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REVIEW ARTICLE

Overview of the molecular mechanisms of migration and invasion in glioblastoma multiforme

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Abstract: Glioblastoma (GBM) is one of the most devastating cancers, with an approximate median survival of only 16 months. Although some new insights into the fantastic heterogeneity of this kind of brain tumor have been revealed in recent studies, all subclasses of GBM still demonstrate highly aggressive invasion properties to the surrounding parenchyma. This behavior has become the main obstruction to current curative therapies as invasive GBM cells migrate away from these foci after surgical therapies. Therefore, this review aimed to provide a relatively comprehensive study of GBM invasion mechanisms, which contains an intricate network of interactions and signaling pathways with the extracellular matrix (ECM). Among these related molecules, TGF-β, the ECM, Akt, and microRNAs are most significant in terms of cellular procedures related to GBM motility and invasion. Moreover, we also review data indicating that Musashi-1 (MSI1), a neural RNA-binding protein (RBP), regulates GBM motility and invasion, maintains stem cell populations in GBM, and promotes drug-resistant GBM phenotypes by stimulating necessary oncogenic signaling pathways through binding and regulating mRNA stability. Importantly, these necessary oncogenic signaling pathways have a close connection with TGF-β, ECM, and Akt. Thus, it appears promising to find MSI-specific inhibitors or RNA interference-based treatments to prevent the actions of these molecules despite using RBPs, which are known as hard therapeutic targets. In summary, this review aims to provide a better understanding of these signaling pathways to help in developing novel therapeutic approaches with better outcomes in preclinical studies.

Keywords: Epithelial-to-mesenchymal transition; Extracellular matrix; Glioblastoma multiforme; Migration signaling; Musashi-1

1. INTRODUCTION

Brain cancers have long been known to be life-threatening malignancies in humans. Despite the fact that all intracranial malignant lesions are termed brain tumors, the specific anatomical location, aggressiveness, and morphology lead to the classification of brain cancers into different subtypes. For example, cancers having astrocyte-like morphologies are known as astrocytomas. Grades I–IV are be further classified according to the standards established by the World Health Organization (WHO).[1] Most grade I and II tandem cell tumors are nonmalignant low-grade tumors, while grade III and IV tandem cell tumors are highly malignant tumors. Grade III astrocytomas are referred to as anaplastic astrocytomas (AAs), whereas all kinds of glioma grade IV astrocytomas are referred to as glioblastoma (GBM), and are the most aggressive and specific subtype. Statistically, GBM has an annual incidence rate of seven cases per 100,000 people, but the average overall lifespan after GBM diagnosis is only 16 months.[2]

GBM is biologically heterogeneous, showing all the classic cancer characteristics, and has certain diversities across patients.[3] Beginning in 1884, when Bennett and Godlee performed the first intracranial surgery to remove a glioma,[4] surgical resection has become the first-line treatment option, while intracranial and distal metastasis often overshadows therapeutic outcomes.[5] Recurrence and metastasis—whereby GBM cells escape by early migration as well as show inherent or acquired resistance to chemotherapy and radiation therapy—still remain serious problems.[6] Despite the specific subclasses of GBM being related to prognosis have been defined through both genetic and epigenetic methods,[7,8] effective therapies aiming at specific pathogenic events or molecular targets are still under robust investigation.

2. ROUTES AND PATTERNS OF GBM CELL INVASION

The routes for GBM invasion have been studied for around 80 years, beginning with its definition by a German pathologist, Hans Joachim Scherer, which was called the Scherer structure.[9] Based on this structure, GBM cells were characterized by their ability to infiltrate along existing structures; for example, white matter tract, subvertebral space, brain parenchyma, and the well-known neovasculature of the brain.[10–14] For years, accumulated reports have highlighted the dynamics

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of GBM invasion and the mode of GBM infiltration. Tamura et al[15] revealed different GBM cell invasion sites in the brain and enriched our knowledge regarding the spatial distribution of GBM. They also noted that GBM cells only spread in one direction to the inside of the corpus callosum, whereas tumor cells do not invade the cortex. GBM infiltration along white matter regions is slower than GBM infiltration along blood vessels, even within the white matter of the brain. On the contrary, based on observations in patients, Alieva et al[16] suggested that GBM invades in a variety of patterns in the brain. Specifically, three morphologies, including well-defined borders, invasive margins, and diffusive infiltrations, help the expansion and growth of a GBM tumor.[16] Notably, the edges of the invasion, respectively, show the continuity of movement or high-speed migration, thereby promoting the overall invasion. In this review, we strive to illustrate an overview of intracranial glioblastoma multiforme migration and invasion, and we aim to draw new attention to the molecular impact of anticancer therapy options.

