Targeting the Epithelial-to-Mesenchymal Transition in Cancer Stem Cells for a Better Clinical Outcome of Glioma

Yu-bao Lu, MS1,*, Tian-Jiao Sun, MS1,*, Yu-tong Chen, MS1, Zong-Yan Cai, MS1, Jia-Yu Zhao, MS1, Feng Miao, MS2, Yong-na Yang, MS3, and Shi-Xin Wang, MS4

Abstract
Glioma is one of the most common malignant tumors of the central nervous system with a poor prognosis at present due to lack of effective treatment options. Its initiation, migration, and multipotency are affected by cancer stem cell's transition. Previous studies imply that changes in the cancer stem cells can affect the malignant differentiation of the tumor. We found that the epithelial-to-mesenchymal transition (EMT)-related regulatory pathway is an important target for tumor therapy. In this review, we discuss the transition factor of EMT and 3 specific pathways that affect the EMT of cancer stem cells during tumor development. We conclude that targeting the EMT process of cancer stem cells can be a feasible approach in the treatment of glioma.

Keywords
EMT, cancer stem cells, glioma, prognosis, targeted therapy

Abbreviations
WHO, World Health Organization; GBM, Glioblastoma multiforme; CSC, Cancer stem cell; EMT, Epithelial-to-mesenchymal transition; TF, Transcription factors; MSC, Mesenchymal stem cell; SNAI-1, Snail Homology-1; Sox2, SRY (sex determination region Y)-box 2; TGF-β, Transforming growth factor-β; HIF-1, mixed-lineage leukemia 1; Shh, Sonic Hedgehog; PTCH1, Patched 1; SMO, Smoothened; GLI, glioma-associated oncogene; ATO, Arsenic trioxide

Received: February 18, 2020; Revised: June 19, 2020; Accepted: July 9, 2020.

Introduction
Glioma is a type of primary brain cancer originating from special non-nerve cells, glial cells, and is associated with poor prognosis at present. It was classified into 4 grades by the World Health Organization (WHO) in 2016. Among them, glioma with clinical grade IV (glioblastoma multiforme, GBM) is still one of the most common and fatal primary brain tumors in neurosurgery. There are no effective treatment methods at present, thereby resulting in poor prognosis. The median survival time is only 12.2 to 18.2 months. Low-grade glioma (WHO grade 1-2) is a well-differentiated glioma; although this type of tumor is not biologically a benign tumor, the patient’s prognosis is relatively good. High-grade gliomas (WHO grades 3,4) are poorly differentiated gliomas; these tumors are malignant tumors, and patients have a poor prognosis. The symptoms and signs caused by gliomas mainly depend on their space-occupying effects and the functions of the affected brain regions. Glioma can cause headache, nausea and vomiting, epilepsy, blurred vision, etc. Stem cells are the origin of the cellular level of organization as they give rise to other cells. At

1 The Second Clinical Medical College, Lanzhou University, Lanzhou, Gansu, China
2 Zhangye People’s Hospital Affiliated to Hexi University, Zhangye, Gansu, China
3 Department of Neurology, The First People’s Hospital of Lanzhou City, Lanzhou, Gansu, China
4 School of Basic Medical Sciences, Lanzhou University, Lanzhou, Gansu, China
*Both authors contributed equally to this study and share first authorship.

Corresponding Author:
Feng Miao, Zhangye People’s Hospital Affiliated to Hexi University, Gansu 734000, China.
Email: 1765757524@qq.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
present, it is generally accepted that stem cells are defined as relatively rare, relatively static, and self-renewing cells. Generally, highly proliferative organs (intestinal tract, skin, etc.) contain at least 2 stem cell pools: one is relatively static, while the other is highly proliferative. At present, the view that gliomas and other primary brain tumors contain self-renewing tumor-causing cells has gradually been widely accepted, suggesting that these tumors contain cancer stem cells (CSCs). Studies indicate that tumor growth is fueled by a small amount of CSCs that are hidden inside the cancer cell pool. Although the subpopulation of CSCs constitute a small minority of tumor cells, their ability enables them to persist, resulting in relapses. Consequently, some anti-cancer treatments focus on suppressing the metastasis and resistance of CSCs.

Epithelial-to-mesenchymal transition (EMT) is an important biological link in the process of tumor occurrence and development, and plays a decisive role in the process of tumor metastasis. The switching of cellular phenotype enables cancer cells to spread more quickly and invade the secondary sites to some extent. EMT progress occurs during various stages of tumor development; we found some related impact factors between EMT and stem cell reprogramming, which influence the progress of glioma and can be used as targets for the development of new treatment strategies. According to the existing wealth of knowledge, some important EMT transcription factor families, including Twist, Snail, and ZEB play a regulatory role in tumor invasiveness and chemotherapy resistance, which is an important reason for the poor prognosis of cancer patients.

Our review is based on the molecular mechanism of GBM tumorigenesis and aims to provide a theoretical basis for the subsequent development of new therapies specific to glioma tumor stem cells in GBM.

**Biological Regulation of EMT**

EMT is a biological process that can promote polarized epithelial cells to undergo a variety of biochemical changes and infer the phenotype of mesenchymal cells. Its biological characteristics mainly include the enhancement of migration ability, invasiveness, and anti-apoptosis ability. EMT is classified into 3 types according to the 3 distinct biological settings encountered.

