Astrocytes, the rising stars of the glioblastoma microenvironment

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Running title: Astrocytes in glioblastoma

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Abstract

Glioblastoma (GBM) is an aggressive primary tumor, causing thousands of deaths worldwide every year. The mean survival of patients with GBM remains below 15 months despite current available therapies. GBM cells' interactions with their stromal counterparts are crucial for tumor development. Astrocytes are glial cells that comprise approximately 50% of all brain cells and are therefore likely to establish direct contact with GBM cells. As other tumor cell types can hijack fibroblasts or immune cells to facilitate tumor growth, GBM cells can actually activate astrocytes, namely the tumor associated astrocytes (TAAs), to promote GBM invasion in the healthy tissue. TAAs have thus been shown to be involved in GBM cells growth and limited response to radiation or chemotherapy (i.e. Temozolomide). Nevertheless, even though the interest in the cancer research community is increasing, the role of TAAs during GBM development is still overlooked. Yet, obtaining an in-depth understanding of the mechanisms by which TAAs influence GBM progression might lead to the development of new therapeutic strategies. The current review therefore reports the different levels of GBM progression at which TAAs have been recently described to be involved in, including tumor cells' proliferation/invasion and resistance to therapies, especially through the activity of extracellular vesicles.

Keywords

Brain, Glioblastoma, Stromal cells, Tumor associated astrocytes, Tumor microenvironment

Main points

Astrocytes get activated into tumor-associated astrocytes (TAAs) during glioblastoma (GBM) growth. The TAAS fraction is a source of potential new targets and biomarkers for future therapeutic strategies against GBM.

Glioblastoma (GBM) is the most lethal malignant tumor arising in the brain for which no cure is currently available (Louis et al., 2016; Ristic, Miric, Jovic, Ristic, & Karic, 2014; Zong, Verhaak, & Canoll, 2012). The standard treatment for post-operative GBM patients is radiotherapy combined with chemotherapy (Stupp et al., 2009). Nevertheless, the median survival for GBM patients remains less than a year from diagnosis (Gittleman et al., 2018). The presence of cells with increased therapeutic resistance and self-renewing properties, such as cancer stem cells within the tumor mass, may underlie the poor patients' response to treatment (Inda, Bonavia, & Seoane, 2014; Toda, 2013). Presence of tumor-associated stromal cells in the GBM microenvironment could also, at least partly, promote tumor progression and consequently participate in the GBM resistance to current therapies observed in patients (O'Brien, Howarth, & Sibson, 2013a).

I. Glioblastoma interactions with the surrounding brain micro-environment

GBM close interaction with their direct microenvironment in the central nervous system is essential to the tumor development, especially considering the specificity of the different brain cell populations and extracellular space (Ferrer, Moura Neto, & Mentlein, 2018; Lorger, 2011).

GBM tumors are highly heterogeneous with respect to the composition of tumor cells and the vast range of tumor associated, non-transformed, parenchymal cells (Charles, Holland, Gilbertson, Glass, & Kettenmann, 2011). Thus, a significant component of GBM is the tumor perivascular niche - the primary location of GBM stem cell-like (GSC) populations (J. T. Chen et al., 2017). GSCs have stem cell-like properties and are thought to highly contribute to the cellular heterogeneity of GBM (Lathia, Mack, Mulkearns-Hubert, Valentim, & Rich, 2015). The perivascular niche is also composed of several stromal cells including microglia, astrocytes, pericytes, fibroblasts and endothelial cells that support tumor progression (X. Zhao et al., 2017). Furthermore, these tumors are also characterized by their ability to interact with extracellular matrix (ECM) of the brain to favor their survival (M. Herrera-Perez, Voytik-Harbin, & Rickus, 2015; Nakod, Kim, & Rao, 2018).

The brain ECM occupies a significant proportion of the CNS and contributes to its normal physiology (Lau, Cua, Keough, Haylock-Jacobs, & Yong, 2013). Most of the components of the brain ECM, namely hyaluronic acid, proteoglycans and associated proteins, are over-expressed by tumor and tumor associated cells during GBM development. Therefore, typical ECM proteins such as fibronectin have been directly linked to tumor invasion by their ability to regulate cell adhesion and migration (Q. K. Chen, Lee, Radisky, & Nelson, 2013). Besides,

accumulating evidences suggest that hyaluronic acid plays a crucial role during GBM invasion in the healthy brain (Misra, Hascall, Markwald, & Ghatak, 2015).

The growth of solid tumors including GBM is highly dependent on neo-angiogenesis through which new blood vessels arise from pre-existing vessels (Vasudev & Reynolds, 2014). During this process, tumor cells secrete pro-angiogenic factors such as vascular endothelial factor-A (VEGF-A) that can affect surrounding endothelial cells in order to promote their proliferation and re-arrangement into blood vessels that consequently integrate into the tumor (Dey, Ulasov, & Lesniak, 2010). This way, a constant supply of oxygen and nutrients is established to counteract hypoxia at the tumor core. Consequently, the use of anti-angiogenic drugs such as bevacizumab that neutralizes VEGF-A has been extensively studied the last two decades in order to decrease tumor growth (Chinot et al., 2014; Gilbert et al., 2014). Nevertheless, these therapies are still of very limited benefits while some of their effects are controversial (Simon et al., 2014; Simon, Gagliano, & Giamas, 2017). In addition to their central role in neo-angiogenesis, endothelial cells have been shown to secrete mediators such as transforming growth factor-β (TGF-β) (Anido et al., 2010), b-FGF (Fessler, Borovski, & Medema, 2015), and epidermal growth factor (EGF) (Schulte et al., 2012) that promote the maintenance of the GSCs self-renewing properties in culture (R. Wang et al., 2010).

Tumor-associated microglia is also observed within the GBM microenvironment (Mildner et al., 2007; Quail & Joyce, 2017). For instance, microglia cells have been reported to promote glioma invasiveness *in vitro* through the action of the matrix metalloproteases, MMP2 and MMP9 (Hu et al., 2014; Markovic, Glass, Synowitz, van Rooijen, & Kettenmann, 2005). In a now well-defined mechanism, tumor associated microglial cells secrete TGF-β that promotes the GBM cells release of pro-MMP2 and versican. In response to versican, the toll-like receptor 2 (TLR2) on tumor associated microglia is activated and consequently induces the activation of the p38-MAPK pathway leading to the expression of membrane-type 1 matrix metalloprotease (MT1-MMP). This is followed by the cleavage and activation of pro-MMP2 by MT1-MMP. Ultimately, MMP2 then degrades ECM components such as gelatins, collagen, and elastins, enhancing GBM cell invasion (Charles et al., 2011).

The role of the endothelial cells (i.e. neo-angiogenesis) and immune/microglia cells during GBM development has already been intensively reported and is now pretty well deciphered. Regarding astrocytes, despite being the most abundant glial cell in the CNS and therefore highly abundant within GBM microenvironment, their impact on the tumor development is yet to be fully understood (Jakel & Dimou, 2017; O'Brien et al., 2013a). Nevertheless, a few recent

reports have acknowledged that question so that the interest about the role of astrocytes on the GBM microenvironment is now increasing (Oushy et al., 2018; Quail & Joyce, 2017).

Indeed, as a correlation between astrocyte subpopulations and GBM subtypes has been noticed, signaling pathways through which astrocytes are able to influence GBM cells invasion, resistance and interactions with their direct cell counterparts have also been thoroughly investigated.

II. Roles of astrocytes in the healthy central nervous system and pathology

1. Astrocytes at the brain blood barrier

Astrocytes are the "star-shaped" cells of the CNS known to play active and dynamic roles in the healthy and diseased brain (Davila, Thibault, Fiacco, & Agulhon, 2013; Reemst, Noctor, Lucassen, & Hol, 2016). Astrocytes are for example directly implicated in the structure and tightness of the brain blood barrier (BBB). The BBB lines the cerebral microvasculature that provides the brain with oxygen and nutrients. Forming the main BBB structure, the end-feet of astrocytes closely associates with the outer surface of the vasculature (Z. Zhao, Nelson, Betsholtz, & Zlokovic, 2015). Through tight junctions, astrocytes directly interact with both endothelial cells and pericytes thus supporting BBB maintenance and dynamic (Alvarez et al., 2011; Horng et al., 2017).

2. Astrocytes modulate synaptic activity and plasticity

Normal brain function is dependent on a balance between inhibitory and excitatory transmission (Farhy-Tselnicker & Allen, 2018). Astrocytes play a central role in regulating this balance as they are able to rapidly and efficiently remove neurotransmitters from the synaptic cleft (Belanger & Magistretti, 2009). Astrocytic processes found in association with excitatory synapses are rich in glutamate transporters such as glutamate aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1) allowing them to maintain the low physiological concentrations of the excitatory neurotransmitter glutamate, thereby limiting neuronal excitation (Chung, Allen, & Eroglu, 2015). In addition, astrocytes activity modulates volume transmission, a process during which neurotransmitters diffuse across the brain extracellular space *via* astrocytes in order to activate extra-synaptic receptors (Vargova & Sykova, 2014).

3. Astrogliosis

In addition to the roles astrocytes play in the healthy CNS, they are also responsive to CNS damage and are subject to astrogliosis, a process whereby astrocytes undergo a number of

molecular, cellular, and functional changes to form reactive astrocytes able to repair the affected tissue (Sofroniew, 2014). During astrogliosis, reactive astrocytes become hypertrophic and they can proliferate and increase in number at the lesion site (Sofroniew, 2009). Also, according to recent publications, the main component of astrocyte intermediate filaments, namely the glial fibrillary acidic protein (GFAP), is found to be up-regulated in reactive astrocytes, along with Nestin and Vimentin, transcription factors (e.g. STAT-3), signaling receptors (e.g. c-MET), growth factors (e.g. brain derived neurotrophic factor (BDNF), growth/differentiation factor 15 (GDF-15)), inflammatory cytokines (e.g. (C-C motif chemokine ligand 2 (CCL2), Interleukin 6 (IL-6)), extracellular matrix components (e.g. collagens, versican) and cell adhesion proteins (e.g. CD44), all in the aim to support tissue repair (Boccazzi & Ceruti, 2016). Among them, some might be considered as potential specific markers of reactive astrocytes (e.g. GFAP) (Zamanian et al., 2012). Therefore, GFAP labeling shows that reactive astrocytes seem to form a border between CNS lesions (e.g. experimental autoimmune encephalitis) and the surrounding tissue (Voskuhl et al., 2009). This lesion "sequestration" may play a role in speeding up clinical stabilization and thereby improving patients' survival (Cregg et al., 2014). Similarly, reactive astrocytes have also been shown to be involved in facilitating BBB repair (Sofroniew, 2009).

III. Role of astrocytes during glioblastoma development

As tumor development involves healthy tissue invasion and subsequent destruction, astrogliosis can also occur in response to GBM growth (O'Brien et al., 2013a). Thus, it has been shown through bioluminescence that astrogliosis peaks three days post-implantation of tumor cell lines in mice (Lee, Borboa, Baird, & Eliceiri, 2011). Nevertheless, even though this process initially aims to aid repairing the healthy brain tissue and fight the progression of the tumor, the same mechanisms might as well support tumor growth under the influence of GBM cells (O'Brien, Howarth, & Sibson, 2013b). Interestingly, the nuclear factor kappa-B (Nf-κB)-dependent signaling might be involved in the activation of astrocytes into tumor-associated astrocytes (TAAs) upon GBM growth (J. K. Kim et al., 2014). Indeed, receptor activator of Nf-κB ligand (RANKL) has been reported to be produced by GBM cells so it can reach its receptor RANK at the astrocytes' surface in order to activate them through the Nf-κB pathway, leading to the rising of TAAs. Those TAAs then produce tumor-promoting factors such as TGF-β enhancing GBM cell invasion (J. K. Kim et al., 2014). In the same way, Priego *et al*.

recently showed that STAT-3 might drive a protumoral program activation in reactive astrocytes in an *in vivo* model of brain metastasis (Priego et al., 2018).

