Cellular and Molecular Neurobiology (2018) 38:1179–1195
https://doi.org/10.1007/s10571-018-0588-3

REVIEW PAPER

Glioma Cell and Astrocyte Co-cultures As a Model to Study Tumor–Tissue Interactions: A Review of Methods

Ivan V. Chekhonin[1] · Dimitry A. Chistiakov[1] · Nadezhda F. Grinenko[1] · Olga I. Gurina[1]

Received: 17 February 2018 / Accepted: 23 April 2018 / Published online: 10 May 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Astrocytes are a dominant cell type that envelopes the glioma bed. Typically, that is followed by formation of contacts between astrocytes and glioma cells and accompanied by change in astrocyte phenotype, a phenomenon known as a 'reactive astrogliosis.' Generally considered glioma-promoting, astrocytes have many controversial peculiarities in communication with tumor cells, which need thorough examination in vitro. This review is devoted to in vitro co-culture studies of glioma cells and astrocytes. Firstly, we list several fundamental works which allow understanding the modalities of co-culturing. Cell-to-cell interactions between astrocytes and glioma cells, the roles of astrocytes in tumor metabolism, and glioma-related angiogenesis are reviewed. In the review, we also discuss communications between glioma stem cells and astrocytes. Co-cultures of glioma cells and astrocytes are used for studying anti-glioma treatment approaches. We also enumerate surgical, chemotherapeutic, and radiotherapeutic methods assessed in co-culture experiments. In conclusion, we underline collisions in the field and point out the role of the co-cultures for neurobiological studies.

Keywords Astrocytes · Glioma · Co-culture · Periglioma zone · Tumorigenesis

Abbreviations

Asn	Asparagine
ATM	Ataxia-telangiectasia mutated
DMSO	Dimethyl sulfoxide
EEAT2	Excitatory amino acid transporter 2
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
GLAST	Glutamate and aspartate transporter
Gln	Glutamine
Glu	Glutamate
GLT-1	Glutamate transporter
GSC	Glioma stem-like cell
GFAP	Glial fibrillary acidic protein
Cx43	Connexin 43
GRO	Growth-related oncogene
JAK	Janus kinase
IL	Interleukin
MCP	Monocyte chemoattractant protein
MMP	Matrix metalloproteinase
mRNA	Matrix RNA
miRNA, miR	MicroRNA
MSC	Mesenchymal stem cells
NRP-2	Neuropilin 2
PCR	Polymerase chain reaction
PI	Propidium iodide
siRNA	Small interfering RNA
SPARC	Secreted protein acidic and rich and cysteine
STAT	Signal transducer and activator
TGF	Transforming growth factor
TIMP	Tissue inhibitor of metalloproteinase
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
VEGF	Vascular endothelial growth factor

✉ Ivan V. Chekhonin
Ivan-Chekhonin@yandex.ru

[1] Department of Fundamental and Applied Neurobiology, V. Serbsky National Medical Research Centre for Psychiatry and Narcology, Kropotkinskiy pereulok 23, Moscow 119034, Russian Federation

Introduction

Gliomas account for about 80% of primary malignant brain tumors. Among those, glioblastoma multiforme, a high-grade glioma type, falls on about 45% of cases, and is the deadliest brain tumor, with a median survival of diseased patients for 12–15 months (Ostrom et al. 2014). One of

the reasons why highly malignant gliomas are characterized by a high recurrence rate after the implication of ‘gold standard’ treatment approaches, is the existence of ‘assistant cells’ including microglia, macrophages (Poon et al. 2017), and astrocytes (Placone et al. 2016) in the tumor tissue, that in turn enhance proliferation and invasion of tumor cells, being literally hijacked by glioma cells and glioma stem-like cells (GSCs) (Gieryng et al. 2017; Kim et al. 2017; Roos et al. 2017). Mechanistically, astrocytoma cells may inhibit T-cell proliferation and promote switching of circulating monocytes from inflammatory to the myeloid-derived suppressor-like phenotype (Jehs et al. 2011; Rodrigues et al. 2010). Astrocytes are a broadly spread cell population in the glioma microenvironment that constitutes almost a half of the brain volume. According to Placone et al. (2016), in contrary to an established role of microglia in gliomagenesis, the precise role of astrocytes in tumor pathogenesis is still not well understood. In tumorigenic conditions, their neurotrophic role is supplemented by a phenomenon called as ‘reactive transformation,’ or ‘reactive astrogliosis,’ which means the up-regulation of glial fibrillary acidic protein (GFAP) (Lee et al. 2011a), growth factors (Kim et al. 2014; Shabtay-Orbach et al. 2015), proinflammatory cytokines (Blank et al. 2015), extracellular matrix proteins (Grau et al. 2015; Jones and Bouvier 2014; Kolar et al. 2015), and some enzymes (Placone et al. 2016). In the turnover, astrocytes, as was shown by Lu et al. (2016), undergo morphological and biochemical changes *via* the epithelial-like-to-mesenchymal-like transition mechanism. These changes were shown to be linked to a canonical Wnt/β-catenin signaling pathway activation. Those astrocytes exhibited an increased paracrine secreting activity, invasiveness, and motility. Earlier on, astrocytes were found to reduce the vulnerability of breast or lung cancer cell lines to chemotherapy and help lymphoma cells to gain resistance to ionizing radiation (Koto et al. 2011). In gliomas, reactive astrocytes contribute to tumor invasion guidance (Gritsenko et al. 2012, 2017). Biasoli et al. (2014) described the consequences of blocking of the p53 gene expression in astrocytes by a glioma-conditioned medium. Further, semi-p53-defective astrocytes secreted extracellular matrix proteins that promoted glioma cell viability. As Rape et al. (2014) mentioned, a tumor microenvironment is characterized by a high degree of heterogeneity. That is a reason why it is difficult to separate the contribution of variable microenvironment components to tumor progression. Nowadays, the number of experiments performed provides an option to suggest using in vitro technologies as potent resources to study crosstalk between tumor and normal brain tissue. In this review, we list models assessing in vitro interactions between glioma cells and astrocytes. Hopefully, two-component co-cultures may appear to be rather simple, and the exclusion of other factors simplifies emphasizing on direct mechanisms of interactions. If needed, peculiarities discovered may be verified in more complicated models.

Principled Co-culturing Studies of Astrocytes and Glioma Cells

As above-mentioned, glioma and astrocytes interact on two-levels—either directly through the cell-to-cell junctions or paracrinally. These two types of contacts may be not distinguished in a direct co-culture. By contrary, using systems of indirect co-culturing may shed light on non-direct cellular interactions, which do not require close tumor microenvironment cellular co-localization (Fig. 1). Lal et al. (1996) developed one of the first co-culture models of glioma and astrocytes. The authors used a rat line S635c15. In a tight contact with glioma cells, astrocytes acquired the reactive phenotype, which was also characterized by elevated GFAP production. Moreover, they formed reactive edging as they do on the periphery of glioma in vivo. The data showed the possibility of tumor-induced astrocyte phenotype switching in vitro. However, direct two-dimensional co-culture studies cannot be fully helpful for attaining the purpose of evaluating spatial peculiarities of tumor microenvironment, and particularly their effects on glioma spreading along cerebral vessels and white matter circuits. As shown previously, 3-D tissue substrates more precisely display tumor–tissue interactions compared with 2-D culture systems (Yamada and Cukierman 2007). Grodecki et al. (2015) employed in vitro strategy to model in vivo glioma–astrocyte interaction in a 3-D manner. The authors utilized electrospun poly-(caprolactone) nanofiber system to mimic real conditions like glioma migration ways. Co-cultures of human glioma cells and rat astrocytes were used. Firstly, the influence of glioma on GFAP production by astrocytes was studied. Then, similarly, an experiment studying the influence of astrocytes on glioma migration was performed. To determine, whether GFAP production alterations were driven by soluble glioma-secreted factors, astrocytes were cultured in a glioma-conditioned medium. Once again, glioma migration in the presence of astrocyte-conditioned medium was also studied. The impact of factors exposed by glioma cells on astrocyte production pattern was examined by co-culturing astrocytes and fixed glioma cells. Glioma cells were co-cultured with fixed astrocytes to study their migration, respectively. In the next experiment, glioma cells were detached after culturing, leaving glioma-produced extracellular matrix on fibers. Addition of astrocytes to such a scaffold could help to study the effect of glioma-secreted proteins on astrocytes. Vice versa, glioma migration in presence of astrocyte-derived extracellular matrix was also investigated. As a result, production of GFAP in astrocytes was

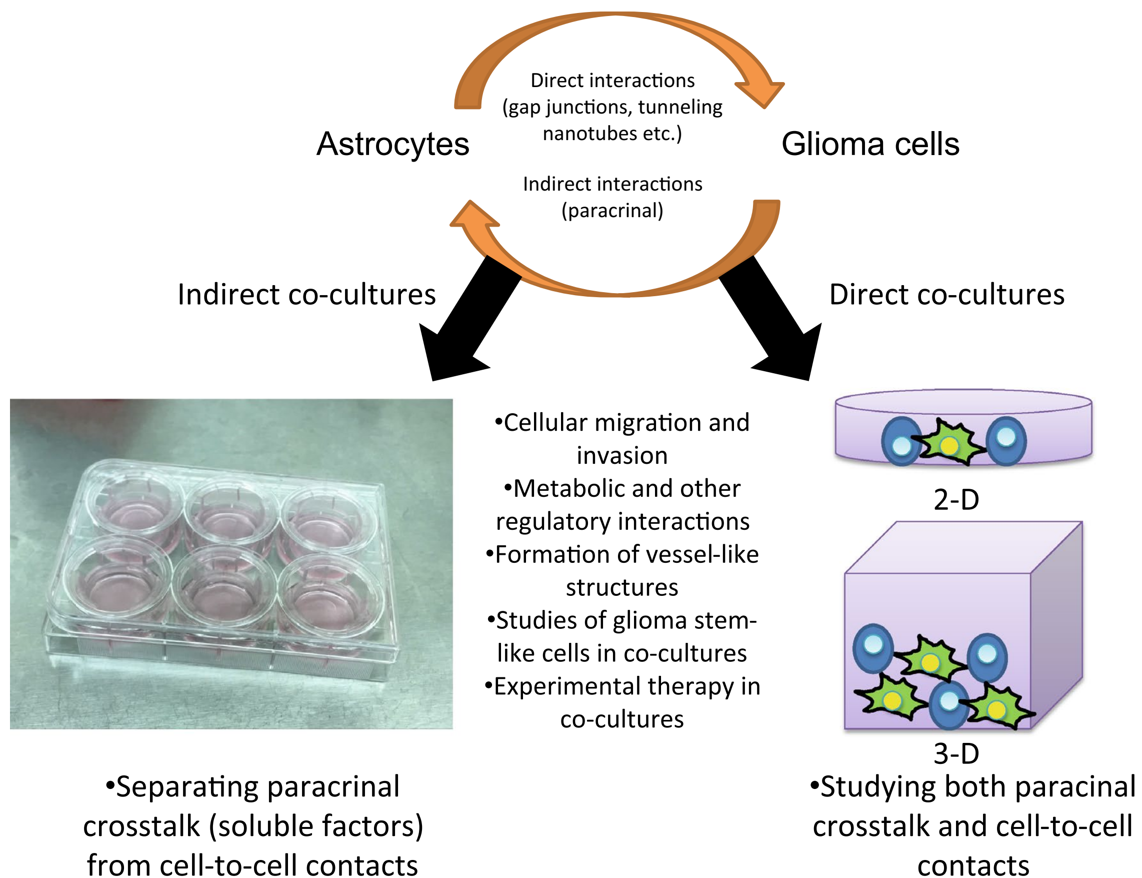

Fig. 1 Types of glioma cell and astrocyte co-cultures and areas of experimental oncology in which they are used

