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Original Articles

IL1RN mediates the suppressive effect of methionine deprivation on glioma proliferation

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ABSTRACT

Metabolic abnormality is one of the hallmarks of cancer cells, and limiting material supply is a potential breakthrough approach for cancer treatment. Increasing researchers have been involved in the study of glioma cell metabolism reprogramming since the significance of IDH1 was confirmed in glioma. However, the molecular mechanisms underlying metabolic reprogramming induced by methionine deprivation regulates glioma cell proliferation remain unclear. Here we demonstrated that methionine deprivation inhibited glioma cell proliferation via downregulating interleukin 1 receptor antagonist (IL1RN) both *in vitro* and *in vivo*, methionine deprivation or knocking down IL1RN induced glioma cell cycle arrest. Moreover, we confirmed that IL1RN is a tumor associated gene and its expression is negatively correlated with the survival time of glioma patients. Altogether these results demonstrate a strong rationale insight that targeting amino acid metabolism such as methionine deprivation/IL1RN related gene therapy may offer novel direction for glioma treatment.

1. Introduction

Glioma is the most common primary malignant intracranial tumor and has a poor prognosis [1,2]. Despite the application of standard therapies—surgical resection, radiotherapy and chemotherapy, the two-year survival rate remains less than 10% after diagnosis of glioblastoma [2]. Though great progress has been made in the treatment of glioma during the last decades, more superior detection methods and therapeutic regimens are still urgently needed [3,4].

Substance metabolism plays a critical role in tumor progression due to high material and energy requirement of tumor cells to maintain their unimpeded proliferation [5]. Recently, researchers demonstrated that restricting nutrients supplementation, such as proteins, glucose and lipids could be a feasible therapy for cancer [6–8]. Methionine is an essential amino acid and is vital to protein synthesis, DNA methylation and polyamine synthesis [9]. Several studies have confirmed that methionine deprivation limits growth and metastasis of different tumors, and prolongs lifespan of experimental animals [10–12]. Similarly, our previous study has indicated that dual deprivation of methionine and cysteine has synergetic inhibitory effect on glioma proliferation by inducing ROS accumulation/autophagy [13], but the exact underlying mechanisms remain to be elucidated.

Under these circumstances, to observe the role of methionine play in glioma proliferation we transplanted primary glioma cells to mice brain

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and deprived methionine supplementation, then performed gene microarray to explore the molecular mechanism of which methionine deprivation inhibits tumor cell proliferation. The gene interleukin 1 receptor antagonist (IL1RN) captured our attention [14]. As a member of the interleukin 1 cytokine family, IL1RN is a well-known immune and pro-inflammatory cytokine that inhibits the activity of interleukin-1 (IL-1) by competitively binding the IL1R1 receptor and preventing its association with the co-receptor interleukin 1 receptor accessory protein (IL1RAP), thereby it modulates lots of IL1 related immune and inflammatory responses [15]. As a potent immune/inflammatory molecule, IL1RN is tightly associated with osteomyelitis, rheumatoid arthritis, traumatic brain injury (TBI) and stroke [16–18]. Recently, an increasing number of studies proved that IL1RN is also actively involved in tumor progression, such as high expression IL1RN increased the risk of bladder cancer and non-cardia gastric carcinoma [19,20], but decreased the risk of lung cancer [21]. These intriguingly contradictory observations drove us to figure out the role of IL1RN in glioma.

In this study, we demonstrated that methionine deprivation inhibited glioma proliferation *in vitro* and *in vivo* and induced cell G1/S phase arrest by suppressing IL1RN expression. Moreover, overexpression of IL1RN could reverse the suppressive effect of methionine deprivation on glioma cell proliferation. The survival curves indicated that high expression of IL1RN predicted a poor prognosis in glioma patients. All together, these results suggest that IL1RN could be a prognostic indicator of and a novel therapeutic target for glioma.

2. Materials and methods

2.1. Patient samples collection and primary glioma cell extraction

Non-tumorous human brain tissues (N = 6) and human glioma tissues (N = 19) were obtained from surgical resection at the Department of Neurosurgery, First Affiliated Hospital of Harbin Medical University. Primary GBM cells were separated from three independent GBM patient tissues respectively and cultured at 37 °C with 5% CO_2 [22]. This study was approved by the Ethics Committee of Harbin Medical University Hospital and carried out in according to the Declaration of Helsinki. All the participants gave written informed consent.

2.2. Cell culture

Human glioma cell lines LN229 and A172 were obtained from China Infrastructure of Cell Line Resource (National Science & Technology Infrastructure, NSTI) and the Institute of Brain Science(Harbin Medical University China). Cell lines were cultured with Dulecco's Modified Eagle medium (DMEM, 11960044, Gibco, USA) or methionine free medium (21013024, Gibco, USA). All cells were maintained at 37 °C in an incubator containing 5% CO_2 [13,23].

2.3. MTT assay

Cells were seeded in 96-well plate at a density of 5000 cells/well. 10 μl of MTT dye (5 mg/ml) was added to each well, after incubation at 37 °C for 4 h the medium was replaced by 200 μl dimethyl sulfoxide. The optical density of each well was measured at a wavelength of 490 nm (BioTek elx800, USA). The cell morphology was observed with a microscope (Leica DMI8, Germany).

2.4. Cell transfection

X-tremeGENE (Roche, Germany) and Lipofectamine 2000 reagent (Invitrogen, USA) was used for siRNA/plasmid transfection according to the manufacturers' instructions. Sequences of the siRNA- NC/IL1RN (GenePharma, China) were shown in Table 1. IL1RN overexpression plasmid pEnter-NC/IL1RN were purchased from Vigene Biosciences. INC (China), Lentivirus Plxv3-shNC, Plxv3-shIL1RN 1–3 were purchased form GenePharma (China).

2.5. Western blot analysis

After extracting from glioma cell/tissues, total proteins were separated on 12.5% SDS-PAGE gel and transferred to polyvinylidene difluoride (PVDF) membrane. The membranes were blocked in 5% skim milk for 2 h and then incubated with primary antibodies at 4 °C overnight. Then membranes were incubated with fluorescently conjugated secondary antibodies (Cat#: 7075, Cell Signaling Technology, USA). Immuno-reactive complexes were visualized using BeyoECL Plus (Beyotime, China). The protein bands were captured using ChemiDoc XRS + Imaging System (Bio-Rad, USA). Primary antibodies were used as follows: IL1RN (ab124962, Abcam, USA), CDK2 (ab32147, Abcam, USA), CDK4 (ab199728, Abcam, USA), Cyclin D1 (ab134175, Abcam, USA), TGF-β (ab179695, Abcam, USA), β-actin (AC004, ABclonal Technology).

2.6. Assessment of cell cycle distribution by flow cytometry

Briefly, cells were collected and incubated with 70% ethyl alcohol overnight. Then cells were washed with PBS and stained with reagents from Cell cycle analysis kit (Beyotime P0010, China). After cells were incubated at 37 °C for 30 min, their fluorescence was visualized by Accuri C6 flow cytometer (BD, USA) and analysed by ModFit LT 5.0.

