University of Groningen

The effect of the tumour microenvironment (TME) on the mesenchymal transition in glioblastoma

Hennie Heida, s2735989
4/7/2021

Supervisor: F.A.E. Kruyt

Abstract

Glioblastoma multiforme (GBM) is the most frequent and malignant type of primary brain tumour. This tumour is difficult to treat, as a result of its invasive nature and heterogeneity. Because of this heterogeneity, GBM has been classified into different subtypes, of which the mesenchymal subtype is the most aggressive type. Since subtypes can shift to one another, it is important to study the mesenchymal transition. Emerging studies imply the importance of targeting the tumour microenvironment (TME), instead of the glioblastoma cells themselves. Importantly, the TME seems to affect the MES transition. Here, the precise mechanism of this is studied for the TAMs, astrocytes and ECs. The TME affects the MES transition by promoting an anti-inflammatory phenotype, and by using hypoxia in their favour. Additionally, it plays a role in the treatment-induced MES transition. Treatment should focus on shifting the phenotype of tumour cells from anti-inflammatory to pro-inflammatory, as well as use anti-angiogenic therapy that is adjusted to the hypoxic environment. In addition, the resistance to the TME-targeted therapies should be assessed. Further studies are necessary to also evaluate the effect of the other TME cell types, as well as to investigate the other subtypes in order to ensure effective treatment of the MES transition.

Table of contents

		Page number
Abstract		2
Table of contents		3
Introduction		4
Role of the TME in MES transition		6
	TAMs	*6*
Promotion of an immunosuppressive TME		6
	Astrocytes	6
Angiogenesis and the role of ECs		7
Treatment-induced MES transition		8
	Radiation-induced MES transition	8
	Anti-angiogenic treatment-induced MES transition	8
Strategy for therapy		9
Shifting the anti-inflammatory phenotype to a pro-inflammatory phenotype		9
	TAMs	9
	Astrocytes	10
Targeting the vasculature		10
Discussion		11
Conclusion		12
References		13

Introduction

Glioblastoma multiforme (GBM) is the most frequent and malignant type of primary brain tumour (Festuccia *et al.*, 2020). This type of tumour is very aggressive and despite the numerous advanced therapies, it remains a challenge to treat this type of tumour effectively (Paw *et al.*, 2015; Soeda *et al.*, 2015), with patients surviving only approximately 15 months after diagnosis (Fanelli *et al.*, 2021).

Current treatment includes surgical resection, followed by radiotherapy combined with either temozolomide or bevacizumab (Davis, 2016; Bajetto *et al.*, 2020). Temozolomide (TMZ) is a DNA-alkylating agent that methylates DNA, whereas bevacizumab is an antibody binding vascular endothelial growth factor-α (VEGF-α), developed to inhibit VEGF signalling (Davis, 2016; Simon *et al.*, 2020; Wang *et al.*, 2021; Xie *et al.*, 2014).

However, due to the spreading of tumour cells into the healthy brain tissue, it is nearly impossible to remove the tumour completely, and a few tumour cells reside after surgery (Monteiro *et al.*, 2017). Radiotherapy is used to remove the remaining cells, but due to the presence of glioma stem-like cells (GSCs) that are resistant to therapy, a subset of resistant tumour cells remains. These GSCs have neural stem-like properties and can self-renew and proliferate, leading to recurrence (Azam *et al.*, 2020; Roos *et al.*, 2017; Shergalis *et al.*, 2018). Thus, despite tumour resection and multiple therapies, GBM still has a high recurrence rate, with more than 90% of GBM patients experiencing recurrence (Wang *et al.*, 2021).

Next to the invasive nature of GBM, treatment is very challenging because of the intra- and intertumoral heterogeneity of the disease (Shergalis *et al.*, 2018; Zinn *et al.*, 2015). In GBM, multiple subpopulations of cancer cells develop within the same tumour, and certain subpopulations are more resistant against treatment than others. Thus, these subpopulations can cause recurrence (Qazi *et al.*, 2017).

To address this heterogeneity, attempts have been made to classify the GBM into different subtypes. Verhaak *et al.* (2010) used genomic profiling and classified GBM into the Proneural, Neural, Classical and Mesenchymal subtype. Interestingly, the subtypes in GBM can shift from one subtype to another, of which the pro-neural to mesenchymal transition (PMT) is believed to be the most outstanding (Azam *et al.*, 2020). This transition can simply happen during tumour progression but is also induced by radiation treatment (Behnan *et al.*, 2019). Importantly, transformation to the mesenchymal subtype is associated with treatment resistance (Azam *et al.*, 2020). Thus, studying the mesenchymal (MES) subtype is important as to understand its resistance and to be able to design effective treatment.

Because of the resistance of GBM, researchers are continuously attempting to discover new targets and ways of treating GBM. It has come to light that perhaps, treatment should not focus on the GBM tumour cells, but rather on the tumour microenvironment (TME) (Lim *et al.*, 2020). This is because evidence is emerging that the TME plays an important role in regulating tumour progression (Quail & Joyce, 2017). It is shown that the components of the TME, under which are multiple cell types, actively take part in tumorigenesis by *e.g.* interacting with the tumour cells (Lim *et al.*, 2020).