increased in co-culture experiments with both glioma cells and astrocytes (increasing glioma–astrocyte ratio increased GFAP production). Conversely, experiments examining the impact of glioma-secreted factors on astrocytes showed a lack of GFAP up-regulation. Furthermore, GFAP production profile was not also altered by factors immobilized on fixed glioma cells. In contrary to the latter, culturing of astrocytes on fibers covered by the glioma-produced extracellular matrix led to a twofold increase in GFAP production compared to the comprehensive control. Glioma cell migration was enhanced by co-cultured astrocytes. Each putative astrocyte-derived glioma-influencing factor was separately studied. Astrocyte-conditioned medium that contained soluble factors strengthened glioma cell migration. By contrast, fixed astrocytes reduced migration of glioma cells in a gradual concentration-dependent manner. The authors proposed that migration was hindered by immobilized astrocyte antigens or (more potentially) by creating a spatial barrier velocity. In conclusion, the 3-D system was managed to reproduce several features of the glioma tumor microenvironment simulating white matter tracts and formation of voluminous (not flat) extracellular matrix deposits. Grodecki et al. suggested that the obtained results correspond to the 2-D co-culture experiments of Le et al. (2003) who showed that glioma cells exploit astrocytes for promoting invasiveness via the matrix metalloproteinase (MMP)-2-dependent pathway. MMP-2 activation in astrocyte–glioma co-cultures may be attributable to the urokinase-type plasminogen activator (uPA), a mechanism essential for both glioma and astrocytes. Interestingly, the MMP-2 precursor was reported to be secreted by astrocytes. Gritsenko et al. (2017) established 3-D frameworks of immortalized murine astrocytes in hyperconfluent culture, enriched by extracellular matrix proteins. The rate of human U251 and E-98 gliomas invasion inversely correlated with a scaffold conditioning time. Notably, the pattern of glioma migration was different from the experiment that involved glioma cell trafficking along the reconstituted basement membrane. As far as glioma migration is taken into account, the 2-D research data also state that co-cultures of glioma and astrocytes may enhance glioma cell migration (Aubert et al. 2008). Schichor et al. (2005) developed a specific model for assessing glioma invasiveness named as a brain slice chamber. In general, that is a modification of the Boyden

chamber. The authors used pig brain tissue slices as a matrix for the tumor cells to invade. The authors studied the invasiveness of human U87 and U373 glioma cells into the brain slices mentioned above. Administration of cytostatics, vincristine, and paclitaxel, inhibited glioma migration. In conclusion, brain slice chamber is a model representing the 3-D architecture of brain tissue that is suitable to study the tumor invasiveness. Gritsenko et al. (2017) used a relative strategy in modeling glioma invasion into the mouse brain slice and observed glioma cells spreading into the mouse brain tissue along the vessels.

An example of using of an astrocyte culture to obtain an indirect co-culture with glioma cells may be found in the study of Gagliano et al. (2009). The original purpose of the work was to analyze the ability of glioma cells to alter an astrocyte phenotype. The authors used a Transwell system to co-culture U87 glioma and astrocytes derived from human neural stem cells. The astrocytes were seeded in the lower chamber while the glioma cells were seeded in the upper chamber. The authors assessed the level of gene expression with help of a real-time polymerase chain reaction (PCR) and protein production using Western blotting including GFAP, MMP-2, tissue inhibitor of MMP-2 (TIMP-2), secreted protein acidic and rich in cysteine (SPARC), transforming growth factor (TGF)-β1, and gap junction protein connexin 43 (Cx43). Accordingly, reactive astrocytes were characterized by increased expression of GFAP, MMP-2, and reduced expression of TIMP-2. SPARC was hallmarked by the down-regulation of mRNA, unaltered protein production in cell lysates, and by increase of the protein concentration in cell culture supernatants. There were no changes in TGF-β1. Cx43 mRNA expression level was reduced after the co-culture experiment. As a protein, Cx43 exists in a non-phosphorylated (open gap junction) and two phosphorylated (closed gap junction) isoforms. Compared to plain astrocytes, co-cultured cells were characterized by decreased ratio between non-phosphorylated and phosphorylated Cx43, showing increase in production of phosphorylated Cx43 (although generally considered inactive, this isoform may reflect a dynamic balance between phosphorylated and non-phosphorylated isoforms). Thus, reactive astrocytes observed in the study were not primarily characterized by a bias towards the malignancy (as genuine malignant astrocytes are described by down-regulated GFAP production) but still had a potential for increased invasiveness as it follows from the changes in MMP-2, TIMP-2 and, partially, SPARC expression and production patterns, being at least hypothetically able to permit the glioma invasion.

Summing up, glioma invasion may be studied in 2-D or 3-D co-cultures with astrocytes, astrocyte-derived or artificial extracellular matrix, and in 3-D brain slice models. Either of the models represents particular features of glioma cell migration pattern. Indirect co-cultures may be used to study phenotype alterations which occur paracrinally.

Assessment of Glioma–Astrocyte Intercellular Communications in Co-cultures

Primary cell-to-cell contacts are a major way of an interaction between glioma cells (Katakowski et al. 2010) and connection with tumor microenvironment (Lee et al. 2013). Gap junctions may be a target for anti-glioma therapy, as they may play a role in tumor expansion (Moinfar et al. 2014). Reactive astrocytes in the periglioma area are characterized by the up-regulation of Cx43, which is critical in forming contacts between malignant cells and reactive astrocytes and promoting glioma expansion and invasion (Sin et al. 2016). Although the study of Gagliano et al. (2009) showed that Cx43 production may be altered in astrocytes even without contact with glioma cells, direct co-culture experiments are also needed to reconstruct the cell-to-cell interaction more precisely.

Oliveira et al. (2005) developed a prominent co-culture to study peculiarities of direct contacts between human glioma cells and astrocytes. The authors established a 3-D model that imitated a brain microenvironment (called as an 'intra-slice implantation system') for glioma invasion studies. Briefly, that is an ex vivo technology, which provides an opportunity for engrafting experimental cells and biopsy specimens into rodent brain slices for subsequent cultivation and examination. That model was also measurable for assessing glioma cell migration in the experimental conditions not so far distant from in vivo studies (de Bouard et al. 2002). Intercellular coupling was studied by Oliveira et al. (2005) in direct glioma–astrocyte co-cultures in vitro. Obviously, glioma–astrocyte interaction could be hampered by carbenoxolone, a blocker of gap junctions. Interestingly, migration in glioma homocultures was not inhibited by carbenoxolone. Furthermore, that was increased. Of the glioma cultures and lines studied, more invasive ones were characterized by a higher level of phosphorylated Cx43 isoform production and a higher level of response to carbenoxolone. Oliveira et al. (2005) suggested that glioma cells exploit astrocytes to form the heterocellular gap junctions thereby enhancing mobility and invasion. Homocellular gap junctions further contribute to maintaining cell adhesion. Zhang et al. (2003) assessed Cx43-transfected (and therefore Cx43-overexpressing) C6 glioma cell migration by use of a Matrigel-coated Transwell invasion chamber. When the experimental cells were co-cultured with astrocytes (by mixture in one compartment), their migration exceeded the control by more than 3 times, an effect likely caused by MMP action. Thus, invasion of glioma cells was enhanced by both Cx43 up-regulation and communication with astrocytes. Obviously,

gap junctions serve as channels to transfer regulatory molecules from cell to cell. Hong et al. (2015) used in vitro co-culture systems to study the effect of gap junction-mediated microRNA transfer between U87 glioma cells and human astrocytes on tumor invasion. The experiment design meant direct co-culturing of cells to obtain gap junction formation. The authors used small interfering RNAs (siRNAs) to inhibit Cx43 expression, as well as 18-α-glycyrrhetinic acid to block the gap junctions. Gap junction-defective glioma cells were also constructed by Cx43 knockdown. As a result, it was found that gap junctions between glioma cells (in U87 monoculture) suppressed glioma invasion, as well as ones between glioma cells and astrocytes and astrocyte–astrocyte gap junctions facilitated tumor expansion. MicroRNAs were found to permeate gap junctions, and the pro-invasive effect was attributable to miR-5096, as its elimination contributed to the reduction of glioma cell invasion. Totally, the authors showed that, in overall, an impact of glioma–astrocyte and interastrocyte gap junction exceeded the glioma–glioma gap junction anti-migratory effect.

Direct cell-to-cell substance-transferring contacts may be mediated not only through gap junctions, but also via tunneling nanotubes. Those nanotubes are thin membrane long-distant structures, which provide an option to transport subcellular structures such as ones having a cell organelle size-like mitochondria (Liu et al. 2014). Zhang and Zhang (2015) reported the development of a co-culture of rat astrocytes and C6 rat glioma cells and found tunneling nanotube formation between the cells. Understandably, in the tube formation experiments, direct co-cultures were used. To exclude the contribution of secreted factors to glioma cell proliferation, Transwell systems were implicated in the control experiments to separate glioma cells from astrocytes. Interestingly, in studies most of the tubes were developed from astrocytes towards C6 glioma cells. Astrocyte-dependent generation of the tunnel nanotubes was regulated by a p53-dependent mechanism. The tube formation activity of both glioma cells and astrocytes could also be stimulated by administration of hydrogen peroxide or blocked by latrunculin A, an F-actin antagonist. Tunneling nanotubes were found to decrease proliferation of C6 glioma cells in the experiment. This observation suggests a promise to develop new anti-glioma strategies.

In general, co-culture studies concerning gap junctions present the direct contact formation between glioma cells and astrocytes as a tumorigenic force. Still, homotype connexin channels may favor resting phenotype of glioma cells (Aubert et al. 2008). Moreover, existence of not fully studied tunneling nanotubes between glioma cells and astrocytes suggests that not every intercellular channel enhances tumor invasiveness.

