Cells	Tumor type	Condition	Experiment	Matrix or scaffold	Effect	Ref.
LN18, GL15, U87, A172	GBM	2D, 3D	Seeding of GBM cells on brain Hi-spots and exposure to anti-proliferative drugs Ara-C, Taxol and TMZ	Brain Hi-Spots	Increased anti-proliferative effect of TMZ on GBM cells maintained on Hi-spots.	[65]
C6 rat, U-87 MG, U-118 MG	Glioma	2D, 3D, Matrigel and chitosan-alginate scaffolds	Comparison of growth and morphology and secretion of VEGF, MMP2, fibronectin and Laminin between cells grown in 2D, in matrigel or on chitosan-alginate scaffolds.	Matrigel, Chitosan-alginate scaffolds	Growth on chitosan-alginate scaffolds reduces growth but increases secretion of VEGF, MMP2, fibronectin and Laminin.	[93]
LN18, F98, F98EGFR- vIII, C6 rat, U-87 MG,	Glioma	2D, 3D spheroid culture, transwell migration	Evaluation of SHA impact on cell growth, collagen I invasion and mRNA expression of genes relevant for cell-cell and cell-matrix interaction.	Collagen I	SAHA treatment causes reduction of invasion and the reorganization of the matrix surrounding the tumor spheroids.	[94]
U178, U251	Glioma	3D transwell	Analysis of transwell invasion and migration after compound inhibition of PKC∂.	Collagen I supplemented with Tenascin C	Tenascin-C deposition triggers glioma invasion in a PKC∂-dependent manner.	[95]
U373	Glioma	3D spheroid	Analysis of growth and dissemination in increasingly stiff collagen I gels.	Collagen I-agarose	Matrix stiffness impacts on glioma cell invasiveness. High stiffness blocks invasiveness.	[19]
U251, U178	Glioma	3D, single cells	Quantification of transwell migration of cells stimulated with TNF-α, IL-1 or a combination of both.	Collagen I	Interleukin-1 beta (IL-1b) and tumor necrosis factor-alpha (TNF-α) increase glioma cell invasiveness in 3D with parallel increased MMP-2 and MMP-9.	[96]
Primary mouse G3 MB	Medulloblastoma	3D neurospheres	Neurosphere compound toxicity assays using FDA-approved drugs and ATP-sensor dye.	None	FDA-approved Pemetrexed and Gemcitabine significantly block proliferation of G3 MB.	[55]
DAOY, UW228	Medulloblastoma	2D, 3D transwell, 3D micro beads	Quantification of collagen invasion after HGF stimulation, small compound kinase inhibitor or siRNA treatment in cells seeded on Micro-beads and embedded in collagen I matrix.	Collagen I	HGF-induced c-Met activation promotes MB cell invasion through the kinase MAP4K4.	[9]
DAOY, UW228, Med PDX1712, MedPDX411, primary MB	Medulloblastoma	2D, 3D micro beads and spheroids	Quantification of collagen invasion and cell migration after growth factor stimulation using invasion counter platform for automated quantification of motile cell behavior in different environments.	Collagen I	HGF, EGF and bFGF are strong promoters of MB cell migration and invasion	[56]

Contind...

ups are a number of organ on a chip technologies that are currently developed for assaying different disease states[69] and testing drug effects and metabolization.[70]

On the opposite side of the spectrum is the need to resolve the mechanisms underlying brain infiltration of single tumor cells, which necessitates approaches allowing the quantitative analysis of molecular events in individual cells. This problem was tackled for the activation status of the important Rho family GTPase's - Rho, Rac and Cdc42 - in glioma cells.[53] Hirata *et al.*[53] used Rho-GTPase-FRET (Förster energy resonance transfer) probes, where spatial activation of the GTPase's was monitored by a shift in fluorescence signal. Rho-family GTPase-FRET fusion protein-expressing glioma cells were orthotopically implanted in rat brains and later analyzed inside brain slice cultures derived of these brains using two-photo microscopy. This study revealed higher Rac1 and Cdc42 and lower RhoA activities in glioblastoma cells penetrating the brain parenchyma than those advancing in the perivascular regions, and suggested that different driver mechanisms could exist for single cell dispersion in glioma.

Together, these studies highlight the need for adapting the model system to the specifics of the biological context, with the consequent inclusion of biophysical or chemical components that best reflect the *in vivo* situation. Besides high-throughput screening platforms for the identification of novel pro-metastatic key players or alternative interference strategies against metastatic dissemination, we also need improved phenotype-based single cell analysis to decipher clonal differences and micro environmental impact on tumor behavior at the single cell level.