Several signals were proved to affect the initiate of EMT. Local expression of TGF-β, EGF, IGF-II, or FGF-2 facilitates EMT by binding epithelial receptors with ligand-inducible intrinsic kinase activity. Autocrine TGFβ requires integrin b1 to induce EMT, and this effect is mediated by p38/MAPK and RhoA. In a normal mouse mammary cell line, the induction of the c-Fos oncogene induces EMT and is associated with downregulation of E-cadherin expression the loss of E-cadherin is pivotal to the further induction of EMT. The effect of Ras mutants on EMT specifically activates either the ERK/MAPK or the PI3K-Akt/PKB pathway.

Classical EMT plays an important role in many stages of embryonic development, including the transformation of epithelial cells to cells with mesenchymal phenotype, with a variety of typical molecular markers, including E-cadherin and Vimentin.

However, partial activation of EMT transcription factors (TFs) can increase the motility of cancer cells, which acts not only on the collective migration process of the cell mass, but even on the migration of individual cancer cells; so partial EMT is conducive to the invasion and spread of cancer cells, which further leads to the growth and metastasis of cancer.

The main executors of EMT are EMT-activated transcription factors (EMT-TF), which mainly include SNAIL, TWIST, and ZEB family members. Some studies have shown that the overexpression of EMT-promoting factor, TWIST is an important factor contributing to the invasive ability of glioma cell lines. Additionally, mesenchymal stem cell (MSC) surface markers, including CD29, CD44, CD90, and CD105 were highly expressed in GBM and its stem cell lines. The knockdown of the chemokine receptor, CXCR4, which was regarded as the specific surface marker for MSC, shows that it is a promoter for the migration of glioma cells and the expression of EMT markers. In addition, the EMT regulatory factor, STAT3, is also important in promoting tumor invasion and growth. Snail Homology-1 (SNAI-1), an EMT-promoting factor, is expressed differently in gliomas with different differentiation, such as high expression in high-grade gliomas and low expression in low-grade gliomas; so SNAI-1 is considered to be related to cell proliferation and infiltration. ZEB1 can promote the transitivity of glioma by recruiting CDH1 promoters and repressing the expression of E-cadherin. In the process of xenotransplantation of human GBM cells in mice, researchers found that SRY (sex determination region Y)-box 2 (Sox2) is the key molecule that maintains the plasticity between tumor stem cells and glioma cells. In short, Sox2 is the key molecule for GBM cells to maintain plasticity.

Transforming growth factor-β (TGF-β) is a powerful endoderm transformation inducer, which can promote tumor progression and metastasis. TGF-β 1 can activate a variety of downstream signal pathways, including PI3 K, Smads, and MAPK, which are involved in TGF-β-induced EMT.

A research shows that primary glioblastoma and its stem cell lines have some of the cellular and molecular characteristics of MSCs. On the other hand, EMT-TFs can maintain the stem cell characteristics and increase the tumorigenicity of cells. This leads us to pay attention to the association between the CSCs and EMT. Some findings are inconsistent with the speculation that EMT is necessary to maintain the phenotype of CSCs, and they imply that EMT is uncoupled from stemness in many contexts. However, these studies did not count on the fact that the EMT in cancer cells is transient, depending mostly on the environmental impact; hence, cancer cells can adopt intermediate mesenchymal states to access the more plastic CSC phenotypes.

Moreover, Schmidt JM et al. explained the comprehensive mechanism of the action of EMT-TF through experiments. This
study found that EMT is contradictory to the induction and inhibition of stem cell-like traits, because these traits can appear as stable traits after the inactivation of Twist1 or Snail1. This suggests that transient Twist1 or Snail1 activation may leave an epigenetic footprint that is easy to deal with, thereby suggesting a closed connection between CSC behavior and the EMT process.

According to the previously reported findings, hypoxia is one of the features of tumors, and the degree of hypoxia increases with the severity of the tumor. It is well known that the hypoxia zone is the hidden area of one of the subsets of CSCs. A recent study shows that a drug combination targeting hypoxia can induce chemoresistance and stemness in glioma cells. The combination of COX-2 inhibitor (NS-398) and BCNU was demonstrated to be an effective therapy, reflected by the decreased production of the inflammatory regulatory factor, PGE2, which is the expression product of COX-2. At the same time, the migration rate of nuclear cells expressing the EMT markers was also significantly inhibited. More importantly, the combination of drugs not only inhibited the formation of glial non-globulin, but also decreased the expression of CD133.

To some extent, COX-2 can be regarded as a regulator of CSC proliferation, and the hypoxic environment promotes the expression of COX-2, thus affecting the proliferative ability of CSCs. It is now well-established that the inflammation triggered by COX-2 is critical in enabling the characteristic of tumorigenesis. In addition, the biological functions of COX-2 include angiogenesis, EMT, and spheroid formation that help in tumor progression.

The main cause of tumor hypoxia is the abnormal structure and distribution of neovascularization in the tumor and the abnormal activation of intravascular coagulation cascade. Recruiting myeloid cells and MSCs that secrete TGF-β is the main way through which hypoxia promotes EMT. Additionally, another important mode by which hypoxia promotes the occurrence of EMT in the tumor microenvironment is by the activation of Notch1 by transcription factors, such as mixed-lineage leukemia 1 (HIF-1) induced by hypoxia, which is known to be very important for the induction and maintenance of hypoxia-induced stem cell phenotype. TGF-β family is also considered to be another important factor for the induction and maintenance of pluripotent stem cells and tumor stem cells. They can upregulate the transcription factors, TWIST or Snail, which are necessary for the EMT process. Notch has already been shown to have the ability of regulating the EMT process. As one of the most important regulatory factors of the EMT process, studies have shown that the expression of ZEB1 is up-regulated in glioma neurospheres under hypoxia; hence, its inhibition can reduce the cell invasion induced by hypoxia. Therefore, the characteristics of stem cells induced by hypoxia through the EMT process promote the destructive and refractory characteristics of GBM to a great extent, and the characteristics of stem cells in turn are the regulatory factors of stem cell characteristics, so they are the main contributors to drug resistance.