3. EPITHELIAL-TO-MESENCHYMAL TRANSITION IN GBM INVASION

Epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) have been recognized as the two main avenues of GBM cell migration and rapid growth.[17] Thus, it becomes essential to comprehend the molecular mechanism of EMT/MET to overcome such a sophisticated disease. Neural development is a unique process, and neuronal cells exhibit a genuine mesenchymal phenotype, which is different from typical somatic cells. As a result, during tumorigenesis, gliomas do not experience a classic EMT, so some have proposed terms such as EMT-like or glial-to-mesenchymal transition (GMT) to describe this unique process.[18] High plasticity and vigorous EMT- or MET-like transformations have been observed in glioma cases, and many findings have suggested that a possible reason for this was due to tumor microenvironment changes.[19,20] In fact, GBM in the mesenchymal subtype has the following characteristics: increased invasiveness, poor clinical prognosis, and significantly shorter recurrence time after initial treatment.

EMT can be initiated by several factors, such as members of the transforming growth factor (TGF) superfamily, hepatocyte growth factor (HGF), hypoxia-inducible factor (HIF), epidermal growth factor (EGF), and fibroblast growth factor (FGF).[21] Moreover, the EMT process involves a variety of signaling pathways, including the mitogen-activated protein kinase (MAPK) as well as the phosphoinositide 3-kinase (PI3K) pathways, some of which effectively promote GBM development.[22] Subsequently, Snail (Snail1 or SNAI1), zinc finger E-box homeobox (ZEB) 1/2, Slug (Snail2 or SNAI2), and Twist1/2, in turn, regulate EMTs by changing the expression patterns of many genes.[23] The genetic profile of mesenchymal mode cells is usually characterized by increased N-cadherin (CDH2), vimentin, fibronectin, and a decreased pattern of epithelial cell surface markers and cytoskeleton, such as E-cadherin (CDH1), occludin, cytokeratin, and claudin.[23] Apart from such genetic alterations, the consequent phenotype changes involve not only motility but also survival, proliferation, and differentiation. These changes serve to increase the ability of GBM cells to invade the surrounding parenchyma.[21,24–26]

3.1. TGF-β regulates the EMT in GBM

In high-grade glioma, the poor clinical prognosis has been found to be associated with increased TGF-β activity, which may activate an EMT.[27–29] Similarly, overexpression of TGF-β in lung, prostate, and breast cancers have all been reported to stimulate EMT followed by cancer expansion.[30–32] Evidently, the enhancement of TGF-β promotes cell growth, infiltration, immunosuppression, angiogenesis, and cell survival.[28,29] Exposure to TGF-β inhibitors, such as LY2109761, results in morphological changes and the suppression of mesenchymal markers both in vivo and in vitro.[33] To summarize the understanding of TGF-β signals, we survey the literature and have listed the representative signaling pathways associated with TGF-associated EMT and invasion in GBM (Fig. 1).

The development and testing of TGF-β-targeted therapy drugs for GBM patients have been focused intensively on interference with TGF-β activation.[34–36] Joseph et al[33] reported they chemically inhibited TGF-β via the A8301 compound, which soundly ceased mesenchymal transition and invasive behavior. It was notable that ZEB1, a TGF-β downstream EMT regulator, was not affected by A8301 treatment, and a bypass signal may have been responsible. Similarly, Xu et al[37] found another way to decrease TGF-β-induced GBM mesenchymal transition by inhibition of PBX3, a pre-B cell leukemia homeobox (PBX) family member. PBX3 is known to mediate EMT in GBM by activating MEK/ERK1/2, resulting in the enhancement of LIN28, which leads to attenuation of let-7b biogenesis. Inhibition of let-7b, in turn, downregulates the genes promoting invasion, such as IL-6 and HMGA2.[37]

Moreover, Kang et al[38] demonstrated that the use of a small molecule inhibitor LY2109761 could selectively upregulate the cell surface Nogo receptor (NgR) and inhibit the activity of TGF-β, thereby reducing GBM infiltration. Furthermore, NgR maturation was limited to the NgR and vimentin interaction, where knockdown of vimentin reduced GBM invasion and migration.[38] Daubon et al[39] demonstrated a novel method to decrease cell invasion using an antagonist peptide (TAX2) to specifically inhibit the interaction of Thrombospondin-1 (THBS1) with CD47, which was shown to rise with the increasing grades of gliomas. THBS1 interacts with the effector protein, CD47, and engages the TGF-β canonical pathway downstream of SMAD3 activation.[40,41]

Apart from increased insights into signaling pathways to identify targets to inhibit TGF-β to reduce GBM invasion, TGF-β was also found to be connected with cell-to-cell communication. In a study by Rodini et al,[42] mesenchymal stem cells (MSCs) were shown to stimulate GBM cell proliferation by TGF-β1-mediated paracrine action, regardless of their direct contacts.