2.7. Quantitative real-time PCR (qRT-PCR)

Briefly, total RNA was isolated from samples with Trizol reagent (Invitrogen, USA). The IL1RN mRNA expression were analysed using a SYBR Green PCR Master Mix kit (Applied Biosystems, USA). GAPDH was used as an internal control. qRT-PCR was performed on ABI Prism 7500 fast thermocycler (Applied Biosystems, CA). The primer sequences of GAPDH and IL1N were in Table 1.

2.8. Orthotopic transplantation experiment

BALB/C nude mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (China). The injection location is 2.5 mm to the right of midline and 0.5 mm posterior to the coronal suture at a depth of 3.5 mm [24]. In diet control assay, human primary glioma cells (2×10^5 cells per mice) were injected into mice brain, after 1 week, mice were randomly divided into standard diet group and the methionine deprivation diet group. After another 3 weeks of diet consumption, the mice were sacrificed. In lentivirus transfection assay, 2×10^5 LN229 cells were injected into mouse brain, after 3 weeks mice were sacrificed. The lentivirus sequences were in Table 1. The standard diet (Cat#: TP 0010G)and methionine free diets(Cat#: TP 01A0401G1) were purchased from Trophic Animal Feed High-tech Co., Ltd. (China). All experimental ethics and animal research were approved by the Ethics Committee of First Affiliated hospital of Harbin Medical University and carried out in according to the Declaration of Helsinki.

2.9. Hematoxylin-eosin (H&E) staining and immunohistochemical (IHC) staining

For H&E staining, tissues were dehydrated and paraffin-embedded in preparation of sectioning, the resulting slices were stained with H&E for histological examination. For IHC assay, tissues were paraffin-embedded and sliced, then stained for interested protein using a streptavidin-biotin peroxidase method. the primary antibodies were used as follows: CDK4 (ab199728, Abcam, USA), Cyclin D1 (ab134175, Abcam, USA), Ki67 (A2094, ABclonal, USA).

Table 1
Sequences of siRNA, primer and lentivirus.

siRNA sequence	
si NC	5′ UUCUCCGAACGUGUCACGUTT 3′, 5′ ACGUGACACGUUCGGAGAATT 3′
IL1RN-si RNA1	5′ GGUACCCAUUGAGCCUCAUTT 3′, 5′ AUGAGGCUCAAUGGGUACCTT 3′
IL1RN-si RNA2	5′ CCUUCUAUCUGAGGAACAATT3′, 5′ UUGUUCCUCAGAUAGAAGGTT 3′
IL1RN-si RNA3	5′ GCGUCAUGGUCACCAAAUUTT 3′, 5′ AAUUUGGUGACCAUGACGCTT 3′
Primer sequences	
GAPDH	F-5′ CACCCACTCCTCCACCTTTGA3′, R-5′ACCACCCTGTTGCTGTAGCCA3′
IL1RN	F-5′TCCAAGCTCCATCTCCACTC3′, R5′GCTGAGTACCTGCCAAGAGC 3′
Lentivirus sequences	
LV-shNC	5′ TTCTCCGAACGTGTCACGT 3′
LV-shIL1RN 1	5′ GGTACCCATTGAGCCTCATGC 3′
LV-shIL1RN 2	5′ GCCCGTCAGCCTCACCAATAT 3′
LV-shIL1RN 3	5′ GCCTGTTCCCATTCTTGCATG 3′

2.10. Bioinformatics analysis

The bioinformatics data were downloaded as follows: gene expression: https://xenabrowser.net/datapages/?hub=https://tcga.xenahubs.net:443, which included data from 515 LGG and 154 GBM patients. Clinical data: http://www.cbioportal.org/, which include clinical data from 521 LGG and 167 GBM patients. Among them, 464 LGG and 116 GBM cases reported disease-free survival (DFS), and the top 75% were chosen for demarcation point.

2.11. Gene microarray and data analysis

Agilent Human Gene Expression (8∗60 K, Design ID:039494)was used to detect the expression of genes. Feature Extraction software (version10.7.1.1, Agilent Technologies) was used to analyze array images to get raw data. Genespring were employed to finish the basic analysis with the raw data. Differentially expressed genes were then identified through fold change as well as *P* value calculated with *t*-test. The fold change ≥ 2.0 and *P* value ≤ 0.05 were set for threshold of up- and down-regulated genes. Bidirectional hierarchical clustering of differentially expressed genes across eight samples was carried out by Cluster 3.0 (http://bonsai.hgc.jp/~mdehoon/software/cluster/) and heatmap was viewed by TreeView 3.0 (https://bitbucket.org/TreeView3Dev/treeview3/). The assay (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL17077) were followed the Minimum Information about a microarray Gene Experiment (MIAME), GEO accession number was GPL17077.

2.12. Statistical analysis

Statistical analysis between groups was performed using Student's *t*-test or one-way ANOVA (Prism version 7.0). $P < 0.05$ was considered statistically significant (*).

3. Results

3.1. Methionine deprivation inhibits glioma cell proliferation in vitro and in vivo

Substance metabolism plays a critical role in tumor progression, and the only way units obtain methionine is from outside as it's an essential amino acid. To figure out whether methionine metabolism plays a critical role in glioma cell proliferation or not we cut off methionine supplement. First, we cultured human primary GBM cells with or without methionine and observed cell sphere formation, as we observed that the cell sphere size of methionine free group (Met (−)) were smaller than those of full medium group obviously (Met (+)) (Fig. 1A), it mean that methionine deprivation inhibited primary glioma cell viability. Moreover, we transplanted primary glioma cells to nude mice brain and found the same inhibitory effect, the results shown that the maximal section area of the methionine deprivation group (Met (−)) was notably smaller than that of the control group (Met (+)) (Fig. 1B). All above, the results demonstrated that methionine deprivation inhibited primary glioma cell viability *in vivo* and *in vitro*.

Meanwhile, methionine deprivation exerted same inhibitory effect in glioma cell lines. We cultured glioma cell line LN229 and A172 present or absence of methionine, next cell viability were detected at indicated time, as the results shown that methionine deprivation significantly reduced LN229 and A172 cell viabilities in a time-dependent manner (Fig. 1C), at the same time we got cell images to got cell morphology at indicated time, the cell number in Met (−) group were smaller than that in Met (+) group which were in accordance with Fig. 1C (Supplementary Fig. S1A). To confirm the suppressive effect were induced by cell death or cell proliferation inhibition, we detected cell death by Tunnel assay and cell proliferation by staining proliferation associated protein Ki67, the results demonstrated that LN229 and A172 cell proliferation were suppressed notably (Fig. 1D and E) and there were no significant cell death (Supplementary Fig. S1 A and B), all above we proved that the glioma cell viability inhibition were induced by cell proliferation suppression. Taken together, these results indicated that methionine deprivation inhibits glioma cell proliferation *in vitro* and *in vivo*.

