The different role of YKL-40 in glioblastoma is a function of MGMT promoter methylation status

Wei-jun Chen1,2, Xiang Zhang1, Hua Han3, Jian-nan Lv1, En-ming Kang1, Yu-lian Zhang4, Wei-ping Liu1, Xiao-sheng He3, James Wang5, Gui-huai Wang5, Yan-bing Yu4 and Wei Zhang6,7

Abstract
Inter- and intratumoral heterogeneity is a hallmark of glioblastoma (GBM) that facilitates recurrence, treatment resistance, and worse prognosis. O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation is a significant prognostic marker for Temozolomide (TMZ) resistance in GBM patients. YKL-40 is a molecular marker for the mesenchymal subtype of GBMs and is responsible for TMZ resistance. However, underlying mechanisms by which MGMT epigenetics impacts patient outcomes and the function of YKL-40 are not fully determined. Herein, we performed in vitro and in vivo experiments, six human IDH1/2 wild-type glioblastoma stem-like cells (GSCs) were established and studied to further determine a potential interaction of YKL-40 and MGMT promoter methylation. We demonstrated that YKL-40 functioned differently in human IDH1/2 wild-type GSCs. In MGMT promoter-methylated (MGMT-m) GSCs, it acted as a tumor suppressor gene. On the other hand, in MGMT promoter-unmethylated (MGMT-um) GSCs, it promoted tumorigenesis. Notably, the reason that YKL-40 played different roles in GSCs could not be interpreted by the molecular classification of each GSCs, but is a function of MGMT promoter methylation status and involves the RAS–MEK–ERK pathway. YKL-40 mediated TMZ sensitivity by activating DNA damage responses (DDRs) in MGMT-m GSCs, and it mediated resistance to TMZ by inhibiting DDRs in MGMT-um GSCs. Our report demonstrated that MGMT promoter methylation status might influence a gene’s function in human cancer. Moreover, our data also highlight the point that gene function should be investigated not only according to the molecular tumor classification, but also the epigenetic signature.

Introduction
Glioblastoma (GBM) is the most lethal and common primary malignant brain tumor in adults. Standard therapy for GBM consists of surgery followed by radiotherapy and chemotherapy with the alkylating agent temozolomide (TMZ). However, the overall survival is <2 years.

YKL-40, also known as CHI3L1, is a 40-kDa secreted glycoprotein that is expressed by both tumor cells and their surrounding tumor-infiltrating macrophages. In patients with solid tumors, elevated serum/plasma YKL-40 was significantly associated with poor overall survival. These tumors included: lung cancer, breast cancer, gastrointestinal cancer, ovarian cancer, urologic cancer, prostate cancer, malignant melanoma, and squamous cell carcinoma of the head and neck. Moreover, elevated serum/plasma concentration of YKL-40 was an independent prognostic variable indicating a short recurrence-free interval and short overall survival. In GBM, based on the molecular classification of GBMs in The Cancer Genome Atlas, YKL-40 is recognized as a marker of the mesenchymal subtype. It was differentially expressed in TMZ-resistant GBM cells and was responsible for TMZ resistance. A limited number of studies have shown that YKL-40 plays a vital role in glioma cell proliferation...
through activation of the MAPK and AKT pathway. However, a more detailed understanding of the role of YKL-40 in GBM remains unclear.

O^6^-methylguanine-DNA methyltransferase (MGMT) promoter methylation predicts a favorable response to alkylating chemotherapy, such as TMZ, in GBM patients. In addition, MGMT promoter methylation status not only mitigates sensitivity or resistance to TMZ, but may also shift the GBM mutational spectrum in the context of alkylating treatment, which may facilitate the emergence of secondary resistance to alkylating agents. All of the mechanisms by which MGMT promoter methylation impacts the survival of GBM patients are not fully understood.

