, magnification ×1,500), cells with intensive microvilli density (E, magnification ×3,000), microvilli length (F, magnification ×10,000).

Figure 3. Morphological properties of 135-BCA metastatic brain carcinoma cell line. Phase-contrast microscopy (A) and SEM topography (B-F). Living cells, at magnification ×100, scale bar 100 µm (A). Morphology of cell line at magnification ×200 (B): clusters of variable shaped cells (flat, spindle,

round). Cells rich in very short microvilli (C magnification ×1,500), cells with intense microvilli density (D, E, magnification ×3,000), microvilli lengths (F, magnification ×10,000).

Figure 4. Morphological properties of short-term glioblastoma cultures. Phase-contrast microscopy (A, B) and SEM topography (C-I). Living cells in 126-GBM culture (A) and 139- GBM culture (B), at magnification ×100, scale bar 100 µm. Morphology of 139-GBM culture at magnification ×200 (C). 159-GBM culture: microvilli-free cells, rare appearance of cells with sparse microvilli (D magnification ×1,500) (E magnification ×3,000), 126-GBM culture: cell without microvilli (F, magnification ×3,000), 139-GBM culture: cells with and without microvilli (G, magnification ×1, 500) (H, magnification ×3,000), microvilli lengths (I, magnification ×10,000).

Figure 5. Morphological properties of short-term adult human brain cultures. Phase-contrast microscopy (A), and SEM topography (B-F). Living cells in 130-NB culture, at magnification ×100, scale bar 100 µm (A), cell morphology in 130-NB culture at magnification ×200 (B). 130-NB culture: microvilli free cells (C, magnification ×1,500) (D, magnification ×3,000), rare appearance of cells with sparse microvilli (E, magnification ×3,000) and microvilli lengths (f, magnification ×10,000).

Figure 6. Indirect immunofluorescence for GFAP. Positively stained cells at passage number 2 in 130-NB cultures (A, B), all GFAP-positive cells disappeared after passage number 3. GFAP+ cells in 126-GBM short-term cultures at passage number 4 (C, D), disappear with passaging until passage number 15. Nuclei were stained with Hoechst.

Figure 7. Schematic representation of infiltrative growth of glioblastoma. Biopsy sample (BS), glioblastoma cells are shown in green and "glia-like" cells in orange. A) culture with a low number of glioblastoma cells, overgrowth of "glia-like" cells. B) culture with GFAP-positive glioblastoma cells, overgrowth of "glia-like" cells. C) culture with a higher number of glioblastoma cells, overgrowth of glioblastoma cells.

Figure 8. Graphic visualization of the percentage of microvilli-free cells and microvilli-bearing cells evaluated by SEM topography. Cells which do not extend the microvilli on their surface, referred as microvilli-free, are in blue diagram and cells with the microvilli on their surface, referred as microvilli-bearing, are in the orange diagram. Short-term cultures of “glia-like” cells have the prevalence of

microvilli-free cells, the short-term cultures from glioblastoma (GBM) do. Glioblastoma cell lines 170-MG-BA, 8-MG-BA and brain metastatic carcinoma 135-BCA cell line have microvilli-bearing cells only.

Table 1. Clinical data of patient with diagnosed *glioblastoma multiforme* (GBM).

Cultures	Age	Sex	DB
126-GBM	35	M	1994
139-GBM	68	M	1994
159-GBM	59	F	1995
388-GBM	47	F	1999
503-GBM	49	F	2001

Abbreviation: DB-date of biopsy

Table 2. Clinical data of patients with non-tumoral diagnoses.

