

| 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 δ . | Collagen I supplemented with Tenascin C | Tenascin-C deposition triggers glioma invasion in a PKC δ -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.

Financial support and sponsorship

A.N. is supported by a grant of the Werner and Hedy Berger-Janser Foundation, M.B. by the Childhood Cancer Research Foundation Switzerland.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, Ellison DW, Lichter P, Gilbertson RJ, Pomeroy SL, Kool M, Pfister SM. Molecular subgroups of medulloblastoma: the current consensus. *Acta neuropathol* 2012;123:465-72.
- Northcott PA, Shih DJ, Peacock J, Garzia L, Morrissy AS, Zichner T, Stütz AM, Korshunov A, Reimand J, Schumacher SE, Beroukheim R, Ellison DW, Marshall CR, Lionel AC, Mack S, Dubuc A, Yao Y, Ramaswamy V, Luu B, Rolider A, Cavalli FM, Wang X, Remke M, Wu X, Chiu RY, Chu A, Chuah E, Corbett RD, Hoad GR, Jackman SD, Li Y, Lo A, Mungall KL, Nip KM, Qian JQ, Raymond AG, Thiessen NT, Varhol RJ, Birol I, Moore RA, Mungall AJ, Holt R, Kawachi D, Roussel MF, Kool M, Jones DT, Witt H, Fernandez-LA, Kenney AM, Wechsler-Reya RJ, Dirks P, Aviv T, Grajkowska WA, Perek-Polnik M, Haberler CC, Delattre O, Reynaud SS, Doz FF, Pernet-Fattet SS, Cho BK, Kim SK, Wang KC, Scheurlen W, Eberhart CG, Fèvre-Montange M, Jouvét A, Pollack IF, Fan X, Muraszko KM, Gillespie GY, Di Rocco C, Massimi L, Michiels EM, Kloosterhof NK, French PJ, Kros JM, Olson JM, Ellenbogen RG, Zitterbart K, Kren L, Thompson RC, Cooper MK, Lach B, McLendon RE, Bigner DD, Fontebasso A, Albrecht S, Jabado N, Lindsey JC, Bailey S, Gupta N, Weiss WA, Bognár L, Klekner A, Van Meter TE, Kumabe T, Tominaga T, Elbabaa SK, Leonard JR, Rubin JB, Liao LM, Van Meir EG, Fouladi M, Nakamura H, Cinalli G, Garami M, Hauser P, Saad AG, Iolascon A, Jung S, Carlotti CG, Vibhakhar R, Ra YS, Robinson S, Zollo M, Faria CC, Chan JA, Levy ML, Sorensen PH, Meyerson M, Pomeroy SL, Cho YJ, Bader GD, Tabori U, Hawkins CE, Bouffet E, Scherer SW, Rutka JT, Malkin D, Clifford SC, Jones SJ, Korbel JO, Pfister SM, Marra MA, Taylor MD. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nat* 2012;488: 49-56.
- Northcott PA, A Korshunov, H Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S, Taylor MD Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 2011;29:1408-14.
- Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, Troost D, Meeteren NS, Caron HN, Cloos J, Mrsic A, Ylstra B, Grajkowska W, Hartmann W, Pietsch T, Ellison D, Clifford SC, Versteeg R. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. *PLoS One* 2008;3:e3088.
- Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, Chintagumpala M, Adesina A, Ashley DM, Kellie SJ, Taylor MD, Curran T, Gajjar A, Gilbertson RJ. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol* 2006;24:1924-31.
- Wang X, Dubuc AM, Ramaswamy V, Mack S, Gendoo DM, Remke M, Wu X, Garzia L, Luu B, Cavalli F, Peacock J, López B, Skowron P, Zagzag D, Lyden D, Hoffman C, Cho YJ, Eberhart C, MacDonald T, Li XN, Van Meter T, Northcott PA, Haibe-Kains B, Hawkins C, Rutka JT, Bouffet E, Pfister SM, Korshunov A, Taylor MD. Medulloblastoma subgroups remain stable across primary and metastatic compartments. *Acta neuropathol* 2015;129:449-57.