Importantly, the composition of the TME has been found to be able to regulate the molecular subtype (Yamini, 2018). Especially interesting are the emerging studies that show that the TME can drive mesenchymal subtype tumours (Behnan *et al.*, 2019). However, the role of the TME in MES transformation is still not fully understood and needs further research (Azam *et al.*, 2020). Therefore, this essay will delve into the mechanisms of how the TME affects the MES transformation.

The research question that will thus be answered in this essay, is:
How does the tumour microenvironment (TME) affect the mesenchymal transition in glioblastoma?

The TME consists of many components. It includes various types of cells, along with soluble factors and extracellular matrix components. The collection of cells includes cancerous as well as non-cancerous cells, namely immune cells, endothelial cells (ECs), GSCs and astrocytes, to name a few (Perus & Walsh, 2019; Simon *et al.*, 2020).

Since there is such a wide variety of TME components, in this essay only certain types of cells will be addressed. These are first off the immune cells, as the TME in GBM is characterized by immunosuppression and this plays an important role in the difficulty of finding effective therapies. In particular the focus will be put on the tumour associated macrophages/microglia (TAMs), since these cells take up most of the volume in the immune system (Azam *et al.*, 2020). Since the GBM is also characterized by hypoxic regions and necrosis, the role of endothelial cells (ECs) will also be researched. The interaction with TAMs will also be researched, as the vasculature is generally known to regulate immunity by controlling lymphocyte trafficking (Allen *et al.*, 2017). Finally, a look will be taken at the role of astrocytes, since these cells recently emerged as potential therapeutic targets (Heiland *et al.*, 2019).

After researching the role of these TME components in MES transition, this information will be used to discuss the strategies for treatment.

The role of the TME in MES transition

The involvement of the TME in the MES subtype was already acknowledged several years ago, when studies showed that the MES subtype displays a high degree of macrophages/microglial infiltration. Bhat *et al.* (2013) further proved their possible involvement in MES differentiation, by showing that the extent of their infiltration correlated with the MES regions. Herein, the macrophages were suggested to be the source of TNF-α, which mediates MES differentiation via NF-kB (Bhat *et al.*, 2013; Fedele *et al.*, 2019; Perus & Walsh, 2019). Additionally, immune cell-deficient glioma spheres were found to be for the large part of the PN subtype, despite originating from MES tumours. This further reinforces the idea that the TME has a role in promoting the MES subtype (Bhat *et al.*, 2013; Perus & Walsh, 2019). As the research so far has mainly focused on the role of TAMs in this promotion, these cells will be discussed first.

TAMs

TAMs are in GBM TME the largest stromal cell population and are mainly enriched with the MES subtype (Azam *et al.*, 2020; Buonfiglioli & Hambardzumyan, 2021). In addition, the TAM amount is negatively correlated with the GBM survival, which further affirms the importance of targeting this group of cells (Peterson *et al.*, 2016). This group of cells is a mixed population of activated brain-resident microglia and infiltrating bone marrow-derived macrophages (BMDMs) (Buonfiglioli & Hambardzumyan, 2021; Kaffes *et al.*, 2019).

The brain-resident microglia are an important group of immune cells of the central nervous system (CNS), where they operate as phagocytic cells and take part in immune surveillance. They are often mentioned as macrophages of the CNS instead (Buonfiglioli & Hambardzumyan, 2021; Roesch *et al.*, 2018). Whereas the microglia reside in the brain, the BMDMs originate from the hematopoietic stem cells. BMDMs infiltrate the tumour following the secretion of soluble factors by GBM cells (Fanelli *et al.*, 2021; Miyauchi & Tsirka, 2018; Roesch *et al.*, 2018).

Since the finding that TAMs potentially mediates MES differentiation, more studies have been performed that research the interaction of TAMs with the GBM. Researchers found that this interaction takes place through the releasing of factors, cytokines, and by regulatory mechanisms. This interaction, which is bilateral, changes the tumour phenotype to a more aggressive state, and can induce mesenchymal transformation (Azam *et al.*, 2020; Buonfiglioli & Hambardzumyan, 2021). The first and foremost important phenomenon that contributes to this change in tumour aggressiveness, is the promotion of an immunosuppressive TME.

Promotion of an immunosuppressive TME

After being recruited to the tumour, the GBM can change the phenotype of TAMs by secreting immunosuppressive factors such as transforming growth factor-β1 (TGF-β1) and colony-stimulating factor-1 (CSF-1) (Cui *et al.*, 2020). Through this process, called polarization, macrophages can be polarized towards either a pro- or an anti-inflammatory phenotype (M1 and M2 TAMs, respectively) (Almahariq *et al.*, 2021). Once polarized, M2 TAMs help in turn promote a pro-tumorigenic environment, and contribute to an immunosuppressive microenvironment (Fanelli *et al.*, 2021; Roesch *et al.*, 2018). The TAMs are *e.g.* able to suppress the T-cell function (Buonfiglioli & Hambardzumyan, 2021). Thus, the GBM can shift the phenotype of TAMs to promote tumour-progression.