Cultures	Age	Sex	Diagnosis	DB
124-NB	45	M	Contusio	1994
130-NB	64	F	Stroke	1994
208-NB	60	M	Contusio	1996
242-NB	48	M	Aneurysm	1996
444-NB	48	M	A-V malf	2000

Abbreviations: DB-date of biopsy, NB-normal brain, A-V malf- arterial-venous malformation

Fig. 1 Download full resolution image

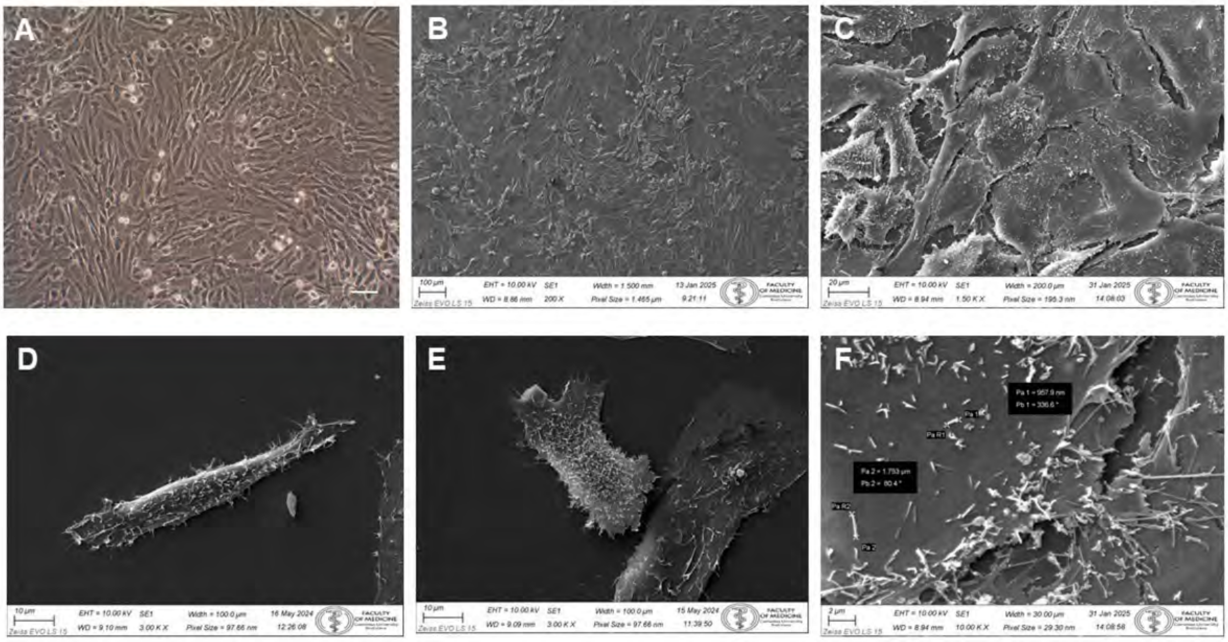