- Angers-Loustau A, Hering R, Werbowetski TE, Kaplan DR, Del Maestro RF. SRC regulates actin dynamics and invasion of malignant glial cells in three dimensions. *Mol Cancer Res* 2004;2:595-605.
- Le PU, Angers-Loustau A, de Oliveira RM, Ajlan A, Brassard CL, Dudley A, Brent H, Siu V, Trinh G, Mölenkamp G, Wang J, Seyed Sadr M, Bedell B, Del Maestro RF, Petrecca K. DRR drives brain cancer invasion by regulating cytoskeletal-focal adhesion dynamics. *Oncogene* 2010;29:4636-47.
- Santhana Kumar K, Tripolitioti D, Ma M, Grahert J, Egli KB, Fiaschetti G, Shalaby T, Grotzer MA, Baumgartner M. The Ser/Thr kinase MAP4K4 drives c-Met-induced motility and invasiveness in a cell-based model of SHH medulloblastoma. *SpringerPlus* 2015;4:19.
- Rottner K, Stradal TE. Actin dynamics and turnover in cell motility. *Curr Opin Cell Biol* 2011;23:569-78.
- Hall A. The cytoskeleton and cancer. *Cancer Metastasis Rev* 2009;28:5-14.
- Numberg A, Kitzing T, Grosse R. Nucleating actin for invasion. *Nat Rev Cancer* 2011;11:177-87.
- Giese A, Bjerkvig R, Berens ME, Westphal M. Cost of migration: invasion of malignant gliomas and implications for treatment. *J Clin Oncol* 2003;21:1624-36.
- Waclaw B, Bozic I, Pittman ME, Hruban RH, Vogelstein B, Nowak MA. A spatial model predicts that dispersal and cell turnover limit intratumour heterogeneity. *Nat* 2015;525:261-4.
- Palmer TD, Ashby WJ, Lewis JD, Zijlstra A. Targeting tumor cell motility to prevent metastasis. *Adv Drug Deliv Rev* 2011;63:568-81.
- Vehlow A, Cordes N. Invasion as target for therapy of glioblastoma multiforme. *Biochim Biophys Acta* 2013;1836:236-44.
- Wells A, Grahovac J, Wheeler S, Ma B, Lauffenburger D. Targeting tumor cell motility as a strategy against invasion and metastasis. *Trends Pharmacol Sci* 2013;34:283-9.
- Wolf K, Friedl P. Extracellular matrix determinants of proteolytic and non-proteolytic cell migration. *Trends Cell Biol* 2011;21:736-44.
- Ulrich TA, Jain A, Tanner K, MacKay JL, Kumar S. Probing cellular mechanobiology in three-dimensional culture with collagen-agarose matrices. *Biomaterials* 2010;31:1875-84.
- Friedl P, Wolf K. Plasticity of cell migration: a multiscale tuning model. *J*

- Cell Biol* 2010;188:11-19.
21. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. *Dis Model Mech* 2011;4:165-78.
 22. Ruser JM, Wu X, Eberhart CG, Taylor MD, Wechsler-Reya RJ. SnapShot: Medulloblastoma. *Cancer Cell* 2014;26:940-40.e1.
 23. Brochner CB, Holst CB, Mollgard K. Outer brain barriers in rat and human development. *Front Neurosci* 2015;9:75.
 24. Saboori P, Sadegh A. Histology and Morphology of the Brain Subarachnoid Trabeculae. *Anat Res Int* 2015;2015:279814.
 25. Zimmermann DR, Dours-Zimmermann MT. Extracellular matrix of the central nervous system: from neglect to challenge. *Histochem Cell Biol* 2008;130:635-53.
 26. Zum AD, Bandtlow CE. Regeneration failure in the CNs: cellular and molecular mechanisms. *Adv Exp Med Biol* 2006;557:54-76.