3.2. Methionine deprivation suppresses IL1RN expression in vitro and in vivo

These markerable inhibitory effect captured our attention and drove us to elucidate the underlying mechanism. We screened differentially expressed genes in Fig. 1B tissues by gene microarray, the result shown that there were 402 differentially expression genes that fold change > 2.0, $P < 0.5$ (Supplementary Fig. S2A), among them 13 genes were significantly upregulated (fold change > 3.0) and 24 genes were significantly downregulated (fold change > 3.0) in methionine deprivation group (Fig. 2A). Afterwards, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment and Gene Ontology (GO) Analysis, and found vast majority differentially expressed genes in KEGG Enrichment signal pathway ($P < 0.05$) or GO Analysis biological process units ($P < 0.05$) were associated with immune/inflammation obviously. KEGG Enrichment signal pathway analysis results shown 12 out of 26 were immune/inflammation pathways (Supplementary Fig. S2B), GO Analysis unit was 12 out of 86 were immune/inflammation units (Fig. 2B), associated representative gene and their fold change were shown in (Supplementary Fig. S2C). Next, we picked out all inflammation/immune genes and draw their disease-free survival (DFS) time of glioma patients using TCGA clinical data. Finally, the results shown that IL1RN was correlated with the disease-free survival time of glioma patients (Fig. 2C). All above, the results verified that methionine deprivation suppressed IL1RN expression *in vivo*.

We also confirmed that IL1RN mRNA and protein expression were downregulated after methionine were deprived in glioma cell lines. The results demonstrated that IL1RN mRNA and protein were

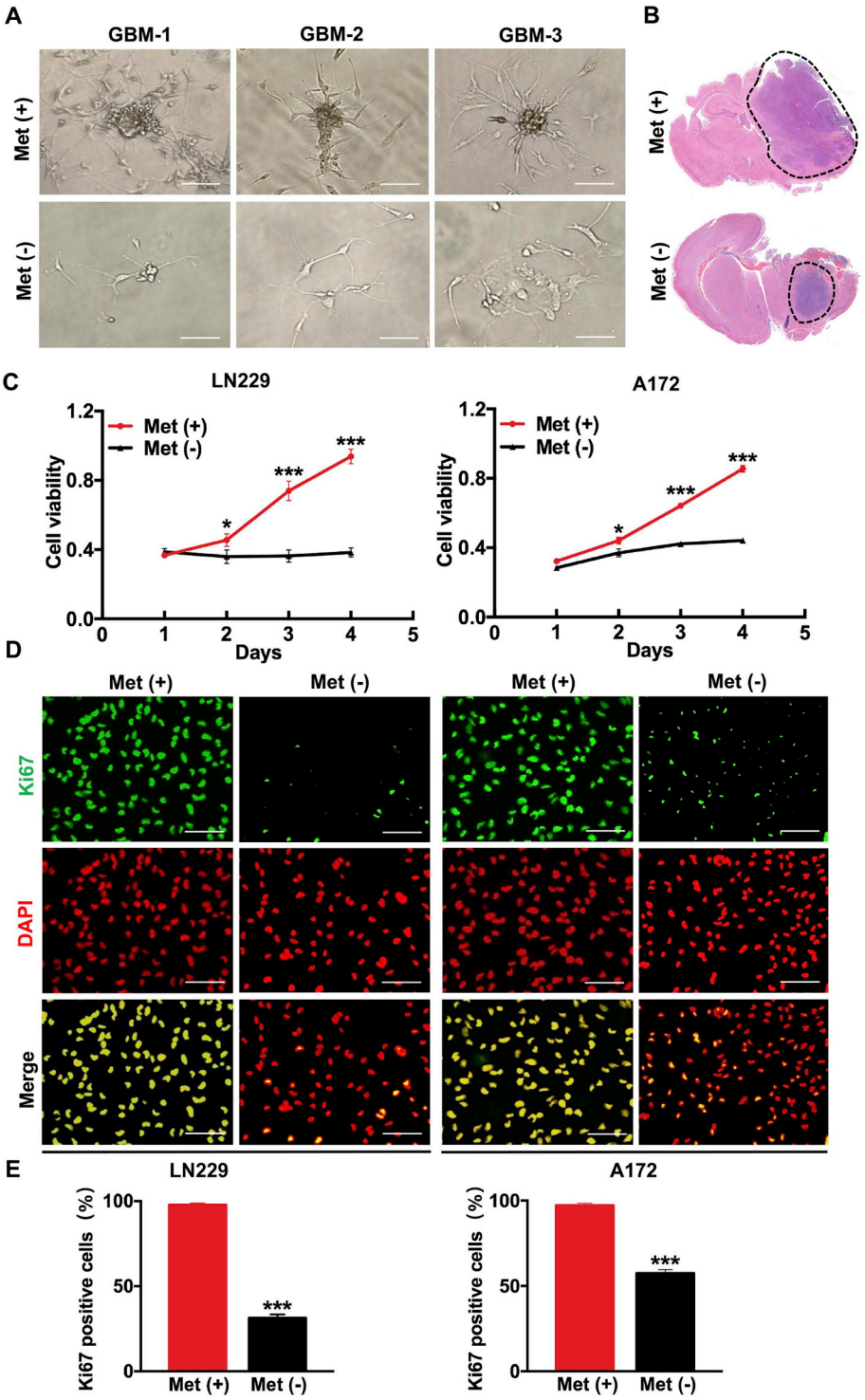

Fig. 1. Methionine deprivation inhibits glioma cell proliferation *in vitro* and *in vivo*. **(A)** Three independents primary GBM cell spheres after cells were cultured with or without methionine for 5 days. Bar: 200 μm. **(B)** Representative images of H&E stained mouse brain sections after intracranial transplanted of primary GBM cells. **(C)** Cell viability of LN229 and A172 cell line after cells were cultured with/withtout methionine for indicate times. **(D and E)** Ki67 protein expression after cell were cultured with or without methionine for 48 h. Bar: 100 μm. Met (+): Standard diet group, Met (−): Methionine free diet group. Data are represented as the mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

downregulated in LN229 and A172 cell line after cells were culture without methionine (Fig. 2D–F). Taken together, these data indicated that the IL1RN expression were suppressed after methionine deprivation both *in vitro* and *in vivo*.

3.3. IL1RN expression is sensitive to methionine deprivation in vivo

To further clarify IL1RN change character we established subcutaneous xenograft model. The results shown that although there was no significance between control group and methionine free group in tumor weight (Fig. 3A and B) and tumor weight/mice weight (Fig. 3C), there was significance in the mRNA (Fig. 3D) and protein expression (Fig. 3E) of IL1RN after 3 days of dividing group. And there was significance between two groups in tumor weight (Fig. 3F and G) and tumor weight/mice weight (Fig. 3H) beside the mRNA (Fig. 3I) and protein expression (Fig. 3J) of IL1RN at day 15. These results indicated that IL1RN expression was sensitive to methionine and was inhibited at early stage after methionine deprivation.