Fig. 2 Download full resolution image

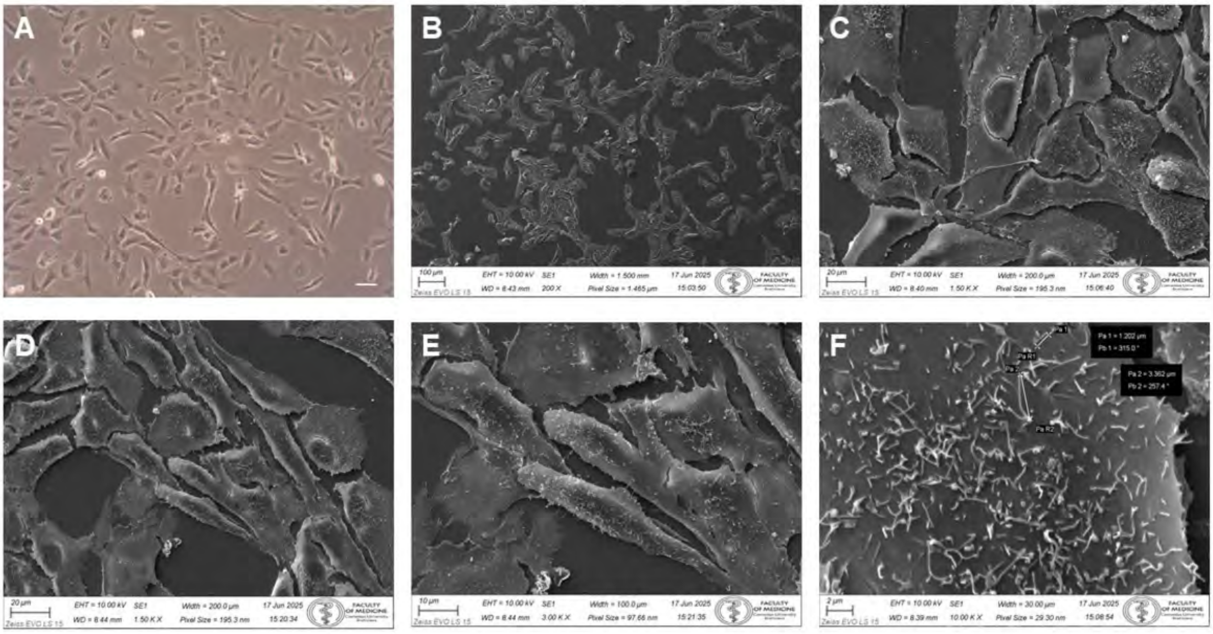

Fig. 3 Download full resolution image

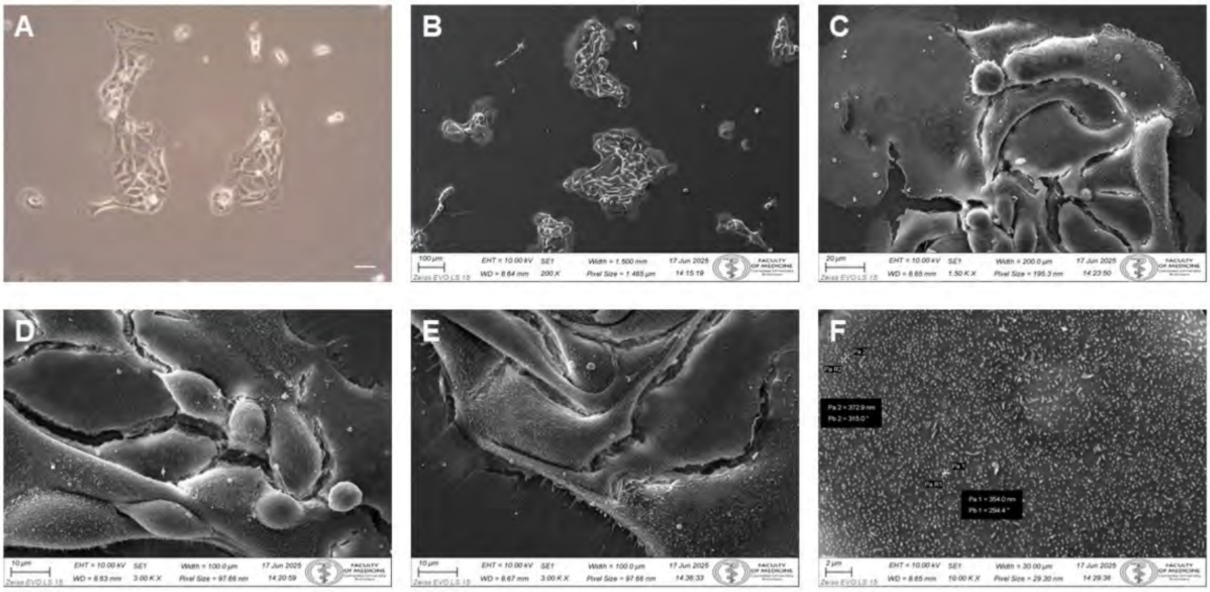