Co-cultures Used to Study Metabolic Interactions Between Astrocytes and Glioma Cells

Glioma cells interact with tumor microenvironment by exchanging metabolites which finally results in tumor progression. Glioma growth and invasion were shown to be dependent on glutamate (Glu) production and induction of excitotoxicity, neurodegeneration, neuroinflammation (Lee et al. 2011b; Takano et al. 2001), and Glu-dependent motility enhancement (Lyons et al. 2007; Piao et al. 2009; van Lith et al. 2014). Still, there is more to discover of how Glu-dependent metabolic pathways can support glioma growth and progression. Astrocytes are also considered to be in a metabolic crosstalk with glioma cells. In particular, a role of L-glutamine (Gln) and Glu as a metabolic pool was established. Yao et al. (2014) studied the Gln/Glu exchange between glioma cells and astrocytes in direct contact. As a result, the authors showed that glutaminase is highly produced in glioma cells and in turn provides an option to intensively convert Gln to Glu. The more glioma cells were in the co-culture, the higher death of neurons subjected to the co-culture medium was observed. Moreover, the authors showed that glioma growth rate was decreased in a direct co-culture with astrocytes at astrocyte/glioma ratios of 0.3:1 or greater reflecting an early glioma stage (many astrocytes, low amount of glioma cells). Several experiments proved that inhibiting deamination of Gln to Glu may reduce tumor aggressiveness (Wang et al. 2010). Particularly, the significance of Gln–Glu metabolism was detected in gliomas (DeBerardinis et al. 2007). Tardito et al. (2015) studied the metabolic network mentioned above. They showed that Gln deprivation may inhibit glioma cell proliferation. One of the study aims was to identify the putative mechanism of metabolic glioma–astrocyte communications. As far as co-culture experiments were performed, rat pup cortical astrocytes were plated and cultured-up to confluence, then Transwells with human LN18 glioma tagged with an infrared fluorescent protein were employed. When LN18 cells were deprived of Gln, the reactive astrocytes still maintained the proliferation and thereby rescued the glioma cells. The salvage factor was diffusible because it exerted its function in a Transwell co-culturing system. Moreover, the administration of L-asparaginase to the cultivating medium, an enzyme that decreases the levels of both asparagine (Asn) and Gln, abrogated the astrocyte-mediated rescue effect. As Asn was available in the medium during the Gln depletion experiment, the data showed that higher glutamine-synthetase-producing astrocytes rescue glioma cells, which lack production of this enzyme and therefore are dependent on Gln supply. The latest indicates a mechanism by which astrocytes contribute to gliomagenesis that is regulated not only by a paracrine

regulatory pathway-mediated signaling, but also metabolically. Jacobs and De Leo (2013) studied the capacity of astrocytes to influence glioma-related Glu metabolism, with the involvement of Glu-dependent signaling. As was shown, excessive Glu concentrations may cause cellular toxicity. Generally, brain cells are defended by Glu transporters that reduce the extracellular Glu concentrations to subtoxic levels. Interestingly, glial cells produce a vast majority of brain Glu transporters and contribute for a valuable portion of Glu clearance (Amara and Fontana 2002). Still, high extracellular Glu concentrations are typical for gliomas, and they are believed to increase tumor invasive capability by interactions with specific receptors such as *N*-methyl-D-aspartate receptors (NMDA receptors) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors) (Piao et al. 2009; Takano et al. 2001). By contrary to the normal brain, glioma tissue is characterized by decreased Glu transporter production, which may be a reason of higher Glu concentration in glioma-affected brains. Furthermore, the less the Glu transporter EEAT2 (excitatory amino acid transporter) production is, the higher the glioma grades develop (de Groot et al. 2005). In the study of Jacobs et al. (2012), rodent astrocytes and CNS-1 glioma cells were implemented. Astrocytes were co-cultured with glioma cells in Boyden chambers in the presence of 5 mM Glu. When propentofylline, an astrocyte-influencing Glu-uptake enhancer by GLT-1 (a glutamate transporter) and GLAST (a glutamate aspartate co-transporter), was administered, the glioma cells were exposed to Glu starvation and their apoptosis was increased. In the next experiment, the expression of Glu transporters GLT-1 and GLAST was inhibited by siRNAs. After inhibition, the experiment was replicated. As a result, the propentofylline effect on glioma cell apoptosis was abrogated (to a greater extent by anti-GLT-1 siRNA) that suggests for the role of astrocyte-related transporters in maintaining Glu clearance. When the authors established co-cultures of human astrocytes and U251 cells to fit more human-like conditions, propentofylline also led to increased apoptosis in glioma cells. These results highlight a need for future investigations as they correlate with the data of propentofylline experimental glioma treatment obtained previously.

Taking into account heterotypic gap junctions mentioned in the previous chapter, we should underline their particular metabolic role in creating structural pathways for intercellular substance exchange. For example, astrocytes may prevent the apoptosis of glioma cells by decreasing the intracellular Ca^{2+} concentrations via the gap junctions (Chen et al. 2015). In conclusion, metabolic interactions between glioma cells and astrocytes, occurring by direct or indirect contact, are generally targeted against tumor growth at early stages of gliomagenesis but then are biased towards glioma expansion as the tumor begins to dominate over its microenvironment.

Studies Assessing the Effects of Astrocytes on Glioma-Related Vessel Formation with Help of Co-cultures

Glioma-to-astrocyte co-cultures became a suitable in vitro tool to study glioma-related angiogenesis due to tumor microenvironment that is suspected to contribute to the capacity of glioma to promote the neovessel generation (Charles et al. 2012; Chen et al. 2014; Rupp et al. 2016). Commonly, angiogenesis-targeting strategies in glioma are broadly implied and studied in clinical trials (Wang et al. 2017). The theory of suppressing tumor vasculature is much questionable, yet to be debated (Tamura et al. 2017). To date, studies of tumor angiogenesis are being designed at the molecular level and also implementing co-cultures. Zheng et al. (2013) performed a study of glioma angiogenesis-related microRNA and their ability to influence the tumor vessel formation. It was shown that NRP-2(neuropilin 2)-specific miR-15b and MMP-3-specific miR-152 could regulate glioma angiogenesis and invasion. The production of NRP-2 and MMP-3 in 9L glioma cells inversely correlated with miR-15b and miR-152, respectively. 9L rat gliomas and astrocytes co-cultures were also developed. Co-culturing of rat astrocytes with 9L cells resulted in decreased miR-15b and miR-152 expression in astrocytes and upregulated NRP-2 and MMP-3. 9L glioma invasiveness was significantly decreased by both miRNAs. In addition, in vitro endothelial tube formation assay showed that miR-15b, but not miR-152, inhibited endothelial tube assembly, and anti-miR-15b microRNA abrogated tube formation inhibition caused by miR-15b. Gliomas are characterized by the formation of perivascular niches responsible for maintenance of GSCs (Chen et al. 2017). Remodeling of the blood–brain barrier microenvironment may play a role in the anti-glioma treatment assistance (Zhao et al. 2017). For example, gliomas are known to exhibit vasculogenic mimicry, which means formation of vessel-like tubes constituted not by endothelial but by tumor cells (Zhang et al. 2017). This is a viewpoint that may explain why gliomas may become resistant to anti-angiogenic therapy (Seftor et al. 2012). Zhang et al. (2016) conducted an in vitro study of galunisertib, a TGF-β receptor inhibitor, especially concerning its anti-angiogenic effects. For assessment of glioma invasion potential in co-cultures, the Transwell system with a 8-μm pore size was chosen. A172 human glioma cells were seeded on the Transwells while human astrocytes were seeded in the lower chamber. In another co-culture experiment, 0.2-μm pore size of Transwells was used, and the cells were seeded in the inverted manner. Accordingly, with help of 0.4-μm pore-size Transwells, glioma cells were conditioned before

the bromodeoxyuridine radioactive proliferation assay. To induce astrocyte-conditioned vascular-mimic tubes, glioma cells were co-cultured with astrocytes before the tube formation assay (on plates covered with Matrigel). Compared to the control, human astrocytes enhanced glioma cell migration and proliferation, a phenomenon that fully reflects the nature of reactive astrocytes. Peculiarity of the experiment was the conclusion that astrocytes also promoted the vessel-like tube formation, i.e., the stimulation of vasculogenic properties. Moreover, it was found out that the glioma tube formation was not inhibited by bevacizumab that indicates the independency of this process from vascular endothelial growth factor (VEGF). By contrary, TGF-β1 was found at relatively high concentration in the co-culture system, and glioma-induced astrocyte origin of TGF-β1 was proved. Astrocyte-derived TGF-β1 also potentiated glioma tube formation. When galunisertib, a TGF-β inhibitor, was administered, an inhibitory effect on the vasculogenic activity of astrocytes and down-regulation of smooth muscle actin-α, vascular endothelial cadherin, matrix metalloproteinase (MMP)-2, and MMP-9 production in glioma cells were observed, most likely due to suppression of Akt and Flk pathways. The authors reported that galunisertib decreased autophagy in glioma–astrocyte co-cultures, proved by Western blotting, immunofluorescence assay (with targeting microtubule-associated protein 1 light chain 3β (LC3B), an autophagy marker), and transmission electron microscopy. As autophagy is in tight relationship with function of GSCs (Galavotti et al. 2013), they also concluded that autophagy is considered to be a source for induction of vasculogenic properties of tumor cells thus enhancing tumor-related formation of vessel-like structures. Glioma–astrocyte co-cultures obtained by Zhang et al. (2016) were characterized by a higher metabolic rate than control, which could be lowered by the addition of galunisertib. Tumor growth and vasculogenic mimicry could also be suppressed by galunisertib in a xenograft tumor model, which confirmed the results of the in vitro co-culture study.

Taken together, angiogenic and vasculogenic strategies of gliomas represent a high degree of reliability allowing the tumors to propagate. As mentioned above, even when endothelial angiogenesis is inhibited, glioma may utilize endothelium-independent strategies of neovessel formation.

Co-cultures Showing a Role of Glioma Stem-Like Cells in Gliomagenesis

GSC population is considered to be one of the major glioma-driving forces (Chen et al. 2012). Characterized by a $CD133^+$ phenotype (Singh et al. 2004a), these cells exhibit the neural stem cell properties (self-renewal and at least partial differentiation combined with $CD133^+$ loss) and tumorigenic capability in vivo (Singh et al. 2004b). Moreover, these cells are found to reside close to the perivascular niche compartment (Chen et al. 2017; Sharma and Shiras 2016), possess an ability to differentiate into pericytes (Cheng et al. 2013) or endothelium (Ricci-Vitiani et al. 2010), and support glioma progression (Jhaveri et al. 2016). GSCs tightly interact with the microenvironment thereby increasing chemo- and radioresistance (Fidoamore et al. 2016). Since a tumor invasion typically implies tumor–tissue interaction, a thorough study of the brain parenchymal impact is needed. Rath et al. (2013) simulated the influence of the brain microenvironment on GSC invasive reactivity. $CD133^+$ GSCs were obtained from the glioblastoma multiforme neurosphere-forming cultures (NSC11, GBAM1) using a fluorescence-activated cell sorting. To study the indirect interaction, GSCs (and $CD133^−$ cells derived from the same line in a corresponding control) were seeded onto a Transwell membrane (8 μm pore size for the migration study, 0.4 μm for gene expression analysis) and then co-cultured with an astrocyte monolayer in the bottom chamber. The second experiment implied monolayer invasion assay, where GSCs (and $CD133^-$ cells, respectively) were seeded on glass-cover slips which were then added to astrocyte monolayers in an inverted manner to provide a direct cell contact. Phenotype changes were assessed using the microarray gene expression analysis (for both direct and indirect experiment) and immunoblotting (for indirect experiment). Cytokine concentrations were also measured in the medium conditioned by astrocytes in co-cultures. The results of indirect co-culture experiments showed that astrocytes and astrocyte-conditioned medium in contrast to indifferent media and normal human fibroblasts enhance the invasiveness of the GSCs, but do not influence non-GSCs. It was also experimentally shown that the effect was not mediated by the autocrine activity of $CD133^+$ cells. For direct co-cultures, the infiltration of astrocyte monolayer by $CD133^+$ cells was higher than the respective infiltration by $CD133^-$ cells. The microarray gene expression study showed that indirect co-culture affected the genes related to cell movement and cell-to-cell signaling and interaction; these results were consistent with the immunoblotting assay. Direct co-culturing altered expression of a greater gene amount, which may be explained by both direct and indirect involvement of astrocytes. Impact of astrocytes on non-GSC was not directly linked with genes responsible for invasion or migration. Finally, the authors noticed that the enhancing effect on $CD133^+$ cells may be mediated by increased levels of various cytokines and chemokines such as monocyte chemoattractant protein (MCP-1), MCP-3, osteopontin, TGFβ-1, VEGF, interleukin (IL)-6, IL-8, etc., compared with the non-conditioned medium. McCord et al. (2009) studied an ability of astrocytes to influence the radiosensitivity of GSCs. Generally, GSCs are more radiosensitive than respective glioma lines, but their exact contribution to glioma radioresistance