1. Significance of different astrocytes sub-populations in the GBM microenvironment

Astrocytes from different CNS regions present vastly distinct morphological, molecular and functional properties, suggesting the existence of heterogeneous subpopulations (Farmer & Murai, 2017). Consequently, TAAs found in the GBM microenvironment might express specific markers that differentiate them from normal astrocytes. Indeed, through establishing the mRNA profile of TAAs in an *in vivo* PDGF-driven model of GBM (proneural GBM subtype), Katz *et al.* have been able to identify genes from the antigen presentation pathway but also CD44 and tenascin-C that are potentially over-expressed by GBM-associated astrocytes exclusively. Consequently, this data suggests very specific roles for TAAs in the GBM interactions with its surrounding microenvironment (Katz et al., 2012). In the same report, authors observed two distinct populations of TAAs in the same tumor bulk with TAAs at the tumor periphery being slightly different to those in the tumor perivascular niche. They noticed that TAAs surrounding the tumor seems similar to 'normal' reactive astrocytes (swollen cell bodies and hyperextended processes) while the CD44 and tenascin-c overexpression was mostly restricted to the perivascular TAAs (Katz et al., 2012). In accordance to this observation, Lin *et al.* reported the correlation between specific astrocyte subpopulations and GBM progression. In this study, intersectional fluorescence-activated cell sorting (FACS) identified five distinct astrocyte subpopulations across the brain (i.e. '1-olfactory bulb', '2-cortex', '3-brainstem', '4-thalamus' and '5-cerebellum'). While these diverse subpopulations were shown to demonstrate different developmental functions and synaptogenesis support, they also separately correlated with different GBM subtypes at the molecular and cellular levels, thus providing new insights for understanding GBM progression dynamics and describing new potential GBM subtypes markers (John Lin et al., 2017). Thus, the 'classical' GBM subtype seemed to correlate with the expression profiles of populations 1-4, the 'neural' GBM subtype with populations 1-3, and the 'mesenchymal' GBM subtype uniquely with population 2. In contrast, the expression profile of the 'proneural' GBM subtype did not coincide with any of these populations (John Lin et al., 2017; Verhaak et al., 2010).

2. Influence of tumor associated astrocytes on glioblastoma cells proliferation

Astrocytes expressing sonic hedgehog (SHH)-pathway components are highly concentrated in the perivascular niche of gliomas (Becher et al., 2008). Clement *et al.* show that deregulation of SHH-Gli signaling results in hyperproliferation of precursor cells and may initiate brain tumors (Clement, Sanchez, de Tribolet, Radovanovic, & Altaba, 2007). In accordance with its role during cell differentiation, the SHH-Gli signaling in astrocytes of the perivascular niche is likely to be involved in initiating gliomas (Komada, 2012). Similarly, the de-regulation of stromal cell-derived factor-1 (SDF1)/CXCR4 signaling in astrocytes is thought to induce aberrant astrocytes proliferation, which can initiate the formation of a GBM tumor (Bonavia et al., 2003).

Regarding reactive astrocytes, they are known to secrete high levels of factors such as tumor necrosis factor-α (TNF-α), tumor growth factor-β (TGF-β), IL-6, and insulin growth factor-1 (IGF-1) in response to CNS injury. All these factors have been shown to significantly increase the *in vitro* proliferation of cancer cells such as brain-metastatic breast or lung cancer cells but also primary brain tumors such as GBM (Nagashima, Suzuki, Asai, & Fujimoto, 2002; Placone, Quinones-Hinojosa, & Searson, 2016; Seike et al., 2011). In the same way, GDF-15, observed as overexpressed in reactive astrocytes, has been shown to increase GBM cells *in vitro* proliferation while GDF-15 depletion decreases *in vivo* tumor growth (Roth et al., 2010; Zamanian et al., 2012). GDF-15, a divergent member of the TGF-β gene family, is also overexpressed in GBM patients and seems to correlate with shorter survival of patients (Shnaper et al., 2009). Overall, those reports highly suggest that, upon GBM rising, surrounding astrocytes might overexpressed proliferation factors such as TGF-β and GDF-15 in order to support the specific aberrant proliferation rates of tumor cells.

In the same picture, ion channels and ion transporters, expressed at the membrane of both astrocytes and GBM cells might then form a bridge between the two cellular types, thus enhancing tumor progression and therapeutic resistance, as suggested by a recent review (Guan, Hasan, Maniar, Jia, & Sun, 2018). Also, the GBM-derived extracellular vesicles (EVs) might significantly alter the intracellular signaling and cytokine profile of normal human astrocytes to push them towards a tumor supporting behavior (Oushy et al., 2018). EVs are membrane-enclosed nanospheres secreted by all cell types (S. M. Kim & Kim, 2017) that are essential players in intercellular communication as they carry proteins, lipids, and nucleic acids between cells (Yanez-Mo et al., 2015). It appears that the exposure of normal astrocytes to GBM EVs generates a growth-stimulant medium loaded with growth factors (VEGF, fibroblast growth factor (FGF), human growth factor (HGF), and EGF), chemokines, and interleukins. Consequently, proliferation of GBM cells has been detected as enhanced when exposed to

conditioned medium derived from astrocytes under the influence of tumor EVs (Oushy et al., 2018).

3. Migration and invasion

In the tumor surrounding microenvironment, GBM invasion mostly occurs along anatomic features such as blood vessels but also astrocytes extensions (Hoelzinger, Demuth, & Berens, 2007; Nakada et al., 2007). This way, in addition to the effects on tumor cell proliferation, TAAs might also directly impact on the migration and invasion of GBM cells (Le et al., 2003b; Rath, Fair, Jamal, Camphausen, & Tofilon, 2013). In order to invade the surrounding tissue, GBM tumors remodel the ECM through the activity of enzymes such as matrix-metalloproteases (MMPs) (Coquerel et al., 2009). Accordingly, a recent study elegantly showed an increase of the migration capabilities of GBM patient-derived cell lines (GBM10, GBM43 and GBAM1) when co-culture with astrocytes in a collagen-hyaluronan 3D matrix (R. M. Herrera-Perez et al., 2018). Le *et al.* also showed that large amounts of reactive astrocytes are found in proximity to the tumor cells to mediate the activation of MMP2 and favor GBM invasion (Le et al., 2003a). In the same study, astrocytes were shown to directly participate in the interaction of the urokinase plasminogen activator (uPA) with its activator uPAR in U251 GBM cells *in vitro*. This, in turn, enhances the activation of plasmin – a serine protease that cleaves and activates MMP2 (Le et al., 2003a). The report then suggests that TAAs in the perivascular niche promote the uPAR-plasmin cascade in order to increase MMPs activation in GBM cells, consequently favoring invasion (**Figure 1**). In a similar way, reactive astrocytes have been observed to secrete IL-6, a protein able to enhance primary GBM cells invasion *in vitro* (W. Chen et al., 2016; Li et al., 2010). Indeed, GBM development, aggressiveness and poor prognosis have been linked to the high expression of IL-6 (Jiang, Han, Cheng, Wang, & Wu, 2017; Tchirkov et al., 2007). Thus, Jiang et al., recently observed that both IL-6 and IL-6-R expression was directly correlated to the highly aggressive mesenchymal GBM subtype and poor survival (Jiang et al., 2017). In the same way, Li *et al.* showed that IL-6 promotes the *in vitro* invasion of U87 GBM cells by up-regulating the expression and secretion of MMP2 and fascin-1, an actin-bundling protein involved in the formation of cellular protrusions enabling cell migration (Li et al., 2010). A separate study also reported a significant increase in the *in vitro* invasiveness of CD133+ GSCs when co-cultured with astrocytes or treated with astrocytes conditioned medium (Rath et al., 2013). Interestingly, those effects appeared to be exclusive to CD133+ GSCs as co-culture or astrocyte conditioned medium failed to stimulate CD133- cell invasion. Furthermore, authors observed well-known mediators of tumor cell

invasion as secreted by astrocytes, such as osteopontin, TGF-β1, IL-6 and IL-8, while GSCs expression of tumor aggressiveness markers such as CD44, CCl2 or hyaluronan synthase 2 (Has2) appeared to increase when co-cultured with astrocytes (Rath et al., 2013). Altogether, these results suggest that TAAs might support the progression of aggressive GBM stem cells.

Similar effects have been observed in response to glial-cell derived neurotrophic factor (GDNF). GDNF- GFRα1 has been implicated in the progression of different types of tumors, including GBM (Wiesenhofer, Weis, & Humpel, 2000). Therefore, using a murine glioma cell line (GL261), Shabtay-Orbach *et al*. showed that astrocyte-derived GDNF enhances GBM migration potential *in vitro* and promotes tumor progression *in vivo* (Shabtay-Orbach, Amit, Binenbaum, Na'ara, & Gil, 2015). This data suggests that astrocyte-derived GDNF may be acting as a chemoattractant to promote GBM cell migration. In addition, through the addition of specific inhibitors to astrocytes conditioned medium, authors could be able to demonstrate that the astrocytes-derived GDNF effect on GBM cell migration was mostly mediated via the transmembrane receptor RET and both the AKT and ERK pathways (Runeberg-Roos & Saarma, 2007; Shabtay-Orbach et al., 2015).

Brain-metastatic cancer cells also exhibit an increased invasion potential in the presence of astrocytes *in vitro*. A study by Marchetti *et al.* suggests that astrocyte-derived NGF promotes the production of the extracellular degrading enzyme heparanase which in turn promotes brain-metastatic specificity invasion of melanoma cells *in vitro* (Marchetti, Li, & Shen, 2000). Transwell assays using a chamber coated with HSPGs show that in the presence of astrocytes conditioned medium, the invasiveness of melanoma cells lines significantly increases (Marchetti et al., 2000). TAAs might thus be critical mediators of tumor metastasis invasion through the brain.

4. Tumor associated astrocytes support glioblastoma cells survival

During GBM development, tumor cells easily adapt to the hypoxic conditions arising in their surrounding microenvironment, thanks to the activation of the HIF-1 signaling and the concomitant help from stromal cells, especially endothelial cells during the neo-angiogenesis process (Quail & Joyce, 2017; Vasudev & Reynolds, 2014). Interestingly, TAAs might also be involved in this GBM cells adaption to hypoxia. Indeed, a recent study using an *in vitro* GBM cells/astrocytes co-culture model reports that the astrocytes secretion of chemokine C-C motif ligand 20 (CCL20) increases in response to hypoxia, leading to an overexpression of HIF-1α

in GBM cells through the binding of the chemokine to its receptor C-C chemokine receptor type 6 (CCR6) and the subsequent activation of the NF-κB pathway. Consequently, under hypoxic conditions, the GBM cells pro-angiogenic potential (VEGF-A secretion and endothelial tubes formation), proliferation and 3D invasion increased in response to conditioned medium from astrocytes previously exposed to hypoxia (Jin et al., 2018). The CCL20/CCR6 axis has recently been associated with poor outcomes of different cancers but also seems to be highly present in high grade gliomas compared to low grade gliomas (Ding et al., 2012; L. Wang et al., 2012). In addition, in line with these *in vitro* results, *in vivo* data showed a direct correlation between CCR6 expression and tumor development (**Figure 2**). Overall, this new study underlines the direct implication of TAAs in GBM cells reaction to hypoxia but also suggests a more indirect involvement in the onset of the neo-angiogenesis process during tumor growth (Jin et al., 2018).