Based on the above conclusions, we speculated that COX-2 / TGF-β / HIF-1α / Zeb1 / GSC axis might be the potential target of glioma treatment.

**γ-SECRETASE SIGNALLING PATHWAY**

Recent studies have shown that the Notch signaling pathway plays an important role in regulating the activity of GSCs. In some preclinical models, the inhibition of Notch signal by γ-secretase signaling pathways was found to reduce the number and / or tumorigenicity of tumor stem cells, suggesting that Notch inhibitors may be used to target tumor stem cells as well as reverse or prevent chemoresistance or radiation resistance.

The Notch family proteins are transmembrane receptor proteins. Their intracellular domains are released from the membrane into the nucleus through the enzymatic hydrolysis of the γ-secretase complex, thereby activating the transcriptional CSL family (CBF1, Suppressor of Hairless, and Lag-1). In many cellular processes, including cell-fate decision, differentiation, proliferation, survival, angiogenesis, and migration, the Notch signaling pathway is known to play an important role.

In GSCs, the Notch signaling pathway plays a key role in the development of tumorigenesis and maintaining the balance between stem cell characteristics and cell differentiation. Notch can enable the CSCs to become resistant to radiation.

Some drugs under clinical trial, such as the γ-secretase signaling pathways to suppress Notch signaling and an oral hedgehog antagonist are awaiting review and approval. The γ-secretase signaling pathways enhance the efficacy of temozolomide in the treatment of human gliomas in vivo and vitro. CD133, Notch-1, and VEGF positive glioma cells were highly expressed in recurrent glioblastoma after radiotherapy and chemotherapy, and Notch was found to regulate the activity of VEGF pathway in GSCs. Research also found that the overall survival was significantly longer in cases with Notch-1 negativity than in those with Notch-1 positivity.

Besides regulating the cell cycle and senescence, Notch also regulates EMT. The experimental data shows that TGF-β plays a role as a key positive effector of Notch1 and EMT in the tumor microenvironment. In the TGF-β-mediated EMT, the expression of Notch ligand, JAG1, is induced by ZEB1, and more and more evidence supports Notch1 as a positive effector of EMT. However, Notch3 was found to limit the expansion of EMT in esophageal keratinocytes. Previous studies have shown that Notch1 activation and EMT coupling promote tumor initiation and tumor heterogeneity in CSCs, while the transcription factor, ZEB1 inhibits Notch3.

We infer that the TGF-β–ZEB1–Notch1-γ-secretase-inhibitor axis could be a potential target for CSC therapy.

**Sonic Hedgehog**

Sonic Hedgehog (Shh) signaling pathway is a very important signal pathway in the process of embryonic development and proliferation, especially in the migration, differentiation, and
survival of neural stem cells.\textsuperscript{77} It has been shown that the ectopic expression of Shh can induce the occurrence of basal cell carcinoma in mice.\textsuperscript{78-79} Most of the solid tumors are characterized by hyperactivation of the Shh pathway.\textsuperscript{80,81} Aberrant Shh signaling accounts for up to 25\% of human cancer-related deaths.\textsuperscript{82} Shh pathway also plays an important role in the regulation of CSC differentiation and maintenance of stemness. Studies have found that Shh signal is very important for the maintenance of CSCs, and inhibition of Shh pathway leads to stem cell proliferation and reduced renewal.\textsuperscript{83,84}

Shh pathway functions by inactivating the 12-transmembrane protein, Patched1 (PTCH1), and suppresses its ability to inhibit the 7-transmembrane protein, Smoothenend (SMO) signal transmission elements, resulting in the nuclear localization of glioma-associated oncogene (GLI) downstream transcription factors.\textsuperscript{85} It has been proved that Gli transcription factors are positively regulated by many molecules, including PI3K-AKT, TGF-\(\beta\), PKC-\(\zeta\), and K-RAS.\textsuperscript{86-89} In vertebrates, GLI transcription factors mainly have 3 members: GLI1, GLI12, and GLI3, of which GLI1 is the only full-length transcriptional activator, while the positive and negative regulation of GLI2 and GLI3 is determined by different processes.\textsuperscript{90}

Activated GLI1 and GLI2 can physically bind the promoter regions of a group of genes, including oncogenes and genes involved in the EMT process, and directly promote their transcriptional expression. These genes are mainly Bmi1, NANOG, and SNAIL1.\textsuperscript{91-95} The TGF-\(\beta\)/SMAD/GLI2 axis has been suggested to be essential for cancer metastasis.\textsuperscript{96} Therefore, the crosstalk between SHH signal pathway and TGF-\(\beta\) signal pathway is the molecular mechanism of endothelial cell transformation.

