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Review Article

Therapeutic Targeting of Cancer Stem Cells in Human Glioblastoma by Manipulating the Renin-angiotensin System

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Abstract: Patients with glioblastoma (GB), a highly aggressive brain tumor, have a median survival of 14.6 months following neurosurgical resection with adjuvant chemoradiotherapy. Quiescent GB cancer stem cells (CSCs) invariably cause local recurrence. These GB CSCs that can be identified by embryonic stem cell markers express components of the renin-angiotensin system and are associated with circulating CSCs. Despite the presence of circulating CSCs, GB rarely develops distant metastasis outside the central nervous system. This paper reviews the current literature on GB growth inhibition in relation to CSCs, circulating CSCs, the RAS and the novel therapeutic approach by repurposing drugs that target the renin-angiotensin system to improve overall symptom-free survival and maintain quality of life.

Keywords: Glioblastoma; Renin-angiotensin system; Cancer stem cells; Drug repurposing

1. Introduction

Human astrocytic tumors are the most common primary intra-axial brain tumors. In the World Health Organization (WHO) classification of central nervous system tumors, grade I astrocytomas include the more well-circumscribed pilocytic astrocytomas, in contrast to grade II to IV diffuse astrocytomas (Louis, Perry et al. 2016). The presence of cytological atypia confers a grade II tumor, and anaplasia and mitotic activity confer a grade III, tumor. Glioblastoma (GB), the most aggressive astrocytic tumor, classified as a grade IV astrocytoma, is characterized by microvascular proliferation and palisading necrosis. Treatment of GB traditionally involves maximal safe surgical resection for

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cytoreduction {Lacroix, 2001 #303} followed by adjuvant chemoradiotherapy with concomitant use of radiotherapy and the alkylating agent temozolomide, extending median survival to 14.6 months (Stupp, Mason et al. 2005). Methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) promoter is associated with better response to temozolomide and prolonged survival (Hegi, 2004 #304). Furthermore, the longstanding obstacle of the delivery of chemotherapy agents to the central nervous system due to the presence of the blood brain barrier, may be overcome by a promising novel drug delivery system that has been developed, involving curcumin-loaded chitosan polylactic-co-glycolic acid nanoparticles modified with sialic acid, to penetrate the blood brain barrier with anti-aldehyde dehydrogenase to target the CSCs (Kuo, Wang et al. 2019).

The recent revision of the WHO classification of central nervous system tumors has incorporated molecular parameters - a paradigm shift that provides dynamic phenotype and genotype classifications, impacts on the prognosis and outcomes. Known intrinsic factors affecting the prognosis of GB include isocitrate dehydrogenase (IDH) mutation and methylation of the MGMT gene. GBs are divided into IDH-wildtype (90% of cases) and IDH-mutant tumors (Louis, Perry et al. 2016). IDH is an enzyme involved in catalyzing oxidative decarboxylation of isocitrate to 2-oxoglutarate. The most common mutation in GB affects IDH1 with a single amino acid missense mutation at arginine 132 replaced by histidine (IDH1 R132H) (Capper, Zentgraf et al. 2009). IDH-wildtype GB tends to arise *de novo*, while IDH-mutants tend to progress from lower-grade precursor lesions and are commonly found in younger patients (Nobusawa, Watanabe et al. 2009). IDH mutants with methylation fingerprints (Paul, Mondal et al. 2017) are associated with a better survival rate due to the accumulation of 2-hydroxyglutarate, secondary to loss of normal enzymatic function (Fathi, Nahed et al. 2016), increasing the sensitivity of the tumors to selective chemoradiotherapy (van den Bent, Weller et al. 2017). Genetic alterations typical of IDH-wildtype GB include TERT promoter mutations (80%), loss of chromosome 10q (70%), homozygous deletion of CDKN2A/DKN2B (60%), loss of chromosome 10p (50%), EGFR alterations (55%), PTEN mutations (40%), TP53 mutations (25-30%), and PI3K mutations (25%) (Louis, Perry et al. 2016).

The Cancer Genome Atlas Network categorizes GB into four subtypes (proneural, neural, classical and mesenchymal) based on the genomic analysis of PDGFRA, IDH1, EGFR and NF1 coupled with a transcriptional profile (Verhaak, Hoadley et al. 2010). Genomic and transcriptomic analysis demonstrate biological heterogeneity between different GB subtypes with important implications for future research. The poor survival rates of GB, together with the recent discovery of key molecular pathways regulating GB cell biology, have fueled intense research to find novel therapeutic targets, particularly at the genomic and molecular levels.