Astrocytes

Similarly, the GBM can change the phenotype of other cells in the TME as well, also using these cells to support the tumour. An example are astrocytes. Astrocytes make up approximately 50% of the cells in the brain (Placone *et al.*, 2016). Normally, astrocytes play an active role in the brain, *e.g.* playing a major role in forming the brain-blood barrier (BBB). Here, the end-feet of astrocytes cover the outer surface of the vasculature. This is also where they directly interact with the ECs and pericytes, supporting the BBB maintenance (Brandao *et al.*, 2019). Furthermore, astrocytes can become activated as response to CNS injury, after which they participate in wound healing of the brain, a process called reactive gliosis. Herein, astrocytes form a protective barrier to limit the tissue damage (Quail & Joyce, 2017).

However, astrocytes can also be reprogrammed by the tumour cells to promote its progression (Brandao *et al.*, 2019; Quail & Joyce, 2017). The effect of reactive astrocytes has been shown before, wherein the factors secreted by normal reactive astrocytes were shown to also increase GBM proliferation. Since the tumour also induces destruction in order to infiltrate into the healthy tissue, tumour progression itself can also lead to the activation of astrocytes (Brandao *et al.*, 2019).

The reprogramming of astrocytes is performed through the gap junctions that astrocytes can form with tumour cells. The tumour cells hereby induce a pro-inflammatory program in the astrocytes, that in turn stimulates the latter to produce cytokines, that in turn aid the tumour by supporting its outgrowth (Quail & Joyce, 2017).

In addition, astrocytes also support an immunosuppressive TME by interacting with TAMs, and this immunosuppression can be promoted through their interaction. This was found by Heiland *et al.* (2019), who also demonstrated that for the increase of GBM-promoting astrocytes, the presence of microglia is crucial (Buonfiglioli & Hambardzumyan, 2021).

Further supporting the notion for the role of astrocytes in MES transition, is the study by Niklasson *et al.* (2019). They found that the induction of reactive astrocyte gene sets is involved in GBM MES transition.

Angiogenesis and the role of ECs

Next to immunosuppression, another hallmark of GBM is angiogenesis. Angiogenesis is crucial for the growth and progression of a GMB tumour (Ahir *et al.*, 2020). Thus, anti-angiogenic therapies are used for treatment. As a result, there is rapid tumour growth and simultaneously inadequate vascularization, leading to hypoxia and necrosis.

However, hypoxia induces the expression of the hypoxia-inducible factor (HIF) family of transcription factors, that mediate the response of the tumour to hypoxia. HIFs are composed of an HIFα and a HIFβ subunit, of which HIFα is stabilized under hypoxic conditions. Joseph and colleagues showed that hypoxia is able to induce a mesenchymal shift, in which HIFα is an essential mediator (Joseph *et al.*, 2015). The effect of hypoxia, together with that of radiation and the interaction of TAMs with GBM cells, is shown in **Figure 1**.

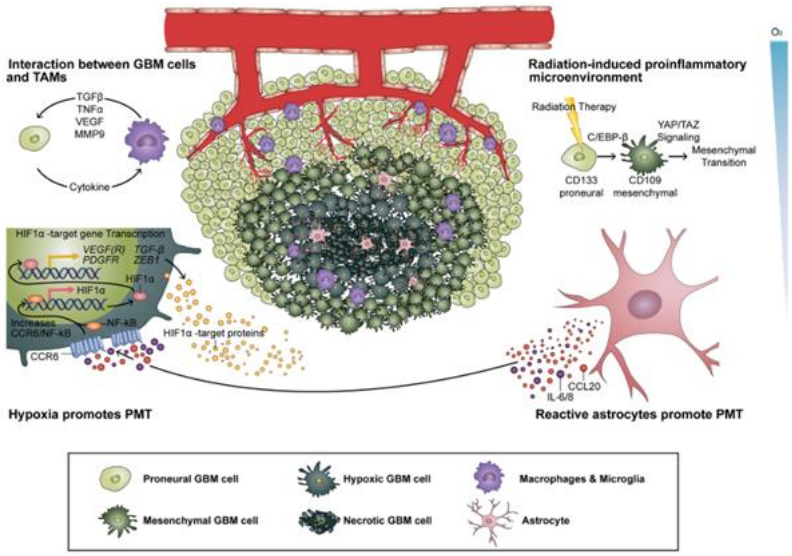

Figure 1. The different mechanisms of how PMT can be induced by microenvironmental factors. Adapted from "Perspective of mesenchymal transformation in glioblastoma" by Kim *et al.*, 2021.

Additionally, TAMs can similar to interacting with astrocytes, also interact with endothelial cells (Buonfiglioli & Hambardzumyan, 2021). TAMs have also been shown to be involved in angiogenesis in brain tumours (Quail & Joyce, 2017). Here, they can sense the hypoxic condition and

control angiogenesis by the production of IL-1β and increase VEGF-A expression. These in turn can adjust the permeability of the vessels and regulate angiogenesis (Buonfiglioli & Hambardzumyan, 2021). In addition, Wang and colleagues showed that tumour-associated ECs can induce macrophage alternative polarization by expressing and releasing IL-6. IL-6 can join with CSF-1 and induce HIF2-a dependent expression of arginase 1 through the activation of peroxisome proliferator-activated receptor-y (PPAR-y) in macrophages, that leads to their polarization (Wang *et al.*, 2018).