3.2. Wnt/β-catenin signaling

The Wnt signaling pathway is a key regulator of the central nervous system (CNS), which functions to adjust cell fate during embryogenesis, migration, and proliferation.[43] However, loss of control of this pathway may result in carcinogenesis. Abnormal activation of the Wnt signaling pathway is associated with many tumors, including GBM.[44,45] In the EMT, β-catenin plays a dual role,[23] and is an important part of the adhesion junction that connects the cytoskeleton. When transported to the nucleus, β-catenin plays a crucial role in driving transcriptional activities. When lacking Wnt, β-catenin is phosphorylated, ubiquitinated, and finally degraded by glycogen synthase kinase 3 β (GSK-3β), reducing the level of β-catenin in the cytoplasm.[24,46,47]

In the invasive border of GBMs, the activation of the Wnt/β-catenin signaling pathway was shown to be higher by Kahlert et al.[44] It was concluded that the increasing infiltration of GBM was caused by the growing level of transcription factors of EMT, such as Twist, ZEB1, Slug, and Snail.[44] In another study by Zhou et al, the increasing invasiveness of GBMs was caused by the suppression of TPD52L2, a functional protein mediating cell heterogeneity, which was promoted by CTNNB1/β-catenin and SNAI1/Snail activating an EMT.[48] However, the chemotherapy

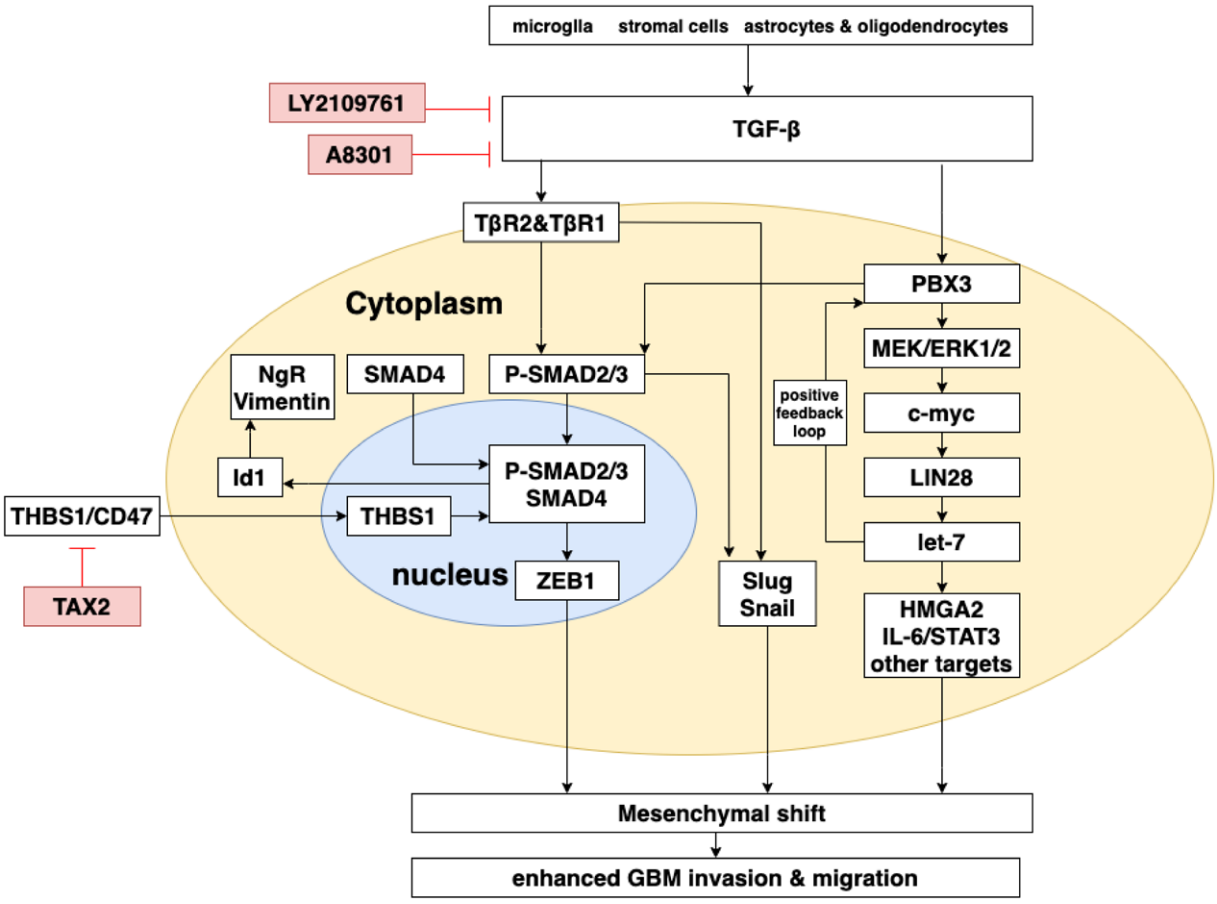

Fig. 1 A summary of representative signals involved in TGF-β-mediated invasion.

sensitization and the inhibited proliferation were both observed in low TPD52L2-expressing GBM cells, indicating that some targets used to prevent cell invasion may not directly slow the development of tumors. Similar conclusions were stated in a study by Cheng et al.[49] The expression of ME2 was shown to be crucial in not only physiological but also pathological functions, for example, in EMT and insulin release.[50,51] This factor was shown to be positively connected with GBM development and behaviors, such as migration, invasion, cell cycle, ROS production, proliferation, and ATP production.