To understand the function of YKL-40 in GSCs, we performed in vitro and in vivo experiments. We demonstrated that YKL-40 impact on GSCs is a function of MGMT promoter methylation status and involves the RAS–MEK–ERK pathway. YKL-40 mediated TMZ sensitivity by activating DNA damage responses (DDRs) in MGMT promoter-methylated (MGMT-m) GSCs, and it mediated resistance to TMZ by inhibiting DDRs in MGMT promoter-unmethylated (MGMT-um) GSCs. Our report demonstrated that MGMT promoter methylation status might influence a gene’s function in human cancer.

Results

Isolation and characterization of six GSC cultures from human GBM specimens

To identify the role of YKL-40 in GSCs, we first established GSC cultures from GBM specimens as previously described. Characteristics of patients were described (Table 1). Tumor tissue cultures yielded typical neurosphere-like structures within 5 days. All tumors were IDH1/2 wild-type primary GBMs, were stable, and could be passaged for more than 3 months. Immunofluorescence staining revealed that the majority of the cells from these cultures were positive for CD133 and Nestin, which are markers for neural stem/progenitor cells (Fig. 1b). To examine the capability of the cells to differentiate into neural cell lineages, we induced the cells to differentiate by exposing them to serum. Differentiated GSCs (DGCs) were grown as adherent monolayers and were positive for astroglial (GFAP) and neuronal (βIII-tubulin) markers (Fig. 1c). Compared with their GSC counterparts, the transcription levels of CD133, Olig2, Vimentin, and protein expression levels of Nestin, Olig2, Vimentin were significantly decreased in all six DGCs (Fig. 1d, e).

YKL-40 played different role in the biological properties of GSCs

To explore the function of YKL-40 in GSCs, we measured baseline levels of YKL-40 in our established GSCs. We observed that WZ05 and WZ12 did not express and secrete YKL-40 at a detectable level, and the remaining GSCs, i.e., WZ27, WZ28, WZ29, and WZ31, expressed and secreted high levels of YKL-40 (Fig. S1). To investigate the biological function of YKL-40 in GSCs, we modified by overexpressing it in WZ05, WZ12 GSCs (Y+) and knocking it down in WZ27, WZ28, WZ29, and WZ31, and WZ31 GSCs (Y−). The effect of overexpression or gene silencing was confirmed (Fig. 2a, b). To explore self-renewal and cell proliferation capabilities in vitro, single-cell spheroid formation and the BrdU assay were performed in both control and modified cells, respectively. YKL-40 significantly enhanced the self-renewal and cell proliferation capabilities of WZ05, WZ28, and WZ29 GSCs (Y+) and knocking it down in WZ27, WZ28, WZ29, and WZ31 GSCs (Y−). The longer overall survival of WZ12, WZ27 and WZ31, even though without statistically significance. This indicated that our GSCs could recapitulate the tumorigenesis of their parental tumors. Furthermore, we observed that YKL-40 overexpression significantly prolonged the overall survival of

Table 1 Characteristics of patients with primary GBM.

| Patient number | Age (Year) | Sex | Location | Histological Diagnosis | Overall survival (month) |
|----------------|------------|-----|----------|------------------------|--------------------------|
| WZ05           | 39         | M   | Frontal  | GBM                    | 13                       |
| WZ12           | 40         | M   | Temporal | GBM                    | 11                       |
| WZ27           | 30         | F   | Frontal  | GBM                    | 9                        |
| WZ28           | 49         | M   | Temporal | GBM                    | 14                       |
| WZ29           | 51         | F   | Frontal  | GBM                    | 12                       |
| WZ31           | 58         | F   | Frontal  | GBM                    | 6                        |
Fig. 1 (See legend on next page.)
nude mice implanted with WZ05 Y+ GSCs while knockdown of YKL-40 shortened the overall survival of nude mice implanted with WZ28 Y− and WZ29 Y− GSCs (Fig. 2g, left), suggesting an inhibitory role of YKL-40 in tumor initiation or progression in these GSCs (WZ05 vs. WZ05 Y+ P = 0.019; WZ28 vs. WZ28 Y− P = 0.004; WZ29 vs. WZ29 Y− P = 0.02). In contrast, YKL-40 overexpression significantly shortened the overall survival of nude mice implanted with WZ12 Y+ GSCs, knockdown of YKL-40 prolonged the overall survival of nude mice implanted with WZ27 Y− and WZ31 Y− GSCs (Fig. 2g, right), suggesting a positive role of YKL-40 in tumor initiation or progression in these GSCs (WZ12 vs. WZ05 Y+ P = 0.032; WZ27 vs. WZ27 Y− P = 0.005; WZ31 vs. WZ31 Y− P = 0.01).