TAAs also facilitate GBM progression through promoting apoptosis evasion. Indeed, Oliveira *et al.* lately reported an increase of glioma cell viability in response to treatment with *in vitro* conditioned medium derived from glial cells that have been previously primed through co-culture with tumor cells (Oliveira et al., 2017). Compared to control, proteins described as being up-regulated in the conditioned medium from primed glial cells are also known to be involved in processes such as cellular homeostasis, cell adhesion, inflammatory responses, and extracellular structure organization (Okolie et al., 2016). In addition, proteins linked with tumor progression such as insulin-like growth factor-binding protein 2 (IBP-2) (Z. Zhang et al., 2014), MMP inhibitor 2 (TIMP2) (K. V. Lu, Jong, Rajasekaran, Cloughesy, & Mischel, 2004), and SPARC-like protein 1 (Turtoi et al., 2012) were also found to be significantly up-regulated (Oliveira et al., 2017).

Moreover, the ECM of astrocytes with mutated p53 has recently been shown to facilitate apoptosis evasion in GBM cells (Biasoli et al., 2014). Accordingly, p53 has also been found to be able to modulate the expression of tumor cells secreted proteins, thus influencing the behavior of neighboring cells (Khwaja et al., 2006). In this way, it appears possible that p53 can be involved in modulating the ECM composition (P. Lu, Weaver, & Werb, 2012). Hence, Biasoli *et al.* reported that the ECM from astrocytes with mutated p53 ($p53^{+/-}$) promotes the survival of GBM cells. Indeed, culturing human T98 and U87 GBM cell lines on freshly immobilized ECM from $p53^{+/-}$ astrocytes had no significant impact on GBM cell proliferation but lowered their apoptotic rate, thus suggesting that this specific ECM promotes GBM survival. Furthermore, the ECM of astrocytes with mutated p53 also showed increased levels

of fibronectin and laminin – proteins known to trigger epithelial to mesenchymal transition (EMT), through which tumor cells acquire a more migratory and invasive phenotype (Q. K. Chen et al., 2013; Zeisberg & Neilson, 2009). Similarly, when co-culturing GBM cells in the ECM of $p53^{+/-}$ astrocytes, there was an increase of the expression of markers (N-cadherin and vimentin) for a mesenchymal phenotype known to be associated with increased tumor cells resistance to apoptosis and migratory potential (Holohan, Van Schaeybroeck, Longley, & Johnston, 2013). In the same study, GBM cells were found to be able to modulate the expression of p53 in astrocytes from the cerebral cortex of new-born mice (Biasoli et al., 2014). Accordingly, this experiment elucidates the idea of an intercellular crosstalk where GBM cells trigger a decrease in the expression of p53 in local astrocytes that in turn prevents apoptosis of GBM cells.

In the same way, astrocyte-derived phosphoprotein enriched in astrocytes of 15kDa (PEA-15) has been shown to increase the survival of GBM cells *in vitro* (Eckert et al., 2008). PEA-15 is a multifunctional protein involved in glucose metabolism and regulating apoptosis (Fiory, Formisano, Perruolo, & Beguinot, 2009). PEA-15 is overexpressed in diffuse/anaplastic astrocytomas and GBM compared to normal brain tissues, more specifically in astrocytes surrounding GBM tumors (Formstecher et al., 2001; Watanabe et al., 2010). Eckert *et al.* demonstrated that PEA-15 protects GBM cells from glucose deprivation-induced apoptosis (Sulzmaier, Opoku-Ansah, & Ramos, 2012). In addition, GBM cell lines overexpressing PEA-15 show PEA-15-dependent protection from low glucose-mediated cell death (Caro-Maldonado et al., 2010). Consequently, suppressing the PEA-15 signaling reversed these protective effects (Eckert et al., 2008). According to this, it is likely that a PEA-15-dependant mechanism might be involved in the astrocytes' support to GBM cells survival.

GBM cells-derived EVs have also been found to modulate surrounding astrocytes signaling, thereby promoting evasion of apoptosis in GBM cells (Oushy et al., 2018). Indeed, Oushy *et al.* showed that the phosphorylation of BAD - a pro-apoptotic member of the Bcl-2 family that becomes inactive when phosphorylated - is observed in astrocytes grown in the presence of GBM cells-derived EVs. Similarly, other key oncogenic mediators such as ERK, AKT, and STATs (Steelman et al., 2008; Steelman et al., 2004) are also found increasingly phosphorylated in GBM EV-stimulated astrocytes. Overall, these studies suggest that a tight GBM/astrocytes crosstalk in the tumor microenvironment is important to limit tumor cells apoptosis, thus promoting aggressiveness (Oushy et al., 2018). Having an in-depth understanding of the related underlying mechanisms may be important for the development of new innovative GBM treatments.

5. Chemoprotection

TAAs might also be implicated in the protection of GBM cells against currently used chemotherapies. A study undertaken by Chen *et al.* showed that *in vitro* co-culture with astrocytes significantly decreases chemotherapy-induced apoptosis of A172 GBM cells (W. L. Chen et al., 2015). However, this protection is dependent on direct contact between GBM cells and astrocytes as separating the two cell populations using a transwell membrane abolished any cytoprotective effects previously observed. In addition to this, dye transfer assays revealed that when in co-culture, functional gap junctions are formed between astrocytes and glioma cells. Consequently, anti-tumor cells effect is increased when combined with carbenoxolone, a potent gap junctions communication inhibitor (W. L. Chen et al., 2015). In addition, another report described the decrease of the intracellular Ca^{2+} burst associated with chemotherapy induced-cytotoxicity when melanoma cells are co-cultured with astrocytes. This effect is abolished in the presence of carbenoxolone (CBX) a specific inhibitor of gap junctions channels (Lin et al., 2010). Similarly, breast and lung cancer cells have been reported to form gap junctions with astrocytes through connexin 43 (Cx43). Indeed *in vitro* data combined with *in vivo* observations showed that formation of Cx43-dependent gap junctions between astrocytes and metastatic tumor cells might provide chemoresistance and enhance cancer growth. The authors reported that metastatic tumor cells are able to transfer the 2'3'-cyclic GMP-AMP second messenger (cGAMP) through the gap junctions in order to stimulate the astrocytes production of pro-inflammatory cytokines such as TNF or IFNα. In return, those cytokines seem to reach back to the metastatic tumor cells and promote their invasion and resistance to therapeutics through the STAT1 and NF-κB pathways. Consequently, they suggested that combining chemotherapies with gap junctions inhibitors might enhance the tumor cells chemosensitivity (Q. Chen et al., 2016). Thus, according to these reports, specific gap junctions between astrocytes and GBM cells can participate in tumor cells resistance to chemotherapeutic agents.

6. Immunoprotection

As cancer cells rise, the immune system gets immediately activated so it can fight the developing tumor through the actions of natural killer cells and T-lymphocytes (Martinez-Lostao, Anel, & Pardo, 2015). Unfortunately, tumor cells get easily resistant to this natural immune reaction. Currently, there is no direct evidence that astrocytes are involved in GBM

cells immunoprotection. Yet, reactive astrocytes secrete factors such as tenascin-C, IL-10 and STAT-3, which have all been linked to mechanisms implicated in immunoprotection of GBM cells (Huang et al., 2010). Similarly, IL-10, an immunomodulatory cytokine with anti-inflammatory properties, has also been shown to stimulate survival of GBM cells, notably *via* inhibition of the expression of pro-inflammatory mediators such as Class II MHC and IFN-γ *in vitro* (Iyer & Cheng, 2012; Qi et al., 2016). Class II MHC is involved in the antigen-presenting ability of monocytes while IFN-γ has been shown to induce the synthesis of IL-6 to support the immune eradication of GBM cell lines *in vitro* (Hotfilder et al., 2000; Lee et al., 2017; Yin et al., 2017). In addition, STAT-3 – another factor found up-regulated in reactive astrocytes (Priego et al., 2018) - plays an essential role in inducing angiogenesis, immunosuppression, and tumor invasion (J. E. Kim, Patel, Ruzevick, Jackson, & Lim, 2014). As a matter of fact, an extensive study by Zhang *et al.* shows that STAT-3 inhibits the activation of microglia and macrophages *in vitro* and *in vivo*, as well as inducing tumor growth (L. Zhang et al., 2009). In addition, Herrera-Perez et al. recently observed that presence of astrocytes prevents the decrease of GBM cells 3D migration upon inhibition of STAT-3 (R. M. Herrera-Perez et al., 2018). In the same way, a STAT-3 expressing subpopulation of reactive astrocytes has been showed to modulate the immune process in the brain during metastasis, thus promoting tumor cells survival and consequently decreasing patients' survival (Priego et al., 2018). Altogether, those reports highlight the potential of TAAs to protect GBM cells against anti-cancer immune reaction by secreting now well-described anti-inflammatory factors (Mostofa, Punganuru, Madala, Al-Obaide, & Srivenugopal, 2017).

7. Tumor associated astrocytes-derived EVs

EVs allows the communication between both neighboring and distant cells (Tkach & Thery, 2016). The composition of astrocyte-derived EVs is heterogeneous and has been involved in both physiological and pathological functions (Fruhbeis, Frohlich, Kuo, & Kramer-Albers, 2013). Yet, some of the content of astrocyte-derived EVs and their roles have been described; Hsp/c70 is linked to neuroprotection (Taylor, Robinson, Gifondorwa, Tytell, & Milligan, 2007); FGF-2, VEGF, endostatin, and PEDF are involved in modulating angiogenesis (Hajrasouliha et al., 2013; Proia et al., 2008) while MMPs mediate ECM remodeling (Sbai et al., 2010). According to this, even though this research is still in its infancy, an EVs-dependent influence of TAAs in GBM tumor supporting processes such as neo-angiogenesis and immune-modulation appears possible. Therefore, through *in vitro* co-culture

methods, it has interestingly been reported that EVs from astrocytes might induce a decrease of the PTEN expression in breast cancer and melanoma metastatic cells in the brain microenvironment, thus enhancing tumor cells proliferation and resistance to apoptosis. Authors showed that exosomal-associated miRNAs are involved in this regulation (Lin Zhang et al., 2015).