 27. Morgenstern DA, Asher RA, Fawcett JW. Chondroitin sulphate proteoglycans in the CNS injury response. *Prog Brain Res* 2002;137:313-32.
 28. Jones EV, Bouvier DS. Astrocyte-secreted matricellular proteins in CNS remodelling during development and disease. *Neural Plast* 2014;2014:321209.
 29. Klekner A, Hutoczek G, Virga J, Remenyi-Puskar J, Toth J, Scholtz B, Cs6sz 6E, Kall6 G, Steiner L, Hortob6gyi T, Bogn6r L. Expression pattern of invasion-related molecules in the peritumoral brain. *Clin Neurol Neurosurg* 2015;139: 138-43.
 30. Ulrich TA, de Juan Pardo EM, Kumar S. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. *Cancer Res* 2009;69:4167-74.
 31. Kim SN, Jeibmann A, Halama K, Witte HT, Walte M, Matzat T, Schillers H, Faber C, Senner V, Paulus W, Kl6mbt C. ECM stiffness regulates glial migration in Drosophila and mammalian glioma models. *Dev* 2014;141:3233-42.
 32. Rao SS, Bentil S, DeJesus J, Larison J, Hissong A, Dupaix R, Sarkar A, Winter JO. Inherent interfacial mechanical gradients in 3D hydrogels influence tumor cell behaviors. *PLoS One* 2012;7:e35852.
 33. Kim Y, Kumar S. CD44-mediated adhesion to hyaluronic acid contributes to mechanosensing and invasive motility. *Mol Cancer Res* 2014;12:1416-29.
 34. Dvorak HF. Tumors: Wounds That Do Not Heal--Redux. *Cancer Immunol Res* 2015;3:1-11.
 35. Halliday JJ, Holland EC. Connective tissue growth factor and the parallels between brain injury and brain tumors. *J Natl Cancer Inst* 2011;103:1141-3.
 36. Yang I, Han SJ, Kaur G, Crane C, Parsa AT. The role of microglia in central nervous system immunity and glioma immunology. *J Clin Neurosci* 2010;17:6-10.
 37. Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H. The brain tumor microenvironment. *Glia* 2011;59:1169-80.
 38. Kerber M, Reiss Y, Wickersheim A, Jugold M, Kiessling F, Heil M, Tchaikovski V, Waltenberger J, Shibuya M, Plate KH, Machein MR. Flt-1 signaling in macrophages promotes glioma growth *in vivo*. *Cancer Res* 2008;68:7342-51.
 39. Strik HM, Stoll M, Meyermann R. Immune cell infiltration of intrinsic and metastatic intracranial tumours. *Anticancer Res* 2004;24:37-42.
 40. Margol AS, Robison NJ, Gnanachandran J, Hung LT, Kennedy RJ, Vali MDhall G, Finlay JL, Erdreich-Epstein A, Krieger MD, Drissi R, Fouladi M, Gilles FH, Judkins AR, Sposto R, Asgharzadeh S. Tumor-associated macrophages in SHH subgroup of medulloblastomas. *Clin Cancer Res* 2015;21:1457-65.
 41. Placone AL, Quinones-Hinojosa A, Searson PC. The role of astrocytes in the progression of brain cancer: complicating the picture of the tumor microenvironment. *Tumour Biol* 2015.
 42. Kim JK, Jin X, Sohn YW, Jin X, Jeon HY, Kim EJ, Ham SW, Jeon HM, Chang SY, Oh SY, Yin J, Kim SH, Park JB, Nakano I, Kim H. Tumoral RANKL activates astrocytes that promote glioma cell invasion through cytokine signaling. *Cancer Lett* 2014;353:194-200.
 43. Rath BH, Fair JM, Jamal M, Camphausen K, Tofilon PJ. Astrocytes enhance the invasion potential of glioblastoma stem-like cells. *PLoS One* 2013;8:e54752.