Next we investigated changes in the stemness of each GSC caused by YKL-40 overexpression or silencing. The transcript levels of CD133, Olig2, and Vimentin were decreased in WZ05 Y+ compared to WZ31 Y− GSCs, while the transcript levels of Nestin were increased in WZ12 Y+ and WZ29 Y− GSCs, both compared to WZ05 Y+ GSCs (Fig. 3a). The protein expression level of Nestin, Olig2, and Vimentin was decreased in WZ05 Y+ compared to WZ31 Y− GSCs and WZ29 Y− GSCs (P < 0.05) but increased in WZ12 Y+ compared to WZ05 Y+ GSCs (P < 0.05) (Fig. 3b).

YKL-40 played different roles in GSCs involved the RAS–MEK–ERK pathway

Our previous study showed that the MAPK pathway was altered by YKL-40 silencing in U87 cells and primary astrocytoma cells. We hypothesized that the MAPK pathway could be involved in the different role of YKL-40 in GSCs. We observed that overexpression of YKL-40 decreased the expression of RAS, pMEK1/2, and pERK1/2 in WZ05. Notably, knockdown of YKL-40 increased the expression of RAS, pMEK1/2, and pERK1/2 in WZ28 and WZ29 (Fig. 4a, left). Conversely, overexpression of YKL-40 increased the expression of RAS, pMEK1/2, and pERK1/2 in WZ12, and its knockdown decreased the expression of RAS, pMEK1/2, and pERK1/2 in WZ27 and WZ31 (Fig. 4a, right). Furthermore, by using single-cell sphere formation and BrdU assays, we demonstrated that PD98059, a specific inhibitor of ERK1/2, significantly inhibited the self-renewal and cell proliferation capabilities of GSCs in WZ12 Y+/PD, WZ28 Y−/PD, and WZ29 Y−/PD GSCs (Fig. 4b, c). Accordingly, results of quantitative PCR (Fig. 4d) and western blotting analyses (Fig. 4e) showed that the transcript levels of CD133, Olig2, Vimentin and protein expression level of Nestin, Olig2, and Vimentin were significantly rescued in WZ12 Y+/PD, WZ28 Y−/PD, and WZ29 Y−/PD GSCs compared with their counterparts.

MGMT promoter methylation status, but not GBM molecular subtypes, is associated with the different role of YKL-40 in GSCs

To explore what could distinguish the difference in the function of YKL-40 in GSCs, we performed RNA-Seq to identify the molecular subclasses of each GSC as previously described. These results indicated that WZ05, WZ27, WZ28, and WZ29 belonged to the classical subtype, whereas WZ12 and WZ31 were of the mesenchymal subtype (Fig. 5a). Thus, the molecular subtypes could not distinguish the different role of YKL-40 in GSCs.