IV. Significance for further diagnosis, prognosis and therapeutic applications

As current therapeutic strategies (chemotherapies and targeted therapies such as anti-angiogenic treatments) are yet to be fully effective, TAAs appear to be new potential targets and/or source of biomarkers for GBM treatment. Both local (i.e. gap junctions) and distant (i.e. cytokines and EVs) roles of TAAs should be exploited. Based on the current review, TAAS-produced IL-6 appears to be at the crossroads of multiple TAAs' effects on GBM development, including promoting proliferation and invasion but also modulating immune response to tumor progression (W. Chen et al., 2016; Jiang et al., 2017; Lee et al., 2017; Seike et al., 2011; Yin et al., 2017; Zamanian et al., 2012). This key cytokine might thus be considered as a target for new therapies but also as a specific biomarker since its expression has been observed as mostly associated with mesenchymal GBM (Jiang et al., 2017). Nevertheless, most of the work that has been done on TAAs-IL-6 was *in vitro*, suggesting that further *in vivo* studies will be needed in order to strengthen its clinical relevance. For the same reasons, STAT-3 and GDNF, respectively implicated in TAAs' effect on immune reaction modulation and tumor migration, should also be considered for future strategies, especially since *in vivo* studies have already validated some of their crucial roles (Priego et al., 2018). In addition, considering that EVs can travel long distances and consequently be found in patients' peripheral blood and urine, further extensive study of the TAAs-derived EVs might also provide with new highly valuable biomarkers (Wendler et al., 2016; Lin Zhang et al., 2015). In the same way, evidences provided by Jon Lin *et al*. or Katz *et al*. describing the specificity of TAAs for distinct GBM subtypes and cell sub-populations suggest a need for more representative TAAs biomarkers as, consequently, GFAP-expressing reactive astrocytes might differ from their tumor supporting counterparts. Furthermore, as these reports confirmed that different subpopulations of astrocytes might co-exist in the same tumor, another potential therapeutic strategy might be to stimulate the 'reactive' phenotype of astrocytes surrounding the tumor, avoiding the rise of the 'tumor associated' phenotype (John Lin et al., 2017; Katz et al., 2012). Accordingly, and since they are in constant interaction with other stromal cells such as endothelial and microglial cells

in the brain, a better control over astrocytes might help the fight against GBM growth through re-organizing the tumor direct microenvironment.

Conclusion

The role of brain stromal cells in GBM progression is being widely reported. Nevertheless, the role of TAAs is still being overlooked. Yet, some studies already conducted *in vitro* and *in vivo* suggested that TAAs have a pro-tumorigenic role in the GBM microenvironment. Indeed conclusive reports directly linked TAAs-dependent mechanisms to GBM proliferation, invasion, apoptosis evasion, and chemoprotection. Other tumor supporting processes such as tumor cells immunoprotection might also involve TAAs action but further studies are needed to address this matter. Moreover, deciphering the mechanisms by which astrocytes crosstalk with other cells of the tumor microenvironment to influence GBM progression might be crucial considering that GBM are now well accepted as being a complex network of both heterogeneous tumor cells and various types of stromal cells interacting with each other, thus altogether supporting tumor growth. Overall, the current overview suggests that deciphering the TAAs-associated underlying signaling might provide new markers and targets for future therapeutic approaches to treat GBM.

References

Alvarez, J. I., Dodelet-Devillers, A., Kebir, H., Ifergan, I., Fabre, P. J., Terouz, S., . . . Prat, A. (2011). The Hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. *Science, 334*(6063), 1727-1731. doi: 10.1126/science.1206936

Anido, J., Saez-Borderias, A., Gonzalez-Junca, A., Rodon, L., Folch, G., Carmona, M. A., . . . Seoane, J. (2010). TGF-beta Receptor Inhibitors Target the CD44(high)/Id1(high) Glioma-Initiating Cell Population in Human Glioblastoma. *Cancer Cell, 18*(6), 655-668. doi: 10.1016/j.ccr.2010.10.023

Becher, O. J., Hambardzumyan, D., Fomehenko, E. I., Momota, H., Mainwaring, L., Bleau, A. M., . . . Holland, E. C. (2008). Gli activity correlates with tumor grade in platelet-derived growth factor-induced gliomas. *Cancer Research, 68*(7), 2241-2249. doi: 10.1158/0008-5472.Can-07-6350

Belanger, M., & Magistretti, P. J. (2009). The role of astroglia in neuroprotection. *Dialogues Clin Neurosci, 11*(3), 281-295.

Biasoli, D., Sobrinho, M. F., da Fonseca, A. C. C., de Matos, D. G., Romao, L., Maciel, R. D., . . . Lima, F. R. S. (2014). Glioblastoma cells inhibit astrocytic p53-expression favoring cancer malignancy. *Oncogenesis, 3*. doi: ARTN e123

10.1038/oncsis.2014.36

Boccazzi, M., & Ceruti, S. (2016). Where do you come from and what are you going to become, reactive astrocyte? *Stem Cell Investigation, 3*(5).

Bonavia, R., Bajetto, A., Barbero, S., Pirani, P., Florio, T., & Schettini, G. (2003). Chemokines and their receptors in the CNS: expression of CXCL12/SDF-1 and CXCR4 and their role in astrocyte proliferation. *Toxicology Letters, 139*(2-3), 181-189. doi: Pii S0378-4274(02)00432-0

Doi 10.1016/S0378-4274(02)00432-0

Caro-Maldonado, A., Tait, S. W. G., Ramirez-Peinado, S., Ricci, J. E., Fabregat, I., Green, D. R., & Munoz-Pinedo, C. (2010). Glucose deprivation induces an atypical form of apoptosis mediated by caspase-8 in Bax-, Bak-deficient cells. *Cell Death and Differentiation, 17*(8), 1335-1344. doi: 10.1038/cdd.2010.21

Charles, N. A., Holland, E. C., Gilbertson, R., Glass, R., & Kettenmann, H. (2011). The brain tumor microenvironment. *Glia, 59*(8), 1169-1180. doi: 10.1002/glia.21136

Chen, J. T., Mao, S. F., Li, H. F., Zheng, M. C., Yi, L. G., Lin, J. M., & Lin, Z. X. (2017). The pathological structure of the perivascular niche in different microvascular patterns of glioblastoma. *Plos One, 12*(8). doi: ARTN e0182183

10.1371/journal.pone.0182183

Chen, Q., Boire, A., Jin, X., Valiente, M., Er, E. E., Lopez-Soto, A., . . . Massague, J. (2016). Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature, 533*(7604), 493-498. doi: 10.1038/nature18268

Chen, Q. K., Lee, K., Radisky, D. C., & Nelson, C. M. (2013). Extracellular matrix proteins regulate epithelial-mesenchymal transition in mammary epithelial cells. *Differentiation, 86*(3), 126-132. doi: 10.1016/j.diff.2013.03.003

Chen, W., Xia, T., Wang, D., Huang, B., Zhao, P., Wang, J., . . . Li, X. (2016). Human astrocytes secrete IL-6 to promote glioma migration and invasion through upregulation of cytomembrane MMP14. *Oncotarget, 7*(38), 62425-62438. doi: 10.18632/oncotarget.11515

Chen, W. L., Wang, D. H., Du, X. W., He, Y., Chen, S. Y., Shao, Q. Q., . . . Li, X. G. (2015). Glioma cells escaped from cytotoxicity of temozolomide and vincristine by communicating with human astrocytes. *Medical Oncology, 32*(3). doi: ARTN 43

10.1007/s12032-015-0487-0

Chinot, O. L., Wick, W., Mason, W., Henriksson, R., Saran, F., Nishikawa, R., . . . Cloughesy, T. (2014). Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med, 370*(8), 709-722. doi: 10.1056/NEJMoa1308345

Chung, W. S., Allen, N. J., & Eroglu, C. (2015). Astrocytes Control Synapse Formation, Function, and Elimination. *Cold Spring Harb Perspect Biol, 7*(9), a020370. doi: 10.1101/cshperspect.a020370

Clement, V., Sanchez, P., de Tribolet, N., Radovanovic, I., & Altaba, A. R. I. (2007). HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Current Biology, 17*(2), 165-172. doi: 10.1016/j.cub.2006.11.033

Coquerel, B., Poyer, F., Torossian, F., Dulong, V., Bellon, G., Dubus, I., . . . Vannier, J. P. (2009). Elastin-derived peptides: matrikines critical for glioblastoma cell aggressiveness in a 3-D system. *Glia, 57*(16), 1716-1726. doi: 10.1002/glia.20884

Cregg, J. M., DePaul, M. A., Filous, A. R., Lang, B. T., Tran, A., & Silver, J. (2014). Functional regeneration beyond the glial scar. *Exp Neurol, 253*, 197-207. doi: 10.1016/j.expneurol.2013.12.024

Davila, D., Thibault, K., Fiacco, T. A., & Agulhon, C. (2013). Recent molecular approaches to understanding astrocyte function in vivo. *Front Cell Neurosci, 7*, 272. doi: 10.3389/fncel.2013.00272

Dey, M., Ulasov, I. V., & Lesniak, M. S. (2010). Virotherapy against malignant glioma stem cells. *Cancer Lett, 289*(1), 1-10. doi: 10.1016/j.canlet.2009.04.045

Ding, X., Wang, K., Wang, H., Zhang, G., Liu, Y., Yang, Q., . . . Hu, S. (2012). High expression of CCL20 is associated with poor prognosis in patients with hepatocellular carcinoma after curative resection. *J Gastrointest Surg, 16*(4), 828-836. doi: 10.1007/s11605-011-1775-4

Eckert, A., Bock, B. C., Tagscherer, K. E., Haas, T. L., Grund, K., Sykora, J., . . . Roth, W. (2008). The PEA-15/PED protein protects glioblastoma cells from glucose deprivation-induced apoptosis via the ERK/MAP kinase pathway. *Oncogene, 27*(8), 1155-1166. doi: 10.1038/sj.onc.1210732

Farhy-Tselnicker, I., & Allen, N. J. (2018). Astrocytes, neurons, synapses: a tripartite view on cortical circuit development. *Neural Dev, 13*(1), 7. doi: 10.1186/s13064-018-0104-y

Farmer, W. T., & Murai, K. (2017). Resolving Astrocyte Heterogeneity in the CNS. *Frontiers in Cellular Neuroscience, 11*. doi: ARTN 300
10.3389/fncel.2017.00300

Ferrer, V. P., Moura Neto, V., & Mentlein, R. (2018). Glioma infiltration and extracellular matrix: key players and modulators. *Glia*. doi: 10.1002/glia.23309

Fessler, E., Borovski, T., & Medema, J. P. (2015). Endothelial cells induce cancer stem cell features in differentiated glioblastoma cells via bFGF. *Mol Cancer, 14*, 157. doi: 10.1186/s12943-015-0420-3

Fiory, F., Formisano, P., Perruolo, G., & Beguinot, F. (2009). Frontiers: PED/PEA-15, a multifunctional protein controlling cell survival and glucose metabolism. *Am J Physiol Endocrinol Metab, 297*(3), E592-601. doi: 10.1152/ajpendo.00228.2009

Formstecher, E., Ramos, J. W., Fauquet, M., Calderwood, D. A., Hsieh, J. C., Canton, B., . . . Chneiweiss, H. (2001). PEA-15 mediates cytoplasmic sequestration of ERK MAP kinase. *Dev Cell, 1*(2), 239-250.