 44. Huang H, Colella S, Kurrer M, Yonekawa Y, Kleihues P, Ohgaki H. Gene expression profiling of low-grade diffuse astrocytomas by cDNA arrays. *Cancer Res* 2000;60:6868-74.
 45. Bhoopathi P, Gondi CS, Gujrati M, Dinh DH, Lakka SS. SPARC mediates Src-induced disruption of actin cytoskeleton via inactivation of small GTPases Rho-Rac-Cdc42. *Cell Signal* 2011;23:1978-87.
 46. Edwards LA, Woolard K, Son MJ, Li A, Lee J, Ene C, Mantey SA, Maric D, Song H, Belova G, Jensen RT, Zhang W, Fine HA. Effect of brain- and tumor-derived connective tissue growth factor on glioma invasion. *J Natl Cancer Inst* 2011;103:1162-78.
 47. Klein A, Schwartz H, Sagi-Assif O, Meshel T, Izraely S, Ben Menachem S, Bengaiev R, Ben-Shmuel A, Nahmias C, Couraud PO, Witz IP, Erez N. Astrocytes facilitate melanoma brain metastasis via secretion of IL-23. *J Pathol* 2015;236 116-27.
 48. Zimmermann M, Box C, Eccles SA. Two-dimensional vs. three-dimensional *in vitro* tumor migration and invasion assays. *Methods Mol Biol* 2013;986:227-52.
 49. Matsusaki M, Case CP, Akashi M. Three-dimensional cell culture technique and pathophysiology. *Adv Drug Deliv Rev* 2014;74:95-103.
 50. Astashkina A, Grainger DW. Critical analysis of 3-D organoid *in vitro* cell culture models for high-throughput drug candidate toxicity assessments. *Ad Drug Deliv Rev* 2014;69:70:1-18.
 51. Weigelt B, Ghajar CM, Bissell MJ. The need for complex 3D culture models to unravel novel pathways and identify accurate biomarkers in breast cancer. *Ad Drug Deliv Rev* 2014;69:70:42-51.
 52. Thoma CR, Zimmermann M, Agarkova I, Kelm JM, Krek W. 3D cell culture systems modeling tumor growth determinants in cancer target discovery. *Ad Drug Deliv Rev* 2014;69:70:29-41.
 53. Hirata E, Yukinaga H, Kamioka Y, Arakawa Y, Miyamoto S, Okada T, Sahai E, Matsuda M. *In vivo* fluorescence resonance energy transfer imaging reveals differential activation of Rho-family GTPases in glioblastoma cell invasion. *J Cell Sci* 2012;125:858-68.
 54. Ivanov DP, Parker TL, Walker DA, Alexander C, Ashford MB, Gellert PR, Garnett MC. *In vitro* co-culture model of medulloblastoma and human neural stem cells for drug delivery assessment. *J Biotechnol* 2015;205:3-13.
 55. Morfouace M, Shelat A, Jacus M, Freeman BB, 3rd, Turner D, Robinson S, Zindy F, Wang YD, Finkelstein D, Ayrault O, Bihannic L, Puget S, Li XN, Olson JM, Robinson GW, Guy RK, Stewart CF, Gajjar A, Roussel MF. Pemetrexed and gemcitabine as combination therapy for the treatment of Group3 medulloblastoma. *Cancer Cell* 2014;25:516-29.
 56. Kumar KS, Pillong M, Kunze J, Burghardt I, Weller M, Grotzer MA, Schneider G, Baumgartner M. Computer-assisted quantification of motile and invasive capabilities of cancer cells. *Sci Rep* 2015;5:15338.
 57. Ansari N, Muller S, Stelzer EH, Pampaloni F. Quantitative 3D cell-based assay performed with cellular spheroids and fluorescence microscopy. *Methods Cell Biol* 2013;113:295-309.