Organotypic brain slice culture (OBSC) in primary brain tumor research

A number of causal gene(s) and associated genetic mutations, molecular changes, probable targets and treatments for a variety of primary brain tumors have been identified. Despite of this, the process of dissemination, metastasis of the tumor cells from the primary site, and tumor recurrence, which is the leading cause for brain tumor related mortality in patients, remain obscure. Total removal of the primary tumor is on many occasions impossible at the microscopic level due to the insidious infiltration of the tumor cells into the surrounding brain tissue.[71] This majorly results in therapeutic failure and urges for model systems that allow addressing brain tumor cell invasion specifically. Standard 3D *in vitro* invasion assays use ECM macromolecules that mimic the basement membrane (e.g. matrigel) as barriers to tumor invasion. These assays (described above and in table 1) although quick, reliable, commercially available and easy to perform, have several limitations. They do not take into account the unique ECM composition in the brain and thus provide artificial environments that fail to closely mimic the normal brain tissue/tumor environment. This is further emphasized by the fact that distinct types of brain tumors localize within specific regions of the brain, highlighting the need for different microenvironments for modeling tumor growth and invasiveness. To circumvent this, mouse models have been generated for studying tumor propagation via orthotopic or subcutaneous xenografting of tumor cells. These experiments, however, are ethically controversial if inappropriately conducted, costly, labor intensive and need lengthy time periods for animal surgery and subsequent tumor development (especially for low grade tumors). These challenges and limitations highlight the need for developing a novel system wherein living brain tissue can be used as an ideal matrix for studying tumor cell growth and invasion.[72] One such system is the organotypic culture, where cellular constituents of organs or parts of organs are allowed to regrow into or persist as organ replacements.

An excellent overview of 3D organotypic cultures has recently been provided,[73] which describes their potentials as experimental systems to visualize cellular mechanisms that drive tissue development, to study the genetic regulation of cell behaviors in tissues and to evaluate the role of micro environmental factors in normal development and disease. One hallmark of organotypic cultures is the tissue environment mimicking the structural and functional specifics of the organ of origin. This turns them into attractive models for cancer research to explore tumor host tissue interactions and to advance therapeutic approaches.

Organotypic brain slice culture for visualization and quantification of brain tumor cell dissemination

OBSCs allow culture, maintenance and long-term survival of sections from any tissue of the CNS. Slices are mostly cultured at an air/liquid interface by either continuous rotation using the roller tube method or on a semi porous membrane using the Stoppini method.[74] Brain tissue slice cultures maintain their normal cytoarchitecture, complex cell relationships and biochemical and electrophysiological properties. OBSCs have been widely used in the field of neurobiology for synaptogenesis, neurogenesis, myelin formation, as models for studying neurodegeneration, for neuroprotective and neurotoxic assays, *etc*.[67] In the field of brain tumor research, they are an ideal platform to access the tumor microenvironment under intact anatomical conditions. Indeed, Jung *et al.*[71] established a brain tumor slice model wherein they used human white matter specimens in the upper chambers of transwell culture dishes. After 24 h, control human astrocytoma cells stably expressing enhanced GFP or GFP-RHAMM (receptor for hyaluronan-mediated motility) transfected astrocytoma cells were placed in a small centrally punched-out hole in the slice. The infiltration and migratory behavior of the GFP-expressing astrocytoma cells could be easily studied using confocal laser scanning microscopy (CF-LSM) up to 30 days post implantation. The authors were able to demonstrate that different astrocytoma cell lines display different degrees of invasion and that the migration of the human astrocytoma cells could be

stimulated or, using antisense targeting strategies, specifically blocked.[71] In an analogous study it was demonstrated that (1) the invasive behavior of the astrocytoma cells in the brain slice co-culture is not always identical to the results obtained from 2D migration studies, (2) the tumor cells spread out multidirectionally, (3) frozen human normal brain tissue can be used for the organotypic culture, (4) there were no obvious signs of necrosis, and (5) the brain cytoarchitecture and viability was preserved for at least 14 days.[72]