Fruhbeis, C., Frohlich, D., Kuo, W. P., & Kramer-Albers, E. M. (2013). Extracellular vesicles as mediators of neuron-glia communication. *Frontiers in Cellular Neuroscience, 7*. doi: ARTN 182
10.3389/fncel.2013.00182

Gilbert, M. R., Dignam, J. J., Armstrong, T. S., Wefel, J. S., Blumenthal, D. T., Vogelbaum, M. A., . . . Mehta, M. P. (2014). A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med, 370*(8), 699-708. doi: 10.1056/NEJMoa1308573

Gittleman, H., Boscia, A., Ostrom, Q. T., Truitt, G., Fritz, Y., Kruchko, C., & Barnholtz-Sloan, J. S. (2018). Survivorship in Adults with Malignant Brain and other Central Nervous System Tumor from 2000-2014. *Neuro Oncol*. doi: 10.1093/neuonc/noy090

Guan, X., Hasan, M. N., Maniar, S., Jia, W., & Sun, D. (2018). Reactive Astrocytes in Glioblastoma Multiforme. *Mol Neurobiol*. doi: 10.1007/s12035-018-0880-8

Hajrasouliha, A. R., Jiang, G. M., Lu, Q. X., Lu, H. Y., Kaplan, H. J., Zhang, H. G., & Shao, H. (2013). Exosomes from Retinal Astrocytes Contain Antiangiogenic Components That Inhibit Laser-induced Choroidal Neovascularization. *Journal of Biological Chemistry, 288*(39), 28058-28067. doi: 10.1074/jbc.M113.470765

Herrera-Perez, M., Voytik-Harbin, S. L., & Rickus, J. L. (2015). Extracellular Matrix Properties Regulate the Migratory Response of Glioblastoma Stem Cells in Three-Dimensional Culture. *Tissue Engineering. Part A, 21*(19-20), 2572-2582. doi: 10.1089/ten.tea.2014.0504

Herrera-Perez, R. M., Voytik-Harbin, S. L., Sarkaria, J. N., Pollok, K. E., Fishel, M. L., & Rickus, J. L. (2018). Presence of stromal cells in a bioengineered tumor microenvironment alters glioblastoma migration and response to STAT3 inhibition. *PLoS ONE, 13*(3), e0194183. doi: 10.1371/journal.pone.0194183

Hoelzinger, D. B., Demuth, T., & Berens, M. E. (2007). Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. *Journal of the National Cancer Institute, 99*(21), 1583-1593. doi: 10.1093/jnci/djm187

Holohan, C., Van Schaeybroeck, S., Longley, D. B., & Johnston, P. G. (2013). Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer, 13*(10), 714-726. doi: 10.1038/nrc3599

Horng, S., Therattil, A., Moyon, S., Gordon, A., Kim, K., Argaw, A. T., . . . John, G. R. (2017). Astrocytic tight junctions control inflammatory CNS lesion pathogenesis. *J Clin Invest, 127*(8), 3136-3151. doi: 10.1172/jci91301

Hotfilder, M., Knupfer, H., Mohlenkamp, G., Pennekamp, P., Knupfers, M., Van Gool, S., & Wolff, J. E. (2000). Interferon-gamma increases IL-6 production in human glioblastoma cell lines. *Anticancer Res, 20*(6B), 4445-4450.

Hu, F., Ku, M.-C., Markovic, D., Dzaye, O. D. a., Lehnardt, S., Synowitz, M., . . . Kettenmann, H. (2014). Glioma associated microglial MMP9 expression is up regulated by TLR2 signalling and sensitive to minocycline. *International journal of cancer. Journal international du cancer, 135*(11), 2569-2578. doi: 10.1002/ijc.28908

Huang, J. Y., Cheng, Y. J., Lin, Y. P., Lin, H. C., Su, C. C., Juliano, R., & Yang, B. C. (2010). Extracellular Matrix of Glioblastoma Inhibits Polarization and Transmigration of T Cells: The Role of Tenascin-C in Immune Suppression. *Journal of Immunology, 185*(3), 1450-1459. doi: 10.4049/jimmunol.0901352

Inda, M. D., Bonavia, R., & Seoane, J. (2014). Glioblastoma Multiforme: A Look Inside Its Heterogeneous Nature. *Cancers, 6*(1), 226-239. doi: 10.3390/cancers6010226

Iyer, S. S., & Cheng, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Crit Rev Immunol, 32*(1), 23-63.

Jakel, S., & Dimou, L. (2017). Glial Cells and Their Function in the Adult Brain: A Journey through the History of Their Ablation. *Frontiers in Cellular Neuroscience, 11*. doi: ARTN 24 10.3389/fncel.2017.00024

Jiang, Y., Han, S., Cheng, W., Wang, Z., & Wu, A. (2017). NFAT1-regulated IL6 signalling contributes to aggressive phenotypes of glioma. *Cell Commun Signal, 15*(1), 54. doi: 10.1186/s12964-017-0210-1

Jin, P., Shin, S. H., Chun, Y. S., Shin, H. W., Shin, Y. J., Lee, Y., . . . Park, J. W. (2018). Astrocyte-derived CCL20 reinforces HIF-1-mediated hypoxic responses in glioblastoma by stimulating the CCR6-NF-kappaB signaling pathway. *Oncogene*. doi: 10.1038/s41388-018-0182-7

John Lin, C. C., Yu, K., Hatcher, A., Huang, T. W., Lee, H. K., Carlson, J., . . . Deneen, B. (2017). Identification of diverse astrocyte populations and their malignant analogs. *Nat Neurosci, 20*(3), 396-405. doi: 10.1038/nn.4493

Katz, A. M., Amankulor, N. M., Pitter, K., Helmy, K., Squatrito, M., & Holland, E. C. (2012). Astrocyte-Specific Expression Patterns Associated with the PDGF-Induced Glioma Microenvironment. *Plos One, 7*(2). doi: ARTN e32453

10.1371/journal.pone.0032453

Khwaja, F. W., Svoboda, P., Reed, M., Pohl, J., Pyrzynska, B., & Van Meir, E. G. (2006). Proteomic identification of the wt-p53-regulated tumor cell secretome. *Oncogene, 25*(58), 7650-7661. doi: 10.1038/sj.onc.1209969

Kim, J. E., Patel, M., Ruzevick, J., Jackson, C. M., & Lim, M. (2014). STAT3 Activation in Glioblastoma: Biochemical and Therapeutic Implications. *Cancers (Basel), 6*(1), 376-395. doi: 10.3390/cancers6010376

Kim, J. K., Jin, X., Sohn, Y. W., Jin, X., Jeon, H. Y., Kim, E. J., . . . Kim, H. (2014). Tumoral RANKL activates astrocytes that promote glioma cell invasion through cytokine signaling. *Cancer Lett, 353*(2), 194-200. doi: 10.1016/j.canlet.2014.07.034

Kim, S. M., & Kim, H. S. (2017). Engineering of extracellular vesicles as drug delivery vehicles. *Stem Cell Investig, 4*, 74. doi: 10.21037/sci.2017.08.07

Komada, M. (2012). Sonic hedgehog signaling coordinates the proliferation and differentiation of neural stem/progenitor cells by regulating cell cycle kinetics during development of the neocortex. *Congenital Anomalies, 52*(2), 72-77. doi: 10.1111/j.1741-4520.2012.00368.x

Lathia, J. D., Mack, S. C., Mulkearns-Hubert, E. E., Valentim, C. L. L., & Rich, J. N. (2015). Cancer stem cells in glioblastoma. *Genes & Development, 29*(12), 1203-1217. doi: 10.1101/gad.261982.115

Lau, L. W., Cua, R., Keough, M. B., Haylock-Jacobs, S., & Yong, V. W. (2013). Pathophysiology of the brain extracellular matrix: a new target for remyelination. *Nat Rev Neurosci, 14*(10), 722-729. doi: 10.1038/nrn3550

Le, D. M., Besson, A., Fogg, D. K., Choi, K. S., Waisman, D. M., Goodyer, C. G., . . . Yong, V. W. (2003a). Exploitation of astrocytes by glioma cells to facilitate invasiveness: A mechanism involving matrix metalloproteinase-2 and the urokinase-type plasminogen activator-plasmin cascade. *Journal of Neuroscience, 23*(10), 4034-4043.

Le, D. M., Besson, A., Fogg, D. K., Choi, K. S., Waisman, D. M., Goodyer, C. G., . . . Yong, V. W. (2003b). 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*(10), 4034-4043.

Lee, J., Borboa, A. K., Baird, A., & Eliceiri, B. P. (2011). Non-invasive quantification of brain tumor-induced astrogliosis. *Bmc Neuroscience, 12*. doi: Artn 9

10.1186/1471-2202-12-9

Lee, J., Tam, H., Adler, L., Ilstad-Minnihan, A., Macaubas, C., & Mellins, E. D. (2017). The MHC class II antigen presentation pathway in human monocytes differs by subset and is regulated by cytokines. *Plos One, 12*(8). doi: ARTN e0183594

10.1371/journal.pone.0183594

Li, R. H., Li, G., Deng, L., Liu, Q. L., Dai, J., Shen, J., & Zhang, J. (2010). IL-6 augments the invasiveness of U87MG human glioblastoma multiforme cells via up-regulation of MMP-2 and fascin-1. *Oncology Reports, 23*(6), 1553-1559. doi: 10.3892/or_00000795

Lin, Q. T., Balasubramanian, K., Fan, D., Kim, S. J., Guo, L. X., Wang, H., . . . Fidler, I. J. (2010). Reactive Astrocytes Protect Melanoma Cells from Chemotherapy by Sequestering Intracellular Calcium through Gap Junction Communication Channels. *Neoplasia, 12*(9), 748-754. doi: 10.1593/neo.10602

Lorger, M. (2011). Tumor microenvironment in the brain. *International Journal of Molecular Medicine, 28*, S40-S40.

Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., . . . Ellison, D. W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol, 131*(6), 803-820. doi: 10.1007/s00401-016-1545-1

Lu, K. V., Jong, K. A., Rajasekaran, A. K., Cloughesy, T. F., & Mischel, P. S. (2004). Upregulation of tissue inhibitor of metalloproteinases (TIMP)-2 promotes matrix metalloproteinase (MMP)-2 activation and cell invasion in a human glioblastoma cell line. *Lab Invest, 84*(1), 8-20. doi: 10.1038/sj.labinvest.3700003

Lu, P., Weaver, V. M., & Werb, Z. (2012). The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol, 196*(4), 395-406. doi: 10.1083/jcb.201102147

Marchetti, D., Li, J., & Shen, R. (2000). Astrocytes contribute to the brain-metastatic specificity of melanoma cells by producing heparanase. *Cancer Research, 60*(17), 4767-4770.

Markovic, D. S., Glass, R., Synowitz, M., van Rooijen, N., & Kettenmann, H. (2005). Microglia stimulate the invasiveness of glioma cells by increasing the activity of metalloprotease-2. *Journal of Neuropathology and Experimental Neurology, 64*(9), 754-762. doi: DOI 10.1097/01.jnen.0000178445.33972.a9

Martinez-Lostao, L., Anel, A., & Pardo, J. (2015). How Do Cytotoxic Lymphocytes Kill Cancer Cells? *Clinical Cancer Research, 21*(22), 5047-5056. doi: 10.1158/1078-0432.Ccr-15-0685

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U. K., Mack, M., . . . Prinz, M. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci, 10*(12), 1544-1553. doi: 10.1038/nn2015

Misra, S., Hascall, V. C., Markwald, R. R., & Ghatak, S. (2015). Interactions between hyaluronan and its receptors (CD44, RHAMM) regulate the activities of inflammation and cancer. *Frontiers in Immunology, 6*. doi: ARTN 201

10.3389/fimmu.2015.00201

Mostofa, A. G. M., Punganuru, S. R., Madala, H. R., Al-Obaide, M., & Srivenugopal, K. S. (2017). The Process and Regulatory Components of Inflammation in Brain Oncogenesis. *Biomolecules, 7*(2), 34. doi: 10.3390/biom7020034

Nagashima, G., Suzuki, R., Asai, J., & Fujimoto, T. (2002). Immunohistochemical analysis of reactive astrocytes around glioblastoma: an immunohistochemical study of postmortem glioblastoma cases. *Clin Neurol Neurosurg, 104*(2), 125-131.