Recently, there have been some medical treatments targeting the shh pathway. Blocking the SHH receptor or interfering with its downstream molecules is the most basic strategy of drug intervention based on SHH signal pathway.\textsuperscript{97} In view of the important role of GLI1/2 in promoting EMT and tumor metastasis in the SHH pathway, blocking GLI1/2 is considered to be a promising strategy in cancer treatment. Compared with blocking the upstream regulatory factors in the SHH pathway, the advantage of targeting GLI protein is that GLI protein is the molecular intersection of multiple signal pathways activated in tumor cells, including TGF-\(\beta\), Wnt, and SHH pathways.\textsuperscript{98}

Treatments using small chemical molecules, GANT58 and/or GANT61, which block GLI1/2 function, have been shown to arrest prostate tumor growth.\textsuperscript{99} The combination of cyclopamine, gemcitabine with vismodegib, and other SMO inhibitors were widely used in clinical therapy for various types of cancers.\textsuperscript{100-102} However, this kind of research has not been widely carried out in gliomas, so it has great limitations. Erismodegib has been proved to induce cell cycle arrest and apoptosis in many tumor cell lines.\textsuperscript{103} It has been shown to effectively inhibit the EMT and invasiveness of a variety of cancers, including glioblastoma, prostate cancer, and renal cell carcinoma, which means that it can act on both tumor epithelial cells and tumor stem cells.\textsuperscript{104-106} Arsenic trioxide (ATO) is an inhibitor of GLI1 and GLI2 transcription factors approved by the FDA. Studies have shown that ATO also shows good therapeutic activity against pancreatic and prostate cancer, emphasizing its effectiveness in killing tumor epithelial cells and tumor initiator cells.\textsuperscript{107-109}

We infer that Shh SMO/ptc—GLI—Norch/TGF-\(\beta\)—snail/EMT-TFs can be a potential pathway that can be targeted in glioma therapy.

**Cross Talk of the 2 Pathway**

Notch Signaling Contributes to Medulloblastoma Growth and Survival. It was proved to be elevated Notch and Shh activity in most medulloblastomas. Notch inhibition with soluble Delta ligand or \(\gamma\)-secretase inhibitors resulted in decreased proliferation and increased apoptosis. Former study pharmacologically inhibited Notch cleavage with the \(\gamma\)-secretase inhibitor, DAPT. The combination of Shh antagonism with cyclopamine and Notch antagonism resulted in a significantly greater response than the use of either agent alone. The effect of combined Shh and Notch pathway inhibition appeared to be additive in these primary tumors.\textsuperscript{110}

Comparing these 2 pathways, It’s not hard to find that both pathways involves Norch signal. We deduce that Norch signal can be the crosstalk connect nodes of the 2 pathways.

**Discussion**

There are already some drugs against GSCs, targeting hypoxia initiators, suppressing the activity of Notch pathway, and regulating the Shh transcription factors. Many drugs are effective in targeting certain molecules, affecting the transcription and expression process in cells. Many of the targeted molecules are related to the EMT process, thereby adjusting the CSC phenotype and changing the CSC process. Although the current median survival time of glioblastoma (grade 4 glioma) remains relatively short and easy to recur, it is undeniable that these drugs or regulatory mechanisms have great therapeutic potential to be discovered.

1. The regulation of EMT is not a separate process, it is a regulatory network regulated by a variety of molecules, and plays an important role in the initial process of tumor development. Therefore, controlling the progression of tumors by controlling the EMT should simultaneously control multiple factors rather than relying on a single pathway. Therefore, we believe that further improving our understanding of the integrated regulatory network and feedback mechanism of the signal pathways in EMT will help bring more reliable evidence for the development of high-precision targeted therapy against glioma. At the same time, the establishment of signal regulatory network can better identify more key hub targets for the research and development of targeted drugs, so as to achieve the purpose of using a single drug to block multiple biological processes.
2. Considering the complexity of the EMT signal regulatory network and the strong adaptability of cancer cells, we, like other researchers, believe that targeting one protein or pathway may not be enough to completely block the EMT. Therefore, anti-EMT therapy should be combined with multi-molecular targeted therapy to combat the EMT transformation. However, we are still skeptical about the prospect of this multi-target and multi-level molecular targeted therapy. Therefore, we believe that the support of stem cell therapy is still needed to facilitate the full use of its advantages (Table 1).

3. At the same time, the factors that play a role in tumor development and progression are complex and diverse. Further studies need to be undertaken to unravel the mechanisms involved in these processes and find more phenotypes that can be suitable prognostic markers or therapeutic targets. We demonstrate some of the currently existing targeted therapies for some of the targets mentioned above. They also aimed at different targets and at different stages of clinical research. (Table 2) It is hoped that in the following research, the comprehensive application of some single-target drugs can be emphasized. Hopefully, in the future, we can identify CSCs and combine the already-tested therapies with newly-discovered drug therapy to control the development of CSCs. We believe that the combination treatment of stem cell therapy with anti-EMT therapy will be one of the effective development directions for future cancer treatment.

In conclusion, further research is needed to reveal the regulatory mechanism of EMT in these nonepithelial tumors and establish a deeper understanding of its molecular regulatory network. On this basis, using signal network analysis and identifying prognostic markers or treatment targets to develop multi-target therapeutic drugs, and combining them with stem cell therapy to improve the clinical outcome of glioma will bring greater improvement in the prognosis of patients with glioma.

**Authors’ Note**

No animal experiments or clinical trials were carried out in the course of this study.

**Acknowledgments**

We would like to thank Editage for English language editing. We thank Dr. Zhijian Ma for his guidance on our manuscript.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by 2019 Scientific Research Project of Traditional Chinese Medicine in Gansu Province (grant no. GZK-2019-46).