As an epigenetic event, MGMT promoter methylation is a favorable prognostic marker, and patients with tumors exhibiting MGMT promoter methylation have a survival benefit from receiving combined radiotherapy and temozolomide chemotherapy. We therefore hypothesized that the MGMT promoter methylation status of GSCs might be associated with the different functions of YKL-40. To investigate this hypothesis, we performed methylation-specific PCR (MSP) to determine the MGMT promoter methylation status of each GSC. MSP is one of the most commonly used DNA-based methods for detecting promoter methylation because of its simplicity and cost-effectiveness. We observed that the MGMT promoter methylation status of GSCs might be associated with the different functions of YKL-40.
Fig. 2 (See legend on next page.)
their control group, respectively (Fig. 5d, e). We further explored whether the gain or loss of MGMT might reverse the effect caused by overexpressing of YKL-40 in WZ05 and WZ12. Notably, by co-overexpressing YKL-40 and MGMT in WZ05 GSC (WZ05 Y+M+), it significantly reversed the inhibited effect of WZ05 Y+ alone by enhancing self-renewal and cell proliferation in vitro (Fig. 5d, e). This finding was confirmed by examining the mRNA transcription and protein expression levels of stemness markers (Fig. 5f, g). In addition, by overexpressing YKL-40 and knocking down the expression of MGMT in WZ12 GSC (WZ12 Y+M−), we observed that...
Fig. 4 The different role of YKL-40 in GSCs involved the RAS–MEK–ERK pathway. a Western blot analyses to measure the protein expression levels of RAS, β-tubulin, pMEK1/2, MEK1/2, pERK1/2, and ERK1/2 in GSC Y+ or Y− compared with controls of each GSC. The ratio has been normalized by β-III-tubulin. b Following the addition of the ERK1/2-specific inhibitor PD98059, data points demonstrated the percentage of single cells capable of sphere formation in serum-free conditions in GSC Y+ or Y− compared with controls of each GSC. PD = PD98059. c With the addition of the ERK1/2-specific inhibitor PD98059, the bar graph indicated the proliferation rates measured using BrdU incorporation in GSC Y+ or Y− compared with controls of each GSC. PD = PD98059. No significant difference was observed between WZ12 C vs. WZ12 Y+/PD, WZ28 C vs. WZ28 Y-/PD, WZ29 C vs. WZ29 Y-/PD. d With the addition of the ERK1/2-specific inhibitor PD98059, the bar graph indicated the transcript levels of CD133, Olig2, Vimentin in GSC Y+ or Y− compared with controls of each GSC. *P < 0.05; **P < 0.01. e With the addition of the ERK1/2-specific inhibitor PD98059, western blotting analyses were performed to measure the protein expression levels of Nestin, Olig2, and Vimentin in GSC Y+ or Y− compared with controls of each GSC. *P < 0.05; **P < 0.01. The ratio has been normalized by β-actin.
WZ12 Y+M− reversed the effect of WZ12 Y+ in vitro (Fig. 5d, e). The altered mRNA transcription and protein expression levels of stemness markers further confirmed the finding (Fig. 5f, g).

**YKL-40 sensitized the response of TMZ in MGMT-m GSCs and accounted for TMZ resistance in MGMT-um GSCs**

To examine the effects of YKL-40 on the sensitivity of GSCs to TMZ, we performed a cell viability assay as...
previously described\(^{21}\). The six GSCs exhibited variable sensitivities to TMZ (Fig. 6a), with three (WZ05, WZ28, and WZ29) being highly sensitive (ED50 < 30 µM) and three (WZ12, WZ27 and WZ31) being highly resistant (ED50 > 200 µM) (Table 2). As predicted, WZ05, WZ28, and WZ29, which are MGMT-m, were highly sensitive; WZ12, WZ27, and WZ31, which are MGMT-um cells, were highly resistant. Notably, in the MGMT-m group (WZ05, WZ28, and WZ29), YKL-40 sensitized the response of GSCs to TMZ (Fig. 6b, c, left). By contrast, in the MGMT-um group (WZ12, WZ27, and WZ31), YKL-40 contributed to GSCs resistance to TMZ (Fig. 6b, c, right). Furthermore, overexpression of MGMT could increase the resistance of WZ05 to TMZ, and co-overexpression of MGMT and YKL-40 attenuated the resistance caused by overexpressing MGMT alone (Fig. 6b, left). Knocking down MGMT also decreased the resistance of WZ12 to TMZ, and overexpressing YKL-40 and knocking down MGMT rescued the effect caused by MGMT silencing alone (Fig. 6b, right).