 58. Eichler M, Jahnke HG, Krinke D, Muller A, Schmidt S, Azendorf R, Robitzki AA. A novel 96-well multielectrode array based impedimetric monitoring platform for comparative drug efficacy analysis on 2D and 3D brain tumor cultures. *Biosens Bioelectron* 2015;67:582-9.
 59. Gritsenko PG, Ilina O, Friedl P. Interstitial guidance of cancer invasion. *J Pathol* 2012;226:185-99.
 60. Fernandez-Fuente G, Mollinedo P, Grande L, Vazquez-Barquero A, Fernandez-Luna JL. Culture dimensionality influences the resistance of glioblastoma stem-like cells to multikinase inhibitors. *Mol Cancer Ther* 2014;13:1664-72.
 61. Pagliarini R, Shao W, Sellers WR. Oncogene addiction: pathways of therapeutic response, resistance, and road maps toward a cure. *EMBO Rep* 2015;16:280-296.
 62. Rao S, DeJesus J, Short AR, Otero JJ, Sarkar A, Winter JO. Glioblastoma Behaviors in Three-Dimensional Collagen-Hyaluronan Composite Hydrogels. *ACS Appl Mater Interfaces* 2013;5:9276-84.
 63. Florzcyk SJ, Wang K, Jana S, Wood DL, Sytsma SK, Sham JG, Kievit FM, Zhang M. Porous chitosan-hyaluronic acid scaffolds as a mimic of glioblastoma microenvironment ECM. *Biomaterials* 2013;34:10143-50.
 64. Pedron S, Becka E, Harley BA. Regulation of glioma cell phenotype in 3D matrices by hyaluronic acid. *Biomaterials* 2013;34:7408-17.
 65. Biggs T, Foreman J, Sundstrom L, Regenass U, Lehembre F. Antitumor

- compound testing in glioblastoma organotypic brain cultures. *J Biomol Screening* 2011;16:805-17.
66. Rutka JT, Dougherty DV, Giblin JR, Edwards MS, McCulloch JR, Rosenblum ML. Growth of a medulloblastoma on normal leptomeningeal cells in culture: interaction of tumor cells and normal cells. *Neurosurgery* 1987;21:872-8.
 67. Humpel C. Organotypic brain slice cultures: A review. *Neurosci* 2015; 305:86-98.
 68. Ma L, Barker J, Zhou C, Li W, Zhang J, Lin B, Foltz G, Küblbeck J, Honkakoski P. Towards personalized medicine with a three-dimensional micro-scale perfusion-based two-chamber tissue model system. *Biomaterials* 2012;33:4353-61.
 69. Huh D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. *Trends Cell Biol* 2011;21:745-54.
 70. Ghaemmaghami AM, Hancock MJ, Harrington H, Kaji H, Khademhosseini A. Biomimetic tissues on a chip for drug discovery. *Drug Discov Today* 2012;17:173-81.
 71. Jung S, Ackerley C, Ivanchuk S, Mondal S, Becker LE, Rutka JT. Tracking the invasiveness of human astrocytoma cells by using green fluorescent protein in an organotypic brain slice model. *J Neurosurg* 2001;94:80-9.
 72. Jung S, Kim HW, Lee JH, Kang SS, Rhu HH, Jeong YI, Yang SY, Chung HY, Bae CS, Choi C, Shin BA, Kim KK, Ahn KY. Brain tumor invasion model system using organotypic brain-slice culture as an alternative to *in vivo* model. *J Cancer Res Clin Oncol* 2002;128:469-76.
 73. Shamir ER, Ewald AJ. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. *Nat Rev Mol Cell Biol* 2014;15:647-64.
 74. Stoppini L, Buchs PA, Muller D. A simple method for organotypic cultures of nervous tissue. *J Neurosci Methods* 1991;37:173-82.
 75. Ohnishi T, Matsumura H, Izumoto S, Hiraga S, Hayakawa T. A novel model of glioma cell invasion using organotypic brain slice culture. *Cancer Res* 1998;58:2935-40.