3.4. IL1RN knockdown inhibits glioma cell proliferation in vitro and in vivo

Next we assessed the role of IL1RN play in glioma cell proliferation.

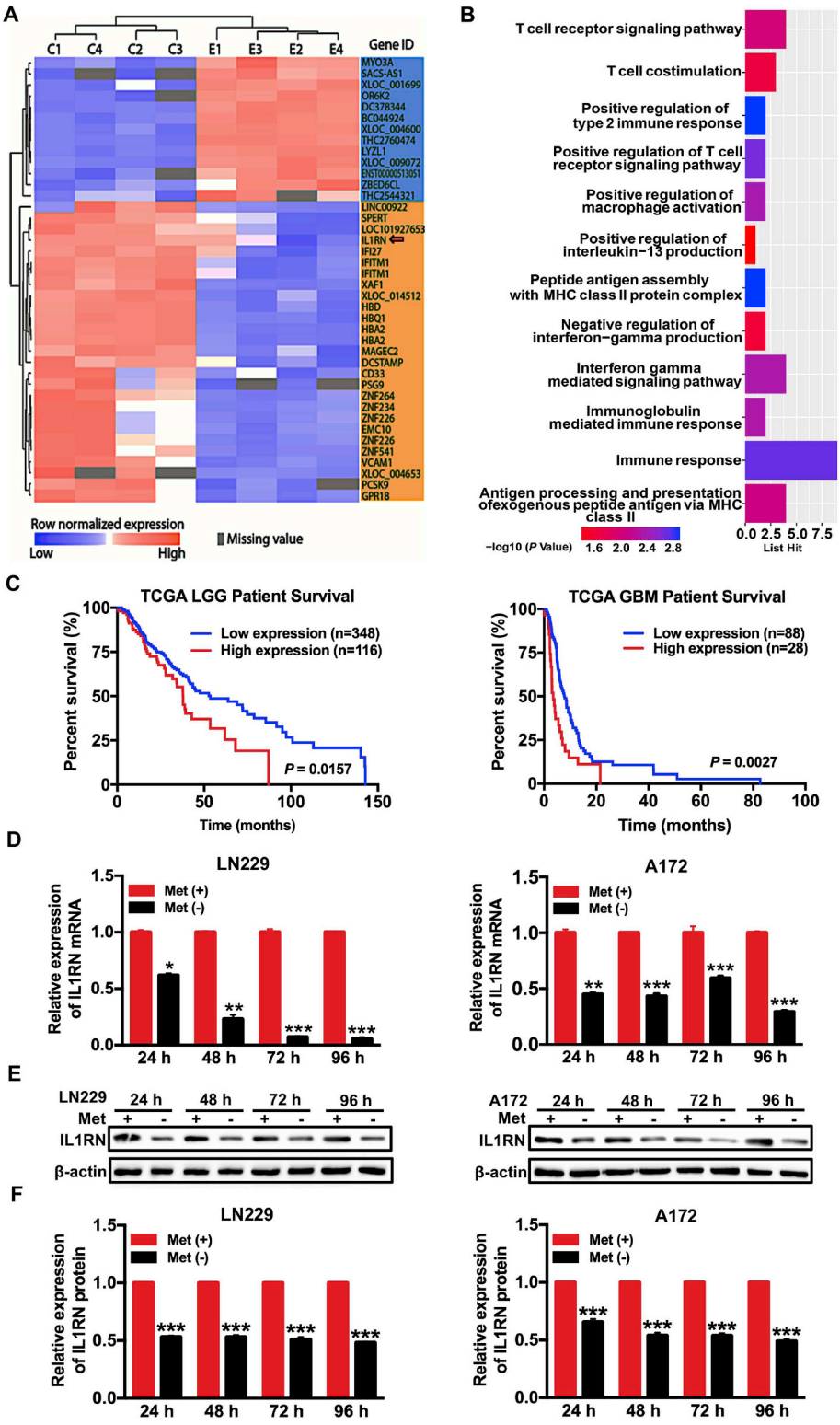

Fig. 2. Methionine deprivation suppresses IL1RN expression *in vitro* and *in vivo*. **(A)** Microarray analysis of orthotopically transplanted human glioma tissues, only show the gene which its fold change > 3.0 and P value < 0.05, red arrow indicates IL1RN, C represents Standard diet group, E represents Methionine free diet group. **(B)** Immune/inflammation related biological process units by GO Analysis. **(C)** Disease-free survival (DFS) curve of TCGA LGG and GBM patients. **(D)** Relative IL1RN mRNA expression after cells were cultured with/without methionine for indicated times, % of Met (+). **(E and F)** IL1RN protein expression after cells were cultured with/without methionine for indicated times. % of Met (+). Met (+): Control group, Met (−): Methionine free group. Data are represented as the mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

First, we knocked down IL1RN by siRNA in LN229 and A172 cell lines, after verifying the siRNA efficiency (Fig. 4A and B and Supplementary Fig. S3A) we measured the cell viability, the results shown that glioma cell viability were suppressed notably when IL1RN were downregulated in glioma cells (Fig. 4C). Meanwhile, cell morphology (Supplementary Fig. S3B) and Ki67 protein expression (Fig. 4D and Supplementary Fig. S3 C and D) indicated that glioma cell proliferation were inhibited when IL1RN was suppressed. All together these results indicated that knocked down IL1RN inhibited glioma cell proliferation *in vitro*.

Furthermore, we knocked down IL1RN by target IL1RN lentivirus *in vivo*. After testing the transfection efficiency (Supplementary Fig. S3 E and F), cells were injected into 5 weeks old nude mice brain, then we measured tumor proliferation by H&E and IHC. H&E results verified that glioma cell proliferation was suppressed notably in IL1RN knockdown group (LV-shIL1RN), and Ki67 protein expression were inhibited in IL1RN knockdown group (Fig. 4E). Additionally, Cyclin D1, CDK4 which are cell cycle related protein expression [25] were inhibited simultaneously (Fig. 4E), this indicated that cell cycle were arrested