**ORCID iD**

Yu-bao Lu [https://orcid.org/0000-0003-4375-8316](https://orcid.org/0000-0003-4375-8316)

**References**

1. Nakada M, Hayashi Y, Hamada J. Role of Eph/ephrin tyrosine kinase in malignant glioma. *Neuro Oncol*. 2011;13(11):1163-1170. doi:10.1093/neuonc/nor102

2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system. *Acta Neuropathol*. 2016;131(2):229-263. doi:10.1007/s00401-015-1504-1

**Table 1.** Key Molecules in Epithelial-to-Mesenchymal (EMT) Transition and Their Effects.

| EMT-TF          | Molecular type                               | Effect                           |
|-----------------|----------------------------------------------|----------------------------------|
| TWIST           | EMT promoting factor                         | enhances tumor invasion         |
| CD29, CD44, CD90, CD105 | mesenchymal stem cell surface markers      | highly expressed in GBM          |
| CXCR4           | chemokine receptor MSC surface marker        | promotes the migration of glioma |
| STATS           | master regulator of EMT                      | promotes the infiltration and growth of the tumor |
| SNAI1           | EMT promoting factor                         | cell proliferation and infiltration |
| ZEB1            | transcriptional repressor                     | promotes transitivity and        |
| Sox2            | stemness related marker                       | represses the expression of E-cadherin. |
| TGF-β1          | transforming growth factor                    | maintains plasticity for the mutual conversion |
|                 |                                               | activates various downstream pathways |

**Table 2.** The Most Important Inhibitor Presently Undergoing Preclinical and Clinical Studies.

| Target          | Candidate therapy                           |
|-----------------|--------------------------------------------|
| EGFR            | EGFRvIII peptide vaccine                   |
| Oncogenic FGFR-TACC | JNJ-42756493 – a highly selective pan-FGFR TKI & other selective FGFR inhibitors (e.g. BGJ398 and AZD4547) (ced) or cediranib + CCNU (CCNU/ced) (PDGFR inhibitor) |
| PDGFR (ced)     | Crizotinib (c-MET/ALK inhibitor)          |
| c-MET/ALK       | bevacizumab plus onartuzumab, MET inhibitor (cabozantinib) |
| MET             | F1KR1/3 inhibitor (JNJ-42756493)           |
| IDH1            | IDH1 inhibitor (AG120)                     |
| SMO             | Cyclopamine                                 |
| GLI             | Arsenic Trioxide (ATO)                     |
| P13k&mtTOR      | NVP-BEZ-235                                 |

... and combine the already-tested therapies with newly-discovered drug therapy to control the development of CSCs. We believe that the combination treatment of stem cell therapy with anti-EMT therapy will be one of the effective development directions for future cancer treatment.

... In conclusion, further research is needed to reveal the regulatory mechanism of EMT in these nonepithelial tumors and establish a deeper understanding of its molecular regulatory network. On this basis, using signal network analysis and identifying prognostic markers or treatment targets to develop multi-target therapeutic drugs, and combining them with stem cell therapy to improve the clinical outcome of glioma will bring greater improvement in the prognosis of patients with glioma.

Authors’ Note

No animal experiments or clinical trials were carried out in the course of this study.

Acknowledgments

We would like to thank Editage for English language editing. We thank Dr. Zhijian Ma for his guidance on our manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by 2019 Scientific Research Project of Traditional Chinese Medicine in Gansu Province (grant no. GZK-2019-46).

ORCID iD

Yu-bao Lu [https://orcid.org/0000-0003-4375-8316](https://orcid.org/0000-0003-4375-8316)

References

1. Nakada M, Hayashi Y, Hamada J. Role of Eph/ephrin tyrosine kinase in malignant glioma. *Neuro Oncol*. 2011;13(11):1163-1170. doi:10.1093/neuonc/nor102

2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system. *Acta Neuropathol*. 2016;131(2):229-263. doi:10.1007/s00401-015-1504-1
system: a summary. Acta Neuropathol. 2016;131(6):803-820. doi: 10.1007/s00401-016-1545-1

3. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NICIC trial. Lancet Oncol. 2009; 10(5):459-466. doi: 10.1016/S1470-2245(09)70025-7

4. Barker N, Bartfeld S, Clevers H. Tissue-resident adult stem cell populations of rapidly self-renewing organs. Cell Stem Cell. 2010; 7(6):656-670. doi: 10.1016/j.stem.2010.11.016

5. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia. 2002;39(3):193-206. doi:10.1002/glia.10094

6. Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci U S A. 2003;100(25):15178-15183. doi:10.1073/pnas.2036535100

7. Brandes AA, Ermani M, Basso U, et al. Monfardini temozolomide a phase II study. Ann Oncol. 2001;12(2):255-257. doi:10.1023/a:1008336723273

8. Lapointe S, Perry A, Butowski NA. Primary brain tumours in adults. Lancet. 2018;392(10145):432-446. doi:10.1016/s0140-6736(18)30990-5

9. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. Nature. 2004;432(7015):296-301. doi:10.1038/nature03128

10. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 2004;64(19):7011-7021. doi:10.1158/0008-5472.CAN-04-1364

11. Ren B, Cui M, Yang G, et al. Tumor microenvironment participates in metastasis of pancreatic cancer. Mol Cancer. 2018;17(1):108. doi:10.1186/s12943-018-0858-1

12. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol. 2006;172(7):973-981. doi:10.1083/jcb.200601018

13. Tarin D, Thompson EW, Newgreen DF. The fallacy of epithelial mesenchymal transition in neoplasia. Cancer Res. 2005;65(14):5996-6001. doi:10.1158/0008-5472.CAN-05-0699

14. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2(6):442-454. doi:10.1038/nrc822

15. Tse JC, Kalluri R. Mechanisms of metastasis: epithelial-to-mesenchymal transition and contribution of tumor microenvironment. J Cell Biochem. 2007;101(4):816-829. doi:10.1002/jcb.21215

16. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer. 2009;9(4):265-273. doi:10.1038/nrc2620

17. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest. 2003;112(12):1776-1784. doi:10.1172/JCI20530

18. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119(6):1420-1428.

19. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer. 2018;18(2):128-134. doi:10.1038/nrc.2017.118

20. Elias MC, Tozer KR, Silber JR, et al. TWIST is expressed in human gliomas and promotes invasion. Neoplasia. 2005;7(9):824-837. doi:10.1593/neo.04352

21. Tso CL, Shintaku P, Chen J, et al. Primary glioblastomas express mesenchymal stem-like properties. Mol Cancer Res. 2006;4(9):607-619. doi:10.1158/1541-7786.MCR-06-0005

22. Zhu Y, Yang P, Wang Q, et al. The effect of CXCR4 silencing on epithelial-mesenchymal transition related genes in glioma U87 cells. Anat Rec. 2013;296(12):1850-1856. doi:10.1002/ar.22821.

23. Priester M, Copanaki E, Vaafazadeh V, et al. STAT3 silencing inhibits gliona single cell infiltration and tumor growth. Neuro Oncol. 2013;15(7):840-852. doi:10.1093/neuonc/noct025

24. Motta FJ, Valera ET, Lucio-Eterovic AK, et al. Differential expression of E-cadherin gene in human neuroepithelial tumors. Genet Mol Res. 2008;7(2):295-304.

25. Han SP, Kim JH, Han ME, et al. SNAI1 is involved in the proliferation and migration of glioblastoma cells. Cell Mol Neurobiol. 2011;31(3):489-496. doi:10.1007/s10571-010-9643-4

26. Sánchez-Tílló E, Lázaro A, Torrent R, et al. ZEB1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1. Oncogene. 2010;29(24):3490-3500. doi:10.1038/onc.2010

27. Berezovsky AD, Poisson LM, Cherda D, et al. Sox2 promotes malignancy in glioblastoma by regulating plasticity and astrocytic differentiation. Neoplasia. 2014;16(3):193-206.e2.06E25. doi:10.1016/j.neo.2014.03.006

28. Pakala SB, Singh K, Reddy SD, et al. TGF-beta1 signaling targets metastasis-associated protein 1, a new effector in epithelial cells. Oncogene. 2011;30(19):2230-2241.

29. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res. 2009;19:156-172.

30. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell. 2008;14(6):818-829.

31. Batlle E, Clevers H. Cancer stem cells revisited. Nat Med. 2017;23(10):1124-1134. doi:10.1038/nm.4409

32. Wellner M, Stupp R, Wick W. Epilepsy meets cancer: when, why, and what to do about it? Lancet Oncol. 2012;13(9):e375-e382.

33. Schmidt JM, Panziulis E, Bartsch HS, et al. Stem-cell-like properties and epithelial plasticity arise as stable traits after transient Twist1 activation. Cell Rep. 2015;10(2):131-139. doi:10.1016/j.celrep.2014.12.032

34. Evans SM, Judy KD, Dunphy I, et al. Hypoxia is important in the biology and aggression of human glial brain tumors. Clin Cancer Res. 2004;10(24):8177-8184.

35. Vinogradov S, Wei X. Cancer stem cells and drug resistance: the potential of nanomedicine. Nanomedicine (Lond). 2012;7(4):597-615.

36. Jalota A, Kumar M, Das BC, Yadav AK, Chosdol K, Sinha S. A drug combination targeting hypoxia induced chemoresistance and stemness in glioma cells. Oncotarget. 2018;9(26):18351-18366. Published 2018 Apr 6. doi:10.18632/oncotarget.24839
37. Herseth JI, Refsnes M, Låg M, Schwarze PE. Role of IL-1 beta and COX2 in silica-induced IL-6 release and loss of pneumocytes in co-cultures. *Toxicol In Vitro*. 2009;23(7):1342-1353.

38. Candido J, Hagemann T. Cancer-related inflammation. *J Clin Immunol*. 2013;33(suppl 1):S79-S84.

39. Bocca C, Ievolella M, Autelli R, et al. Expression of Cox-2 in human breast cancer cells as a critical determinant of epithelial-to-mesenchymal transition and invasiveness. *Expert Opin Ther Targets*. 2014;18(2):121-135.

40. Gately S. The contributions of cyclooxygenase-2 to tumor angiogenesis. *Cancer Metastasis Rev*. 2000;19(1-2):19-27.

41. Neil JR, Johnson KM, Nemenoff RA, Schiennmann WP. Cox-2 inactivates Smad signaling and enhances EMT stimulated by TGF-beta through a PGE2-dependent mechanisms. *Carcinogenesis*. 2008;29(11):2227-2235.

42. Majumder M, Xin X, Liu L, et al. COX-2 induces breast cancer stem cells via EP4/PJ3/AKT/NOTCH/WNT AXIS. *Stem Cells*. 2016; 34(9):2290-2305.

43. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science*. 2005;307(5706):58-62. doi:10.1126/science.1104819

44. Sahlgren C, Gustafsson MV, Jin S, Poellinger L, Lendahl U. Hypoxia-induced mixed-lineage leukemia 1 regulates glioma stem cell tumorigenic potential. *Cell Death Differ*. 2012;19(3):428-439. doi:10.1038/cdd.2011.109

45. Fan X, Khaki L, Zhu TS, et al. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells*. 2010;28(1):5-16. doi:10.1002/stem.254

46. Ye XZ, Xu SL, Xin YH, et al. Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGFB1 signaling pathway. *J Immunol*. 2012;189(1):444-453. doi:10.4049/jimmunol.1103248

47. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420-1428. doi:10.1172/JCI39104 [published correction appears in *J Clin Invest*. 2010;120(5):1786.]