 76. Matsumura H, Ohnishi T, Kanemura Y, Maruno M, Yoshimine T. Quantitative analysis of glioma cell invasion by confocal laser scanning microscopy in a novel brain slice model. *Biochem Biophys Res Commun* 2000;269:513-20.
 77. de Bouard S, Christov C, Guillamo JS, Kassas-Duchossoy L, Palfi S, Leguerinel C, Masset M, Cohen-Hagenauer O, Peschanski M, Lefrançois T. Invasion of human glioma biopsy specimens in cultures of rodent brain slices: a quantitative analysis. *J Neurosurg* 2002;97:169-76.
 78. Palfi S, Swanson KR, De Bouard S, Chretien F, Oliveira R, Gherardi RK, Kros JM, Peschanski M, Christov C. Correlation of *in vitro* infiltration with glioma histological type in organotypic brain slices. *Br J Cancer* 2004;91:745-52.
 79. Chadwick EJ, Yang DP, Filbin MG, Mazzola E, Sun Y, Behar O, Pazyra-Murphy MF, Goumnerova L, Ligon KL, Stiles CD, Segal RA. A brain tumor/organotypic slice co-culture system for studying tumor microenvironment and targeted drug therapies. *J Vis Exp* 2015;e53304.
 80. Moser KV, Schmidt-Kastner R, Hinterhuber H, Humpel C. Brain capillaries and cholinergic neurons persist in organotypic brain slices in the absence of blood flow. *Eur J Neurosci* 2003;18:85-94.
 81. Hutter-Schmid B, Kniewallner KM, Humpel C. Organotypic brain slice cultures as a model to study angiogenesis of brain vessels. *Front Cell Dev Biol* 2015;3:52.
 82. Caspani EM, Echevarria D, Rottner K, Small JV. Live imaging of glioblastoma cells in brain tissue shows requirement of actin bundles for migration. *Neuron Glia Biol* 2006;2:105-14.
 83. Baskin R, Woods NT, Mendoza-Fandino G, Forsyth P, Egan KM, Monteiro AN. Functional analysis of the 11q23.3 glioma susceptibility locus implicates PHLDB1 and DDX6 in glioma susceptibility. *Sci Rep* 2015;5:17367.
 84. Kessler J, Guttler A, Wichmann H, Rot S, Kappler M, Bache M, Vordermark D. IDH1(R132H) mutation causes a less aggressive phenotype and radiosensitizes human malignant glioma cells independent of the oxygenation status. *Radiother Oncol* 2015;116:381-7.
 85. Jung JH, Kim AA, Chang DY, Park YR, Suh-Kim H, Kim SS. Three-dimensional assessment of bystander effects of mesenchymal stem cells carrying a cytosine deaminase gene on glioma cells. *Am J Cancer Res* 2015;5:2686-96.
 86. Smith SJ, Ward JH, Tan C, Grundy RG, Rahman R. Endothelial-like malignant glioma cells in dynamic three dimensional culture identifies a role for VEGF and FGFR in a tumor-derived angiogenic response. *Oncotarget* 2015;6:22191-205.
 87. Huang M, Ke Y, Sun X, Yu L, Yang Z, Zhang Y, Du M, Wang J, Liu X, Huang S. Mammalian target of rapamycin signaling is involved in the vasculogenic mimicry of glioma via hypoxia-inducible factor-1alpha. *Oncol Rep* 2014;32:1973-80.
 88. Boutin ME, Hoffman-Kim D. Application and assessment of optical clearing methods for imaging of tissue-engineered neural stem cell spheres. *Tissue Eng Part C Methods* 2015;21:292-302.
 89. He W, Kuang Y, Xing X, Simpson RJ, Huang H, Yang T, Chen J, Yang L, Liu E, He W, Gu J. Proteomic comparison of 3D and 2D glioma models reveals increased HLA-E expression in 3D models is associated with resistance to NK cell-mediated cytotoxicity. *J Proteome Res* 2014;13:2272-81.