is still unclear. Moreover, the impact of brain microenvironment on GSCs was confirmed by the fact that GSCs are more radioresistant when being grown under intracerebral growth conditions rather than grown in vitro (Jamal et al. 2012). In the second research of Rath et al. (2015), the influence of astrocytes on GSC radiosensitivity was studied. Like in the previous work (Rath et al. 2013), the authors used GSC-astrocyte indirect co-cultures, then a radiation survival assay (a single irradiation) was performed. To detect the rate of a loss of tumor cells, intracellular ATP content was quantified. Another promising way of assessing the radiosensitivity was to examinate histone H2AX (γH2AX) foci with the immunofluorescent analysis. Cytokine concentrations in the conditioned media were also evaluated, and gene expression analysis was performed. For these experiments, GSCs were cultured with astrocytes (indirectly) or alone (control). Similarly, GSCs alone or after co-culturing with astrocytes were subject to an immunoblotting analysis with antibodies against signal transducer and activator 3 (STAT3), activated form of STAT3 (phospho-STAT3), and β-actin, a non-muscle contractile protein responsible for cell motility and integrity. As was shown, astrocytes were able to reduce vulnerability of GSCs to ionizing radiation. The latter effect was achieved due to induction of DNA repair in comparison with the control. The number of histone H2A (γH2AX) foci was greater in a GSC monoculture than in a co-culture with astrocytes. Taken into account that γH2AX foci counts correlate with the number of radiation-induced double-strand breaks, experimentally derived data suggest that astrocytes are able to potentiate GSC radioresistance by reducing DNA damage. In line with this, it was found that astrocytes co-cultured with GSCs release various cytokines (IL-4, IL-6, growth-related-oncogene (GRO), MCP-3, etc.) that can influence the GSC radioresistance improvement (of the 80 cytokines assessed, 27 and 31 were increased in NSC11 and GBAM1 co-cultures, respectively, compared to stem cell media). In GSCs co-cultured with astrocytes, 112 transcripts were upregulated at least by twofold, 90 transcripts were down-regulated at the level of least 0.5-fold. Notably, in GSCs co-cultured with astrocytes, enhanced STAT3 activation was detected since total STAT3 levels in a GSC monoculture and a co-culture did not significantly variate while the level of the phospho-STAT3 was elevated in co-cultured GSCs in comparison with the control. In addition, the inhibition of Janus kinase (JAK)/STAT3-dependent pathway partially abrogated the reduction of astrocyte-induced radiosensitivity. These data were then confirmed in athymic mice intracranially implanted with NSC11 GSC xenografts and then treated with WP1066, an inhibitor of JAK/STAT3 pathway. The inhibition of this signaling resulted in down-regulation of phospho-STAT3 in xenografts and increased sensitivity to irradiation. Indeed, the co-culture system suggested also showed that an in vitro GSC–astrocyte interaction model may be helpful to find agents for tumor radiosensitization and to identify the role of the glioma microenvironment in the development of radioresistant mechanisms. Guo et al. (2012) studied the ability of different tumor environment cells to enhance the proliferation of GSCs in vitro. The authors performed co-culturing of CD133^{+}-cells with different glioma cells or normal astrocytes and then assessed the impact of the glioma microenvironment on GSCs and compared production of the growth factors in co-cultures and monocultures. Indirect co-cultures were obtained using filter chambers with 0.4-µm pore-size inserts. GSCs were cultured in lower chambers, while feeder cells including rat primary astrocytes (from rat pups), U87 human glioma cells, unsorted glioblastoma tumor cells, and U87-glioblastoma-derived CD133^{-} cells were cultured in the upper compartment. Actually, no reference for astrocytes was selected since they served as a control in co-cultures. Paracrine factors contributed to the behavior of GSCs in co-cultures. Thus, after standard technique of co-culturing, CD133^{+} cells showed higher proliferation capacity in co-cultures with tumor cells including primary patient-derived glioblastoma, U87 cells, or glioma-derived CD133^{-} cells rather than after co-culture with primary astrocytes. This finding at first glance may seem controversial to the results of Rath et al. (2013, 2015) who showed that astrocytes may be the promoters of CD133^{+} cells invasion and radioresistance. Still, their second study underlined that astrocytes seeded on Transwell inserts did not alter GSC proliferation as well, thus underlining the validity of radioresistance study. What is more, different abilities of astrocytes to secrete CD133^{+} cell-driving cytokines were revealed, ranging from the significant level in studies of Rath et al. (2013, 2015) to the negligible one in a work of Guo et al. (2012). As far as experimental peculiarities were concerned, in the study of Rath et al. (2013) fibroblasts were selected as a feeder control for invasion studies and in the next study (Rath et al. 2015) astrocytes served as a control in a co-culture with U251 glioma showing inability of astrocytes to potentiate radioresistance of an established glioma line. To overcome the discrepancy between Rath et al. (2013, 2015) and Guo et al. (2012), further studies involving GSC co-cultures should be performed, taking all variants of feeder cells for CD133^{+} cells (glioma cell lines, primary glioma-derived cells, astrocytes, and non-brain-derived cells like fibroblasts) into account.

Experimental Anti-glioma Therapy and In Vitro Co-cultures

Chemotherapy and Recombinant Technologies

In this chapter, we list co-culture experiments used to evaluate the impact of astrocytes on glioma response to different

therapeutic approaches. Nowadays, the role of astrocytes in modulating brain tumor response to chemotherapy is vigorously studied. Generally, different chemotherapeutic agents are studied in plain 2-D glioma cultures. Nonetheless, these models do not completely reflect a real state of a problem (Mehta et al. 2012). Yang et al. (2014) established a 3-D bioluminescence co-culture model to demonstrate the role of astrocytes in glioblastoma drug-resistance induction. Glioblastoma cells (human U251, rat C6, human A172 and LN18, biopsy-derived P3 glioma line) were transfected with enhanced green fluorescent protein (eGFP) and luciferase-encoding lentiviral vectors. The bioluminescence intensities were observed to highly correlate with a cell number and to be more sensitive than traditional tetrazolium colorimetric assays. As a second co-culture component, TNC-1 immortalized astrocytes were used. Then, experimental co-cultures and monocultures (as a control) were treated by temozolomide or doxorubicin. The presence of immortalized astrocytes in a co-culture improved the U251, C6, A172, P3 cell survival after temozolomide treatment and the U251 and LN18 survival after doxorubicin treatment. These observations are in line with studies concerning other tumor cell types, which became more invasive in the presence of fibroblasts thereby suggesting that glioma therapy resistance mechanisms have much in common with similar strategies of other tumors (Flach et al. 2011). Lin et al. (2016) employed an in vitro model to study the role of astrocytes in protecting glioma from chemotherapy. The authors used human glioma cell lines (U87, U251, and A172) co-cultured directly with immortalized murine astrocytes. For chemoprotection analysis, astrocytes were labeled with GFP and then co-cultured with glioma cells at a ratio of 1:1 in the presence of chemotherapeutic agents (temozolomide, cisplatin, 5-fluorouracil). After fixation, cells were detached and stained with propidium iodide (PI). To assess the cell cycles, the authors used the PI–fluorescein isothiocyanate protocol. For all three glioma lines mentioned above, the level of apoptosis as a measure of a chemotherapeutic effect was chosen. Electron microscopy showed direct astrocyte–glioma cell-to-cell contacts. The rates of apoptosis in all three glioma lines separately treated with each of all three chemotherapeutic agents were significantly reduced. To exclude the influence of the human-murine interspecific interaction, murine fibroblasts were also co-cultured with U87 glioma as a control, and the fibroblasts did not protect glioma cells from the drugs. Basically, the drugs did not increase apoptosis in astrocytes. The role of a paracrine cell-to-cell interaction was studied using spatially separated astrocyte and glioma co-cultures, which did not allow the direct contacts to form. Astrocytes were seeded into the upper compartment of the Boyden chamber (a pore size of 0.4 μm), and the glioma cells were seeded into the lower compartment. Interestingly, separation of astrocytes from glioma cells abrogated the protective effects in all studied glioma cell lines. Then, the role of gap junctions in the drug resistance was examined. Administration of carbenoxolone reversed the astrocyte-driven chemoprotection of U87 against temozolomide. Further, an RNA microarray analysis showed that among genes that were influenced by astrocytes in a carbenoxolone-dependent manner, there were genes involved in cell survival and drug resistance such as mitogen-activated protein kinase, tyrosine-protein kinase, and B-cell lymphoma 2 (Bcl-2). These data indicate that astrocytes protected glioma cells from the toxic effect of all studied chemotherapeutic drugs through direct gap junction-mediated contacts that resulted in significant decrease of drug-induced apoptosis of the glioma cells. This drug-resistance mechanism may be one of the most important reasons that limits the efficiency of glioma chemotherapy. Astrocytes transmit their protective properties to neighboring glioma cells and stimulate chemoresistance. In a suggestive work, Chen et al. (2015) employed in vitro glioma–astrocyte co-cultures to study mechanisms of glioma resistance to chemical therapy. Human A172 glioma and normal human astrocytes were exploited. Post-incubating, the cultures were subjected to the administration of temozolomide and vincristine. Both direct and non-direct technologies of co-culturing were used. In a co-culture with glioma cells, astrocytes were observed to reduce tumor cell apoptosis caused by temozolomide and vincristine. The protective effect was exerted through Cx43-mediated gap junctions in direct co-cultures, whereas implementation of carbenoxolone led to increased glioma cell apoptosis when co-cultured with astrocytes. For indirect co-cultures, the glioma cell apoptosis decreased slightly but not substantially.

In malignant gliomas, steroids as the anti-inflammatory agents are generally used for shrinking edema although they may potentiate a radio- and chemoresistance likely due to the gap junction inhibition (Robe et al. 2005). Hinkerohe et al. (2011) studied the effect of dexamethasone on glioma cells and astrocytes in vitro. In the study, mixed co-culture models of astrocytes and microglia were developed. Normal conditions were mimicked by adding of 5% microglia while pathological patterns were created by adding of 30% microglia to the co-culture. Previously, similar co-cultures were used to prove the anti-inflammatory properties of levetiracetam, an anti-convulsant agent (Haghikia et al. 2008). All co-cultures were incubated with dexamethasone. After administration of dexamethasone to human U87 and rat F98 and C6 cell lines, a dose-dependent decrease in cell number of coupled glioma cells, dose-dependent depolarization (partially significant), and decrease of Cx43 production was observed. Concomitant treatment with mifepristone and dexamethasone resulted in the prevention of reduction of coupled glioma cells number, Cx43 production, and at least in the partial restoration of glioma cell membrane resting potential. 'Pathological'

glial cultures treated with dexamethasone were characterized by increased cell coupling (non-blockable with mifepristone), gap junction formation (resistant to the blockage by mifepristone), and microglia branchement (reduced by mifepristone). In contrary, dexamethasone was not significantly altering a 'physiological' co-culture. Although direct glioma–astroglia interactions were not studied, the work sheds light on the interaction between different cell subpopulations present in affected brains. Briefly, dexamethasone mediates the increase of coupling of astrocytes and Cx43 production in astroglia but reduces coupling of glioma cells and tumor Cx43 production. As microglia was settled down by dexamethasone, it should be noted that this observation can be attributed to general immunosuppressive properties of the drug. In conclusion, the authors suggested that dexamethasone might hamper the chemotherapeutic efficacy thereby underlining the need of more studies related to the problem (Hinkerohe et al. 2011).