It is well known that TMZ induces DDR, and phosphorylation of H2AX (p-H2AX) is closely associated with the process as a sensitive molecular marker of DNA double strand breaks (DSBs)\(^{22}\). Thus, we hypothesized that the effect of YKL-40 on response to TMZ could be mediated via DDR. Western blotting results clearly demonstrated that YKL-40 sensitized the response to TMZ in MGMT-m GSCs (WZ05, WZ28, WZ29) via an activation of p-H2AX and p-ATM (Fig. 6d). In MGMT-um GSCs (WZ12, WZ27, WZ31), YKL-40 inhibited the activation of p-H2AX and p-ATM (Fig. 6d). Notably, overexpression of MGMT inhibited the activation of DDRs in TMZ-treated WZ05, and co-overexpression of MGMT and YKL-40 attenuated the effect caused by overexpression of MGMT alone (Fig. 6d). Furthermore, knocking down of MGMT could induce DDRs in WZ12 to TMZ, and overexpressing YKL-40 and knocking down MGMT attenuated the effect caused by MGMT silencing alone (Fig. 6d).

Thus, these in vitro and in vivo experiments demonstrated that MGMT promoter methylation status of GSCs is associated with different roles of YKL-40 in these cells. This supports the notion that a gene can act either as a tumor promoter or tumor suppressor gene depending on the epigenetic context.

### Discussion

We here demonstrated that the YKL-40 gene, which is recognized as a molecular marker of the mesenchymal subtype of GBMs\(^{6,7}\), functioned differently in human IDH1/2 wild-type GSCs. In MGMT-m GSCs, it acted as a tumor suppressor gene and sensitized GSCs’ response to TMZ by activating DDRs. On the other hand, in MGMT-um GSCs, it promoted tumorigenesis and accounted for TMZ resistance by inhibiting DDRs. Notably, the reason that YKL-40 played different roles in GSCs could not be interpreted by the molecular classification of each GSCs, but was closely related with their MGMT promoter methylation status. Our report demonstrated that MGMT promoter methylation status could influence a cancer-relevant gene’s function. These data also highlight the point that gene function should be investigated not only according to the molecular tumor classification, but also the epigenetic signature.

Based on prior work, we realized it is not by chance that YKL-40’s different role could not be interpreted by the molecular classification in GSCs. These molecular classifications of GBMs are based on the DNA and RNA profiles of bulk tumors mixed with various cell types, GSCs are only a subpopulation among those various cells\(^{6,7,23,24}\). Single-cell RNA-seq revealed that individual tumors contained a spectrum of GBM subtypes and hybrid cellular states\(^{25}\). Cells from the same tumor may harbor different mutations or epigenetic states. Therefore,
Fig. 6 (See legend on next page.)
molecular classification does not completely recapitulate intratumoral heterogeneity, which has been increasingly appreciated as a determinant of treatment resistance and tumor recurrence in GBM. Interestingly, a comprehensive approach involving DNA methylation-based classification of central nervous system tumors across all entities and age groups, indicated that a combined DNA methylation signature with histology and molecular tumor classification could improve the accuracy of diagnosis. In another study it was demonstrated that epigenetic subtypes were significant independent predictors of survival in multivariate analysis in 1122 adult diffuse grade II, III, and IV gliomas. All of these data emphasize the point that the epigenetic context is critical in neuropathology and in a patient’s prognosis. Specifically, our study demonstrates that the epigenetic context, in the form of MGMT promoter methylation status and that these differential roles are involved in the RAS–MEK–ERK pathway in GSCs. The precise interaction between the YKL-40 gene and RAS–MEK–ERK signaling requires further investigation.