Nakada, M., Nakada, S., Demuth, T., Tran, N. L., Hoelzinger, D. B., & Berens, M. E. (2007). Molecular targets of glioma invasion. *Cellular and Molecular Life Sciences, 64*(4), 458-478. doi: 10.1007/s00018-007-6342-5

Nakod, P. S., Kim, Y., & Rao, S. S. (2018). Biomimetic models to examine microenvironmental regulation of glioblastoma stem cells. *Cancer Lett, 429*, 41-53. doi: 10.1016/j.canlet.2018.05.007

O'Brien, E. R., Howarth, C., & Sibson, N. R. (2013a). The role of astrocytes in CNS tumors: pre-clinical models and novel imaging approaches. *Front Cell Neurosci, 7*, 40. doi: 10.3389/fncel.2013.00040

O'Brien, E. R., Howarth, C., & Sibson, N. R. (2013b). The role of astrocytes in CNS tumors: pre-clinical models and novel imaging approaches. *Frontiers in Cellular Neuroscience, 7*. doi: ARTN 40
10.3389/fncel.2013.00040
Okolie, O., Bago, J. R., Schmid, R. S., Irvin, D. M., Bash, R. E., Miller, C. R., & Hingtgen, S. D. (2016). Reactive astrocytes potentiate tumor aggressiveness in a murine glioma resection and recurrence model. *Neuro-Oncology, 18*(12), 1622-1633. doi: 10.1093/neuonc/now117
Oliveira, A. I., Anjo, S. I., de Castro, J. V., Serra, S. C., Salgado, A. J., Manadas, B., & Costa, B. M. (2017). Crosstalk between glial and glioblastoma cells triggers the "go-or-grow" phenotype of tumor cells. *Cell Communication and Signaling, 15*. doi: ARTN 37
10.1186/s12964-017-0194-x
Oushy, S., Hellwinkel, J. E., Wang, M., Nguyen, G. J., Gunaydin, D., Harland, T. A., . . . Graner, M. W. (2018). Glioblastoma multiforme-derived extracellular vesicles drive normal astrocytes towards a tumour-enhancing phenotype. *Philosophical Transactions of the Royal Society B-Biological Sciences, 372*(1737). doi: ARTN 20160477
10.1098/rstb.2016.0477
Placone, A. L., Quinones-Hinojosa, A., & Searson, P. C. (2016). The role of astrocytes in the progression of brain cancer: complicating the picture of the tumor microenvironment. *Tumour Biol, 37*(1), 61-69. doi: 10.1007/s13277-015-4242-0
Priego, N., Zhu, L., Monteiro, C., Mulders, M., Wasilewski, D., Bindeman, W., . . . Valiente, M. (2018). STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. *Nature Medicine, 24*(7), 1024-1035. doi: 10.1038/s41591-018-0044-4
Proia, P., Schiera, G., Mineo, M., Ingrassia, A. M. R., Santoro, G., Savettieri, G., & Di Liegro, I. (2008). Astrocytes shed extracellular vesicles that contain fibroblast growth factor-2 and vascular endothelial growth factor. *International Journal of Molecular Medicine, 21*(1), 63-67.
Qi, L., Yu, H., Zhang, Y., Zhao, D., Lv, P., Zhong, Y., & Xu, Y. (2016). IL-10 secreted by M2 macrophage promoted tumorigenesis through interaction with JAK2 in glioma. *Oncotarget, 7*(44), 71673-71685. doi: 10.18632/oncotarget.12317
Quail, D. F., & Joyce, J. A. (2017). The Microenvironmental Landscape of Brain Tumors. *Cancer Cell, 31*(3), 326-341. doi: 10.1016/j.ccell.2017.02.009
Rath, B. H., Fair, J. M., Jamal, M., Camphausen, K., & Tofilon, P. J. (2013). Astrocytes enhance the invasion potential of glioblastoma stem-like cells. *PLoS One, 8*(1), e54752. doi: 10.1371/journal.pone.0054752
Reemst, K., Noctor, S. C., Lucassen, P. J., & Hol, E. M. (2016). The Indispensable Roles of Microglia and Astrocytes during Brain Development. *Front Hum Neurosci, 10*, 566. doi: 10.3389/fnhum.2016.00566
Ristic, S., Miric, M., Jovic, S., Ristic, S., & Karic, J. (2014). Histological characteristics and markers of proliferation and differentiation in rat brain with experimental glioma. *Vojnosanit Pregl, 71*(9), 828-832.
Roth, P., Junker, M., Tritschler, I., Mittelbronn, M., Dombrowski, Y., Breit, S. N., . . . Wischhusen, J. (2010). GDF-15 Contributes to Proliferation and Immune Escape of Malignant Gliomas. *Clinical Cancer Research, 16*(15), 3851-3859. doi: 10.1158/1078-0432.Ccr-10-0705
Runeberg-Roos, P., & Saarma, M. (2007). Neurotrophic factor receptor RET: structure, cell biology, and inherited diseases. *Annals of Medicine, 39*(8), 572-580. doi: 10.1080/07853890701646256

Sbai, O., Ould-Yahoui, A., Ferhat, L., Gueye, Y., Bernard, A., Charrat, E., . . . Khrestchatisky, M. (2010). Differential Vesicular Distribution and Trafficking of MMP-2, MMP-9, and Their Inhibitors in Astrocytes. *Glia, 58*(3), 344-366. doi: 10.1002/glia.20927

Schulte, A., Gunther, H. S., Martens, T., Zapf, S., Riethdorf, S., Wulfing, C., . . . Lamszus, K. (2012). Glioblastoma stem-like cell lines with either maintenance or loss of high-level EGFR amplification, generated via modulation of ligand concentration. *Clin Cancer Res, 18*(7), 1901-1913. doi: 10.1158/1078-0432.ccr-11-3084

Seike, T., Fujita, K., Yamakawa, Y., Kido, M. A., Takiguchi, S., Teramoto, N., . . . Noda, M. (2011). Interaction between lung cancer cells and astrocytes via specific inflammatory cytokines in the microenvironment of brain metastasis. *Clinical & Experimental Metastasis, 28*(1), 13-25. doi: 10.1007/s10585-010-9354-8

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. *International Journal of Cancer, 137*(5), 1012-1020. doi: 10.1002/ijc.29380

Shnaper, S., Desbaillets, I., Brown, D. A., Murat, A., Migliavacca, E., Schluep, M., . . . Hegi, M. E. (2009). Elevated levels of MIC-1/GDF15 in the cerebrospinal fluid of patients are associated with glioblastoma and worse outcome. *Int J Cancer, 125*(11), 2624-2630. doi: 10.1002/ijc.24639

Simon, T., Coquerel, B., Petit, A., Kassim, Y., Demange, E., Le Cerf, D., . . . Vannier, J. P. (2014). Direct effect of bevacizumab on glioblastoma cell lines in vitro. *Neuromolecular Med, 16*(4), 752-771. doi: 10.1007/s12017-014-8324-8

Simon, T., Gagliano, T., & Giamas, G. (2017). Direct Effects of Anti-Angiogenic Therapies on Tumor Cells: VEGF Signaling. *Trends Mol Med, 23*(3), 282-292. doi: 10.1016/j.molmed.2017.01.002

Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci, 32*(12), 638-647. doi: 10.1016/j.tins.2009.08.002

Sofroniew, M. V. (2014). Astrogliosis. *Cold Spring Harb Perspect Biol, 7*(2), a020420. doi: 10.1101/cshperspect.a020420

Steelman, L. S., Abrams, S. L., Whelan, J., Bertrand, F. E., Ludwig, D. E., Basecke, J., . . . McCubrey, J. A. (2008). Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. *Leukemia, 22*(4), 686-707. doi: 10.1038/leu.2008.26

Steelman, L. S., Pohnert, S. C., Shelton, J. G., Franklin, R. A., Bertrand, F. E., & McCubrey, J. A. (2004). JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. *Leukemia, 18*(2), 189-218. doi: 10.1038/sj.leu.2403241

Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J. B., Janzer, R. C., . . . Trials, N. C. I. C. C. (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncology, 10*(5), 459-466. doi: 10.1016/S1470-2045(09)70025-7

Sulzmaier, F., Opoku-Ansah, J., & Ramos, J. W. (2012). Phosphorylation is the switch that turns PEA-15 from tumor suppressor to tumor promoter. *Small GTPases, 3*(3), 173-177. doi: 10.4161/sgtp.20021

Taylor, A. R., Robinson, M. B., Gifondorwa, D. J., Tytell, M., & Milligan, C. E. (2007). Regulation of heat shock protein 70 release in astrocytes: Role of signaling kinases. *Developmental Neurobiology, 67*(13), 1815-1829. doi: 10.1002/dneu.20559

Tchirkov, A., Khalil, T., Chautard, E., Mokhtari, K., Veronese, L., Irthum, B., . . . Verrelle, P. (2007). Interleukin-6 gene amplification and shortened survival in glioblastoma patients. *British Journal of Cancer, 96*(3), 474-476. doi: 10.1038/sj.bjc.6603586

Tkach, M., & Thery, C. (2016). Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell, 164*(6), 1226-1232. doi: 10.1016/j.cell.2016.01.043

Toda, M. (2013). Glioma stem cells and immunotherapy for the treatment of malignant gliomas. *ISRN Oncol, 2013*, 673793. doi: 10.1155/2013/673793

Turtoi, A., Musmeci, D., Naccarato, A. G., Scatena, C., Ortenzi, V., Kiss, R., . . . Castronovo, V. (2012). Sparc-Like Protein 1 Is a New Marker of Human Glioma Progression. *Journal of Proteome Research, 11*(10), 5011-5021. doi: 10.1021/pr3005698

Vargova, L., & Sykova, E. (2014). Astrocytes and extracellular matrix in extrasynaptic volume transmission. *Philos Trans R Soc Lond B Biol Sci, 369*(1654), 20130608. doi: 10.1098/rstb.2013.0608

Vasudev, N. S., & Reynolds, A. R. (2014). Anti-angiogenic therapy for cancer: current progress, unresolved questions and future directions. *Angiogenesis, 17*(3), 471-494. doi: 10.1007/s10456-014-9420-y

Verhaak, R. G., Hoadley, K. A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M. D., . . . Cancer Genome Atlas Research, N. (2010). Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell, 17*(1), 98-110. doi: 10.1016/j.ccr.2009.12.020

Voskuhl, R. R., Peterson, R. S., Song, B., Ao, Y., Morales, L. B., Tiwari-Woodruff, S., & Sofroniew, M. V. (2009). Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. *J Neurosci, 29*(37), 11511-11522. doi: 10.1523/JNEUROSCI.1514-09.2009

Wang, L., Qin, H., Li, L., Zhang, Y., Tu, Y., Feng, F., . . . Gao, G. (2012). Overexpression of CCL20 and its receptor CCR6 predicts poor clinical prognosis in human gliomas. *Med Oncol, 29*(5), 3491-3497. doi: 10.1007/s12032-012-0314-9

Wang, R., Chadalavada, K., Wilshire, J., Kowalik, U., Hovinga, K. E., Geber, A., . . . Tabar, V. (2010). Glioblastoma stem-like cells give rise to tumour endothelium. *Nature, 468*(7325), 829-833. doi: 10.1038/nature09624

Watanabe, Y., Yamasaki, F., Kajiwara, Y., Saito, T., Nishimoto, T., Bartholomeusz, C., . . . Kurisu, K. (2010). Expression of phosphoprotein enriched in astrocytes 15 kDa (PEA-15) in astrocytic tumors: a novel approach of correlating malignancy grade and prognosis. *J Neurooncol, 100*(3), 449-457. doi: 10.1007/s11060-010-0201-1

Wendler, F., Favicchio, R., Simon, T., Alifrangis, C., Stebbing, J., & Giamas, G. (2016). Extracellular vesicles swarm the cancer microenvironment: From tumor-stroma communication to drug intervention (In Press). *Oncogene*.