A relatively new approach to glioma therapy is based on utilizing recombinant technologies. For example, usage of oncolytic viruses provides an option to selectively infect tumor cells, while normal cells remain non-infected (Foreman et al. 2017). Kober et al. (2015) studied the roles of astrocytes and microglia in experimental oncolytic viral vaccine therapy of GL261 murine glioma. A reduced viral replication in astrocytes and microglia monocultures was detected thereby showing a capacity of these cells to serve as viral traps which may distract viruses from the tumor cells and hence reduce efficient virus infection of the tumor cells. Co-culture experiments in this study concerned only microglia and glioma, not astrocytes. Germano et al. (2006) developed mouse embryonic stem cell-derived astrocytes transformed with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and then assessed their proapoptotic effect on human glioma. As a result, modified astrocytes were able to induce apoptosis of A172 human glioma cells even more efficiently than recombinant TRAIL itself. Glioma-killing effect was selective and did not involve non-malignant astrocytes.

Co-culture experiments concerning chemotherapy mainly indicate the role of gap junctions in mediating drug resistance. Still, there are problems to be solved. An in vivo study of antibodies to extracellular Cx43 fragment showed that monotherapy led to significant tumor regression, while concomitant use of temozolomide neutralized the anti-tumor effect (Yusubalieva et al. 2014). As temozolomide, like other chemotherapeutic agents (Cottin et al. 2010), may demand gap junctions to penetrate the tumor cells, the latter experiment resulted in competition between the two drugs. Here, we face the problem that heterocellular (astrocyte–glioma) gap junctions should be blocked in order to inhibit tumor outgrowth and facilitate chemotherapy while homocellular (glioma–glioma) channels may help the drug to spread within the tumor and thus need to be spared at least to some extent.

Surgery

As surgery is a major component of current glioma treatment, intense research was undertaken to determine the exact mechanisms of tumor recurrence after resection and the role of glioma microenvironment in these processes. Among the variety of methods, in vitro models were also being employed. Okolie et al. (2016) developed a complex murine glioma resection and recurrence model, which included both in vitro and in vivo experiments. In in vivo studies, the authors created photodetectable reporter-producing murine glioma cells and then implanted those cells as allografts. The tumors were then resected with help of a microsurgical image-guided technique. Even though more than 90% of a tumor volume was surgically removed, the tumor relapse occurred. Notably, the reactive post-resection astrocyte phenotype was detected by immunostaining. Generally, astrocytes surrounding gliomas highly produced GFAP, a phenomenon, which was confirmed by the results of earlier experimental periglioma zone studies (Chekhonin et al. 2012). According to the immunostaining after glioma resection, the astrocytes co-produced both GFAP and nestin. These observations indicate that surgical intervention causes a switch of a reactive astrocyte phenotype towards a more primitive one and may even induce stem cell-like features. In in vitro studies, the authors constructed co-cultures of gliomas and astrocytes (i.e., immortalized astrocytes and astrocytes derived from the in vivo model) by a separate seeding of cells into adjacent wells in 2-chamber culture inserts, placed in culture plates. After incubation, inserts were removed and resection was modeled by scratch injuring. Then, migration, proliferation, and expression patterns of cells in co-cultures were assessed. Scratch injury of immortalized astrocytes was followed by a significant increase of nestin production. In co-cultures, injuring of immortalized astrocytes led to an increased migration of murine glioma cells. Uninjured astrocytes derived from the in vivo model also potentiated migration of the mouse glioma cells, but injury of astrocytes did not affect glioma cell migration. Of note, injury caused to glioma cells did not increase their own migration potential. The migration-activating effect of immortalized astrocytes could be also reproduced in established murine (GL261) and human (U251, U87) gliomas. Scratch injury of astrocytes increased murine ex vivo glioma proliferation as well as proliferation of GL261, U251, and U87 glioma cells. Paracrine ability of injured immortalized astrocytes to enhance ex vivo murine glioma proliferation was confirmed by adding conditioned medium from scratched astrocyte to glioma cells (as a control, medium conditioned by uninjured astrocytes was

used). When gene expression and protein production profiles of injured astrocytes were combined, transcriptome and secretome changes of cytokine production were detected, and C-X-C motif chemokine 5 (Cxcl5) was defined as the most suitable change-driving candidate. Thus, blunt force surgical trauma (simulated in vivo or in vitro) may induce reactive peritumoral astrocytes to dedifferentiate partially and therefore promote tumor migration and proliferation (Okolie et al. 2016).

Radiotherapy

Mentioned above experiments concerning influence of astrocytes on GSC radiosensitivity (Rath et al. 2015) were preceded by co-culturing studies devoted to irradiation of astrocytes and glioma cells. In the studies mentioned below, astrocytes were generally regarded as components of brain microenvironment. Nakamura et al. (2007) used human gliomas and astrocytes to assess changes of glioma response to irradiation in a co-culture model. Human malignant glioma cells, immortalized astrocytes, and oncogene-transformed immortalized human astrocytes labeled with enhanced GFP were chosen. The studied cells were plated onto a layer of normal human astrocytes and their invasion through the confluent astrocyte layer was assessed. Immortalized astrocytes failed to migrate through the astrocyte monolayer probably due to the cell-to-cell contact inhibition. All the glioma cells and transformed astrocytes demonstrated an ability to penetrate through the monolayer. Irradiation by single fractions up to 3 Gy did not change the invasiveness of U87, A172, and U373 human gliomas and transformed astrocytes. However, a dose-dependent decrease of invasiveness of U251 cells was found. Interestingly, the production of MMP-2 was increased in parallel with the radiation dose rise. This finding may partially explain the mechanism of post-irradiation glioma invasion through the involvement of MMP-2-dependent pathway. As for the effect of irradiation on cellular invasion, the question is whether astrocytes in the layer prevented the decrease in migration or that was an intrinsic feature of the glioma cell lines studied. Burdak-Rothkamm et al. (2007) employed astrocyte–glioma co-cultures to assess the influence of radiation-induced bystander effect, i.e., the transmitting of the radiation-induced response to the unirradiated cells. T98G glioma cells or normal human astrocytes have been exposed to targeted irradiation and then co-cultured directly with one another. As a result of co-culturing, both cell types were found to be able to provide bystander effect to each other, increasing the amount of γH2AX foci in unirradiated cells. What is more, bystander effect in glioma could be inhibited by dimethyl sulfoxide (DMSO, a reactive oxygen species antagonist) and Filipin (caveolae-mediated endocytosis inhibitor), while in astrocytes only Filipin abrogated the effect. Golding et al. (2009) undertook attempts to increase glioma radiosensitivity in experiment targeting ATM kinase (ataxia-telangiectasia mutated), which is involved in the induction of the DNA damage response and thus in radioresistance. ATM inhibitor KU-60019 showed therapeutic properties as a quite potent glioma radiosensitizer and inhibitor of glioma invasion and growth, also in the presence of temozolomide. The administration of this inhibitor did not influence human embryonic stem cell-derived astrocytes but in combination with temozolomide led to the limitation of the U1242 glioma cell growth in an unirradiated co-culture with human astrocytes, thus simulating synergism of two drugs outside of the irradiated field (Golding et al. 2012). We have already mentioned this issue while talking about concomitant use of anti-Cx43-antibodies and temozolomide. Here, the problem of drug combination in presence of another treatment factor (irradiation) is discussed again, as temozolomide did not show antagonism with ATM inhibitors when applied to glioma cells before irradiation. We also think that further co-culture studies of astrocytic impact on combined glioma treatment regimens may reveal new putative tumor-resistance mechanisms.

Disadvantages of Two-Component Models and Methods of Overcoming Them

As discussed above, glioma cells in vivo are tightly incorporated into the complex microenvironment, which consists not only of tumor cells and astrocytes, but also of other glial cells, stem cells, vessels and perivascular niches, metabolites, and extracellular matrix (Xiao et al. 2017). Taking this fact into account, co-cultures of only glioma cells and astrocytes have several methodological disadvantages. Here, we shortly discuss some ways to expand the two-component model.

The first means is to use additional extracellular matrix proteins for plate coating in co-cultures. Coating of culture plates with extracellular matrix proteins may be also used for studying the interactions between astrocytes and glioma cells. Indeed, membrane-associated MMP-14 was found to interact with CD44, a hyaluronic acid receptor, hereby potentiating tumor migration and invasion (Zarrabi et al. 2011). This metalloproteinase also exerts its functions through changes in levels of MMP-2 and MMP-9 (Ulasov et al. 2014), which have been already discussed. For these reasons, the process of glioma invasion should be better observed in presence of original extracellular substrates. Chen et al. (2016) established Transwell chamber co-cultures of human A172 or U251 glioma cells and normal human astrocytes to study the ability of astrocytes to alter glioma invasion and migration pattern. To assess invasion, the upper chamber was covered by Matrigel and seeded with glioma cells after astrocytes were seeded in the lower compartment. The expression pattern (transcripts of metalloproteinase genes)

and protein production pattern of glioma cells stimulated by astrocyte-conditioned medium was also studied using real-time PCR and Western blot, respectively. Migration was higher in the co-culture system than in control. What is more, several genes and their products were upregulated after co-cultivation of glioma cells with astrocyte-conditioned medium. Among those, MMP-14 demonstrated the highest expression growth, verified cytometrically, as this protein is membrane associated. The experimental analysis showed that action of MMP-14 was exerted more through MMP-2 pathway, than through cleavage of CD44. The astrocyte-induced changes in glioma expression and production pattern were also shown to be IL-6-dependent. Similar strategies for studying migration in presence of extracellular matrix proteins or artificial scaffolds were applied in the studies discussed earlier, which also reflect a movement towards making glioma and astrocyte co-cultures closer to in vivo conditions. Still, the usage of commercial extracellular matrices which are not fully adaptable to experimental conditions represents a substantial shortcoming. Souza et al. (2010) proposed 3-D glioma cell cultures and co-cultures of cells loaded with bacteriophages (targeting integrins) plus magnetic iron oxide and gold nanoparticles forming hydrogels, as all two-dimensional cultures have the disadvantage of being planar, thus not reflecting the exact intercellular contacts and signaling pathways. Experimentally modified cells when subject to magnetic field formed spherical structures able to levitate. The cellular conglomerates obtained in the work showed exponential growth in contrary to 2-D cultures. Human glioma cells formed N-cadherin-dependent cell-to-cell contacts similar to ones in xenografts and more plentiful than in a 2-D model. The established technology could provide designing co-cultures. As the authors showed, levitating spheres of astrocytes and glioma cells could reproduce the model of glioma invasion (a confrontation assay). Souza et al. (2010) suggest this model being more tailored in context of particular experiment than ones using standard extracellular scaffold protein mixtures and being rather simple, as it needs 2-D cultures initially.

Another means of enriching the methodological significance of co-cultures is to add new cellular components usual for brain or glioma microenvironment, such as neurons and microglia. There are limited data about artificial three-component or multicomponent co-cultures, as most of the studies use two cell types, for example, glioma cells and neurons or glioma cells and microglia, or ex vivo tissue slices. Again, we face the important role of the extracellular matrix. Faria et al. (2006) studied whether glioma cells could maintain neuronal growth, which is dependent on glial-derived laminin. As a result, glioma cells, but not other tumors, were able to support the metabolism of neurons and neuritic development, though malignant astrocytes could not distinguish between embryonic and post-natal neurons. In addition, neurons were also interacting with glioma cells in co-culture affecting the pattern of glioma-derived laminin deposition. Glioma cells exerted some normal features of astrocytes favoring not only neurodegeneration, but also neuronal growth. Another step studied in two-component co-cultures lacking astrocytes is experimental cellular anti-glioma therapy. It was shown that microglia activated by polyinosinic:polycytidylic acid obtained anti-tumor activity (Kees et al. 2012), and CD3/CD28-activated peripheral blood mononuclear cells increase the expression and the production of proinflammatory cytokines by glioma cells (Jehs et al. 2011). To date, popular direction of cell therapy research area is studying bone marrow-derived mesenchymal stem cells (MSCs), which have tropism towards injury area (e.g., tumor) (Doucette et al. 2011). Ho et al. (2013) found that MSCs inhibited outgrowth of patient-derived glioma cells and glioma cell lines by apoptosis in direct in vitro co-cultures and also in vivo when injected subcutaneously into mice. Medium conditioned by MSC-glioma co-cultures impaired recruitment of endothelial progenitor cells showing the effect of experimental therapy on angiogenesis inhibition. In a work of our colleagues, MSCs were shown to form gap junctions with glioma cells, reflecting a putative mechanism of anti-tumor activity (Gabashvili et al. 2016). So far, the main question to be solved in future is how astrocytes may influence the interactions between glioma and other cells of tumor microenvironment and whether astrocytes in situ may affect cellular anti-glioma treatment.