3.3. Other molecules involved in EMT in GBM invasion

Apart from the TGF-β and Wnt/β-catenin pathways, there are other molecules that have been reported to be involved in EMT regulating GBM invasion, which provides an increasing number of potential targets for defining new intervention strategies (Fig. 2). For example, EphB2, a tyrosine kinase receptor or ephrin ligand, is upregulated and crucial in many tumors, functioning in abilities such as facilitating an EMT and triggering pathological features in GBM.[52] Also, under the condition of hypoxia, EphB2 stabilization was enhanced by hypoxia-inducible factor 2α (HIF-2α), which is needed for cellular adaptation to hypoxia and effects the potential infiltration in GBM.[53] The upregulation of EphB2 in hypoxia indeed regulates the phosphorylation of paxillin, helping cell adhesion to the ECM. This suggested not only the connection of each component in promoting GBM invasion but also revealed the importance of studying the tumor microenvironment to prevent GBM motility.

4. THE ECM IN GBM INVASION

To enter the circumambient environment and participate in cell movement and contraction successfully, GBM cells must break away from a primary mass, adhere to the ECM, and then degrade this.[54] Moreover, it has been demonstrated that the ECM connected with tumors is essentially different from the composition of normal brain tissue.[55,56] The ECM contains plentiful molecules that play a key role in the process of GBM invasion,[57] such as fibronectin, integrins, Tenascin C, and proteases such as metalloproteases (ADAM), disintegringrin, cathepsin B, and urokinase (uPA).[58]

Metalloproteinases (MMPs) play an important role in regulating GBM invasion, and one of its members, matrix metalloproteinase 2 (MMP2), was shown to be applied by GBM cells to invade the ECM. Kegelman et al[54] reported that expression of MMP2 and interleukin-8 was enhanced by overexpression of melanoma differentiation-associated gene 9 (MDA-9/syntenin), which could develop motility and invasiveness. A high expression of MDA-9/syntenin also promoted the growing activation of other molecules, including nuclear factor kappa-B, c-Src, and p38 mitogen-activated protein kinase. This provides new targets for regulating EMT by facilitating GBM infiltration. Invadopodia facilitates ECM degradation by promoting tumor cell motility through the parenchyma microenvironment, and the invading and metastasizing capabilities of tumor cells are also connected with the formation of invadopodia.[59,60] Focal adhesions and multiprotein complexes link the ECM with the cytoskeleton using integrins.[61] When cells move, integrin-ECM complexes continually form and break down.[62]

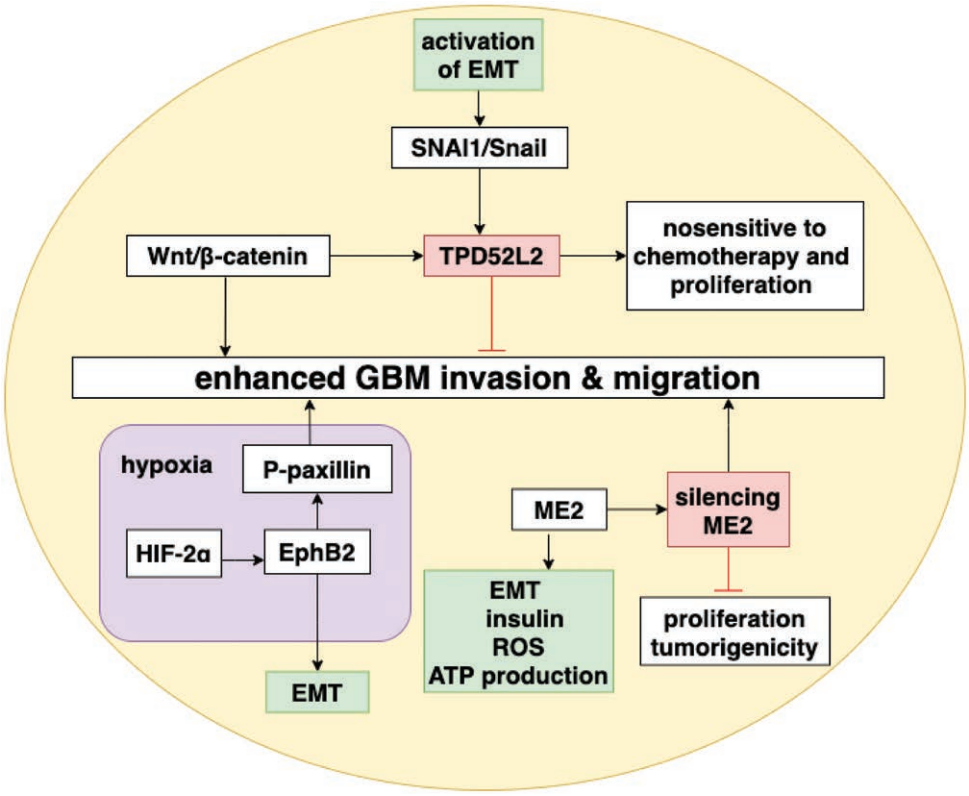

Fig. 2 A summary of signaling pathways involved in Wnt/β-catenin and other molecules in GBM invasion. GBM = glioblastoma.