48. Ye XZ, Xu SL, Xin YH, et al. Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGFB1 signaling pathway. *J Immunol*. 2012;189(1):444-453. doi:10.4049/jimmunol.1103248

49. Kahlert U, Suwala A, Raabe E, et al. ZEB1 promotes invasion in human fetal neural stem cells and hypoxic glioma neurospheres. *Brain Pathol*. 2015;25(6):724-732. doi:10.1111/bpa.12240

50. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756-760. doi:10.1038/nature05236

51. Myśczyszyn A, Czarnecka AM, Matak D, et al. The role of hypoxia and cancer stem cells in renal cell carcinoma pathogenesis. * Stem Cell Rev*. 2015;11:919-943.

52. Kanamori M, Kawaguchi T, Negro JM, et al. Contribution of notch signaling activation to human glioblastoma multiforme. *J Neurosurg*. 2007;106(3):417-427. doi:10.3171/jns.2007.106.3.417

53. Purow BW, Haque RM, Noel MW, et al. Expression of Notch-1 and its ligands, delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. *Cancer Res*. 2005;65(6):2353-2363. doi:10.1158/0008-5472.CAN-04-1890

54. Zhu TS, Costello MA, Talsma CE, et al. Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells. *Cancer Res*. 2011;71(18):6061-6072. doi:10.1158/0008-5472.CAN-10-4269

55. Fan X, Khaki L, Zhu TS, et al. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells*. 2010;28(1):5-16. doi:10.1002/stem.254

56. Kopan R. Notch: a membrane-bound transcription factor. *J Cell Sci*. 2002;115(Pt 6):1095-1097.

57. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell*. 2009;137(2):216-233. doi:10.1016/j.cell.2009.03.045

58. Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. *Dev Cell*. 2009;16(5):633-647. doi:10.1016/j.devcel.2009.03.010

59. Kageyama R, Ohtsuka T. The notch-hes pathway in mammalian neural development. *Cell Res*. 1999;9(3):179-188. doi:10.1038/sj.cr.7290016

60. Wang J, Wakeman TP, Lathia JD, et al. Notch promotes radioresistance of glioma stem cells. *Stem Cells*. 2010;28(1):17-28. doi:10.1002/stem.261

61. Fan X, Khaki L, Zhu TS, et al. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells*. 2010;28(1):5-16. doi:10.1002/stem.254

62. Gilbert CA, Daou MC, Moser RP, Ross AH. Gamma-secretase inhibitors enhance temozolomide treatment of human gliomas by inhibiting neurosphere repopulation and xenograft recurrence. *Cancer Res*. 2010;70(17):6870-6879. doi:10.1158/0008-5472.CAN-10-1378

63. Saito N, Fu J, Zheng S, et al. A high notch pathway activation predicts response to γ secretase inhibitors in proneural subtype of glioma tumor-initiating cells. *Stem Cells*. 2014;32(1):301-312. doi:10.1002/stem.1528

64. Gilbert CA, Daou MC, Moser RP, Ross AH. Gamma-secretase inhibitors enhance temozolomide treatment of human gliomas by inhibiting neurosphere repopulation and xenograft recurrence. *Cancer Res*. 2010;70(17):6870-6879. doi:10.1158/0008-5472.CAN-10-1378

65. Saito N, Fu J, Zheng S, et al. Effect of Notch expression in glioma stem cells on therapeutic response to chemo-radiotherapy in recurrent glioblastoma. *Brain Tumor Pathol*. 2015;32(3):176-183. doi:10.1007/s10014-015-0215-7

66. Rangarajan A, Talora C, Okuyama R, et al. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO J*. 2001;20(13):3427-3436. doi:10.1093/emboj/20.13.3427

67. Kagawa S, Natsuizaka M, Whelan KA, et al. Cellular senescence checkpoint function determines differential Notch1-dependent oncogenic and tumor-suppressor activities. *Oncogene*. 2015;34(18):2347-2359. doi:10.1038/onc.2014.169
68. Ronchini C, Capobianco AJ. Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): implication for cell cycle disruption in transformation by Notch(ic). *Mol Cell Biol*. 2001; 21(17):5925-5934. doi:10.1128/mcb.21.17.5925-5934.2001

69. Zavadil J, Cermak L, Soto-Nieves N, Böttiger EP. Integration of TGF-beta/Smad and Jagged1/Notch signalling in epithelial-mesenchymal transition. *EMBO J*. 2004;23(5):1155-1165. doi: 10.1038/sj.emboj.7600069

70. Niessen K, Fu Y, Chang L, Karsan A. Slug is a direct notch target required for initiation of cardiac cushion cellularization. *J Cell Biol*. 2008;182(2):315-325. doi: 10.1083/jcb.200710067

71. Sahlgren C, Gustafsson MV, Jin S, Poellinger L, Lendahl U. Notch signalling mediates hypoxia-induced tumor cell migration and invasion. *Proc Natl Acad Sci U S A*. 2008;105(17):6392-6397. doi:10.1073/pnas.0802047105

72. Natsuiizaka M, Whelan KA, Kagawa S, et al. Interplay between Notch1 and Notch3 promotes EMT and tumor initiation in squamous cell carcinoma. *Nat Commun*. 2017;8(1):1758. Published 2017 Nov 24. doi: 10.1038/s41467-017-01500-9

73. Brabletz S, Bajdak K, Meidhof S, et al. The ZEB1/miR-200 feedback loop controls notch signalling in cancer cells. *EMBO J*. 2011;30(4):770-782. doi:10.1038/emboj.2010.349