2. Glioblastoma Cancer Stem Cells

Cancer stem cells (CSCs) in human brain tumors were initially discovered by the identification of cells expressing the cell surface marker CD133, a cell surface pentaspan transmembrane glycoprotein located in plasma membrane protrusions (Singh, Clarke et al. 2003). This observation was further extended by a study demonstrating stem-like neural precursor cells in GB, which can initiate growth

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and recurrence of the tumor even following multiple serial transplantations (Galli, Binda et al. 2004). CSCs divide asymmetrically giving rise to identical, highly tumorigenic CSCs, and non-tumorigenic cancer cells which form the bulk of the tumor, contributing to intra-tumoral heterogeneity. The aggressive nature of GB is attributed to the presence of small subpopulations of CSCs and the potential molecular treatment options for targeting these GB CSCs have been reviewed extensively (Kalkan 2015). Quiescent GB CSCs have the capacity for perpetual self-renewal and proliferation supported by tumor microenvironmental factors including TGF-β and hypoxia, to promote tumor recurrence, providing a potential explanation for resistance to conventional treatments (Tejero, Huang et al. 2019). The CSC markers expressed in GB are categorized according to the cellular localization which include cell surface markers (e.g., CD133, CD15, A2B5, L1CAM), cytoskeletal proteins (e.g., nestin), transcription factors (e.g., SOX2, NANOG, OCT4), post-transcriptional factors (e.g., Musashi1), and polycomb transcriptional suppressors (e.g., Bmi1, Ezh2) (Kalkan 2015).

Yamanaka *et al* achieved a significant breakthrough with the discovery that mature mouse embryonic cells and adult fibroblasts can be reprogrammed to form pluripotent stem cells by adding a combination of key transcription factors OCT4, SOX2, c-MYC and KLF4 (Takahashi and Yamanaka 2006). These factors are known to be expressed by embryonic stem cells (ESCs), and overexpression of these transcription factors can result in the transformation of somatic cells into induced-pluripotent stem cells (iPSCs) (Bradshaw, Wickremesekera et al. 2016, Chhabra 2017). Primitive populations expressing ESC markers such as NANOG4, KLF4, c-MYC, OCT4 and SOX2 have been identified in GB. Importantly, NANOG has been identified as an independent prognostic factor in predicting survival for GB (Elsir, Edqvist et al. 2014). We have previously proposed the presence of a CSC hierarchy in GB, implicating that OCT4+ cells represent the most primitive CSCs, which can differentiate to form SOX2+ and SALL4+ progenitor cells (Bradshaw, Wickremsekera et al. 2016). Invariant stem cell hierarchy is seen in GB with slow-cycling stem cells giving rise to fast-cycling progenitor cells which in turn generate non-proliferative cells, with the presence of outlier stem cells where chemotherapy facilitates proliferation of drug resistant stem cells (Lan, Jorg et al. 2017, Koh, Wickremesekera et al. 2018).

Transcription factors, including OCT4 and SOX2 may play a critical role in perpetual self-renewal of GB CSCs (Kalkan 2015). For example, SOX2 which is highly expressed in GB (Bradshaw, Wickremsekera et al. 2016) is considered a master transcription factor crucial in maintaining pluripotency of mammalian ESCs and is exponentially correlated with the expression of CD133 (Lee, Kotliarova et al. 2006). In addition, SOX2 silencing in GB tumor-initiating cells has been shown to inhibit tumor proliferation (Gangemi, Griffero et al. 2009), one of several strategies targeting SOX2 in GB (Garros-Regulez, Garcia et al. 2016). Tunicamycin, an inhibitor of N-linked glycosylation which acts as an endoplasmic reticulum stress inducer, has been shown to cause cell cycle arrest in G1 phase, blocking the self-renewal capability of glioma CSCs by reducing the expression of SOX2 (Xing, Ge et al. 2016).

Traditionally, the contrast-enhancing components of GB seen on MRI thought to be the moving front of tumor progression and invasion, were targeted for neurosurgical resection. However, multimodal

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MRI techniques such as diffusion tensor imaging coupled with magnetic resonance spectroscopy confirm the presence of tumor cells beyond the contrast enhancing rim (Yan, Li et al. 2019). These infiltrating tumor edges that show contrast enhancement harbor significantly higher percentages of CD133+ cells and are associated with a higher proliferative index (Kim, Kim et al. 2017). Furthermore, tumor cells found in normal brain, beyond the margin of contrast enhancement, also show the presence of CD133+ and SOX2+ cells (Peng, Fu et al. 2019), confirming the infiltrative nature of GB and that these CSCs are a reservoir for the initiation of tumor recurrence following surgical resection and adjuvant chemoradiation.