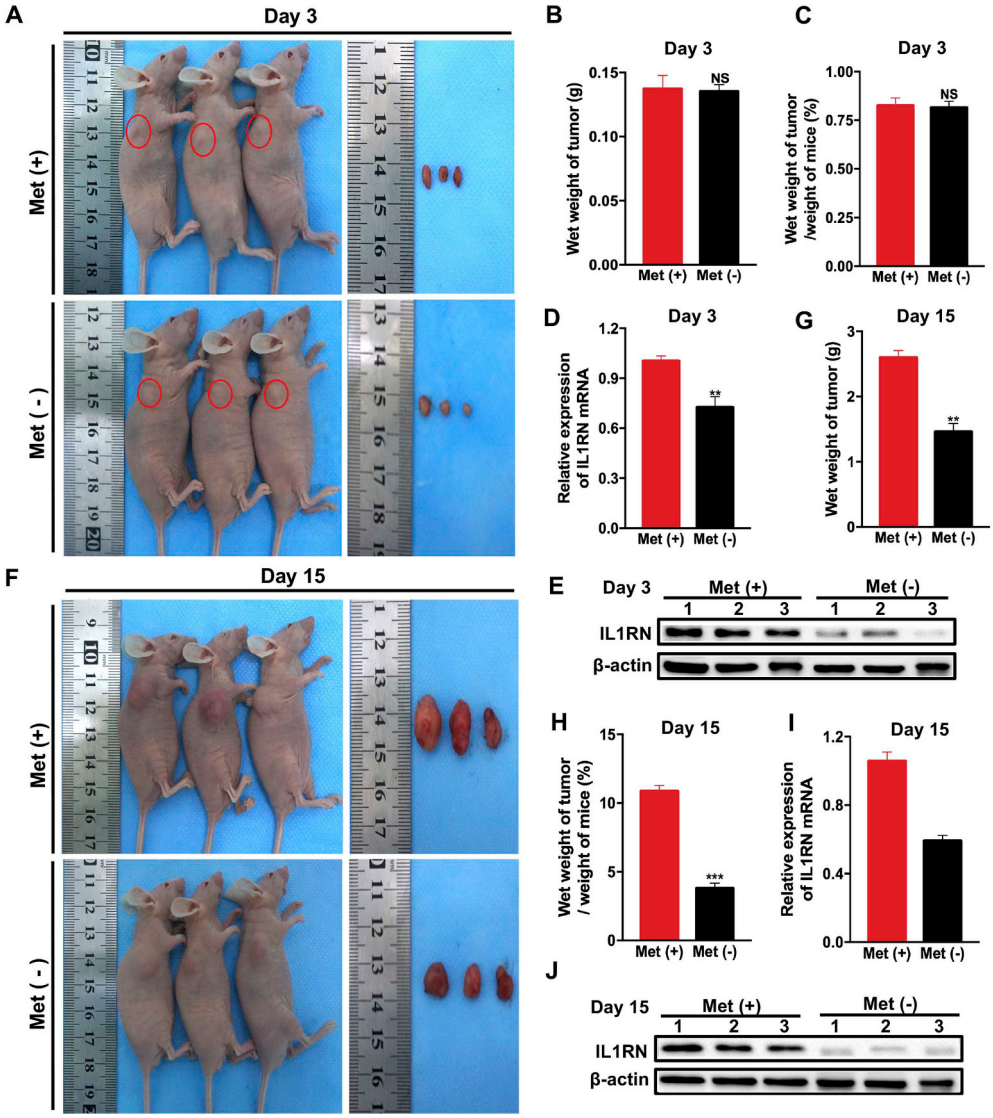

Fig. 3. IL1RN expression is sensitive to methionine deprivation *in vivo*. (A–E) Tumor pictures, wet weight of tumor (g), wet weight of tumor/mouse weight and the relative expression of IL1RN mRNA and protein after divided into standard diet group and methionine free diet group for 3 days. **(F–J)** Tumor pictures, wet weight of tumor (g), wet weight of tumor/mouse weight and the relative expression of IL1RN mRNA and protein after divided into standard diet group and methionine free diet group for 15 days. Data are represented as the mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

when methionine were deprived. All together, these results demonstrated that silencing IL1RN expression suppressed glioma cell proliferation both *in vitro* and *in vivo*.

3.5. Methionine deprivation inhibits glioma cell proliferation by suppressing IL1RN expression

To verify the hypothesis that methionine deprivation suppresses glioma proliferation by downregulating IL1RN, glioma cells were transfected with IL1RN- siRNA glioma cells and cultured with methionine free medium. Although we found that IL1RN protein expression were obviously suppressed (Fig. 5A and B), the cell viability were no significance change between these methionine deprived groups (Fig. 5C), these results demonstrated that IL1RN knockdown and methionine deprivation have no synergistic effect on inhibiting glioma cell viability.

Meanwhile, we upregulated IL1RN expression in glioma cells and cultured cell with methionine free medium, as we expected cell viability were reversed belong with IL1RN upregulation (Fig. 5 D and E), this mean upregulated IL1RN expression could reversed the suppressive effect of methionine deprivation on glioma cell proliferation (Fig. 5F and Supplementary Fig. S4). In all, these data suggested that methionine deprivation and IL1RN knockdown exert the same inhibitory effect on glioma cell proliferation and functioned in the same pathway in this assay.

3.6. Methionine deprivation/IL1RN downregulation induces glioma cell G1/S phase arrest

As we found cell cycle related protein were suppressed when methionine were deprived *in vivo* (Fig. 4E), so we hypothesized that the inhibitory effect of methionine deprivation or IL1RN suppression on cell proliferation were induced by cell cycle arrest. In this circumstance, we detected cell cycle phase distribution by flow cytometry. The results shown that glioma cells were blocked at G1/S stage when methionine were deprived, and IL1RN overexpression could reverse this arrest effect (Fig. 6A and B). Meanwhile, cell cycle related proteins expression were coincident with the cell population in cell cycle distribution (Fig. 6C and D). Together, these data demonstrated that methionine deprivation or IL1RN suppression induced glioma cell proliferation