In addition to hypoxia, the necrotic microenvironment may be involved in the transition to the MES subtype as the tumours progress. This is indicated by the finding that there is a high level of necrosis in the MES subtype, as well as by the results of Cooper *et al.* (2012) who showed that an increase in level of necrosis led non-MES GBMs to become more transcriptionally similar to the MES subtype (Verhaak *et al.*, 2010).

Treatment-induced MES transition

As mentioned before, the mesenchymal transition not only happens during tumour progression but is also suggested to be induced by treatment, *e.g.* through radiation, anti-angiogenic treatment and DNA damaging agents like TMZ (Behnan *et al.*, 2019; Kim *et al.*, 2021).

Radiation-induced MES transition

Minata and colleagues have shown that after radiation, glioma stem-like cells of the Proneural subtype lose expression of CD133 and acquire CD109 expression instead, which drives oncogenic signalling and subsequently leads the cells to express mesenchymal features (Minata *et al.*, 2019). Radiation also affects components of the TME, although the underlying mechanisms are still not fully understood (Ali *et al.*, 2021). Still, enough results imply the importance of studies researching this subject.

One rather self-explanatory change is the increase in infiltration of immune cells into the brain parenchyma, as a result of the radiation-induced increase in vessel permeability. Thus, radiation hereby indirectly affects inflammation. If radiation results in chronic inflammation, this can stabilize HIF-1 through high levels of intracellular NO in GBM cells. HIF-1 in turn induces the expression of stromal-derived factor 1 (SDF-1) which promotes the recruitment of macrophages upon radiation (Gupta & Burns, 2018).

Ali *et al.* (2021), however found that radiation therapies led to a decrease in the accumulation of T-cells and of different myeloid cell populations in the TME (Ali *et al.*, 2021). This was unexpected, as the MES subtype is characterized by a high level of TAMs and increased levels of T-cells (Kaffes *et al.*, 2019). They suggested that this decrease is a result of the disrupted blood vessels that otherwise would serve as a way of delivering the T-cells to the tumour. They also suggested that this disruption is most presumably the result of radiation-induced necrosis in tumours that leads to the death of GBM cells (Ali *et al.*, 2020). Thus, this suggests that immune cell infiltration depends on the amount of radiation. Whereas radiation can increase the infiltration of immune cells, this is not the case if radiation additionally induces necrosis and immune cells lack vasculature to reach an area in the tumour to infiltrate.

In addition to inducing the infiltration of immune cells, radiation can also directly affect the TAMs. In their study, Akkari *et al.* (2020) showed that radiation altered the transcriptional program of brain-resident microglia and monocyte-derived macrophages and increased the number of TAMs at several time points during radiation treatment. Together with the finding that irradiation is also followed by a change in mRNA expression, associated with a positive regulation of macrophage chemotaxis (Doan *et al.*, 2018; Grégoire *et al.*, 2020; Kim *et al.*, 2021), this suggests that radiotherapy results in TAM recruitment. And since TAM recruitment can in turn cause MES transition, and thus an increase in radio-resistance, these findings indicate that MES transition is a response to treatment (Kim *et al.*, 2021).

Anti-angiogenic treatment-induced MES transition

Chronic anti-angiogenic therapy can also ultimately promote MES transition, at least that of PMT. It was found that this kind of therapy led to the shortening of tumour vessels and hereby

potentiated hypoxia. This subsequently worsened the inflammation and angiogenesis in the TME and thus promoted PMT (Kim *et al.*, 2021). This is supported by the finding that anti-VEGF therapy is able to raise the recruiting of TAMs in preclinical GBM models (Peterson *et al.*, 2016).

Furthermore, similar to how MES transition is indicated to promote resistance to radiotherapy, it has also been shown to promote anti-angiogenic therapy resistance (Chandra *et al.*, 2020). An example is through the workings of TAMs, which in addition to their aforementioned role in tumour angiogenesis, also have been implicated in resistance to anti-angiogenic treatment (Quail & Joyce, 2017).

Additionally, similar to TAMs, astrocytes are also seen to potentially protect the tumour against treatment. Reactive astrocytes are suggested to play a role in immunoprotection, based on the fact that they secrete factors, *e.g.* tenascin-C (TNC), IL-10 and STAT-3, which all have been shown to be involved in GBM immunoprotection (Brandao *et al.*, 2018; Placone *et al.*, 2016). Interestingly, TNC has been shown to be overexpressed in MES GBM, where it promotes the cancer cell plasticity (Angel *et al.*, 2020).

Strategy for therapy

Taking all the events in mind that take place in the TME and subsequently affect the GBM phenotype and MES transition, various methods for treatment seem necessary to ensure efficient treatment of GBM.

Shifting the anti-inflammatory phenotype to a pro-inflammatory phenotype

One such strategy is shifting the anti-inflammatory phenotype to a pro-inflammatory one, for example the phenotype of the TAMs (Heiland *et al.*, 2019). This strategy has a higher chance of being efficient than depleting the TAM populations altogether (Quail & Joyce, 2017). As mentioned, the pro-inflammatory TME plays a major factor in the resistance to therapy, switching the phenotype would thus be truly convenient for successful treatment.

TAMs

One way to create a pro-inflammatory phenotype is through inhibition of colony-stimulating factor-1 receptor (CSF-1R) (Quail & Joyce, 2017). CSF1 is required for the recruitment of TAMs into the TME, and TAMs depend on CSF for their survival and differentiation, as mentioned previously (Xu *et al.*, 2020). Correspondingly, inhibiting CSF-1R either depletes or depolarizes the TAMs (Quail & Joyce, 2017).