STAT3 is a transcription factor essential for self-renewal of ESCs {Kiger, 2001 #305}. The JAK-STAT3 signaling pathway involves activation of JAK, phosphorylation of STAT proteins, and their translocation into the nucleus, where the STAT proteins act as transcription factors. Pharmacological inhibition of the STAT3 activator JAK leads to decreased STAT3 transcriptional activation and reduced levels of associated matrix metalloproteinases (MMPs), potentially impacting on the extracellular matrix degrading ability of invadopodia (Stylli, Kaye et al. 2008), impeding the migratory and invasive potential of GB (Senft, Priester et al. 2011). STAT3 binds to the Notch1 promoter leading to the activation of Notch signaling which also activates the transcription of stem cell markers in astrocytomas (Zhu, Costello et al. 2011). Inhibition of the Notch signaling pathway also impedes the maintenance of glioma stem cells and tumorsphere formation, in addition to reducing the expression of the glioma stem cell markers CD133, SOX2 and nestin (Yahyanejad, King et al. 2016).

Curcumin, a naturally occurring component of turmeric, has been shown to inhibit JAK signaling, inducing reactive oxygen species, and downregulating STAT3 phosphorylation, resulting in reduced proliferation of the tumor cells (Weissenberger, Priester et al. 2010). Curcumin-induced reactive oxygen species promote cytotoxicity, DNA damage and apoptosis (Seyithanoğlu, Abdallah et al. 2019). Rather than relying only on the development of novel compounds, repurposing existing FDA-approved drugs to target GB would be a faster route to target oncogenic GB cell functions and improved therapy, as shown by targeting invadopodia activity in GB cell lines (Whitehead, Nguyen et al. 2018).

3. Circulating Cancer Stem Cells

The concept of circulating CSCs and 'liquid biopsy" has been proposed as an alternative to obtaining histological specimens for diagnosis and molecular typing of the tumors (van Schaijik, Wickremesekera et al. 2019). It presents an alternative mechanism local recurrence of GB, implicating epithelial-to-mesenchymal (EMT) and mesenchymal-to-epithelial (MET) transformational pathways (Fedele, Cerchia et al. 2019), a paradigm counterintuitive to the concept of activation of regional non-circulating quiescent GB CSCs causing local recurrence of GB. Despite the invasive nature of GB and the presence of circulating CSCs, the reasons for the reported rarity of distant metastatic GB (Johansen, Rochat et al. 2016, Lewis, Rivera et al. 2017, Wu, Zhong et al. 2017, Rosen, Blau et al. 2018), are unknown.

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Historically, the concept of circulating CSCs is supported by studies demonstrating immunosuppressed patients who had received transplanted organs from donors with GB (Collignon, Holland et al. 2004) developed metastatic GB in lymph nodes and distant organs (Pasquier, Pasquier et al. 1980), and identifying circulating CSCs in peripheral blood of GB patients (Muller, Holtschmidt et al. 2014). Early commentary on ultrastructural features suggested two potential factors that refute the possibility of circulating GB CSCs. Firstly, neoplastic glial cells are excluded from extravasation by the vascular basal laminae of the brain, and secondly, even if the neoplastic cells manage to escape into the vascular system, they are prevented from binding to the endothelium of the target organs, due to lack of appropriate cell adhesion molecules (Pilkington 1997). More recent suggested reasons include mesenchymal plasticity exhibited by GB CSCs which are more differentiated and unable to find a suitable *niche* other than the brain (Ricci-Vitiani, Pallini et al. 2008).