Fig. 4 Download full resolution image

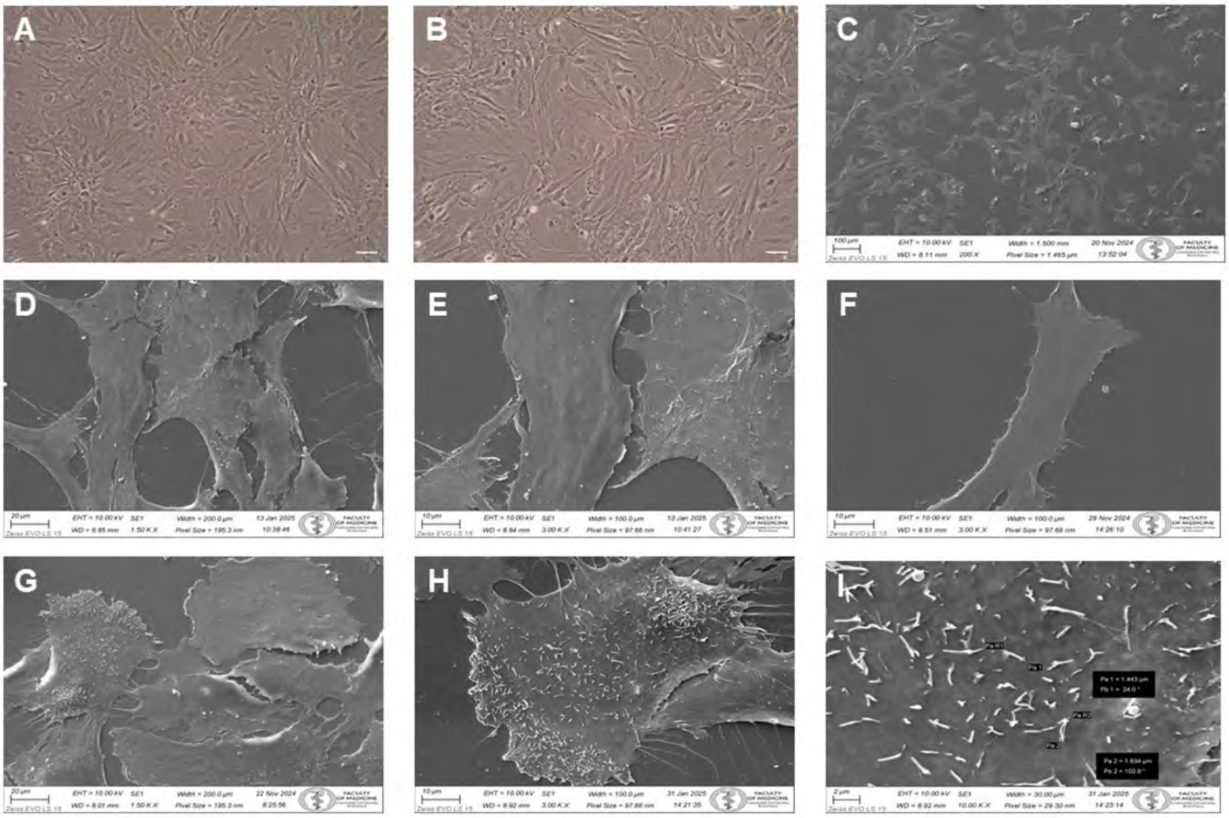

Fig. 5 Download full resolution image

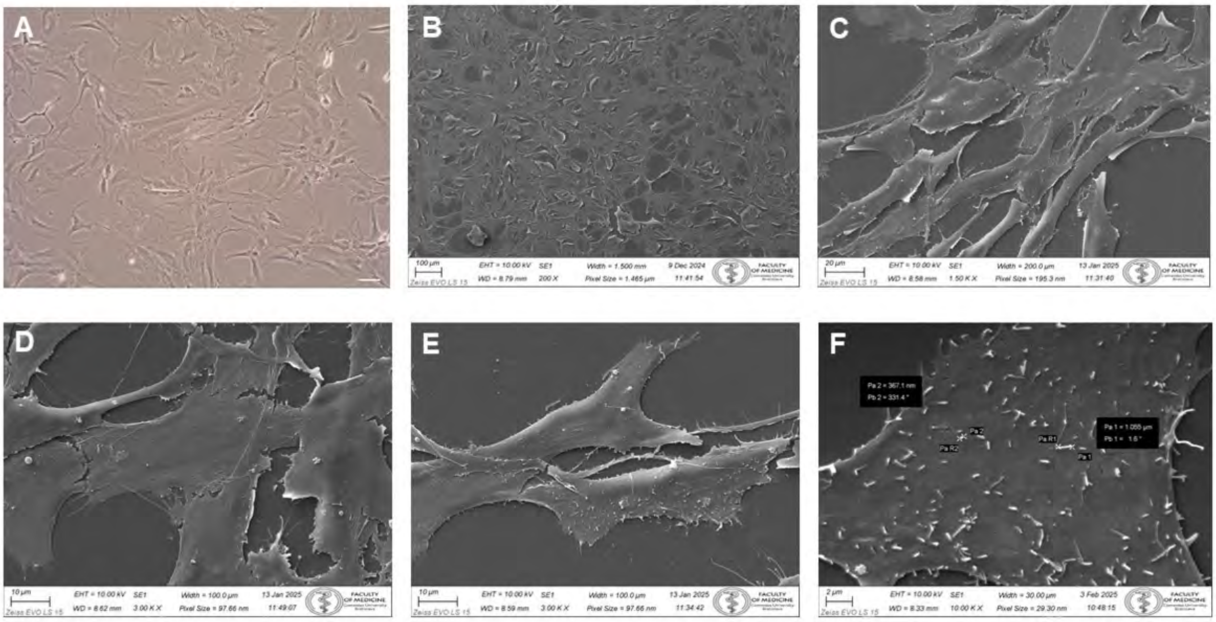

Fig. 6 Download full resolution image