GBM cells, thus, should have less motility after stabilizing focal adhesions and decreasing the expression of Crk-associated substrate (Cas) phosphorylation.[63] The stability of focal adhesions and the phosphorylation of Cas is brought about by the activities of PTP-PEST, which is a cytoplasmic protein tyrosine phosphatase. Another key member regulating GBM invasion in ECM is integrin, which is a receptor of the cell membrane, and composed by α and β subunits. Upon activation, integrin transmits ECM and tumor microenvironment signals to the inside of cells, and stimulates cell migration, invasion, proliferation, differentiation, and apoptosis.[64–67] GBM motility was also found to be enhanced by ILK through the activation of MMP13, Rho-associated kinase 1 (ROCK1), and fascin actin-bundling protein 1 (FSCN1), in a stepwise manner.[68] In view of the engagement of THBS family members, a signal intermediary caused by the TGF-β superfamily of cytokines suggests that ECM shares the same signaling pathways as EMT to control GBM invasion. Therefore, these shared molecules are more attractive as therapeutic targets for creating a combined effect to attenuate GBM invasion and motility. We have summarized these ECM-associated signals in an illustrated scheme below (Fig. 3).

5. AKT SIGNALING IN GBM INVASION

The Akt signaling pathway is often activated by extracellular stimulation, including stimulation by growth factors, and mediates cell survival, growth, and metabolism, via certain receptor tyrosine kinases.[1] There are plenty of studies that have illustrated that tumor development, like cancer growth, was regulated by the activation of the Akt signaling pathway.[69–72] Hence, it has become a hot topic in research to find a way to inactivate the Akt signaling pathway. Along with Akt signaling, the attenuation of CWCY and Kazal-like domains proteoglycan 1 (SPOCK1) inhibits the migration of GBM cells.[73–76] Metformin (N, N-dimethylbiguanide) treatment was found to effectively suppress Akt activation and subsequently downgrade GBM malignancy.[77] Numerous small molecules have been gradually developed to abrogate Akt signaling, such as MK-2206, which is effective in many cancers.[78,79] Apart from directly targeting Akt in this signaling pathway, its upstream and downstream molecules are also targeted. HSF1 or HuR silencing can lead to decreased GBM invasion and growth through the inhibition of the expression of Rictor,[80] while higher Rictor promotes growth and invasion in GBM. Moreover, the expression level of Rictor is mediated by two means. One is HuR activity regulated by HSF1, which mediates the level of Rictor mRNA, and the other is a feed-forward cascade caused by increasing the mTORC2 activity, which is caused by the activation of HSF1 through mTORC2/Akt signaling. We have arranged the Akt-associated signals together for an organized view of the comprehensive Akt signaling found in GBM (Fig. 4).

6. MUSASHI-1 SIGNALING IN GBM INVASION

Musashi is a neuronal RNA binding protein discovered in 1994 and plays an important role in neurodevelopment.[81] This gene is named after the legendary Japanese samurai Miyamoto Musashi who used two swords to fight.[82,83] In vertebrates, the family of Musashi proteins has two highly conserved homologous proteins, Musashi 1 (MSI1) and Musashi 2 (MSI2).[84,85] Given the RNA-binding ability of MSI1 and its abundance in neuronal tissues, accumulated studies have indicated the pro-tumoral characteristics of MSI1.[86,87] Moreover, many studies have highlighted the connection between the Wnt, Hedgehog, and Akt signaling cascades.[88–91]