More recently, EMT has gained increased recognition and momentum, as a process determining the presence or absence of metastases. Transcription factors and signaling pathways involved in EMT in gliomas have been described (Du, Tang et al. 2017). Through EMT, an epithelial cell assumes increasing migratory ability and infiltrative capacity by transforming into a more immature mesenchymal cell type. The Hedgehog signaling pathway is shown to regulate the self-renewal of CD133-positive glioma CSCs (Clement, Sanchez et al. 2007). Activation of this pathway leads to increased expression of the transcription factors Snail and Slug, suppressing expression of E-cadherin, resulting in reduced junctional adherence between epithelial cells and increased capacity of cell migration (Song, Chen et al. 2018). GB cells have been shown to be devoid of cell junctions while peri-tumoral cells display fully organized desmosomes and junctional complexes (Angelucci, D'Alessio et al. 2018). Nuciferine has been shown to inhibit EMT by decreasing Slug expression via the AKT and STAT3 signaling pathways in GB (Li, Chen et al. 2019). In another study, a combination of an antagonist of the Hedgehog signal transducer Smoothened and an ATP competitor have been shown to reduce the expression of Snail, Slug and Zeb1, thus inhibiting EMT, suggesting that combined inhibition of the PI3K/AKT/mTOR and Sonic Hedgehog pathways can be exploited together to suppress the growth of GB (Nanta, Shrivastava et al. 2019). TGF-β1 has been shown to induce EMT in GB cells by decreasing the expression of E-cadherin, inducing upregulation of mesenchymal markers (e.g., N-cadherin, vimentin), crucial regulators (e.g., Twist1, β-catenin), EMT-activating transcription factors (e.g., Snail, Slug, Zeb1); and activating various downstream pathways including PI3K, Smads and MAP kinase (Zhang 2009).

An *in vitro* study has shown that metformin inhibits TGF-β1 and suppresses the self-renewal capacity of GB CSCs and expression of CSC markers by decreasing the phosphorylation of AKT and mTOR (Song, Chen et al. 2018). Resveratrol, a natural phenol found in grapes, berries and peanuts, has also been found to suppress EMT by suppressing the levels of MMPs and associated invadopodia activity, in addition to decreasing secondary gliosphere formation and expression of CSC markers via regulation of Smad-dependent signaling pathway (Song, Chen et al. 2019).

In summary, the concept of circulating CSCs in GB introduces novel etiological pathways and may

provide explanations for the resistance to traditional therapies and high rate of tumor recurrence. A comprehensive review of the many current studies on GB CSCs and EMT-MET in glioma is beyond the scope of this review. However, further characterization may lead to the development of targeted systemic therapies based on the modulation of the renin-angiotensin system (RAS).

4. The Renin-angiotensin System

The RAS (Figure 1) is a hormone system physiologically important in cardiovascular homeostasis and regulation of blood pressure in humans. Renin, which is physiologically secreted by the renal juxtaglomerular apparatus, acts to convert angiotensinogen, normally produced by the liver, to angiotensin I. Angiotensin I is then converted to angiotensin II (ATII) by angiotensin-converting enzyme (ACE), largely produced in the lungs. ATII receptor 1 (ATIIR1) and ATII receptor 2 (ATIIR2) are G protein-coupled receptors with antagonistic effects. Activation of ATIIR1 induces cellular proliferation, inflammation and angiogenesis, whereas activation of ATIIR2 inhibits cell growth and enhances programmed cell death and cellular differentiation (Perdomo-Pantoja, Mejía-Pérez et al. 2018).

Renin is formed by the cleavage of its inactive precursor, pro-renin, to active renin, by binding to pro-renin receptor (PRR) (Cousin, Bracquart et al. 2010), as well as by various enzymes including cathepsin B (Neves, Duncan et al. 1996), cathepsin D and cathepsin G (Munro, M., Wickremesekera, A.C., et al. 2017) (Figure 1). COX-2 causes the upregulation of PRR (Wang, Lu et al. 2014) (Figure 1). β-blockers reduce the production of pro-renin (Holmer, Hengstenberg et al. 2001) (Figure 1). Insulin growth factor (IGF) activates insulin growth factor receptor-1 (IGFR-1) to promote conversion of pro-renin to active renin (Standen, Sferruzzi-Perri et al. 2015) (Figure 1). The action of ATII on ATIIR1 can be blocked by angiotensin receptor blockers (ARBs) (Pinter and Jain 2017) (Figure 1). The RAS has been implicated in the hallmarks of cancer (George, Thomas et al. 2010, Wegman-Ostrosky, Soto-Reyes et al. 2015). We have demonstrated the expression of components of the RAS: PRR, ACE, ATIIR1 and ATIIR2 by CSCs in different cancer types including head and neck cutaneous squamous cell carcinoma (SCC) (Nallaiah, Lee et al. 2019), oral cavity SCC (OCSCC) affecting the lip (Ram, Brasch et al. 2017), buccal mucosa (Featherston, Yu et al. 2016) and oral tongue (Itinteang, Dunne et al. 2016), liver metastases from colon adenocarcinoma (Narayanan, Wickremesekera et al. 2019) and metastatic melanoma to the brain (Wickremesekera, Brasch et al. 2019). More importantly, components of the RAS: PRR, ATIIR1 and ATIIR2 have been shown to be expressed by the CSCs in GB with ACE and also PRR, ATIIR1 and ATIIR2 localizing to the endothelium of the microvessels (Bradshaw, Wickremesekera et al. 2016) (Figure 2). These findings suggest CSCs within GB and other types of cancers, may be a novel therapeutic target by modulation of the RAS (Roth, Wickremesekera et al. 2019)