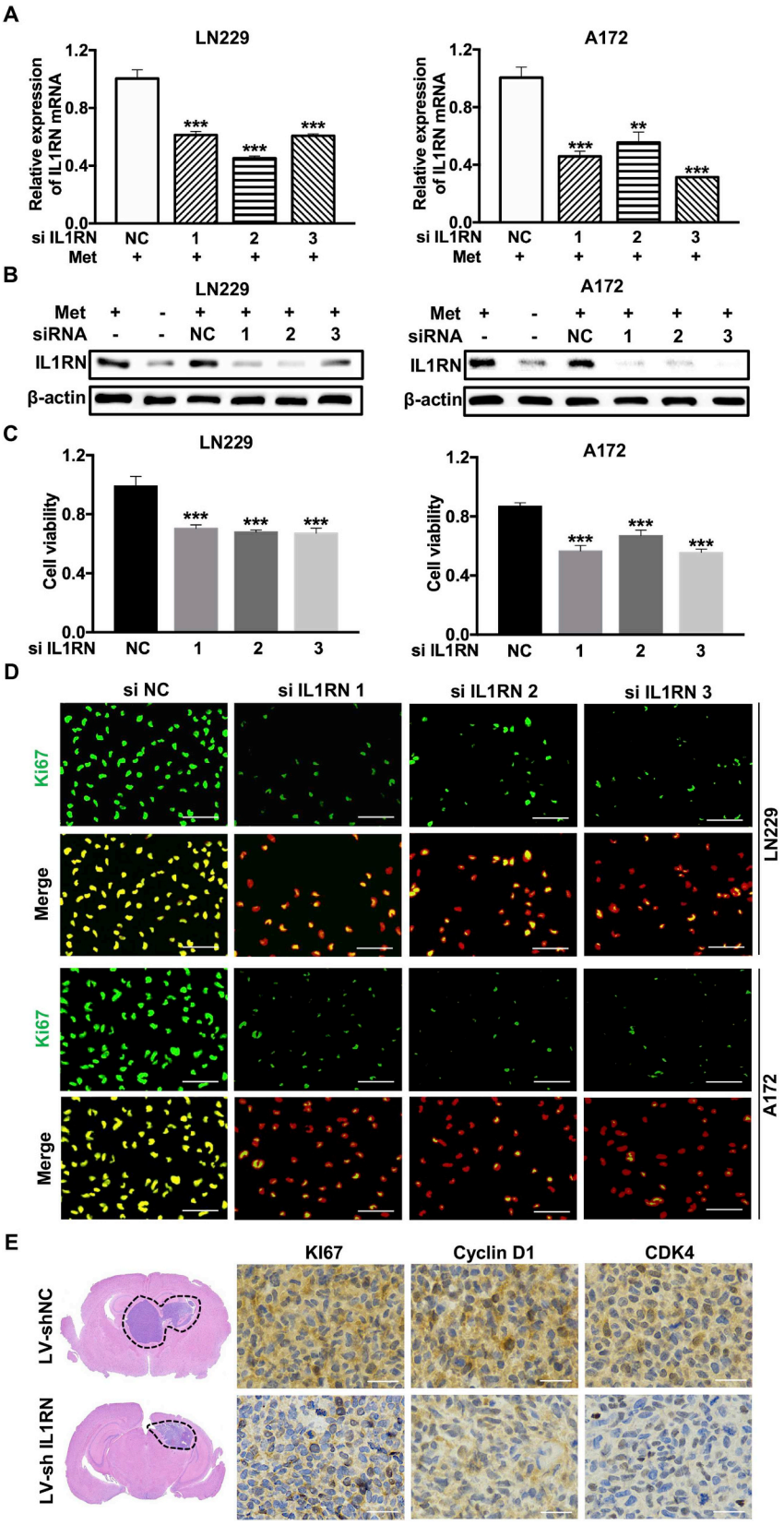

Fig. 4. IL1RN knockdown inhibits glioma cell proliferation in vitro and in vivo. **(A)** Relative IL1RN mRNA expression after transfection of IL1RN-siRNA for 48 h, cells were cultured with methionine. **(B)** Relative IL1RN protein expression after transfection of IL1RN-siRNA for 48 h. **(C)** Cell viability of LN229 and A172 cell line after transfection of IL1RN siRNA for 48 h, cells were cultured with methionine. **(D)** Ki67 protein expression in LN229 and A172 cell lines after transfection of IL1RN siRNA for 48 h, bar: 100 μm. **(E)** Representative images of H&E-stained mouse brain sections after intracranial transplantation of glioma cells, and representative images of IHC-stained mouse brain sections for Ki67, CDK4 and Cyclin D1, bar: 100 μm. LV- shNC: Negative control lentivirus, LV- shIL1RN: IL1RN knockdown lentivirus. All data are represented as the mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

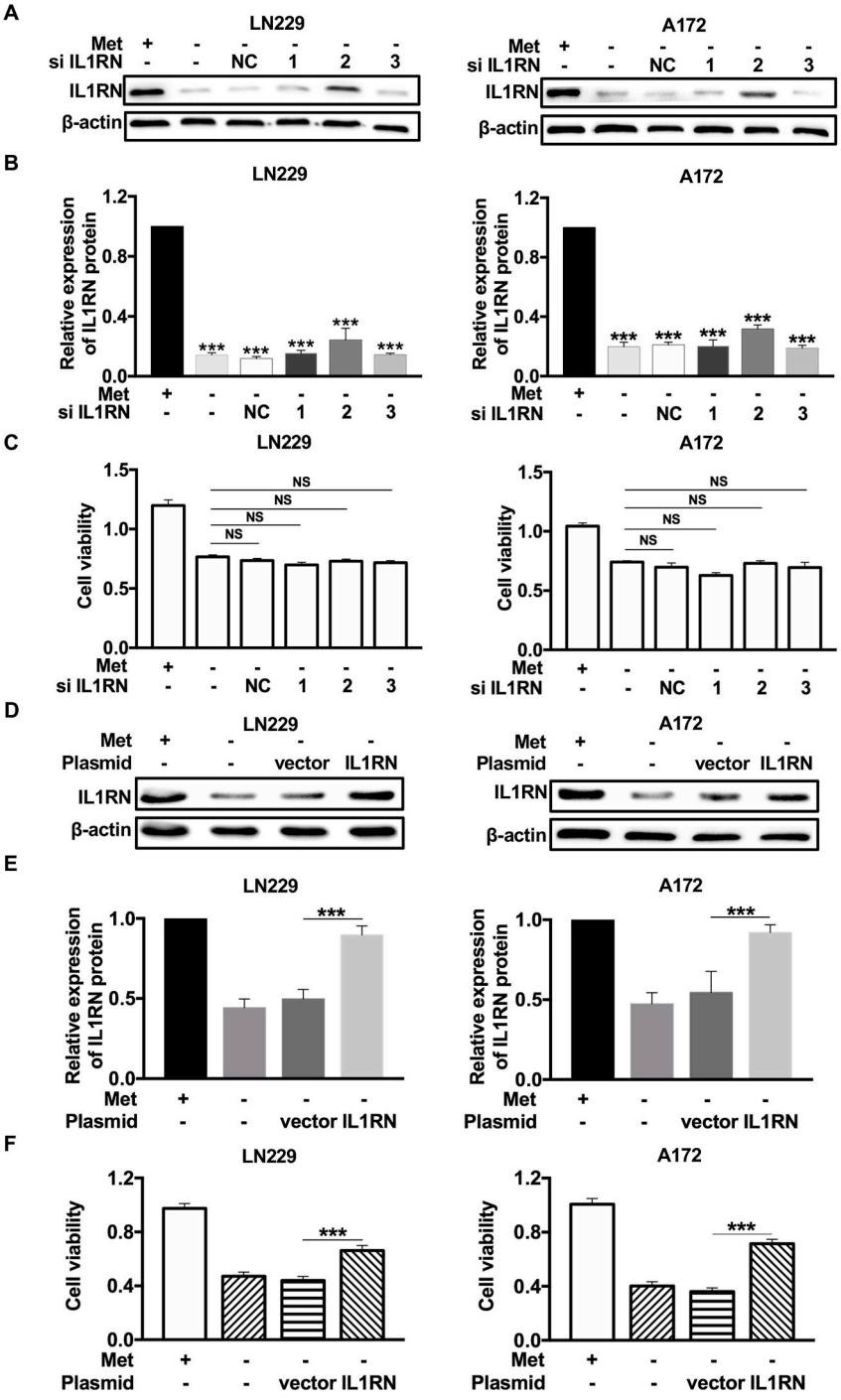

Fig. 5. Methionine deprivation inhibits glioma cell proliferation by suppressing IL1RN expression. (A and B) IL1RN protein expression in LN229 and A172 cell line after transfection of IL1RN siRNA for 48 h. **(C)** Cell viability of LN229 and A172 cell line after transfection of IL1RN siRNA for 48 h. NS: no significance. **(D and E)** IL1RN protein expression in LN229 and A172 cell lines after transfection of IL1RN overexpression plasmid for 48 h. **(F)** Cell viability of LN229 and A172 cell line after transfection of IL1RN overexpression plasmid for 48 h. Vector: Negative control plasmid, IL1RN: IL1RN overexpression plasmid. Cells were cultured as indicated. Data are represented as the mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

inhibition via G1/S phase cell cycle arresting *in vivo* and *in vitro*.