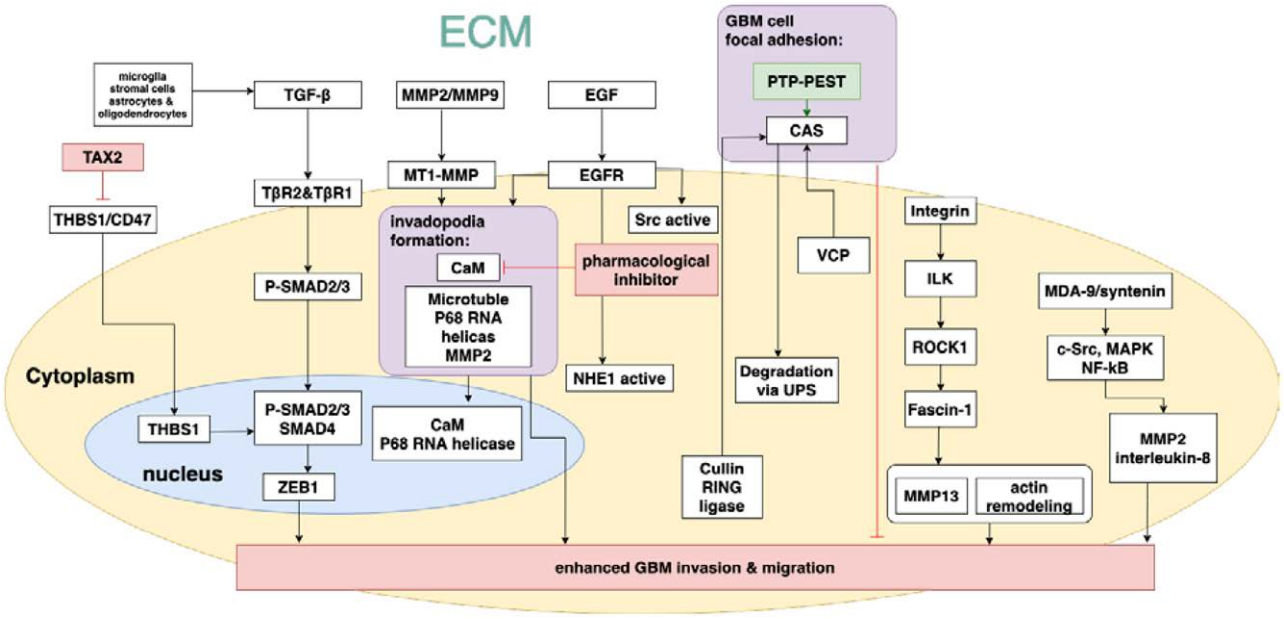

Fig. 3 A summary of signaling pathways involved in the extracellular matrix (ECM) in GBM invasion. The green color highlights an interaction previously mentioned in the text. GBM = glioblastoma.

In terms of the MSI1-mediated cell motility, MSI1 may promote GBM migration through ICAM1 and VCAM1, and MSI1 regulates radioresistance by increasing the role of VCAM1 in repairing homologous recombination, evading apoptosis, and upregulating DNA damage responses.[92,93] Moreover, MSI1 inhibits the translation of Tensin 3 (TNS3) by directly combining with the 3′ UTR of its mRNA. This process changes GBM cell morphology, enhancing GBM cell invasion and viscoelasticity as TNS3 inhibits cell invasion.[94,95] TNS3 and MSI1 are mutually exclusively expressed in metastatic tumors. Patients suffering GBM with low MSI1/TNS3 have been shown to have poor clinical prognosis.

7. DISCUSSION AND FUTURE WORK

Chen et al[96] found that malignant cancers show elevated cytoplasmic MSI1 levels, which can metastasize in the cytoplasm in reaction to stress and enhance cancer development. The argonaute (AGO) proteins are also members of the RBP family and are important in silencing RNA through regulating the process of decay and translationally suppressing targets.[97–99] In many cancers, AGO2 is found to be ectopically overexpressed.[97] Many studies have shown that AGO2 can directly participate in the development of cancer through its interaction with oncogenic factors.[100] The combination of MSI1 translocation and MSI1/AGO2 could be a key to help understand the development of

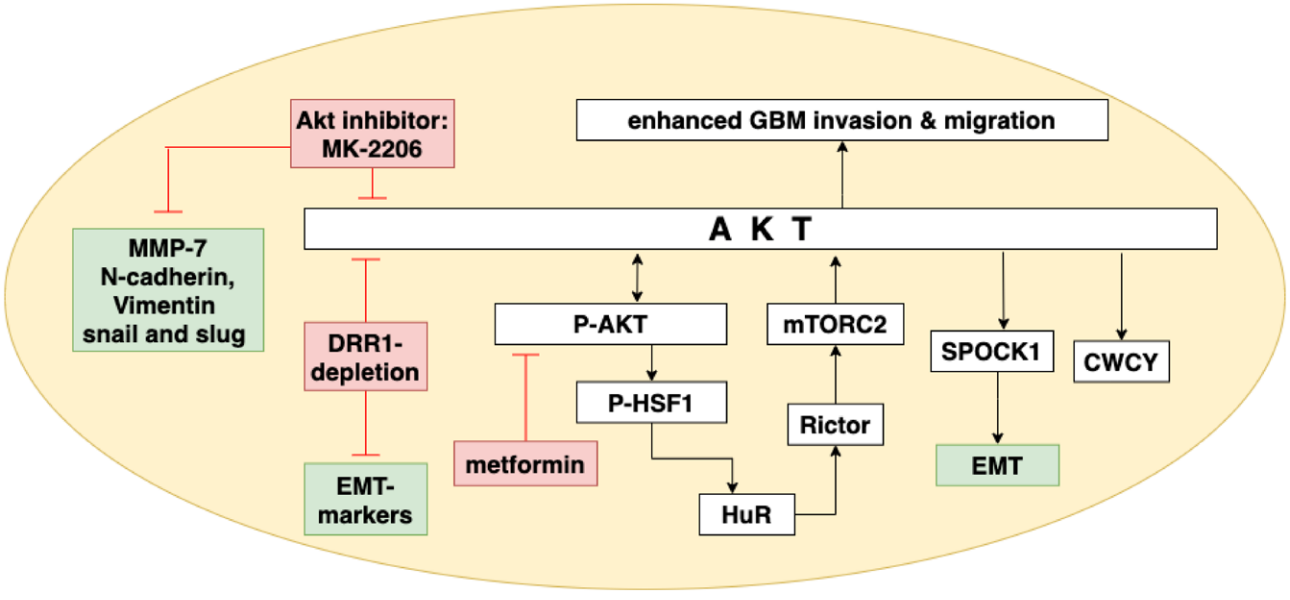

Fig. 4 A summary of signaling pathways involved in Akt in GBM invasion. The green color highlights interactions previously mentioned in the text. GBM = glioblastoma.