Lysosomal cysteine protease cathepsin B is increased six-fold in GB, compared to normal brain tissues (Rempel, Rosenblum et al. 1994), which is further confirmed by studies demonstrating increased cathepsin B expression in GB, compared to anaplastic astrocytomas, low-grade gliomas and normal brain tissues (Sivaparvathi, Sawaya et al. 1995, Konduri, Lakka et al. 2001). Greater cathepsin B

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immunoreactivity in primary brain tumors and endothelial cells is associated with shorter survival times (Strojnik, Kos et al. 1999). Another analysis reveals that cathepsin B and plasminogen activator inhibitor type 1 are important biomarkers for predicting overall survival of patients with GB (Colin, Voutsinos-Porche et al. 2009). Activation of cathepsins induces cell-membrane associated urokinase plasminogen activator (uPA), causing extracellular release of plasmin from plasminogen. Plasmin activates various MMPs capable of degrading basal lamina proteins (Levicar, Strojnik et al. 2002), increasing the motility of glioma cancer cells. We undertook an analysis of GB based studies within the online Oncomine® platform for datasets that contained mRNA expression levels of cathepsin B. Oncomine (version 4.5—www.oncomine.org, Compendia Bioscience™, Ann Arbor, MI, USA, Thermo Fisher) is an online tool that contains 715 mRNA and copy number expression datasets from 86,733 cancer and normal tissue samples (12,764 samples are normal tissue samples). Our datamining of the brain/central nervous system datasets deposited in the Oncomine Compendium examined the relative mRNA levels of cathepsin B in both GB and normal brain tissue. As can be observed by the data presented in Table 1, there is an elevation of cathepsin B in GB tissue, relative to normal brain in three studies (TCGA Brain, Bredel Brain 2, Sun Brain) (Rhodes, Yu et al. 2004).

Table 1 Cathepsin B overexpression in glioblastoma compared to normal brain

Number of Glioblastoma Samples	Number of Corresponding Normal Brain Samples	Total number of Measured Gene	Mean Fold Change (Log2)	p-value	Sample Type	Platform	Study
542	10	12,624	2.0662	1.96E-8	mRNA	Human Genome U133A Array	TCGA Brain
27	4	14,836	1.819	1.84E-5	mRNA	Not Defined	Bredel Brain 2
81	23	19,574	1.543	4.02E-7	mRNA	Human Genome U133 Plus 2.0 Array	Sun Brain

Cathepsin B mRNA expression was examined in glioblastoma tissue within the Oncomine database. Displayed in the table are the mean fold changes vs. corresponding normal tissue in each study and overall p-value. Gene expression data are log transformed and normalized as previously described (Rhodes et al, 2004).

Upregulation of cathepsin B and uPA receptors induces SOX2 and Bmi1 expression, both critical for maintaining the stemness of glioma CSCs, whilst knockdown of cathepsin B and uPA receptors suppresses expression of SOX2, Bmi1 and nestin, *in vivo* (Gopinath, Malla et al. 2013). Caffeine has been found to suppress proliferation of GB cell lines, and is associated with decreased activity of cathepsin B and upregulation of tissue inhibitor of metalloproteinase-1 via the MAPK signaling pathway (Cheng, Ding et al. 2016). RNA sequencing of a radio-resistant pediatric GB cell line following radiation revealed the over-expression of pro-cathepsin B, implicating the potential for

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alternative therapies that target metalloproteinases or cathepsin B (Alhajala, Nguyen et al. 2018). Expression of cathepsin B and cathepsin D has been demonstrated in OCT4+ and SALL4+ CSCs in IDH-wildtype GB (Koh, Wickremesekera et al. 2017) (Figure 2). These cathepsins constitute bypass loops of the RAS, contributing to the production of RAS peptides which promote proliferation of CSCs in GB (Figure 2). Therefore, targeting the RAS and its bypass loops in GB CSCs may potentially control the growth of GB tumors.