Wiesenhofer, B., Weis, C., & Humpel, C. (2000). Glial cell line-derived neurotrophic factor (GDNF) is a proliferation factor for rat C6 glioma cells: evidence from antisense experiments. *Antisense & Nucleic Acid Drug Development, 10*(5), 311-321. doi: DOI 10.1089/oli.1.2000.10.311

Yanez-Mo, M., Siljander, P. R., Andreu, Z., Zavec, A. B., Borras, F. E., Buzas, E. I., . . . De Wever, O. (2015). Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles, 4*, 27066. doi: 10.3402/jev.v4.27066

Yin, Y., Qiu, S., Li, X., Huang, B., Xu, Y., & Peng, Y. (2017). EZH2 suppression in glioblastoma shifts microglia toward M1 phenotype in tumor microenvironment. *J Neuroinflammation, 14*(1), 220. doi: 10.1186/s12974-017-0993-4

Zamanian, J. L., Xu, L. J., Foo, L. C., Nouri, N., Zhou, L., Giffard, R. G., & Barres, B. A. (2012). Genomic Analysis of Reactive Astrogliosis. *Journal of Neuroscience, 32*(18), 6391-6410. doi: 10.1523/Jneurosci.6221-11.2012

Zeisberg, M., & Neilson, E. G. (2009). Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest, 119*(6), 1429-1437. doi: 10.1172/JCI36183

Zhang, L., Alizadeh, D., Van Handel, M., Kortylewski, M., Yu, H., & Badie, B. (2009). Stat3 inhibition activates tumor macrophages and abrogates glioma growth in mice. *Glia, 57*(13), 1458-1467. doi: 10.1002/glia.20863

Zhang, L., Zhang, S., Yao, J., Lowery, F. J., Zhang, Q., Huang, W.-C., . . . Yu, D. (2015). Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth. *Nature, 527*(7576), 100-104. doi: 10.1038/nature15376

Zhang, Z., Yang, M., Chen, R., Su, W., Li, P., Chen, S., . . . Hu, C. (2014). IBP regulates epithelial-to-mesenchymal transition and the motility of breast cancer cells via Rac1, RhoA and Cdc42 signaling pathways. *Oncogene, 33*(26), 3374-3382. doi: 10.1038/onc.2013.337

Zhao, X., Chen, R. J., Liu, M., Feng, J. F., Chen, J., & Hu, K. L. (2017). Remodeling the blood-brain barrier microenvironment by natural products for brain tumor therapy. *Acta Pharmaceutica Sinica B, 7*(5), 541-553. doi: 10.1016/j.apsh.2017.07.002

Zhao, Z., Nelson, A. R., Betsholtz, C., & Zlokovic, B. V. (2015). Establishment and Dysfunction of the Blood-Brain Barrier. *Cell, 163*(5), 1064-1078. doi: 10.1016/j.cell.2015.10.067

Zong, H., Verhaak, R. G., & Canoll, P. (2012). The cellular origin for malignant glioma and prospects for clinical advancements. *Expert Rev Mol Diagn, 12*(4), 383-394. doi: 10.1586/erm.12.30

Legends

Table Of Contents Image: Astrocytes, the rising stars of the glioblastoma microenvironment (Servier Medical Art)

Under the influence of glioblastoma cells, tumor associated astrocytes support tumor progression and resistance to therapeutic strategies through the secretion of cytokines, formation of gap junctions, production of extracellular vesicles but also modification of the brain extracellular matrix.

Figure 1: Tumor associated astrocytes support glioblastoma invasion through the activation of MMP-2 (Inspired by Le *et al,* 2003 – Servier Medical Art).

In the GBM microenvironment, pro-uPA produced by both tumor cells and TAAs can reach its receptor at the astrocytes surface and get activated. Plasminogen is consequently activated into plasmin that, in turn, cleaves pro-MMP-2 to produce the active MMP-2. Through the partial degradation of the brain ECM by MMP-2, GBM cells invasion is thus promoted. ECM: Extra Cellular Matrix; MMP-2: Matrix Metalloprotease 2; uPA: urokinase Plasminogen Activator; uPA-R: uPA-Receptor.

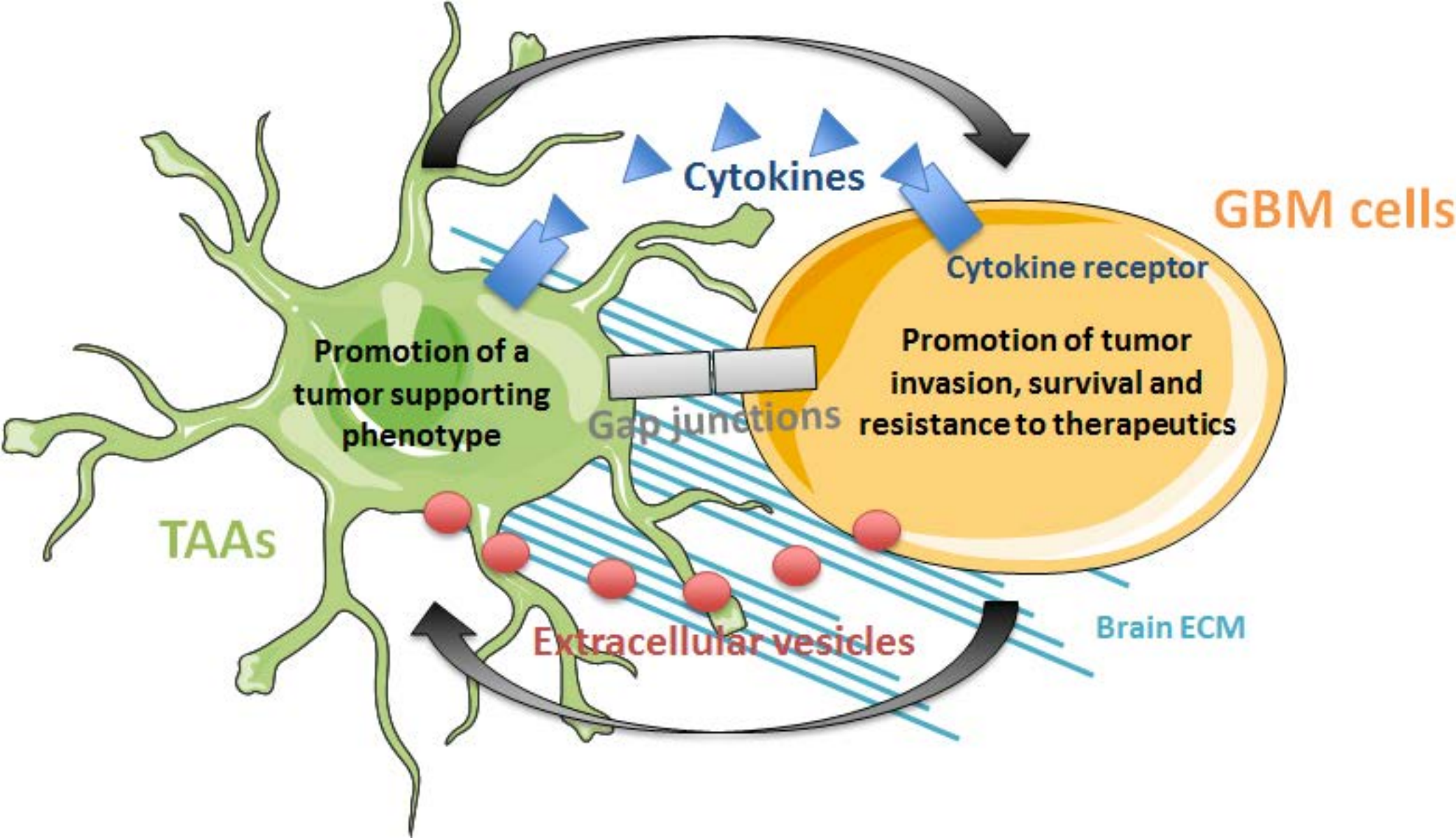
Figure 2: Tumor growth-associated hypoxia triggers astrocytes-dependent support of glioblastoma cells invasion and aggressiveness via the CCL20/CCR6 signaling (Inspired by Jin *et al,* 2018 – Servier Medical Art).

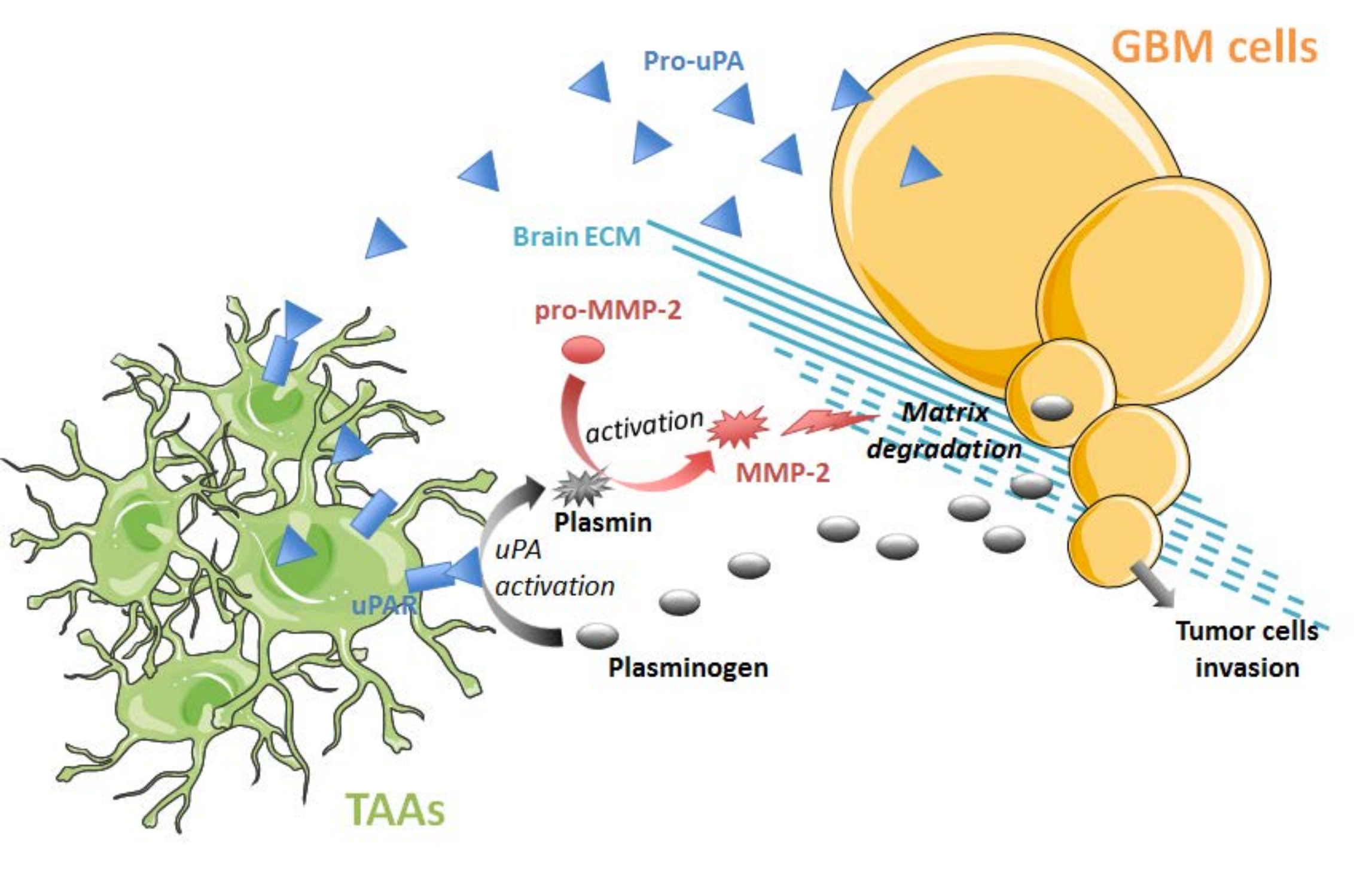
TAAs secretion of CCL20 is stimulated upon the rise of hypoxic conditions in the GBM microenvironment. CCL20 can then bind its receptor CCR6 at the GBM cell surface, thus leading to the activation of the NF-κB pathway that contributes to stabilize HIF-1α. Consequently, HIF-1 dependent signaling pathways get activated, then promoting pro-angiogenic and tumor cell invasion mechanisms. CCL20: C-c motif Ligand 20; CCR6: C-C chemokine receptor type 6; GBM: Glioblastoma; TAAs: Tumor Associated Astrocytes.