Almahariq *et al.* (2021) found that the CSF-1R inhibitor BLZ-945 enhances the efficacy of radiotherapy as well as reduces the immune suppression in GBM. In another study, it is furthermore showed that targeting TAMs with BLZ-945 combined with radiotherapy increased the survival in a preclinical model considerably (Akkari *et al.*, 2020).

Although these studies seem promising, a previous study did find resistance to some extent. The study in which this resistance to BLZ-945 was found, was carried out by Quail *et al.* (2016). Using a mouse model, they found that although general survival was prolonged, more than 50% of tumours recurred after treatment with BLZ-945. They found that this resistance was tumour cell-extrinsic and thus driven by the TME, as the sensitivity to BLZ945 treatment was re-established in the tumour cells when they were transplanted in a naïve setting. Further investigation led to the conclusion that activation of PI3K underlies this resistance, together with the activation of IGF1R. This activation is a result of IGF1 secretion into the extracellular environment by TAMs, in which IGF-1 is upregulated as response to long-term CSF-1R inhibition. Fortunately, the efficiency of BLZ-945 was increased with combinatorial treatment. Blockade of insulin-like growth factor-1 receptor (IGF-1R) concomitant with treatment with BLZ-945 extended the median survival in a mouse model (Quail *et al.*, 2016).

When investigating the effect of the TME in CSF-1R inhibition, Quail *et al.* (2016) also found that the rebounding tumours were consistently appearing next to regions of glial scarring. This led to the hypothesis that the response to brain injury possibly contributes to a TME that likely provokes

recurrence. Consistently, they found that compared to vehicle TAMs, the gene sets of rebound TAMs were enriched for interleukin 4 (IL4) and TGFβ, IL4 being a cytokine that is known to mediate alternate activation of macrophages with a wound-healing phenotype. Thus, CSF-1R resistance may be triggered by the glial scarring, that subsequently induces IGF1 secretion, leading to resistance (Quail *et al.*, 2016; Quail & Joyce, 2017).

Astrocytes

This suggests that glial scarring plays a role in triggering resistance to CSF-1R inhibition. This suggestion, together with the aforementioned finding that astrocytes also interact with TAMs, which promotes immune-suppression (Heiland *et al.*, 2019; Quail & Joyce, 2017), highlight the astrocytes as a potential target for GBM treatment. Unfortunately, so far there is still very little known about the effects of astrogliosis on brain tumours. Since there is, however, sufficient information about the interaction between astrocytes and microglia (Buonfiglioli & Hambardzumyan, 2021; Schiffer *et al.*, 2018), here the possible treatment targeting this interaction will be discussed instead.

Since the increase in reactive pro-tumorigenic astrocytes in GBM also depends on the presence of microglia (Buonfiglioli & Hambardzumyan, 2021; Heiland *et al.*, 2019), the blockage of CSF-1R is one proposed approach to targeting the interaction (Matias *et al.*, 2018). Furthermore, researchers aim to target the chemokines and their receptors in microglial cells. These form potentially a good target as they are highly expressed and play a major role in tumour progression. One example is the inhibition of the receptor of SDF-1 with AMD3100. Similar to CSF1, inhibiting SDF1 targets the macrophages (Matias *et al.*, 2018).

Furthermore, targeting the gap junctions and their connexins (CXs) could be a good approach for decreasing the interaction. Matias *et al.* also suggest this, based on the evidence from the study by Hong *et al.* (2015). Herein, researchers showed that gap junctions can also be used by GBM cells to transfer micro ribonucleic acids (miRs) to astrocytes (Matias *et al.*, 2018).

Targeting the vasculature

Since anti-angiogenic therapy has so far not been successful, attempts continue to be made to improve its efficacy. One such approach is using combinatorial treatment, similar to how the blockade of IGF1R concomitantly with BLZ-945 increased the effect of the latter. An example is the study by Peterson *et al.* (2016). Here, the researchers combined cediranib, which inhibits VEGFR tyrosine kinase, with MEDI3617, an antibody that is Angiopoietin-2 (Ang-2)-neutralizing and thus targets Ang2. Ang-2 is just like VEGF upregulated in GBMs and thought to take part in its angiogenesis. The combination of these treatments improved the survival in murine GBM models, as well as improved the vascular normalization. In addition, when immunocompetent mice were treated with the dual therapy, this led to a difference in the recruitment and polarization of the TAMs (Peterson *et al.*, 2016).

The normalization of the vasculature is an important goal, since this is required for the delivery of anti-cancer drugs and immune cells into the tumour. Thus, although it is important to decrease the aberrant vasculature in recurrent tumours, one should take caution not to block the blood flow too much so that new detrimental problems can occur, such as extreme hypoxia in the TME that only helps the tumour progression by *e.g.* speeding up invasion and metastasis (Lee *et al.*, 2020).