5. Repurposing Drugs that Target the RAS

Numerous drugs have been demonstrated to promote GB cell apoptosis *in vitro* and *in vivo*, by modulating the RAS (Rivera, Arrieta et al. 2001, Ramírez-Expósito and Martínez-Martos 2019). ACE inhibitors reduce production of ATII, while ARBs selectively block ATIIR1 (Figure 1). The anti-neoplastic action of the RAS-modulating drugs is primarily due to the inhibition of ATII (Arrieta, Guevara et al. 2005). The ARB losartan, a selective inhibitor of ATIIR1, has been shown to suppress growth of C6 rat glioma and induce apoptosis in C6 glioma cells (Arrieta, Guevara et al. 2005). Nonetheless, the ASTER study, a randomized placebo-controlled trial investigating the addition of losartan to the standard of care (concomitant use of radiotherapy and temozolomide) for patients with GB failed to show a difference in steroid requirement or significant improvement in median overall survival in patients with newly diagnosed GB (Ursu, Thomas et al. 2019). Other studies have shown that selective synthetic renin inhibitors decrease DNA synthesis and induce apoptosis in GB cells (Juillerat-Jeanneret, Celerier et al. 2004), and that ARBs are associated with statistically improved progression-free survival and overall survival in 81 patients with GB (Januel, Ursu et al. 2015). Auranofin, an inhibitor of cathepsin B, and captopril, an ACE inhibitor, are included in the coordinated undermining of survival paths (CUSP9) treatment protocol – a trial targeting recurrent GB by combining nine repurposed drugs with temozolomide, highlighting the six themes important to cancer therapy, accepting that cytotoxic drugs alone have been futile in prolonging survival of GB patients and these drugs may improve the efficacy of chemotherapeutic agents such as temozolomide (Kast, Karpel-Massler et al. 2014). The study drugs, which included aprecipitant (antiemetic), auranofin (anti-rheumatoid disease drug), captopril (ACE inhibitor), celecoxib (COX-2 inhibitor), disulfiram (alcohol aversion), itraconazole (anti-fungal), minocycline (antibiotic), quetiapine (anti-psychotic), sertraline (anti-depressant) and temozolomide, had divergent mechanisms, caused toxicities and was ineffective as the patients had advanced disease with poor Karnofsky Performance Scores, the subject of much debate {Purow, 2016 #310}.

Numerous epidemiological studies have demonstrated a lower incidence of cancer and/or improved survival rate of cancer patients taking medications that modulate the RAS. These include a one-third reduction of the risk of developing skin SCC, in patients who were treated with ACE inhibitors or ARBs (Christian, Lapane et al. 2008); reduced risk of developing head and neck, gastric, colon and prostate cancers in patients receiving propranolol (Chang, Huang et al. 2015). Treatment with aspirin, a COX-1 and COX-2 inhibitor (Qiao, Yang et al. 2018) and ketorolac, a specific COX-2 inhibitor, (Viegas, Manso et al. 2011), are associated with a reduction in the risk of developing bowel cancer (Ferrandez, Prescott et al. 2003) and reduction of recurrence and death in breast cancer patients

(Forget, Vandenhende et al. 2010), respectively. Improved survival has been observed in ovarian cancer patients who are administered non-selective β-blockers (Watkins, Thaker et al. 2015), and patients with multiple myeloma receiving propranolol (Hwa, Shi et al. 2017). Cathepsin B over-expression is associated with higher tumor grades and reduced overall survival in patients with OCSCC (Yang, Ho et al. 2016). Importantly, improved survival of OCSCC patients after administration of curcumin, an inhibitor of cathepsin B, has been reported (Wilken, Veena et al. 2011). More specifically, a recent study shows that the use of RAS inhibitors is associated with survival benefit in glioma patients (Levin, Chan et al. 2017).

Repurposing drugs, including anti-depressants, anti-convulsants, anti-hypertensives, statins, singly or in combination for the treatment of GB has been reviewed recently (Rundle-Thiele, Head et al. 2016) with positive effects. The understanding of the regulation of the RAS and CSCs in GB, in particular the expression and function of cathepsin B (Itinteang, Chudakova et al. 2015) and the IGF/IGFR-1 pathway (Al Hassan, Fakhoury et al. 2018) lead us to propose modulating the RAS, a singular systemic homeostatic pathway, using a combination of drugs (Figure 1), to simultaneously inhibit key steps of the RAS, its bypass loops and crosstalk signaling pathways interacting with the RAS, may offer a novel therapeutic approach for patients with GB (Roth, Wickremesekera et al. 2019), to potentially increase overall survival whilst preserving their quality of life and avoid toxicities. Currently, we are undertaking a drug repurposing study using a cocktail consisting of propranolol (a β-blocker), metformin (an IGF/IGFR-1 blocker), curcumin (a cathepsin B blocker), aliskiren (a renin blocker) and cilazapril (an ACE inhibitor), losartan (an ATRB) to treat GB (Tan 2018).