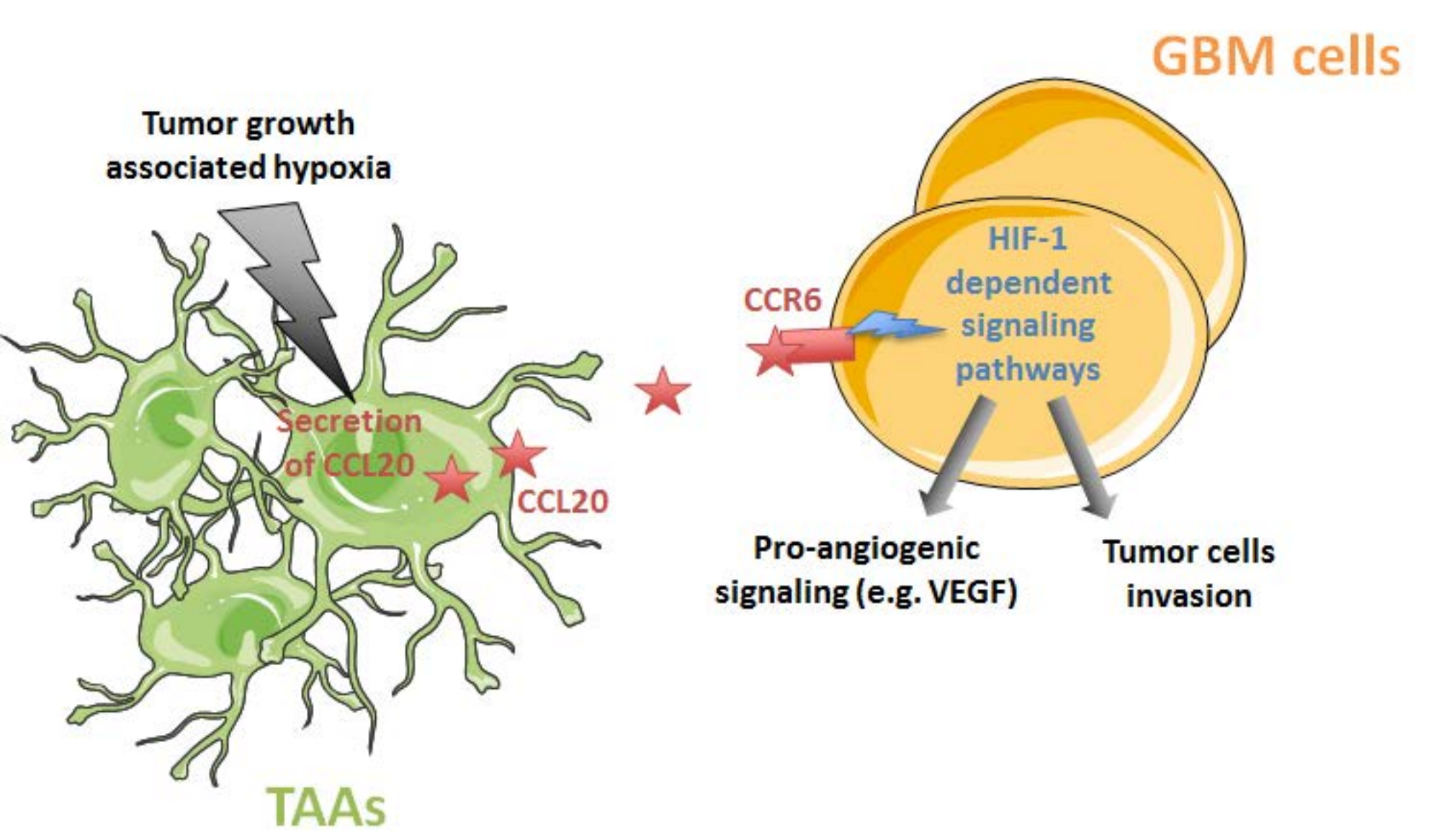
Figure 3: GAP junctions allow the transfer of cGAMP from glioblastoma cells to surrounding tumor associated astrocytes (Inspired by Chen *et al,* 2016 – Servier Medical Art).

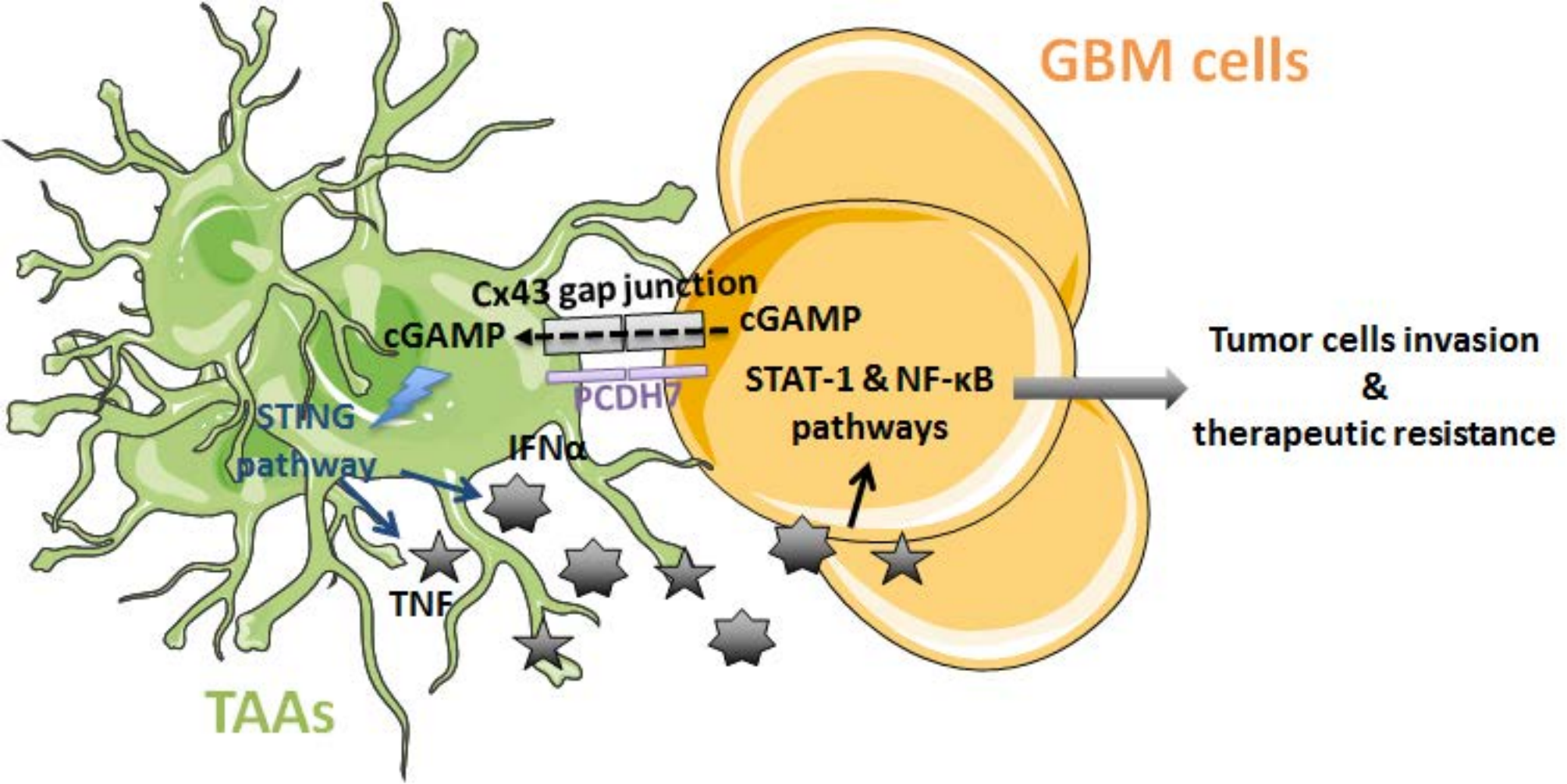
Gap junctions established through Cx43 and PCDH7 allow the transfer of cGAMP from GBM cells to TAAs. The STING pathway is stimulated by cGAMP in TAAs, thus promoting the cell

production and release of factors such as TNF and IFNα in the GBM microenvironment. Those factors can, in turn, activate the STAT-1 and NF-κB in GBM cells, consequently supporting tumor cell invasion and resistance to chemotherapies. cGAMP: 2'3'-cyclic GMP-AMP second messenger; Cx43: connexin 43; IFNα: Interferon alpha; PCDH7: Protocadherin-7; STAT-1: Signal transducer and activator of transcription 1; STING: Stimulator of interferon genes, TNF: Tumor necrosis factor.


Cytokines
GBM cells
Cytokine receptor
Promotion of a tumor supporting phenotype
Promotion of tumor invasion, survival and resistance to therapeutics
Gap junctions
TAAs
Extracellular vesicles
Brain ECM


GBM cells
Pro-uPA
Brain ECM
pro-MMP-2
activation
MMP-2
Matrix degradation
Plasmin
uPA activation
uPAR
Plasminogen
Tumor cells invasion
TAAs


GBM cells
Tumor growth associated hypoxia
Secretion of CCL20
CCL20
TAAs
CCR6
HIF-1 dependent signaling pathways
Pro-angiogenic signaling (e.g. VEGF)
Tumor cells invasion


GBM cells
Cx43 gap junction
cGAMP
cGAMP
PCDH7
STAT-1 & NF-κB
pathways
Tumor cells invasion
&
therapeutic resistance
STING
pathway
IFNα
TNF
TAAs