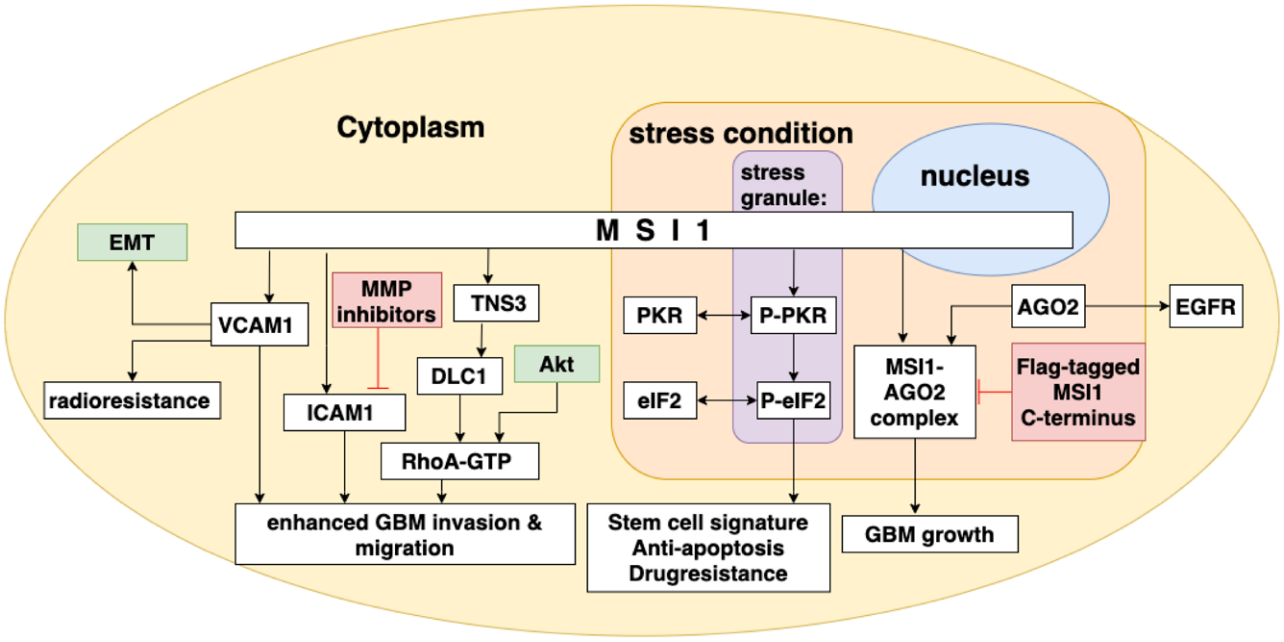

Fig. 5 A summary of signaling pathways involved with Musashi-1 (MSI1) in GBM invasion. The green box represents an interaction previously mentioned in the text. GBM = glioblastoma.