6. Conclusions

The prognosis for patients with GB remains poor despite intensive research over the last 50 years. New therapeutic regimens are necessary to improve the overall survival and the quality of life of these patients. Further research into CSCs and the role of the RAS and its bypass loops and signaling pathways that converge onto the RAS, in the regulation of the CSCs in cancer, may underscore a potential paradigm shift in the treatment of GB. Randomized controlled trials incorporating repurposed drugs targeting these mechanisms are needed to demonstrate the efficacy of this novel therapeutic approach that may enhance the efficacy of current treatment protocols.

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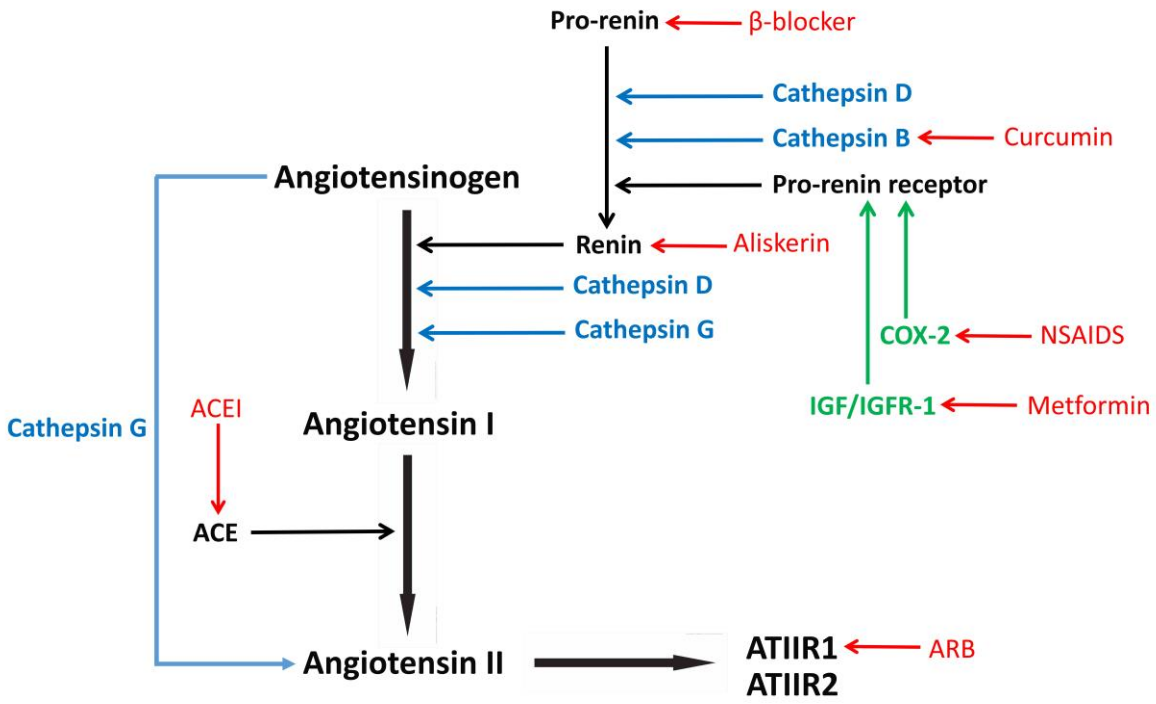