Although the human origin of the biopsies used as the host tissue in these studies excludes species-specific effects in the co-culture, slices from newborn rat or mouse brains are excellent alternatives. They offer several advantages: brain regions corresponding to the *in vivo* tumor localization can be chosen, developmental stage of the brain slice can be adjusted, multiple replicas from same brain region can be generated, and the use of transgenic animals allows modification of the cellular microenvironment. Ohnishi *et al.*[75] established OBSCs from 2-day-old neonatal rat brains, which were transferred on double-layered membranes consisting of two different membrane types and maintained at an interface between the air and the culture medium. The slices were then co-cultured with C6 glioma cells labeled with PKH2 fluorescent dye. After 2 days of co-culture, the exogenous application of the chemotactic stimulator neural cell adhesion molecule L1 triggered tumor cell migration from the upper to the bottom membrane through the brain slice.[75] Since this study lacked CF-LSM analysis, OBSCs were subsequently performed by the slightly modified Stoppini method, which allowed quantifying glioma cell invasion using confocal microscopy.[76] This study revealed that the migrating cells showed a strong increase in immunoreactivity for matrix metalloproteinase 2 and 9.[76] Analogous OBSC technology was later used for mouse brain slices to quantify the invasiveness of glioma[77] and to correlate it with histological type.[78] Both studies used human, DiI-stained glioma biopsy tumor fragments and GFP-expressing spheroids directly implanted in the cortex of brain slices derived from 7 day old mice. This intraslice implantation system could be maintained in culture for 2 to 4 weeks. Quantification of the distance and density of the tumor cell invasion revealed that GBMs were 2-4 times more invasive than the lower grade glioma cells (LGGs). Within the different groups and grades of GBMs and LGGs, heterogeneity in terms of invasion was seen. It was also observed that the spheroids were less invasive in comparison to the directly grafted fragments. Overall using this system, Palfi *et al.*[77,78] and de Bouard *et al.*[77] could successfully recapitulate, monitor and quantify the invasion of single cells and the dissemination of glioma *ex vivo*. Recently, Chadwick *et al.*[79] developed OBSCs from postnatal day 6 mice and cultured the whole brain slices on membrane inserts coated with laminin. Tumor cells (astrocytoma and medulloblastoma) were stained with Cm-DiI for monitoring, and dispensed on the center of the slice. This co-culture system remained viable for one week and effects of drug therapies on tumor cell proliferation, cell death or changes in protein expression were successfully analyzed. Thus, Chadwick *et al.*[79] used the OBSC system as a qualitative and quantitative assay to calculate the fold change in the number of cells during the period of slice culture. Furthermore, they investigated either the whole brain or specific regions within the brain, to assess environmental impact on primary brain tumor cell growth.

Organotypic brain slice culture to study the micro environmental impact

Malignant astrocytoma/GBM cause mortality by local tumor growth and brain invasion rather than systemic metastasis. GBM tumor cells diffusely infiltrate the brain parenchyma within and along the white matter tracts or around cerebral blood vessels,[53] and rarely penetrate basal lamina structures at the glial limitans externa. Analogously, malignant medulloblastoma must also infiltrate cerebellar tissue for distal dissemination. Moreover, resection of MB tumors is inevitably followed by relapse if the patients are not treated with cranio-spinal radiotherapy and chemotherapy, suggesting the occurrence of local dissemination of tumor cells from the primary medulloblastoma. *In vitro* studies aiming at better understanding the local invasion process have been hampered by the lack of identification of the brain ECM macromolecules involved and the only poorly understood implication of the cellular microenvironment. *In vivo* approaches on the other hand, offer too little spatial and temporal resolution to monitor tumor-microenvironment interactions appropriately. Thus, OBSCs could provide an important platform to study the cross-talk between the tumor cells and normal cells in a physiologically relevant environment. OBSCs can be used for investigating the microenvironment and its impact on the growth and spread of primary brain tumors, and for testing the measures that could be taken to prevent or treat it effectively.[79] Although, there is a lack of vascular supply to the tissue in the slices, capillaries do survive in these sections without any circulation.[80] Despite of the fact that there is no blood flow and that the capillaries are not functional, it is likely that they are still capable of expressing and secreting various molecules,[81] which could affect other cell types in the slice culture including the tumor cells. In addition, the intriguing exchange between tumor cells and astrocytes and the suspected tumor promoting functions of astrocytes [41-43] urges for novel studies addressing the therapeutic potential of the astrocyte-tumor interaction, for which organotypic slice culture would be an ideal system.

Along with their use for monitoring tumor dissemination, OBSCs have also been used for high resolution imaging of cytoskeletal structures in living glioblastoma cells. For this, glioblastoma cells were transfected with GFP-actin and placed onto murine brain slices and spinal cord explants. Using live-cell imaging to visualize the cytoskeleton of the tumor cells, a major change in the gross morphology from a solid, two dimensional state to a three dimensional substrate was noted. This morphological change was characterized by long, dendritic-like processes that displayed regions

of ruffling activity and filopodial protrusions and by down regulation of stress-fibers.[82]

Thus, OBSC is an excellent technology to address a wide range of topics in primary brain tumor research, ranging from growth- and dissemination-promoting signaling, to the intricate interrelations between the tumor and its surrounding host tissue to the evaluation of efficaciousness of novel targeting strategies.

FUTURE PERSPECTIVES

Main emphasis for improving current *in vitro* technologies should be given to the cellular composition and the biophysical and chemical environment conditions under which the experiment is performed. The microenvironment of the *in vivo* location of the tumor and the composition of the neuronal and interstitial cells resident in this location should guide the choice of the components. At the single cell and population levels, molecular sensors for specific cell functions should be used for probing tumor cell behavior and therapeutic efficacy. Finally, an increased output should be strived for to enable pharmacological and genetic screening approaches for drug target identification. Thus, an organotypic environment, specific read-outs and the high throughput capability will be the three pillars of future *in vitro* approaches. A great potential lies in organotypic slice culture, and when this technology is combined with state-of-the-art microscopy, it will allow to reveal fundamental aspects of local tumor cell infiltration, the interaction of neuronal and brain interstitial cell populations with the tumor cells and the evaluation of the efficaciousness of novel treatments.

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Conflicts of interest
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