To tackle this problem, Jain *et al.* proposed a theory in which vessels are normalized by simply decreasing the intensity of anti-angiogenic therapy to an amount that could lead to a balance in anti- and pro-angiogenic signals. Hereby, delivery of oxygen, drugs, and antitumorigenic immune cells into the tumour is still possible, without inducing intra-tumoural hypoxia or necrosis (Lee *et al.*, 2020). Combining for example CSF-1 with anti-angiogenic therapy adjusted to the tumour thus seems like a great approach to treating GBM. Hereby, the normalized vessels enable the infiltration of immune cells, which are now instead of tumour-promoting nature, rather immune cells that help target the GBM cells thanks to CSF-1.

Discussion

In this essay, the effect of the TME in the mesenchymal transition in glioblastoma is researched. The TME affects this transition by promotion of an immunosuppressive environment and by using hypoxia in their favour to support the tumour progression. In addition, MES transition is induced by treatment and seems to be a mechanism against resistance, wherein the TME plays an important role. Treatment should be focused on shifting the anti-inflammatory phenotype of the environment to a pro-inflammatory one, and focus on the normalization of the vessels.

These obtained findings suggest that the TME should indeed be targeted during GBM treatment. The cells that are discussed here, namely the TAMs, astrocytes and ECs, are not simply bystanders but actively interact with GBM cells and each other, affecting the course of tumour progression. Thus, it is of purpose to further research the role of the TME in GBM progression, so that treatment can be adjusted to the effect of TME.

Although the most important processes in which the TME plays a role in the transition have been discussed, there are still many factors that have not been explored here. For one, the role of other cell types in the TME is also essential to investigate. One example is that GSCs interact a lot with ECs, and can even differentiate into ECs (Brooks & Parrinello, 2017), thus targeting the ECs in anti-angiogenic therapy is simply not sufficient.

Furthermore, although the mesenchymal subtype is the most aggressive subtype, it is of importance to study the other subtypes as well. This is first off because of the fact that different subtypes can coexist within the same tumour (Niklasson *et al.*, 2019). And second, because of the suggestion that MES transition is a two-way phenomenon, further implying the necessity of combinatorial therapy, in this case targeting both the Proneural and the Mesenchymal subtype (Azam *et al.*, 2020). In future studies, researchers should also look further into the many factors that can further affect the MES transition or the effectivity of the treatment.

An important example is the study by Quail *et al.*, in which resistance to BLZ-945 was found. This stresses that in addition to finding an efficient TME-targeted therapy, it is also important to research the resistance that can appear. The mechanisms for resistance to current treatment is already known well, whereas TME-targeted therapies are fairly new and resistance to these is so far barely researched (Quail *et al.*, 2016). Thus, it is essential to define how resistance can evolve to the new therapies, so that the therapies can also be used over a long period of time (Quail *et al.*, 2017). Additionally, combinatorial treatment should be investigated that prevents this resistance and improves the outcome, similar to how IGF-1R inhibition helped the outcome of GBM treatment with CSF-1R inhibitor BLZ-945.

Currently, there are two Phase 1 trials going on that investigate the combinatorial effect of CSF1R with a different drug. One study researches the effect of combining anti-CSF1R antibody Cabiralizumab with anti-Programmed Cell Death Ligand 1 (PD-1) antibody Nivolumab, in GBM patients with advanced malignant glioma. In the other trail, anti-CSF1R monoclonal antibody SNDX-6352 its effect is investigated, as well as its combination with the anti-PD-1 antibody Duravalumab. This is researched in patients that have solid tumours (Buonfiglioli & Hambardzumyan, 2021).

Conclusion

In conclusion, the tumour microenvironment does in fact affect the MES transition in GBM. This is mediated through the promotion of an pro-inflammatory environment, as well as through hypoxia. In addition, not only affects the TME a role in the MES transition during tumour progression, it also plays a role in the MES transition that happens as a result of treatment, such that of radiation or anti-angiogenic therapy. Strategy should first and foremost shift the anti-inflammatory phenotype back to a pro-inflammatory phenotype, and combine the treatment for this with adjusted anti-angiogenic therapy. Hereby, cells of the TME acquire a pro-inflammatory phenotype and are also able to enter the microenvironment where they can target the GBM cells.

References

Ahir BK, Engelhard HH, Lakka SS. 2020. Tumor Development and Angiogenesis in Adult Brain Tumor: Glioblastoma. *Mol Neurobiol.* 57(5):2461-2478.

Akkari L, Bowman RL, Tessier J, Klemm F, Handgraaf SM *et al.*. 2020. Dynamic changes in glioma macrophage populations after radiotherapy reveal CSF-1R inhibition as a strategy to overcome resistance. *Sci Transl Med.* 12(552): eaaw7843.

Ali S, Borin TF, Piranlioglu R, Ara R, Lebedyeva I *et al.*. 2021. Changes in the tumor microenvironment and outcome for TME-targeting therapy in glioblastoma: A pilot study. *PLoS One.* 16(2):e0246646.

Allen E, Jabouille A, Rivera LB, Lodewijckx I, Missiaen R *et al.*. 2017. Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. *Science Translational Medicine.* 9(385).

Almahariq MF, Quinn TJ, Kesarwani P, Kant S, Miller CR, Chinnaiyan P. 2021. Inhibition of Colony-Stimulating Factor-1 Receptor Enhances the Efficacy of Radiotherapy and Reduces Immune Suppression in Glioblastoma. *In Vivo.* 35(1):119-129.