 90. Jiguet Jiglaire C, Baeza-Kallee N, Denicolai E, Baretts D, Metellus P, Padovani L, Chinot O, Figarella-Branger D, Fernandez C. *Ex vivo* cultures of glioblastoma in three-dimensional hydrogel maintain the original tumor growth behavior and are suitable for preclinical drug and radiation sensitivity screening. *Exp Cell Res* 2014;321:99-108.
 91. Lee KH, Lee KH, Lee J, Choi H, Lee D, Park Y, Lee SH. Integration of microfluidic chip with biomimetic hydrogel for 3D controlling and monitoring of cell alignment and migration. *J Biomed Mater Res A* 2014;102:1164-72.
 92. Levin VA, Panchabhai S, Shen L, Baggerly KA. Protein and phosphoprotein levels in glioma and adenocarcinoma cell lines grown in normoxia and hypoxia in monolayer and three-dimensional cultures. *Proteome Sci* 2012;10:5.
 93. Kievit FM, Florczyk SJ, Leung MC, Veisheh O, Park JO, Disis ML et al. Chitosan-alginate 3D scaffolds as a mimic of the glioma tumor microenvironment. *Biomaterials* 2010;31:5903-10.
 94. An Z, Gluck CB, Choy ML, Kaufman LJ. Suberoylanilide hydroxamic acid limits migration and invasion of glioma cells in two and three dimensional culture. *Cancer Lett* 2010;292:215-27.
 95. Sarkar S, Yong VW. Reduction of protein kinase C delta attenuates tenascin-C stimulated glioma invasion in three-dimensional matrix. *Carcinogenesis* 2010;31:311-7.
 96. Sarkar S, Yong VW. Inflammatory cytokine modulation of matrix metalloproteinase expression and invasiveness of glioma cells in a 3-dimensional collagen matrix. *J Neurooncol* 2009;91:157-64.
 97. Jamison S, Lin Y, Lin W. Pancreatic endoplasmic reticulum kinase activation promotes medulloblastoma cell migration and invasion through induction of vascular endothelial growth factor A. *PLoS One* 2015;10:e0120252.
 98. Bhatia S, Baig NA, Timofeeva O, Pasquale EB, Hirsch K, MacDonald TJ, Dritschilo A, Lee YC, Henkemeyer M, Rood B, Jung M, Wang XJ, Kool M, Rodriguez O, Albanese C, Karam SD. Knockdown of EphB1 receptor decreases medulloblastoma cell growth and migration and increases cellular radiosensitization. *Oncotarget* 2015;6:8929-46.
 99. Dudu V, Able RA Jr, Rotari V, Kong Q, Vazquez M. Role of Epidermal Growth Factor-Triggered PI3K/Akt Signaling in the Migration of Medulloblastoma-Derived Cells. *Cell Mol Bioeng* 2012;5:502-413.
 100. Yuan L, Zhang H, Liu J, Rubin JB, Cho YJ, Shu HK, Schliederjan M, MacDonald TJ. Growth factor receptor-Src-mediated suppression of GRK6 dysregulates CXCR4 signaling and promotes medulloblastoma migration. *Mol Cancer* 2013;12:18.
 101. Werbowetski-Ogilvie TE, Seyed Sadr M, Jabado N, Angers-Loustau A, Agar NY, Wu J, Bjerkvig R, Antel JP, Faury D, Rao Y, Del Maestro RF. Inhibition of medulloblastoma cell invasion by Slit. *Oncogene* 2006;25:5103-12.
 102. Morrison LC, McClelland R, Aiken C, Bridges M, Liang L, Wang X, Di Curzio D, Del Bigio MR, Taylor MD, Werbowetski-Ogilvie TE. Deconstruction of medulloblastoma cellular heterogeneity reveals differences between the most highly invasive and self-renewing phenotypes. *Neoplasia* 2013;15:384-98.