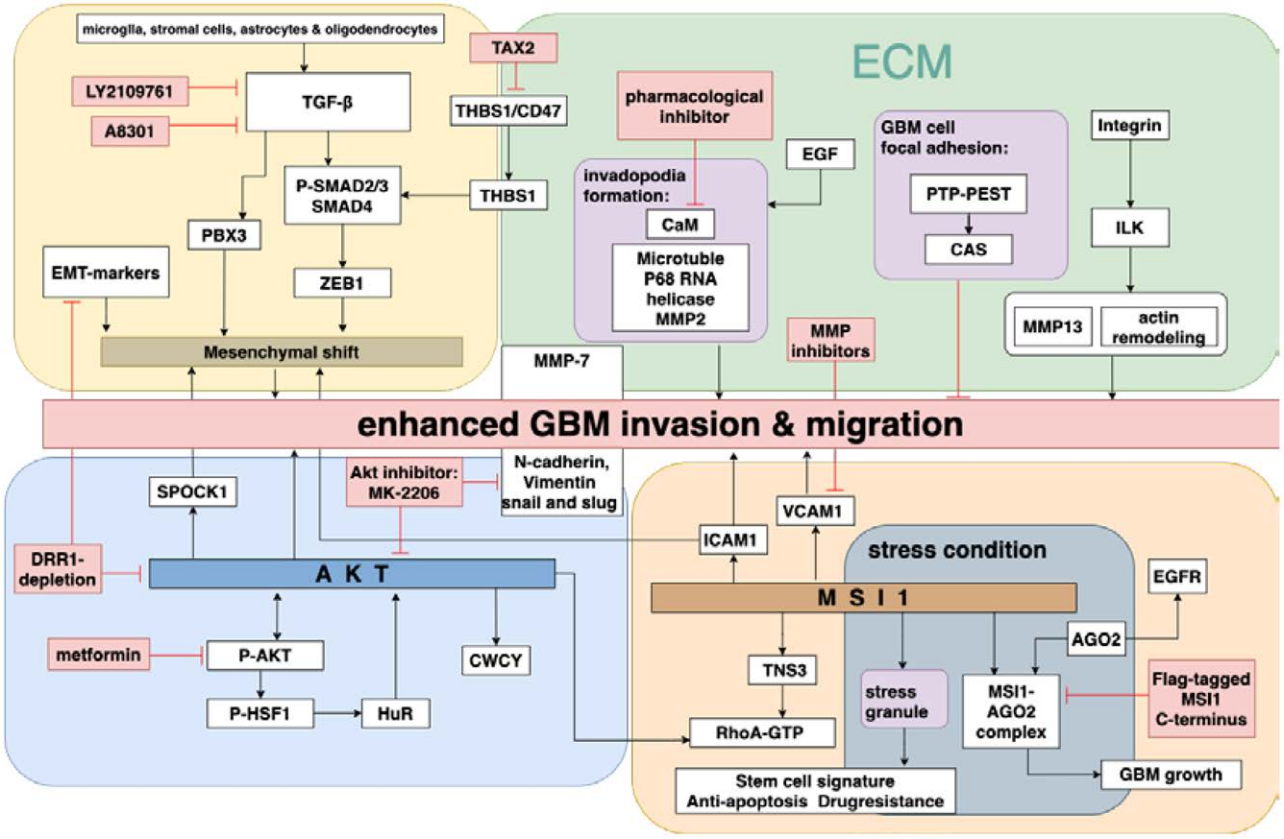

Fig. 6 Schematic of the relationship between EMT, ECM, Akt, and MSI in promoting GBM invasion and migration. ECM = extracellular matrix; EMT = epithelial-to-mesenchymal transition; GBM = glioblastoma; MSI = Musashi-1.

cancer. Indeed, MSI1 might be an important therapeutic target. However, MSI1 inhibitors are still a long way from clinical work, and these drugs need to be shown to be useful in tumor treatment.[101]

Given the activity and expression of MSI1 in cancers, such as GBM, it is possible to gain insight into the connection between the signature of a transcriptome and the actual signal network that can be manipulated in GBM. Like mechanisms of epigenetic regulation, posttranscriptional proteins (such as MSI1) have become important hubs for extensive control of carcinogenic signal networks, and the role of MSI1 in GBM needs to be refined to reduce its associated pathological conditions. As MSI1 plays an important role in DNA repair, chemotherapy resistance, and cancer invasion in normal tissues, targeting MSI1 in different ways may by itself have potential applications as a trustworthy tactic to ameliorate GBM.[81] It is expected that fruitful results will be achieved in the near future testing this.

In conclusion, more work is necessary to comprehend the mechanism of GBM invasion and motility to find novel intervention approaches for this destructive carcinoma, and possibly treat specific subtypes of GBM. With this in mind, to carry out an appropriate treatment plan, it is necessary for neuroscientists, neurologists, neurosurgeons, and oncologists to thoroughly understand the most significant signal transduction processes in glioma motility and invasion and comprehend the clinical manifestations of GBM infiltration in detail. In recent years, some singling pathways have been reported to be connected with GBM infiltration and represent potential therapeutic targets and prognostic biomarkers. Among these, the most representative signaling pathways were shown to be EMT, ECM, Akt, and MSI1. In GBM cells, many signals are caused by the EMT involved in GBM invasion, such as Wnt/β-catenin, SMAD, Ras, and Akt, and ECM also leads to different composition regulation in GBM motility, such as, MMPs, invadopodia, and focal adhesions. A large amount of research has demonstrated that the Akt signaling pathway links many aspects of cancer development, including promoting the expression of EMT.[102] Moreover, it has been demonstrated that MSI1 is related to many signaling pathways, such as the Notch signaling pathway and Wnt signaling. Apart from that, recent studies have also exposed some signaling pathways that connect with EMT, ECM, and the PDK1-Akt axis. These results suggest that each signaling pathway involved in GBM invasion is not independent, but rather, they act as points of a huge network (Fig. 6). In this article, we reviewed the essential cellular pathways and processes that regulate GBM infiltration, and we also described their correlation as potential therapeutic targets for GBM management.

Although our understanding of GBM has made important progress lately, the prognosis of patients is still poor. GBM recurrence is inevitable, because in GBM, highly aggressive cells have spread beyond primary cancer and can be seen by modern imaging techniques, meaning they still exist after surgery.[57] Therefore, new therapies and novel treatment strategies are needed in the process of targeting GBM. Uncontrolled proliferation is a prerequisite for the progression of GBM. However, the proliferation mechanism of GBM alone does not ameliorate the prognosis of patients.[57] This treatment cannot solve the unique characteristics of GBM, which are a result of extensive migration to the surrounding tissue of the brain. Therefore, it is critical to find novel treatment methods based on basic research. An in-depth understanding of the pathogenesis of GBM may be a more useful approach to produce a paradigm shift in treatment strategies.

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