Angel I, Pilo Kerman O, Rousso-Noori L, Friedmann-Morvinski D. 2020. Tenascin C promotes cancer cell plasticity in mesenchymal glioblastoma. *Oncogene.* 39(46):6990-7004.

Azam Z, To ST, Tannous B. 2020. Mesenchymal Transformation: The Rosetta Stone of Glioblastoma Pathogenesis and Therapy Resistance. *Advanced Science.* 7(22):2002015.

Bajetto A, Thellung S, Dellacasagrande I, Pagano A, Barbieri F, Florio T. 2020. Cross talk between mesenchymal and glioblastoma stem cells: Communication beyond controversies. *Stem Cells Transl Med.* 9(11):1310-1330.

Behnan J, Finocchiaro G, Hanna G. 2019. The landscape of the mesenchymal signature in brain tumours. *BRAIN.* 142:847-866.

Bhat KPL, Balasubramaniyan V, Vaillant B, Ezhilarasan R, Hummelink K *et al.*. 2013. Mesenchymal differentiation mediated by NF-κB promotes radiation resistance in glioblastoma. *Cancer Cell.* 24(3):331-46.

Brandao M, Simon T, Critchley G, Giamas G. 2019. Astrocytes, the rising stars of the glioblastoma microenvironment. *Glia.* 67(5):779-790.

Brooks LJ, Parrinello S. 2017. Vascular regulation of glioma stem-like cells: a balancing act. *Current Opinion in Neurobiology.* 47:8-15.

Buonfiglioli A, Hambardzumyan D. 2021. Macrophages and microglia: the cerberus of glioblastoma. *Acta Neuropathological Communications.* 9(54):1-21.

Chandra A, Jahangiri A, Chen W, Nguyen AT, Yagnik G *et al.*. 2020. Clonal ZEB1-Driven Mesenchymal Transition Promotes Targetable Oncologic Antiangiogenic Therapy Resistance. *Cancer Res.* 80(7):1498-1511.

Cooper LA, Gutman DA, Chisolm C, Appin C, Kong J *et al.*. 2012. The tumor microenvironment strongly impacts master transcriptional regulators and gene expression class of glioblastoma. *Am J Pathol.* 180(5):2108-19.

Cui X, Ma C, Vasudevaraja V, Serrano J, Tong J *et al.*. 2020. Dissecting the immunosuppressive tumor microenvironments in Glioblastoma-on-a-Chip for optimized PD-1 immunotherapy. *eLife.* 9:e52253.

Davis ME. 2016. Glioblastoma: Overview of Disease and Treatment. *Clin J Oncol Nurs.* 20(5 Suppl):S2-8.

Doan NB, Nguyen HS, Alhajala HS, Jaber B, Al-Gizawiy MM *et al.*. 2018. Identification of radiation responsive genes and transcriptome profiling via complete RNA sequencing in a stable radioresistant U87 glioblastoma model. *Oncotarget.* 9(34):23532-23542.

Fanelli GN, Grassini D, Ortenzi V, Pasqualetti F, Montemurro N *et al.*. 2021. Decipher the Glioblastoma Microenvironment: The First Milestone for New Groundbreaking Therapeutic Strategies. *Genes*. 12(3):445.

Fedele M, Cerchia L, Pegoraro S, Sgarra R, Manfioletti G. 2019. Proneural-Mesenchymal Transition: Phenotypic Plasticity to Acquire Multitherapy Resistance in Glioblastoma. *Int J Mol Sci.* 20(11):2746.

Festuccia C, Biordi AL, Tombolini V, Hara A, Baily D. 2020. Targeted Molecular Therapy in Glioblastoma. *Journal of Oncology.*

Grégoire H, Roncali L, Rousseau A, Chérel M, Delneste Y *et al.*. 2020. Targeting Tumor Associated Macrophages to Overcome Conventional Treatment Resistance in Glioblastoma. *Front Pharmacol.* 11:368.

Gupta K, Burns TC. 2018. Radiation-Induced Alterations in the Recurrent Glioblastoma Microenvironment: Therapeutic Implications. *Front Oncol.* 8:503.

Heiland DH, Ravi VM, Behringer SP, Frenking JH, Wurm J *et al.*. 2019. Tumor-associated reactive astrocytes aid the evolution of immunosuppressive environment in glioblastoma. *Nature Communications.* 10(2541).

Hong X, Sin WC, Harris AL, Naus CC. 2015. Gap junctions modulate glioma invasion by direct transfer of microRNA. *Oncotarget*. 6:15566–15577.

Joseph JV, Conroy S, Pavlov K, Sontakke P, Tomar T *et al.*. 2015. Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the HIF1α-ZEB1 axis. *Cancer Lett.* 359(1):107-16.

Kaffes I, Szulzewsky F, Chen Z, Herting CJ, Gabanic B *et al.*. 2019. Human Mesenchymal glioblastomas are characterized by an increased immune cell presence compared to Proneural and Classical tumors. *OncoImmunology.* 8(11).

Kim Y, Varn F, Park S, Yoon BW, Park HR, Lee C, Verhaak RGW, Paek SH. 2021. Perspective of mesenchymal transformation in glioblastoma. *Acta Neuropathological Communications.* 9:50.