3.7. IL1RN is upregulated in human glioma tissues and correlated with the prognosis of glioma patients

All above, the results demonstrated that methionine deprivation inhibited glioma cell proliferation by suppressing IL1RN expression. However, the role of IL1RN play in glioma patients is remain unclear. Therefore, we caught glioma clinical data to draw survival curve of glioma patients, the disease free survival curve indicated that IL1RN expression was negatively correlated with the survival time of glioma patients (Fig. 2C), the hazard ratios (High/Low) were 1.517 (LGG) and 1.964 (GBM), the 95% confidence interval were 1.033–2.227 (LGG) and 1.125 to 3.428 (GBM), the statistical significance were 0.0157 (LGG) and 0.0027 (GBM) (Fig. 7A). The IL1RN expression data in TCGA database, it shown that IL1RN expression were upregulated in GBM than that in LGG (Fig. 7B). We also collected nontumor tissues and primary glioma tissues for detecting IL1RN mRNA expression, the data demonstrated that IL1RN mRNA expression were upregulated in glioma tissues compared to that in nontumor tissues, which was consistent with TCGA data (Fig. 7C). Moreover, the IL1RN protein expression in the human glioma tissues were also upregulated compared to nontumor brain tissues (Fig. 7D and E). Collectively, these results demonstrated that IL1RN expression is upregulated in human glioma tissues and high IL1RN expression predicted a poor prognosis in glioma patients.

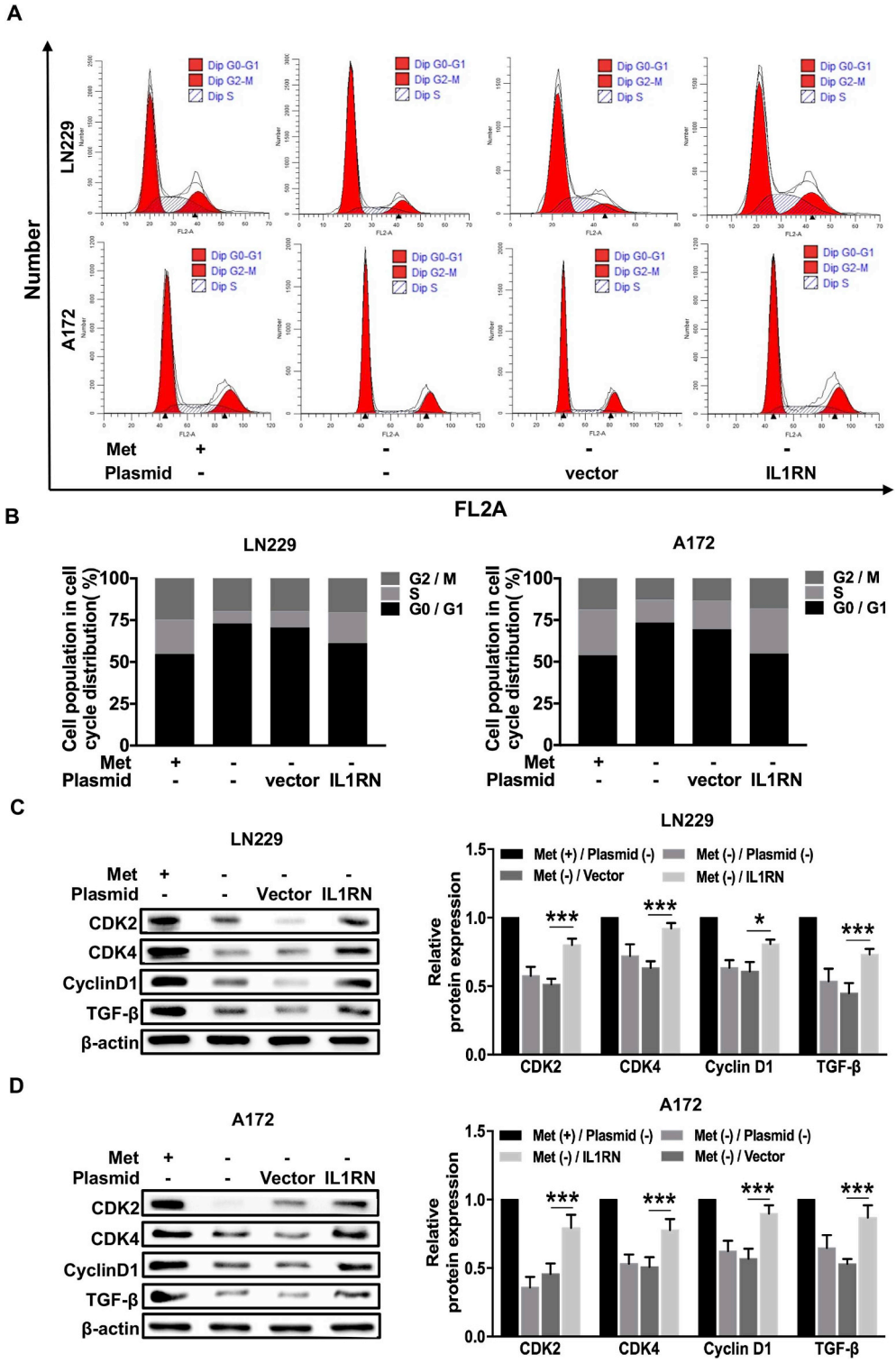

Fig. 6. Methionine deprivation/IL1RN downregulation induces glioma cell G1/S phase arrest. **(A and B)** Cell cycle distribution after transfection of IL1RN overexpression plasmid for 48 h, cells were cultured as indicated. **(C)** Expression of cell cycle associated proteins in LN229 cell line after transfection of IL1RN overexpression plasmid for 48 h. **(D)** Expression of cell cycle associated proteins in A172 cell line after transfection of IL1RN overexpression plasmid for 48 h. Data are represented as the mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

4. Discussion

Although many efforts have been made for the treatment of glioma, its prognosis remains extremely dismal [26,27]. Unbridled proliferation is the major characteristic of glioma and main reason for its poor outcome, therefore, maximal restriction of cell proliferation is vital for glioma therapy [28,29]. An increasing number of studies have confirmed that substance metabolism, including protein, glucose and lipid metabolism, plays a critical role in tumor proliferation [30–33], thus, metabolic reprogramming is a potential breakthrough approach for glioma treatment.