Conclusion

Revealing all hidden pitfalls and understanding the specific features of glioma-related reactive astrocytosis still represents a challenge for both clinicians and investigators. Rivera-Zengotita and Yachnis (2012) noted that distinguishing between gliosis and glioma is a problem even for surgical neuropathologists. Being a universal reaction of the central nervous system to the brain injury, gliosis may mimic glioma tumorigenesis morphologically. As for glioma-related gliosis, most of studies mentioned above generally support the idea that astrocytes promote the growth and invasion of the tumor at advanced stages. Still, interaction of glioma cells and astrocytes is not unidirectional and tumorigenic. Co-culture systems provide a unique ability to highlight particular pathways of cell-to-cell communication. As we mentioned above, Yao et al. (2014) showed that the ability of astrocytes to inhibit the growth rate of glioma cells depended on cell concentrations in co-cultures thereby underlining a potential anti-tumor activity of astrocytes under specific conditions. Despite being quite simple, two-component co-cultures may represent the first step of the complicated multistage research and obviously should stimulate further studies with addition

of new cell types to the co-cultures. For example, Iwadate et al. (2016) proposed an existence of an anti-glioma cellular network, with the main roles of glia and neurons, which initially create a non-permissive anti-tumor microenvironment. Extracellular matrix proteins and glycoproteins are the major astrocyte-derived anti-glioma factors at the early steps of gliomagenesis. Thus, it makes a sense of underlining a role of the extracellular matrix in co-culture experiments. To date, several reviews considered this topic (Rape et al. 2014; Xiao et al. 2017). The trend is emerging to characterize astrocytes that secrete either pro- or anti-glioma-directed extracellular matrix. We also mentioned Cx43 among the molecules responsible for communications between reactive astrocytes and glioma cells during glioma migration and invasion. Still, the role of Cx43 in glioma progression needs to be discussed. For instance, Gonzalez-Sanchez et al. (2016) found that Cx43 could inhibit the oncogenic activity of c-Src by recruiting tyrosine kinase CSK and phosphatase and tensin homolog (PTEN) thus showing a rationale of Cx43-up-regulating strategies for glioma treatment. A therapeutic potential of Cx43 was confirmed in a study involving a Cx43-derived peptide that mimics c-Src-controlling region (Jaraiz-Rodriguez et al. 2017). Despite promising perspectives, the researchers should take into account possible pro-tumorigenic effects of Cx43 especially considering that this protein is produced by reactive peritumoral astrocytes. In vitro co-culture models exert a unique opportunity to evaluate all the problems that look enigmatic now. As the tumor microenvironment is involved in promoting tumor survival and progression, astrocytes represent quite a well-recognized hallmark of a crosstalk between glioma and non-glioma cells thereby underlining a need in novel in vitro and in vivo studies.

Acknowledgements The work was carried out within the Grant Number 17-00-00161 issued by the Russian Foundation For Basic Research.

Author Contributions IVC: source selection, analytical discussion, manuscript composing; OIG: analytical discussion; DAC and NFG: scientific editing.

Compliance with Ethical Standards

Conflict of interest I.V. Chekhonin declares that he has financial relationship with the funding organization. O.I. Gurina declares that she has financial relationship with the funding organization. D.A. Chistiakov declares that he has no potential conflict of interest. N.F. Grinenko declares that she has no potential conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Amara SG, Fontana AC (2002) Excitatory amino acid transporters: keeping up with glutamate. Neurochem Int 41:313–318

Aubert M, Badoual M, Christov C, Grammaticos B (2008) A model for glioma cell migration on collagen and astrocytes. J R Soc Interface 5:75–83. https://doi.org/10.1098/rsif.2007.1070

Biasoli D, Sobrinho MF, da Fonseca AC, de Matos DG, Romao L, de Moraes Maciel R, Rehen SK, Moura-Neto V, Borges HL, Lima FR (2014) Glioblastoma cells inhibit astrocytic p53-expression favoring cancer malignancy. Oncogenesis 3:e123. https://doi.org/10.1038/oncsis.2014.36

Blank AE, Baumgarten P, Zeiner P, Zachskorn C, Loffler C, Schittenhelm J, Czupalla CJ, Capper D, Plate KH, Harter PN, Mittelbronn M (2015) Tumour necrosis factor receptor superfamily member 9 (TNFRSF9) is up-regulated in reactive astrocytes in human gliomas. Neuropathol Appl Neurobiol 41:e56-67. https://doi.org/10.1111/nan.12135

Burdak-Rothkamm S, Short SC, Folkard M, Rothkamm K, Prise KM (2007) ATR-dependent radiation-induced gamma H2AX foci in bystander primary human astrocytes and glioma cells. Oncogene 26:993–1002. https://doi.org/10.1038/sj.onc.1209863

Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H (2012) The brain tumor microenvironment. Glia 60:502–514

Chekhonin VP, Baklaushev VP, Yusubalieva GM, Belorusova AE, Gulyaev MV, Tsitrin EB, Grinenko NF, Gurina OI, Pirogov YA (2012) Targeted delivery of liposomal nanocontainers to the peritumoral zone of glioma by means of monoclonal antibodies against GFAP and the extracellular loop of Cx43. Nanomedicine 8:63–70. https://doi.org/10.1016/j.nano.2011.05.011

Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488:522–526. https://doi.org/10.1038/nature11287

Chen X, Zhang L, Zhang IY, Liang J, Wang H, Ouyang M, Wu S, da Fonseca ACC, Weng L, Yamamoto Y, Yamamoto H, Natarajan R, Badie B (2014) RAGE expression in tumor-associated macrophages promotes angiogenesis in glioma. Cancer Res 74:7285–7297. https://doi.org/10.1158/0008-5472.can-14-1240

Chen W, Wang D, Du X, He Y, Chen S, Shao Q, Ma C, Huang B, Chen A, Zhao P, Qu X, Li X (2015) Glioma cells escaped from cytotoxicity of temozolomide and vincristine by communicating with human astrocytes. Med Oncol 32:43. https://doi.org/10.1007/s12032-015-0487-0

Chen W, Xia T, Wang D, Huang B, Zhao P, Wang J, Qu X, Li X (2016) Human astrocytes secrete IL-6 to promote glioma migration and invasion through upregulation of cytomembrane MMP14. Oncotarget 7:62425–62438. https://doi.org/10.18632/oncotarget.11515

Chen J, Mao S, Li H, Zheng M, Yi L, Lin JM, Lin ZX (2017) The pathological structure of the perivascular niche in different microvascular patterns of glioblastoma. PLoS ONE 12:e0182183. https://doi.org/10.1371/journal.pone.0182183

Cheng L, Huang Z, Zhou W, Wu Q, Donnola S, Liu JK, Fang X, Sloan AE, Mao Y, Lathia JD, Min W, McLendon RE, Rich JN, Bao S (2013) Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell 153:139–152. https://doi.org/10.1016/j.cell.2013.02.021

Cottin S, Ghani K, de Campos-Lima PO, Caruso M (2010) Gemcitabine intercellular diffusion mediated by gap junctions: new implications for cancer therapy. Mol Cancer 9:141. https://doi.org/10.1186/1476-4598-9-141

de Bouard S, Christov C, Guillamo JS, Kassar-Duchossoy L, Palfi S, Leguerinel C, Masset M, Cohen-Hagenauer O, Peschanski M, Lefrancois T (2002) Invasion of human glioma biopsy

specimens in cultures of rodent brain slices: a quantitative analysis. J Neurosurg 97:169–176. https://doi.org/10.3171/jns.2002.97.1.0169

de Groot JF, Liu TJ, Fuller G, Yung WK (2005) The excitatory amino acid transporter-2 induces apoptosis and decreases glioma growth in vitro and in vivo. Cancer Res 65:1934–1940. https://doi.org/10.1158/0008-5472.can-04-3626

DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci USA 104:19345–19350. https://doi.org/10.1073/pnas.0709747104

Doucette T, Rao G, Yang Y, Gumin J, Shinojima N, Bekele BN, Qiao W, Zhang W, Lang FF (2011) Mesenchymal stem cells display tumor-specific tropism in an RCAS/Ntv-a glioma model. Neoplasia (New York NY) 13:716–725

Faria J, Romao L, Martins S, Alves T, Mendes FA, de Faria GP, Hollanda R, Takiya C, Chimelli L, Morandi V, de Souza JM, Abreu JG, Moura Neto V (2006) Interactive properties of human glioblastoma cells with brain neurons in culture and neuronal modulation of glial laminin organization. Differentiation 74:562–572. https://doi.org/10.1111/j.1432-0436.2006.00090.x

Fidoamore A, Cristiano L, Antonosante A, d'Angelo M, Di Giacomo E, Astarita C, Giordano A, Ippoliti R, Benedetti E, Cimini A (2016) Glioblastoma stem cells microenvironment: The paracrine roles of the niche in drug and radioresistance. Stem Cells Int 2016:6809105. https://doi.org/10.1155/2016/6809105

Flach EH, Rebecca VW, Herlyn M, Smalley KS, Anderson AR (2011) Fibroblasts contribute to melanoma tumor growth and drug resistance. Mol Pharm 8:2039–2049. https://doi.org/10.1021/mp200421k

Foreman PM, Friedman GK, Cassady KA, Markert JM (2017) Oncolytic virotherapy for the treatment of malignant glioma. Neurotherapeutics 14:333–344. https://doi.org/10.1007/s13311-017-0516-0

Gabashvili AN, Baklaushev VP, Grinenko NF, Mel'nikov PA, Cherepanov SA, Levinsky AB, Chehonin VP (2016) Antitumor activity of rat mesenchymal stem cells during direct or indirect co-culturing with C6 glioma cells. Bull Experim Biol Med 160:519–524. https://doi.org/10.1007/s10517-016-3211-y

Gagliano N, Costa F, Cossetti C, Pettinari L, Bassi R, Chiriva-Internati M, Cobos E, Gioia M, Pluchino S (2009) Glioma-astrocyte interaction modifies the astrocyte phenotype in a co-culture experimental model. Oncol Rep 22:1349–1356

Galavotti S, Bartesaghi S, Faccenda D, Shaked-Rabi M, Sanzone S, McEvoy A, Dinsdale D, Condorelli F, Brandner S, Campanella M, Grose R, Jones C, Salomoni P (2013) The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells. Oncogene 32:699–712. https://doi.org/10.1038/onc.2012.111

Germano IM, Uzzaman M, Benveniste RJ, Zaurova M, Keller G (2006) Apoptosis in human glioblastoma cells produced using embryonic stem cell-derived astrocytes expressing tumor necrosis factor-related apoptosis-inducing ligand. J Neurosurg 105:88–95. https://doi.org/10.3171/jns.2006.105.1.88

Gieryng A, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B (2017) Immune microenvironment of gliomas. Lab Invest 97:498–518. https://doi.org/10.1038/labinvest.2017.19