74. Zhang T, Guo L, Creighton CJ, et al. A genetic cell context-dependent role for ZEB1 in lung cancer. *Nat Comm*. 2016;7:12231. Published 2016 Jul 26. doi:10.1038/ncomms12231

75. Wang T, Xuan X, Pian L, et al. Notch-1-mediated esophageal carcinoma EC-9706 cell invasion and metastasis by inducing epithelial-mesenchymal transition through Snail. *Tumour Biol*. 2014;35(2):1193-1201. doi:10.1007/s13277-013-1159-3

76. Ohashi S, Natsuiizaka M, Naganuma S, et al. A NOTCH3-mediated squamous cell differentiation program limits expansion of EMT-competent cells that express the ZEB transcription factors. *Cancer Res*. 2011;71(21):6836-6847. doi:10.1158/0008-5472.CAN-11-0846

77. Chiang C, Litingtung Y, Lee E, et al. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature*. 1996;383(6599):407-413. doi:10.1038/383407a0

78. Fan H, Oro AE, Scott MP, Khavari PA. Induction of basal cell carcinoma features in transgenic human skin expressing Sonic Hedgehog. *Nat Med*. 1997;3(7):788-792. doi:10.1038/nm0797-788

79. Oro AE, Higgins KM, Hu Z, Bonifas JM, Epstein EH Jr, Scott MP. Basal cell carcinomas in mice overexpressing sonic hedgehog. *Science*. 1997;276(5313):817-821. doi:10.1126/science.276.5313.817

80. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature*. 2003;422(6929):313-317. doi:10.1038/nature01493

81. Dierks C, Beigi R, Guo GR, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. *Cancer Cell*. 2008;14(3):238-249. doi: 10.1016/j.ccr.2008.08.003

82. Lum L, Beachy PA. The hedgehog response network: sensors, switches, and routers. *Science*. 2004;304(5678):1755-1759. doi: 10.1126/science.1098020

83. Liu S, Dontu G, Mantle ID, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res*. 2006;66(12):6063-6071.
98. Zhang J, Tian XJ, Xing J. Signal transduction pathways of EMT induced by TGF-β, SHH, and WNT and their crosstalks. J Clin Med. 2016;5(4):E41. doi:10.3390/jcm5040041
99. Lauth M, Bergström A, Shimokawa T, Toftgård R. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. Proc Natl Acad Sci U S A. 2007;104(20):8455-8460. doi:10.1073/pnas.0609699104
100. LoRusso PM, Rudin CM, Reddy JC, et al. Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors. Clin Cancer Res. 2011;17(8):2502-2511. doi:10.1158/1078-0432.CCR-10-2745
101. Kaye SB, Fehrenbacher L, Holloway R, et al. A phase II, randomized, placebo-controlled study of vismodegib as maintenance therapy in patients with ovarian cancer in second or third complete remission. Clin Cancer Res. 2012;18(23):6509-6518. doi:10.1158/1078-0432.CCR-12-1796
102. Singh BN, Fu J, Srivastava RK, Shankar S. Hedgehog signaling antagonist GDC-0449 (Vismodegib) inhibits pancreatic cancer stem cell characteristics: molecular mechanisms. PLoS One. 2011;6(11):e27306. doi:10.1371/journal.pone.0027306
103. Pan S, Wu X, Jiang J, et al. Discovery of NVP-LDE225, a potent and selective smoothened antagonist. ACS Med Chem Lett. 2010;1(3):130-134.
104. D’Amato C, Rosa R, Marciano R, et al. Inhibition of Hedgehog signalling by NVP-LDE225 (Erismodegib) interferes with growth and invasion of human renal cell carcinoma cells. Br J Cancer. 2014;111(6):1168-1179. doi:10.1038/bjc.2014.421
105. Fu J, Rodova M, Nanta R, et al. NPV-LDE-225 (Erismodegib) inhibits epithelial mesenchymal transition and self-renewal of glioblastoma initiating cells by regulating miR-21, miR-128, and miR-200. Neuro Oncol. 2013;15(6):691-706. doi:10.1093/neuonc/not011
106. Nanta R, Kumar D, Meeker D, Rodova M, Van Veldhuizen PJ, Shankar S, Srivastava RK. NVP-LDE-225 (Erismodegib) inhibits epithelial-mesenchymal transition and human prostate cancer stem cell growth in NOD/SCID IL2Rγ null mice by regulating Bmi-1 and microRNA-128. Oncogenesis. 2013;2(4):e42. doi:10.1038/oncsis.2013.5
107. Bansal N, Farley NJ, Wu L, Lewis J, Youssoufian H, Bertino JR. Darinaparsin inhibits prostate tumor-initiating cells and DU145 xenografts and is an inhibitor of hedgehog signaling. Mol Cancer Ther. 2015;14(1):23-30. doi:10.1158/1535-7163.MCT-13-1040
108. Han JB, Sang F, Chang JJ, et al. Arsenic trioxide inhibits viability of pancreatic cancer stem cells in culture and in a xenograft model via binding to SHH-Gli. Onco Targets Ther. 2013;6:1129-1138. doi:10.2147/OTT.S49148
109. Kahlert UD, Joseph JV, Kruyt FAE. EMT- and MET-related processes in nonepithelial tumors: importance for disease progression, prognosis, and therapeutic opportunities. Mol Oncol. 2017;11(7):860-877. doi:10.1002/1878-0261.12085
110. Andrew RH, Joel IP, Stacey H, et al. The smoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas. Cancer Res. 2004;64(21):7794-7800. doi:10.1158/0008-5472.CAN-04-1813