In summary, our findings have emphasized the critical role of epigenetic status in cancer-related gene function. It also sheds light on the notion that gene expression quantification based on molecular tumor classifications should consider the epigenetic context in the future. The clinical implications of our work include: (1) the potentially beneficial role of combined gene targeting therapies based on the underlying epigenetic context; (2) the potential role that epigenetic context may have in cancer gene function in other human malignancies.

Materials and methods
Isolation and culture of WZs
Surgical specimens of GBMs were collected at Xijing Hospital by W.Z. (WZ series) with approval by the Institutional Review Board. Tissues were processed as previously described. See Supplementary Material for details.

Differentiation induction and immunocytochemistry
See Supplementary Material for details.

Viral infections
See Supplementary Material for details.

### Table 2  Median-effect doses of TMZ in vitro.

| GSC line | ED$_{50}$ TMZ μM |
|----------|------------------|
| WZ05     | 22               |
| WZ28     | 10               |
| WZ29     | 25               |
| WZ12     | 900              |
| WZ27     | 850              |
| WZ31     | 250              |

As a critical epigenetic event, promoter methylation of the DNA repair enzyme gene MGMT could compromise DNA repair mechanisms and increase sensitivity to TMZ. The value of MGMT promoter methylation for predicting a favorable response to alkylating chemotherapy in GBM has been established. MGMT promoter methylation was identified as a positive prognostic marker for overall (21.2 vs. 14 months) and progression-free survival (8.7 vs. 5.7 months) in newly diagnosed GBM patients. Our results provide new insights into the potential mechanisms by which GBM patients benefit from MGMT promoter methylation. In this study, we focused on the YKL-40 gene only, more genes should be evaluated for their function in the context of MGMT-m vs. MGMT-um.

As a critical pathway in IDH1/2 wild-type gliomas, alterations in the RAS–MEK–ERK signaling pathway have been detected in most cases. This pathway plays a critical role in maintaining stemness and promoting tumorigenesis in GSCs. It has been shown that YKL-40 expression has a positive association with the expression of phosphor-ERK1/2, which is strongly correlated with a poor response to radiotherapy and poor clinical outcome. Our previous study indicated that the vital role that YKL-40 played in established glioma cell proliferation was through the activation of MAPK and AKT pathway. In this new study, we identified the different roles that YKL-40 can play as a function of MGMT promoter methylation status and that these differential roles are involved in the RAS–MEK–ERK pathway in GSCs. The precise interaction between the YKL-40 gene and RAS–MEK–ERK signaling requires further investigation.

**Fig. 6** YKL-40 sensitized the response of TMZ in MGMT-m GSCs and accounted for TMZ resistance in MGMT-um GSCs. a TMZ dose–response curves. GSCs were cultured in triplicate wells in vitro measured using BrdU 5 days after the addition of TMZ. Error bars represent 95% confidence intervals. Dotted line indicates 50% cell viability relative to mock-treated cells. TMZ temozolomide b With the addition of ED50 TMZ to WZ05 and WZ12 series (T+), the bar graph indicated the proliferation rates measured by BrdU incorporation in each GSC (*P < 0.05; **P < 0.01). No significant difference was observed between WZ05 C T+ vs. WZ05 Y+M+T+, WZ12 C T+ vs. WZ12 Y+M−T+. c With the addition of ED50 TMZ to WZ28, WZ29, WZ27, and WZ31 GSCs (T+), the bar graph indicated the proliferation rates measured by BrdU incorporation in each GSCs (*P < 0.05; **P < 0.01). d Western blotting analyses were performed to quantify the protein expression levels of YKL-40, Actin, p-ATM, ATM in each GSC series. The ratio has been normalized by Vinculin.
GBM pathology review, DNA extraction, and MGMT promoter methylation analysis

All tissue samples from primary tumors were confirmed by pathology review to represent GBM according to the World Health Organization classification of tumors of the central nervous system. MGMT promoter methylation was determined by MSP. See Supplementary Material for details.