Lee WS, Yang H, Chon HJ, Kim C. 2020. Combination of anti-angiogenic therapy and immune checkpoint blockade normalizes vascular-immune crosstalk to potentiate cancer immunity. *Exp Mol Med.* 52(9):1475-1485.

Lim E, Kim S, Oh Y, Suh Y, Kaushik N *et al.*. 2020. Crosstalk between GBM cells and mesenchymal stemlike cells promotes the invasiveness of GBM through the C5a/p38/ZEB1 axis. *Neuro-oncology.* 22(10):1452-1462.

Matias D, Balça-Silva J, da Graça GC, Wanjiru CM, Macharia LW *et al.*. 2018. Microglia/Astrocytes-Glioblastoma Crosstalk: Crucial Molecular Mechanisms and Microenvironmental Factors. *Front Cell Neurosci.* 12:235.

Minata M, Audia A, Shi J, Lu S, Bernstock J *et al.*. 2019. Phenotypic Plasticity of Invasive Edge Glioma Stem-like Cells in Response to Ionizing Radiation. *Cell Rep.* 26(7):1893-1905.

Miyauchi JT, Tsirka SE. 2018. Advances in Immunotherapeutic Research for Glioma Therapy. *J Neurol.* 265(4):741-756.

Monteiro AR, Hill R, Pilkington GJ, Madureira PA. 2017. The Role of Hypoxia in Glioblastoma Invasion. *Cells.* 6(4):45.

Niklasson M, Bergström T, Jarvius M, Sundström A, Nyberg F *et al.*. 2019. Mesenchymal transition and increased therapy resistance of glioblastoma cells is related to astrocyte reactivity. *J Pathol.* 249(3):295-307.

Paw I, Carpenter R, Watabe K, Debinski W, Lo H. 2015. Mechanisms Regulating Glioma Invasion. *Cancer Letters.* 362(1):1-7.

Perus L, Walsh L. 2019. Microenvironmental Heterogeneity in Brain Malignancies. *Frontiers in Immunology.* 10:2294.

Peterson TE, Kirkpatrick ND, Huang Y, Farrar CT, Marijt KA *et al.*. 2016. Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. Proc Natl Acad Sci U S A. 113(16):4470-5.

Placone AL, Quiñones-Hinojosa A, Searson PC. 2016. The role of astrocytes in the progression of brain cancer: complicating the picture of the tumor microenvironment. *Tumour Biology.* 37(1):61-9.

Qazi MA, Vora P, Venugopal C, Sidhu SS, Moffat J, Swanton C, Singh SK. 2017. Intratumoral heterogeneity: pathways to treatment resistance and relapse in human glioblastoma. 28(7):1448-1456.

Quail DF, Bowman RL, Akkari L, Quick ML, Schuhmacher AJ *et al.*. 2016. The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. *Science.* 352(6288):aad3018.

Quail DF, Joyce JA. 2017. The Microenvironmental Landscape of Brain Tumors. *Cancer Cell.* 31:326-341.

Roesch S, Rapp C, Dettling S, Herold-Mende C. 2018. When Immune Cells Turn Bad - Tumor-Associated Microglia/Macrophages in Glioma. *Int J Mol Sci.* 19(2):436.

Roos A, Ding Z, Loftus JC, Tran NL. 2017. Molecular and Microenvironmental Determinants of Glioma Stem-Like Cell Survival and Invasion. *Frontiers in Oncology.* 7(120).

Schiffer D, Annovazzi L, Casalone C, Corona C, Mellai M. 2018. Glioblastoma: Microenvironment and Niche Concept. *Cancers (Basel).* 11(1):5.

Shergalis A, Bankhead A, Luesakul U, Muangsin N, Neamati, N. 2018. Current Challenges and Opportunities in Treating Glioblastoma. *Pharmacological Reviews.* 70(3):412-445.

Simon T, Jackson E, Giamas G. 2020. Breaking through the glioblastoma micro-environment via extracellular vesicles. *Oncogene.* 39(23):4477-4490.

Soeda A, Hara A, Kunisada T, Yoshimura S, Iwama T, Park DM. 2015. The Evidence of Glioblastoma Heterogeneity. *Scientific Reports.* 5:7979.

Verhaak RGW, Hoadley KA, Purdom E, Wang V, Qi Y *et al.*. 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.

Wang Q, He Z, Huang M, Liu T, Wang Y *et al.*. 2018. Vascular niche IL-6 induces alternative macrophage activation in glioblastoma through HIF-2α. *Nat Commun.* 9(1):559.

Wang X, Lu J, Guo G, Yu J. 2021. Immunotherapy for recurrent glioblastoma: practical insights and challenging prospects. *Cell Death Dis.* 12(4):299.

Xie Q, Mittal S, Berens ME. 2014. Targeting adaptive glioblastoma: an overview of proliferation and invasion. *Neuro Oncol*. 16(12):1575-84.

Xu S, Tang L, Li X, Fan F, Liu Z. 2020. Immunotherapy for glioma: Current management and future application. *Cancer Lett.* 476:1-12.

Yamini B. 2018. NF-kB, Mesenchymal Differentiation and Glioblastoma. *Cells.* 7:125.

Zinn PO, Singh SK, Colen RR. 2015. Shedding light on glioblastoma cellular heterogeneity. *Neuro-oncology*. 17(3):327-328.