Golding SE, Rosenberg E, Valerie N, Hussaini I, Frigerio M, Cockcroft XF, Chong WY, Hummersone M, Rigoreau L, Menear KA, O'Connor MJ, Povirk LF, van Meter T, Valerie K (2009) Improved ATM kinase inhibitor KU-60019 radiosensitizes glioma cells, compromises insulin, AKT and ERK prosurvival signaling, and inhibits migration and invasion. Mol Cancer Ther 8:2894–2902. https://doi.org/10.1158/1535-7163.mct-09-0519

Golding SE, Rosenberg E, Adams BR, Wignarajah S, Beckta JM, O'Connor MJ, Valerie K (2012) Dynamic inhibition of ATM kinase provides a strategy for glioblastoma multiforme radiosensitization and growth control. Cell Cycle 11:1167–1173. https://doi.org/10.4161/cc.11.6.19576

Gonzalez-Sanchez A, Jaraiz-Rodriguez M, Dominguez-Prieto M, Herrero-Gonzalez S, Medina JM, Tabernero A (2016) Connexin43 recruits PTEN and Csk to inhibit c-Src activity in glioma cells and astrocytes. Oncotarget 7:49819–49833. https://doi.org/10.18632/oncotarget.10454

Grau SJ, Trillsch F, Tonn JC, Goldbrunner RH, Noessner E, Nelson PJ, von Luettichau I (2015) Podoplanin increases migration and angiogenesis in malignant glioma. Int J Clin Exp Pathol 8:8663–8670

Gritsenko PG, Ilina O, Friedl P (2012) Interstitial guidance of cancer invasion. J Pathol 226:185–199. https://doi.org/10.1002/path.3031

Gritsenko P, Leenders W, Friedl P (2017) Recapitulating in vivo-like plasticity of glioma cell invasion along blood vessels and in astrocyte-rich stroma. Histochem Cell Biol. https://doi.org/10.1007/s00418-017-1604-2

Grodecki J, Short AR, Winter JO, Rao SS, Winter JO, Otero JJ, Lannutti JJ, Sarkar A (2015) Glioma-astrocyte interactions on white matter tract-mimetic aligned electrospun nanofibers. Biotechnol Prog 31:1406–1415. https://doi.org/10.1002/btpr.2123

Guo J, Niu R, Huang W, Zhou M, Shi J, Zhang L, Liao H (2012) Growth factors from tumor microenvironment possibly promote the proliferation of glioblastoma-derived stem-like cells in vitro. Pathol Oncol Res 18:1047–1057. https://doi.org/10.1007/s12253-012-9543-7

Haghikia A, Ladage K, Hinkerohe D, Vollmar P, Heupel K, Dermietzel R, Faustmann PM (2008) Implications of antiinflammatory properties of the anticonvulsant drug levetiracetam in astrocytes. J Neurosci Res 86:1781–1788. https://doi.org/10.1002/jnr.21639

Hinkerohe D, Wolfkuhler D, Haghikia A, Meier C, Faustmann PM, Schlegel U (2011) Dexamethasone differentially regulates functional membrane properties in glioma cell lines and primary astrocytes in vitro. J Neurooncol 103:479–489. https://doi.org/10.1007/s11060-010-0456-6

Ho IA, Toh HC, Ng WH, Teo YL, Guo CM, Hui KM, Lam PY (2013) Human bone marrow-derived mesenchymal stem cells suppress human glioma growth through inhibition of angiogenesis. Stem Cells (Dayton Ohio) 31:146–155. https://doi.org/10.1002/stem.1247

Hong X, Sin WC, Harris AL, Naus CC (2015) Gap junctions modulate glioma invasion by direct transfer of microRNA. Oncotarget 6:15566–15577. https://doi.org/10.18632/oncotarget.3904

Iwadate Y, Fukuda K, Matsutani T, Saeki N (2016) Intrinsic protective mechanisms of the neuron-glia network against glioma invasion. J Clin Neurosci 26:19–25. https://doi.org/10.1016/j.jocn.2015.07.024

Jacobs VL, De Leo JA (2013) Increased glutamate uptake in astrocytes via propentofylline results in increased tumor cell apoptosis using the CNS-1 glioma model. J Neurooncol 114:33–42. https://doi.org/10.1007/s11060-013-1158-7

Jacobs VL, Landry RP, Liu Y, Romero-Sandoval EA, De Leo JA (2012) Propentofylline decreases tumor growth in a rodent model of glioblastoma multiforme by a direct mechanism on microglia. Neuro-oncology 14:119–131. https://doi.org/10.1093/neuonc/nor194

Jamal M, Rath BH, Tsang PS, Camphausen K, Tofilon PJ (2012) The brain microenvironment preferentially enhances the radioresistance of CD133(+) glioblastoma stem-like cells. Neoplasia (New York NY) 14:150–158

Jaraiz-Rodriguez M, Tabernero MD, Gonzalez-Tablas M, Otero A, Orfao A, Medina JM, Tabernero A (2017) A short region of

Connexin43 reduces human glioma stem cell migration, invasion, and survival through Src, PTEN, and FAK. Stem Cell Rep 9:451–463. https://doi.org/10.1016/j.stemcr.2017.06.007
Jehs T, Faber C, Juel HB, Nissen MH (2011) Astrocytoma cells upregulate expression of pro-inflammatory cytokines after co-culture with activated peripheral blood mononuclear cells. APMIS 119:551–561. https://doi.org/10.1111/j.1600-0463.2011.02773.x
Jhaveri N, Chen TC, Hofman FM (2016) Tumor vasculature and glioma stem cells: contributions to glioma progression. Cancer Lett 380:545–551. https://doi.org/10.1016/j.canlet.2014.12.028
Jones EV, Bouvier DS (2014) Astrocyte-secreted matricellular proteins in CNS remodelling during development and disease. Neural Plast 2014:321209. https://doi.org/10.1155/2014/321209
Katakowski M, Buller B, Wang X, Rogers T, Chopp M (2010) Functional microRNA is transferred between glioma cells. Cancer Res 70:8259–8263. https://doi.org/10.1158/0008-5472.can-10-0604
Kees T, Lohr J, Noack J, Mora R, Gdynia G, Todt G, Ernst A, Radlwimmer B, Falk CS, Herold-Mende C, Regnier-Vigouroux A (2012) Microglia isolated from patients with glioma gain antitumor activities on poly (I:C) stimulation. Neuro-oncology 14:64–78. https://doi.org/10.1093/neuonc/nor182
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 (2014) Tumoral RANKL activates astrocytes that promote glioma cell invasion through cytokine signaling. Cancer Lett 353:194–200. https://doi.org/10.1016/j.canlet.2014.07.034
Kim Y, Jeon H, Othmer H (2017) The role of the tumor microenvironment in glioblastoma: a mathematical model. IEEE Trans Biomed Eng 64:519–527. https://doi.org/10.1109/tbme.2016.2637828
Kober C, Rohn S, Weibel S, Geissinger U, Chen NG, Szalay AA (2015) Microglia and astrocytes attenuate the replication of the oncolytic vaccinia virus LIVP 1.1.1 in murine GL261 gliomas by acting as vaccinia virus traps. J Transl Med 13:216. https://doi.org/10.1186/s12967-015-0586-x
Kolar K, Freitas-Andrade M, Bechberger JF, Krishnan H, Goldberg GS, Naus CC, Sin WC (2015) Podoplanin: a marker for reactive gliosis in gliomas and brain injury. J Neuropathol Exp Neurol 74:64–74. https://doi.org/10.1097/nen.0000000000000150
Koto M, Cho H, Riesterer O, Giri U, Story MD, Ha CS, Raju U (2011) Human lymphoma cells develop resistance to radiation in the presence of astrocytes in vitro. Anticancer Res 31:33–38
Lal PG, Ghirnikar RS, Eng LF (1996) Astrocyte-astrocytoma cell line interactions in culture. J Neurosci Res 44:216–222
Le DM, Besson A, Fogg DK, Choi KS, Waisman DM, Goodyer CG, Rewcastle B, Yong VW (2003) Exploitation of astrocytes by glioma cells to facilitate invasiveness: a mechanism involving matrix metalloproteinase-2 and the urokinase-type plasminogen activator-plasmin cascade. J Neurosci 23:4034–4043
Lee J, Borboa AK, Baird A, Eliceiri BP (2011a) Non-invasive quantification of brain tumor-induced astrogliosis. BMC Neurosci 12:9. https://doi.org/10.1186/1471-2202-12-9
Lee SG, Kim K, Kegelman TP, Dash R, Das SK, Choi JK, Emdad L, Howlett EL, Jeon HY, Su ZZ, Yoo BK, Sarkar D, Kim SH, Kang DC, Fisher PB (2011b) Oncogene AEG-1 promotes glioma-induced neurodegeneration by increasing glutamate excitotoxicity. Cancer Res 71:6514–6523. https://doi.org/10.1158/0008-5472.can-11-0782
Lee HK, Finniss S, Cazacu S, Bucris E, Ziv-Av A, Xiang C, Bobbitt K, Rempel SA, Hasselbach L, Mikkelsen T, Slavin S, Brodie C (2013) Mesenchymal stem cells deliver synthetic microRNA mimics to glioma cells and glioma stem cells and inhibit their cell migration and self-renewal. Oncotarget 4:346–361. https://doi.org/10.18632/oncotarget.868
Lin Q, Liu Z, Ling F, Xu G (2016) Astrocytes protect glioma cells from chemotherapy and upregulate survival genes via gap junctional communication. Mol Med Rep 13:1329–1335. https://doi.org/10.3892/mmr.2015.4680
Liu K, Ji K, Guo L, Wu W, Lu H, Shan P, Yan C (2014) Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. Microvasc Res 92:10–18. https://doi.org/10.1016/j.mvr.2014.01.008
Lu P, Wang Y, Liu X, Wang H, Zhang X, Wang K, Wang Q, Hu R (2016) Malignant gliomas induce and exploit astrocytic mesenchymal-like transition by activating canonical Wnt/beta-catenin signaling. Med Oncol 33:66. https://doi.org/10.1007/s12032-016-0778-0
Lyons SA, Chung WJ, Weaver AK, Ogunrinu T, Sontheimer H (2007) Autocrine glutamate signaling promotes glioma cell invasion. Cancer Res 67:9463–9471. https://doi.org/10.1158/0008-5472.can-07-2034
McCord AM, Jamal M, Williams ES, Camphausen K, Tofilon PJ (2009) CD133+ glioblastoma stem-like cells are radiosensitive with a defective DNA damage response compared with established cell lines. Clin Cancer Res 15:5145–5153. https://doi.org/10.1158/1078-0432.ccr-09-0263
Mehta G, Hsiao AY, Ingram M, Luker GD, Takayama S (2012) Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. J Control Release 164:192–204. https://doi.org/10.1016/j.jconrel.2012.04.045
Moinfar Z, Dambach H, Faustmann PM (2014) Influence of drugs on gap junctions in glioma cell lines and primary astrocytes in vitro. Front Physiol 5:186. https://doi.org/10.3389/fphys.2014.00186
Nakamura JL, Haas-Kogan DA, Pieper RO (2007) Glioma invasiveness responds variably to irradiation in a co-culture model. Int J Radiat Oncol Biol Phys 69:880–886. https://doi.org/10.1016/j.ijrobp.2007.06.052
Okolie O, Bago JR, Schmid RS, Irvin DM, Bash RE, Miller CR, Hingtgen SD (2016) Reactive astrocytes potentiate tumor aggressiveness in a murine glioma resection and recurrence model. Neuro-oncology 18:1622–1633. https://doi.org/10.1093/neuonc/now117
Oliveira R, Christov C, Guillamo JS, de Bouard S, Palfi S, Venance L, Tardy M, Peschanski M (2005) Contribution of gap junctional communication between tumor cells and astroglia to the invasion of the brain parenchyma by human glioblastomas. BMC Cell Biol 6:7. https://doi.org/10.1186/1471-2121-6-7
Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, Wrensch MR, Barnholtz-Sloan JS (2014) The epidemiology of glioma in adults: a "state of the science" review. Neuro-oncology 16:896–913. https://doi.org/10.1093/neuonc/nou087
Piao Y, Lu L, de Groot J (2009) AMPA receptors promote perivascular glioma invasion via β1 integrin-dependent adhesion to the extracellular matrix. Neuro-oncology 11:260–273. https://doi.org/10.1215/15228517-2008-094
Placone AL, Quinones-Hinojosa A, Searson PC (2016) The role of astrocytes in the progression of brain cancer: complicating the picture of the tumor microenvironment. Tumour Biol 37:61–69. https://doi.org/10.1007/s13277-015-4242-0
Poon CC, Sarkar S, Yong VW, Kelly JJP (2017) Glioblastoma-associated microglia and macrophages: targets for therapies to improve prognosis. Brain 140:1548–1560. https://doi.org/10.1093/brain/aww355
Rape A, Ananthanarayanan B, Kumar S (2014) Engineering strategies to mimic the glioblastoma microenvironment. Adv Drug Deliv Rev 79–80:172–183. https://doi.org/10.1016/j.addr.2014.08.012
Rath BH, Fair JM, Jamal M, Camphausen K, Tofilon PJ (2013) Astrocytes enhance the invasion potential of glioblastoma stem-like cells. PLoS ONE 8:e54752. https://doi.org/10.1371/journal.pone.0054752