RNA isolation and quantitative real-time PCR

Total RNA was isolated from cultured cells using TRIzol (TaKaRa, Tokyo, JPN) and purified using miRNeasy columns (Qiagen). All RNA isolation and quantitative real-time PCR were performed according to our previous studies. See Supplementary Material for details.

ELISA for YKL-40 in culture medium

ELISA was performed according to our previous study. See Supplementary Material for details for details.

Western blotting analyses

Western blotting analyses were performed according to our previous study. See Supplementary Material for details.

Single-cell sphere-formation assay and BrdU

TMZ (Merck Sharp & Dohme Ltd, Whitehouse, NJ, USA) was dissolved in 5% dimethyl sulfoxide (DMSO) in phosphate-buffered saline (PBS). The doses of TMZ ranged from 0.01 to 1000 µM. Dose–response curves and effective dose (ED50) values were obtained and compared at day 5. See Supplementary Material for details.

Inhibitor

The ERK pathway specific inhibitor PD98059 was purchased from Calbiochem (Millipore). See Supplementary Material for details.

RNA-Seq and data analysis

RNA sample collection and preparation: total cellular RNA was extracted using TRIzol (TaKaRa, Tokyo, JPN). RNA degradation and contamination were monitored on 1% agarose gels. RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). See Supplementary Material for details. Data generated for this study are available through the Gene Expression Omnibus (GEO: GSE153794).

Identification of gene expression-based subtypes

We downloaded the Verhaak’s gene expression signatures of 840 genes (ClaNC840_centroids.xls) to determine the subtype of each sample according to four known molecular subtypes (neural, proneural, classical, mesenchymal). Training and prediction were performed using an R implementation of ClaNC software, a nearest centroid-based classifier. A training set consisting of 173 samples and 840 genes was used to predict subtypes in our samples as described by Verhaak.

Tumorigenicity studies

Female athymic nu/nu mice aged 6–8 weeks were obtained from the Fourth Military Medical University Experimental Center (Shaanxi, CHN) and were anesthetized with pentobarbital according to our previous study. All animal procedures were performed with the approval of the Subcommittee on Research Animal Care (SRAC) at Xijing Hospital. To generate intracerebral xenografts, 1 × 10^5 WZs in 2 µl of PBS were stereotactically implanted into the right cerebrum (2 mm lateral to the bregma at a depth of 3 mm) as previously described. Mice were monitored and euthanized when they developed significant neurological symptoms. Formalin-fixed paraffin-embedded sections were stained with H&E. All animal procedures were performed with the approval of the SRAC at Xijing Hospital.

Statistics

Comparisons of data obtained from real-time PCR, cell survival, single sphere-formation assay, and MGMT promoter methylation were performed using two-tailed Student’s t tests (unpaired). Survival analysis was performed with Kaplan–Meier curves, and their comparisons were examined with log-rank tests. P values < 0.05 were considered significant.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81302174, 81672909, 81671302, 81872062), the China Postdoctoral Science Foundation (2014M552633).

Author details

1Department of Neurosurgery, Xijing Hospital, The Fourth Military Medical University, Xi’an, Shaanxi, PR China. 2Department of Emergency Medicine, Jinling Hospital, Medical School of Nanjing University, Nanjing, PR China. 3Department of Medical Genetics and Developmental Biology, The Fourth Military Medical University, Xi’an, Shaanxi, PR China. 4Department of Neurosurgery, China-Japan Friendship Hospital, Beijing, PR China. 5Department of Neurosurgery, Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, PR China.

Conflict of interest

The authors declare that they have no conflict of interest.