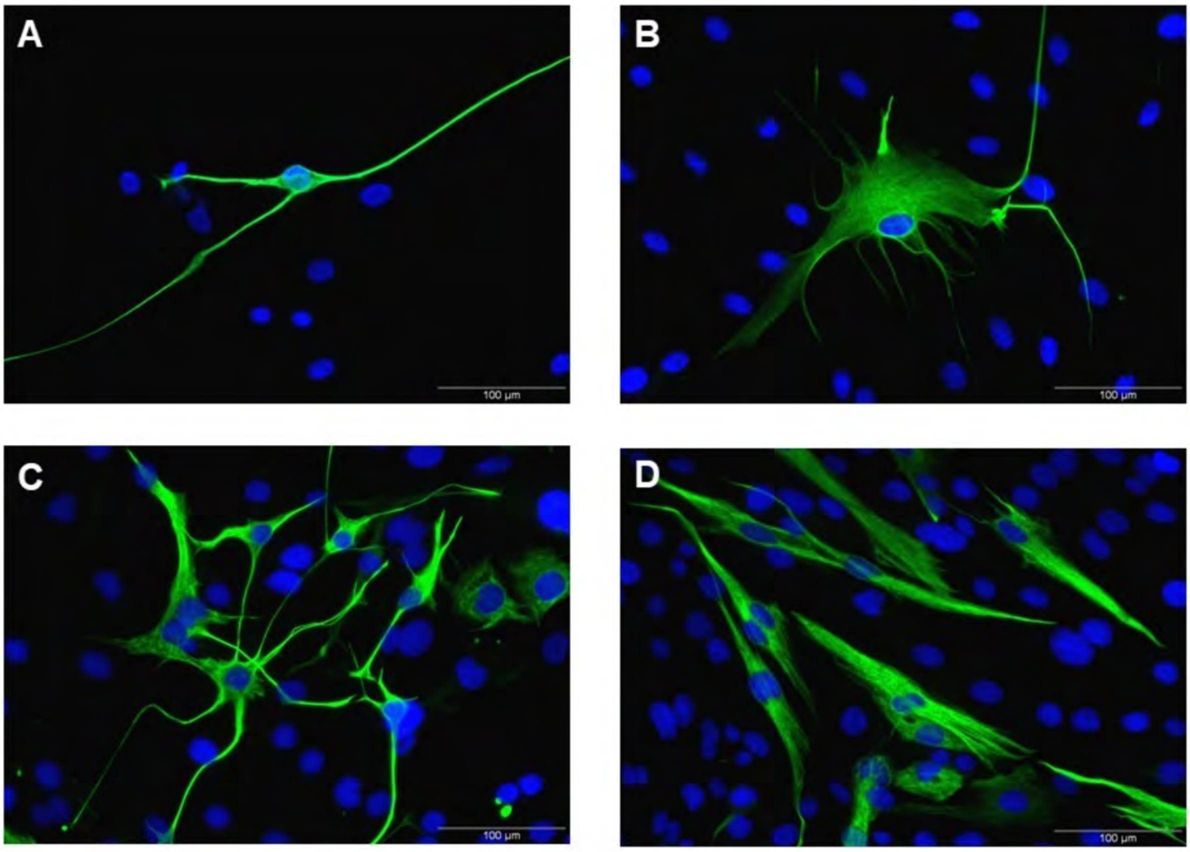

Fig. 7 Download full resolution image

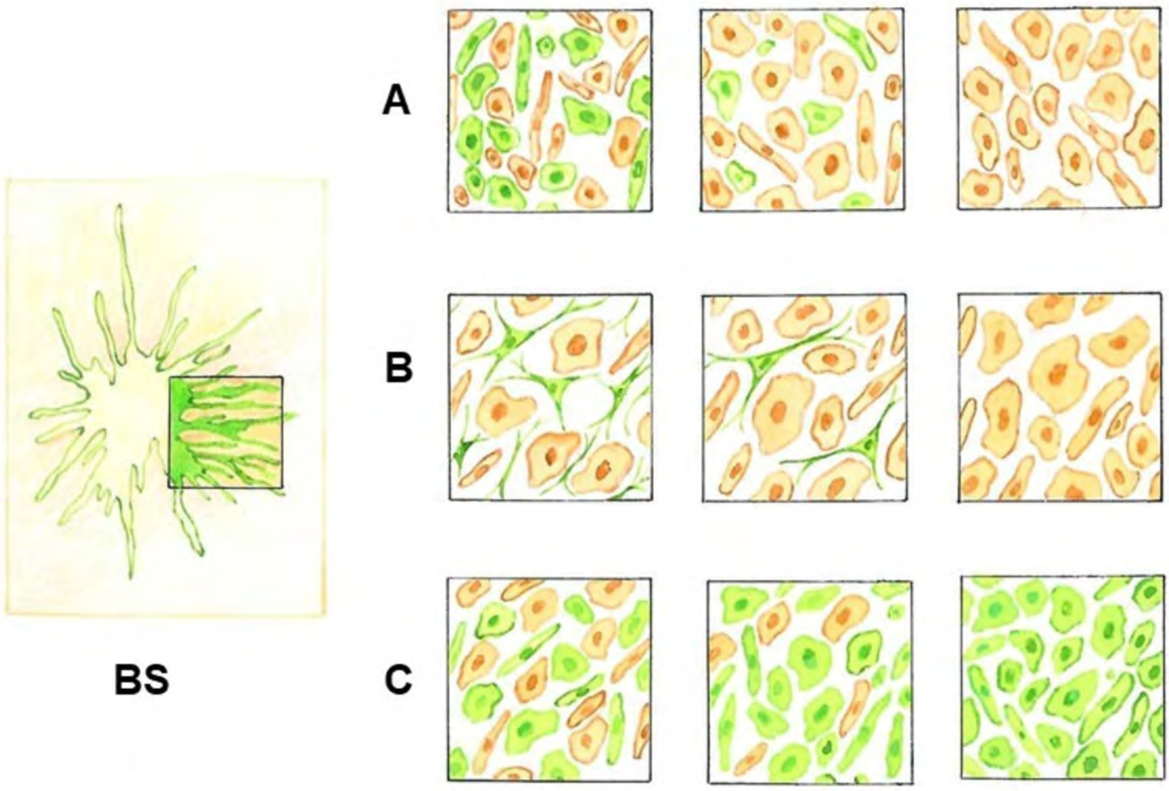

Fig. 8 Download full resolution image