Figure 1: The renin-angiotensin system (RAS), its bypass loops and convergent signaling pathways, and medications that target key steps of these pathways. The classical RAS, highlighted in black, regulates blood pressure, stem cells and tumor development. Bypass loops of the RAS, highlighted in blue, involving enzymes such as cathepsins B, D and G provide redundancy, while other signaling pathways such as the COX-2 pathway and the IGF/IGFR-1 pathway, highlighted in green, converge on the RAS, to activate the pro-renin receptor. Key steps of the RAS and related pathways can be inhibited by commonly available medications, highlighted in red. Angiotensinogen (AGN) is physiologically synthesized and released by the liver and is cleaved by renin which is released by the kidneys, to form angiotensin I (ATI). Renin is formed following binding of pro-renin to the pro-renin receptor. Production pro-renin is reduced by β-blockers, and renin can be directly blocked using aliskerin. ATI is converted to angiotensin II (ATII) by angiotensin-converting enzyme (ACE), normally produced by the lungs. ACE can be blocked using ACE inhibitors (ACEI). ATII interacts with the G-protein coupled receptors ATII receptor 1 (ATIIR1) and ATII receptor 2 (ATIIR2), to restore homeostasis. ATIIR1 can be blocked using an ATIIR1 blocker (ARB). Cathepsins B and D are also renin-activating enzymes that convert pro-renin to renin. Curcumin inhibits the activities of cathepsin B. Cathepsin D also converts AGN to ATI, and cathepsin G converts ATI to ATII or AGN directly, to ATII. The COX-2 pathway and the IGF/IGFR-1 pathway can be blocked using non-steroidal anti-inflammatory drugs (NSAIDS) and metformin, respectively.

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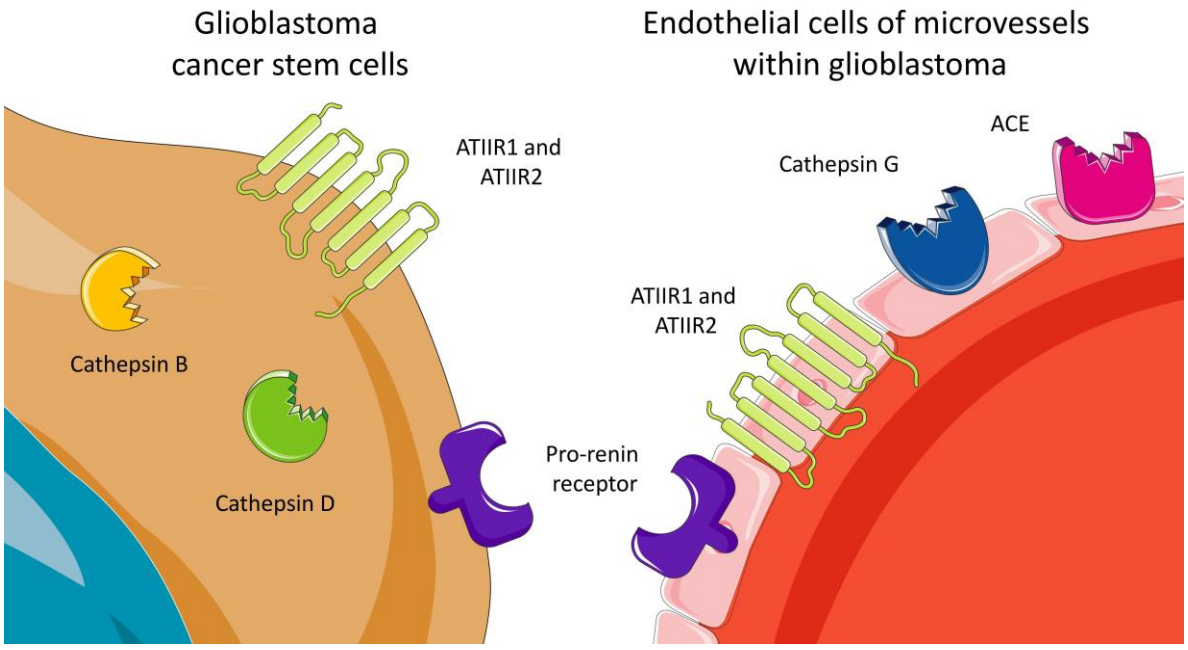

Figure 2: Expression of components of the renin-angiotensin system and proteins that constitute bypass loops of the renin-angiotensin system by cancer stem cells and microvessels within glioblastoma. Cancer stem cells in glioblastoma express ATIIR1, ATIIR2, pro-renin receptor, cathepsin B and cathepsin D. The endothelium on the microvessels within glioblastoma express ACE, ATIIR1, ATIIR2 and cathepsin G.

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Conflicts of Interest: PD and ST are inventors of the of the patent Cancer Stem Cells (US15/503025) and the PCT patent Cancer Therapeutic (PCT/NZ2018/050006), and the provisional patent application Novel Pharmaceutical Compositions for Cancer Therapy (US/62/711709). All other authors declare no conflict of interest.

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