Rath BH, Wahba A, Camphausen K, Tofilon PJ (2015) Coculture with astrocytes reduces the radiosensitivity of glioblastoma stem-like cells and identifies additional targets for radiosensitization. Cancer Med 4:1705–1716. https://doi.org/10.1002/cam4.510

Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, Maira G, Parati EA, Stassi G, Larocca LM, De Maria R (2010) Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature 468:824–828. https://doi.org/10.1038/nature09557

Rivera-Zengotita M, Yachnis AT (2012) Gliosis versus glioma?: don't grade until you know. Adv Anat Pathol 19:239–249. https://doi.org/10.1097/PAP.0b013e31825c6a04

Robe PA, Nguyen-Khac M, Jolois O, Rogister B, Merville MP, Bours V (2005) Dexamethasone inhibits the HSV-tk/ganciclovir bystander effect in malignant glioma cells. BMC Cancer 5:32. https://doi.org/10.1186/1471-2407-5-32

Rodrigues JC, Gonzalez GC, Zhang L, Ibrahim G, Kelly JJ, Gustafson MP, Lin Y, Dietz AB, Forsyth PA, Yong VW, Parney IF (2010) Normal human monocytes exposed to glioma cells acquire myeloid-derived suppressor cell-like properties. Neuro-oncology 12:351–365. https://doi.org/10.1093/neuonc/nop023

Roos A, Ding Z, Loftus JC, Tran NL (2017) Molecular and microenvironmental determinants of glioma stem-like cell survival and invasion. Front Oncol 7:120. https://doi.org/10.3389/fonc.2017.00120

Rupp T, Langlois B, Koczorowska MM, Radwanska A, Sun Z, Hussenet T, Lefebvre O, Murdamoothoo D, Arnold C, Klein A, Biniossek ML, Hyenne V, Naudin E, Velazquez-Quesada I, Schilling O, Van Obberghen-Schilling E, Orend G (2016) Tenascin-C orchestrates glioblastoma angiogenesis by modulation of pro- and anti-angiogenic signaling. Cell Rep 17:2607–2619. https://doi.org/10.1016/j.celrep.2016.11.012

Schichor C, Kerkau S, Visted T, Martini R, Bjerkvig R, Tonn JC, Goldbrunner R (2005) The brain slice chamber, a novel variation of the Boyden Chamber Assay, allows time-dependent quantification of glioma invasion into mammalian brain in vitro. J Neurooncol 73:9–18. https://doi.org/10.1007/s11060-004-3341-3

Seftor RE, Hess AR, Seftor EA, Kirschmann DA, Hardy KM, Margaryan NV, Hendrix MJ (2012) Tumor cell vasculogenic mimicry: from controversy to therapeutic promise. Am J Pathol 181:1115–1125. https://doi.org/10.1016/j.ajpath.2012.07.013

Shabtay-Orbach A, Amit M, Binenbaum Y, Na'ara S, Gil Z (2015) Paracrine regulation of glioma cells invasion by astrocytes is mediated by glial-derived neurotrophic factor. Int J Cancer 137:1012–1020. https://doi.org/10.1002/ijc.29380

Sharma A, Shiras A (2016) Cancer stem cell-vascular endothelial cell interactions in glioblastoma. Biochem Biophys Res Commun 473:688–692. https://doi.org/10.1016/j.bbrc.2015.12.022

Sin WC, Aftab Q, Bechberger JF, Leung JH, Chen H, Naus CC (2016) Astrocytes promote glioma invasion via the gap junction protein connexin43. Oncogene 35:1504–1516. https://doi.org/10.1038/onc.2015.210

Singh SK, Clarke ID, Hide T, Dirks PB (2004a) Cancer stem cells in nervous system tumors. Oncogene 23:7267–7273. https://doi.org/10.1038/sj.onc.1207946

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004b) Identification of human brain tumour initiating cells. Nature 432:396–401. https://doi.org/10.1038/nature03128

Souza GR, Molina JR, Raphael RM, Ozawa MG, Stark DJ, Levin CS, Bronk LF, Ananta JS, Mandelin J, Georgescu MM, Bankson JA, Gelovani JG, Killian TC, Arap W, Pasqualini R (2010) Three-dimensional tissue culture based on magnetic cell levitation. Nat Nanotechnol 5:291–296. https://doi.org/10.1038/nnano.2010.23

Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M (2001) Glutamate release promotes growth of malignant gliomas. Nat Med 7:1010–1015. https://doi.org/10.1038/nm0901-1010

Tamura R, Tanaka T, Miyake K, Yoshida K, Sasaki H (2017) Bevacizumab for malignant gliomas: current indications, mechanisms of action and resistance, and markers of response. Brain Tumor Pathol 34:62–77. https://doi.org/10.1007/s10014-017-0284-x

Tardito S, Oudin A, Ahmed SU, Fack F, Keunen O, Zheng L, Miletic H, Sakariassen PO, Weinstock A, Wagner A, Lindsay SL, Hock AK, Barnett SC, Ruppin E, Morkve SH, Lund-Johansen M, Chalmers AJ, Bjerkvig R, Niclou SP, Gottlieb E (2015) Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma. Nat Cell Biol 17:1556–1568. https://doi.org/10.1038/ncb3272

Ulasov I, Yi R, Guo D, Sarvaiya P, Cobbs C (2014) The emerging role of MMP14 in brain tumorigenesis and future therapeutics. Biochimica et biophysica acta 1846:113–120. https://doi.org/10.1016/j.bbcan.2014.03.002

van Lith SA, Navis AC, Verrijp K, Niclou SP, Bjerkvig R, Wesseling P, Tops B, Molenaar R, van Noorden CJ, Leenders WP (2014) Glutamate as chemotactic fuel for diffuse glioma cells: are they glutamate suckers? Biochimica et biophysica acta 1846:66–74. https://doi.org/10.1016/j.bbcan.2014.04.004

Wang JB, Erickson JW, Fuji R, Ramachandran S, Gao P, Dinavahi R, Wilson KF, Ambrosio AL, Dias SM, Dang CV, Cerione RA (2010) Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. Cancer Cell 18:207–219. https://doi.org/10.1016/j.ccr.2010.08.009

Wang N, Jain RK, Batchelor TT (2017) New directions in anti-angiogenic therapy for glioblastoma. Neurotherapeutics 14:321–332. https://doi.org/10.1007/s13311-016-0510-y

Xiao W, Sohrabi A, Seidlits SK (2017) Integrating the glioblastoma microenvironment into engineered experimental models. Futur Sci OA 3:Fso189. https://doi.org/10.4155/fsoa-2016-0094

Yamada KM, Cukierman E (2007) Modeling tissue morphogenesis and cancer in 3D. Cell 130:601–610. https://doi.org/10.1016/j.cell.2007.08.006

Yang N, Yan T, Zhu H, Liang X, Leiss L, Sakariassen PO, Skaftnesmo KO, Huang B, Costea DE, Enger PO, Li X, Wang J (2014) A co-culture model with brain tumor-specific bioluminescence demonstrates astrocyte-induced drug resistance in glioblastoma. J Transl Med 12:278. https://doi.org/10.1186/s12967-014-0278-y

Yao PS, Kang DZ, Lin RY, Ye B, Wang W, Ye ZC (2014) Glutamate/glutamine metabolism coupling between astrocytes and glioma cells: neuroprotection and inhibition of glioma growth. Biochem Biophys Res Commun 450:295–299. https://doi.org/10.1016/j.bbrc.2014.05.120

Yusubalieva GM, Baklaushev VP, Gurina OI, Zorkina YA, Gubskii IL, Kobyakov GL, Golanov AV, Goryainov SA, Gorlachev GE, Konovalov AN, Potapov AA, Chekhonin VP (2014) Treatment of poorly differentiated glioma using a combination of monoclonal antibodies to extracellular connexin-43 fragment, temozolomide, and radiotherapy. Bull Experim Biol Med 157:510–515. https://doi.org/10.1007/s10517-014-2603-0

Zarrabi K, Dufour A, Li J, Kuscu C, Pulkoski-Gross A, Zhi J, Hu Y, Sampson NS, Zucker S, Cao J (2011) Inhibition of matrix metalloproteinase 14 (MMP-14)-mediated cancer cell migration. J Biol Chem 286:33167–33177. https://doi.org/10.1074/jbc.M111.256644

Zhang L, Zhang Y (2015) Tunneling nanotubes between rat primary astrocytes and C6 glioma cells alter proliferation potential of glioma cells. Neurosci Bull 31:371–378. https://doi.org/10.1007/s12264-014-1522-4

Zhang W, Nwagwu C, Le DM, Yong VW, Song H, Couldwell WT (2003) Increased invasive capacity of connexin43-overexpressing

malignant glioma cells. J Neurosurg 99:1039–1046. https://doi.org/10.3171/jns.2003.99.6.1039

Zhang C, Chen W, Zhang X, Huang B, Chen A, He Y, Wang J, Li X (2016) Galunisertib inhibits glioma vasculogenic mimicry formation induced by astrocytes. Sci Rep 6:23056. https://doi.org/10.1038/srep23056

Zhang L, Xu Y, Sun J, Chen W, Zhao L, Ma C, Wang Q, Sun J, Huang B, Zhang Y, Li X, Qu X (2017) M2-like tumor-associated macrophages drive vasculogenic mimicry through amplification of IL-6 expression in glioma cells. Oncotarget 8:819–832. https://doi.org/10.18632/oncotarget.13661

Zhao X, Chen R, Liu M, Feng J, Chen J, Hu K (2017) Remodeling the blood-brain barrier microenvironment by natural products for brain tumor therapy. Acta Pharm Sin B 7:541–553. https://doi.org/10.1016/j.apsb.2017.07.002

Zheng X, Chopp M, Lu Y, Buller B, Jiang F (2013) MiR-15b and miR-152 reduce glioma cell invasion and angiogenesis via NRP-2 and MMP-3. Cancer Lett 329:146–154. https://doi.org/10.1016/j.canlet.2012.10.026