Publisher’s note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41419-020-02909-9).
References

1. Zhang W. et al. Bevacizumab with angiostatin-armed oHSV increases antiangiogenesis and decreases bevacizumab-induced invasion in U87 glioma. Mol. Ther. 20, 37–45 (2012).
2. Zhang W. et al. Association between YKL-40 and adult primary astrocytoma. Cancer 116, 2689–2697 (2010).
3. Ismail H. et al. Measured and genetically predicted plasma YKL-40 levels and melanoma mortality. Eur. J. Cancer 121, 74–84 (2019).
4. Johansen J. et al. Serum YKL-40 in risk assessment for colorectal cancer: a prospective study of 4,946 subjects at risk of colorectal cancer. Cancer Epidemiol. Biomark. Prev. 24, 621–626 (2015).
5. Krogh M. et al. Prognostic and predictive value of YKL-40 in stage IIB-III melanoma. Melanoma Res. 26, 367–376 (2016).
6. Verhaak R. G. et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFA, IDH1, EGFR, and NF1. Cancer Cell 17, 98–110 (2010).
7. Phillips H. S. et al. Molecular subtypes of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9, 157–173 (2006).
8. Yasuto A. et al. Novel cancer-testis antigen expression on glioma cell lines derived from high-grade glioma patients. Oncol. Rep. 31, 1683–1690 (2014).
9. Stupp R. et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N. Engl. J. Med. 352, 987–996 (2005).
10. Hegi M. E. et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N. Engl. J. Med. 352, 997–1003 (2005).
11. Herg M. E. & Stupp, R. Withholding temozolomide in glioblastoma patients with unmethylated MGMT promoter — still a dilemma? Neuro-oncol. 17, 1425–1427 (2015).
12. Gilbert M. R. et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 31, 4085–4091 (2013).
13. Malmstrom A. et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. Lancet Oncol. 13, 916–926 (2012).
14. Walter M. et al. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat. Rev. Neurol. 6, 39–51 (2010).
15. Hagi M. E. et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 26, 4189–4199 (2008).
16. Gorlia T. et al. Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981–22981/CE.3. Lancet Oncol. 9, 29–38 (2008).
17. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455, 1061–1068 (2008).
18. Jacinto, F. V. & Esteller, M. Mutator pathways unleashed by epigenetic silencing in human cancer. Mutagenesis 22, 247–253 (2007).
19. Wick W. et al. MGMT testing—the challenges for biomarker-based glioma treatment. Nat. Rev. Neurol. 10, 372–385 (2014).
20. Zhang W. et al. Combination of oncolytic herpes simplex viruses armed with angiostatin and IL-12 enhances antitumor efficacy in human glioblastoma models. Neoplasia 15, 591–599 (2013).
21. Kanai R. et al. Oncolytic virus-1 mediated manipulation of DNA damage responses: synergy with chemotherapy in killing glioblastoma stem cells. J. Natl Cancer Inst. 104, 42–55 (2012).
22. Mah, L. J., El-Osta, A. & Karagiannis, T. C. gammaH2AX: a sensitive molecular marker of DNA damage and repair. Leukemia 24, 679–686 (2010).
23. Brennan C. W. et al. The somatic genomic landscape of glioblastoma. Cell 155, 462–477 (2013).
24. Nooshin H. et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17, 510–522 (2010).
25. Patel A. P. et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344, 1396–1401 (2014).
26. Capper D. et al. DNA methylation-based classification of central nervous system tumours. Nature 555, 469–474 (2018).
27. Ceccharelli M. et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell 164, 550–563 (2016).
28. Sato A. et al. MEK-ERK signaling dictates DNA-repair gene MGMT expression and temozolomide resistance of stem-like glioblastoma cells via the MDM2-protein kinase and Akt pathways in glioblastoma. Clinical cancer research; an official journal of the American Association for. Cancer Res. 12, 3935–3941 (2006).
29. Waki H. et al. Human glioblastoma-derived cancer stem cells: establishment of invasive glioma models and treatment with oncolytic herpes simplex virus vectors. Cancer Res. 69, 3472–3481 (2009).