Among numerous substances, methionine has captured a large

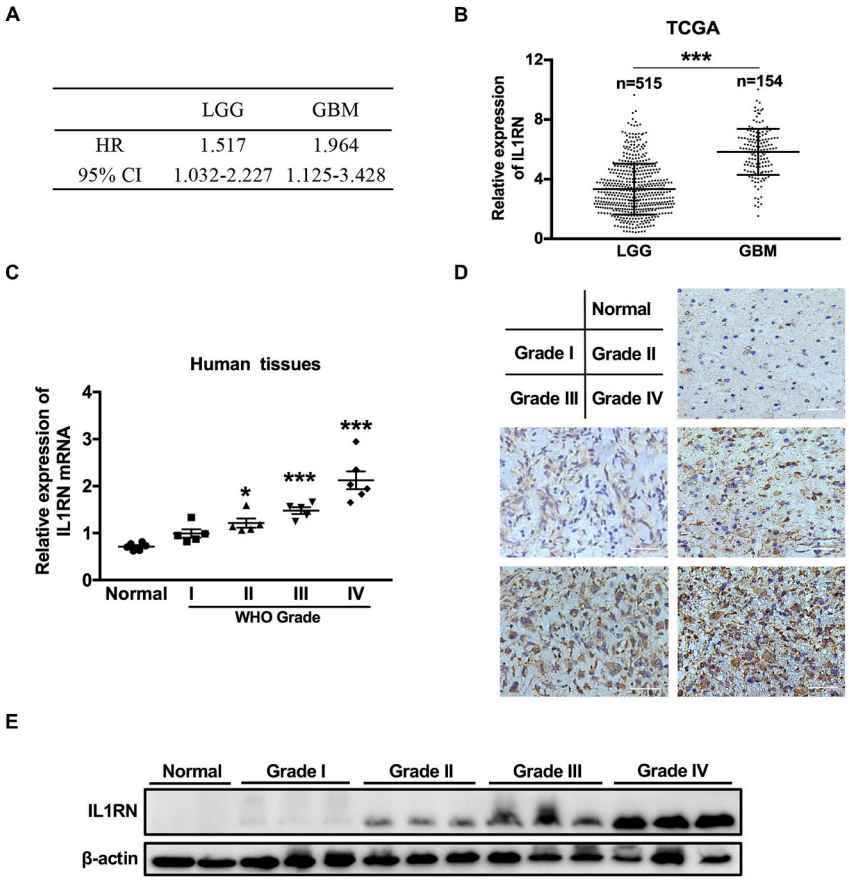

Fig. 7. IL1RN is upregulated in human glioma tissues and correlated with prognosis of glioma patients. **(A)** Statistics of glioma patients' diseases free curve. **(B)** Relative IL1RN mRNA expression in TCGA glioma patients' tissues. **(C)** Relative IL1RN mRNA expression in normal human tissues and WHO Grade I – IV glioma tissues. **(D)** Representative IHC images of IL1RN protein in different grade human glioma tissues and normal brain tissues, bar: 50 μm. **(E)** IL1RN protein expression in normal brain tissues and different grade human glioma tissues. Normal tissues n = 2, glioma WHO I - IV n = 3. Data are represented as the mean ± SD, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

number attentions due to its important role in protein synthesis, DNA methylation and polyamine synthesis. Many studies have demonstrated that methionine deprivation could suppress tumor proliferation [9,10,34], however, the underlying molecular mechanism remains unclear. In this study, we demonstrated that glioma proliferation is methionine-dependent, which suggests that restricting the methionine supply could inhibit glioma cell proliferation. Upon screening differentially expression genes, we first found that IL1RN was obviously downregulated *in vitro* and *in vivo* when methionine were deprived. Furthermore, our results demonstrated that upregulating IL1RN could reverse the inhibitory effect of methionine deprivation on glioma. Altogether, IL1RN plays an important role in glioma cell metabolic reprogramming which induced by methionine deprivation.

Although we haven't figure out how methionine deprivation regulates those differentially expression genes including IL1RN, we found the expression of transcription factor TGF-β and NF-κB were changed under those stress, this maybe a reason why genes expression was changed. In addition, methionine can provide methyl for lots of biology progresses including DNA and histone methylation [9,10], and genes expression could be regulated by DNA or/and histone methylation/demethylation. Moreover, as an essential amino acid methionine is material for protein synthesis, these proteins maybe participate in modification change of chromatin [12]. All above maybe potential mechanism of methionine deprivation regulates gene expression, and we will explore the mechanism in future work.

Inflammation and immune disorder are the hallmarks of cancer and are vital for cancer cell progress [35]. IL1RN is an immune and inflammatory cytokine and now is well known as a proinflammatory cytokine [36]. Several researchers proved that IL1RN is associated with tumor progression and exerts diverse effects on different tumor types, for example, high expression of IL1RN increased the risk of bladder cancer and gastric carcinoma but decreased the risk of lung cancer [19–21]. Our team was tremendously fascinated by the contradictory results and drove us to explore the function of IL1RN in glioma. In this assay we demonstrated that IL1RN is upregulated in glioma tissues, which is in accordance with the TCGA data, and IL1RN knockdown suppressed glioma cell viability *in vitro* and *in vivo*. In all, these results suggest that IL1RN is vital for glioma survival.

Tada et al. suggest a potential role of IL1RN in regulation of the IL-1 autocrine loop in glioblastomas [37], it means that IL1RN also play a vital role in IL1 mediate inflammation/immunity pathway beside in metabolic reprogramming, therefore these results highlight that IL1RN may be a potential hub between IL-1 mediate inflammation/immunity pathway and methionine deprivation induced metabolic reprogramming pathway in glioma. Although we screened IL1RN from inflammation/immunity gene by KEGG enrichment and GO analysis, it is unclear whether IL1RN is fully function as an IL1 receptor blocker or not in glioma metabolic reprogramming [38,39], therefore, our future studies will explore the functional mechanism of IL1RN in glioma. However, our study provides a potential key to regulate both IL1 mediate inflammation/immunity pathway and methionine deprivation induced metabolic reprogramming pathway in glioma, it means IL1RN will be a potential breakthrough point in glioma therapy. Meanwhile we also proved that inhibiting IL1RN expression induced glioma cell cycle arrest, in further study we will explore the underlying mechanism including of its interactions with cell cycle protein. At the same time, we also found downregulating IL1RN inhibited glioma migration, it is also an important work to explore the molecular mechanism.

Clinically, high IL1RN expression predicted poor prognosis in LGG and GBM, but its use as a biomarker for glioma still requires verification, further more we will collect serum and/or cerebrospinal fluid from glioma patient to detect the IL1RN mRNA/protein level [40]. As approved by Food and Drug Administration (FDA, USA), adeno-associated virus (AAVs) show great potential in clinical application [41,42], we

will deliver IL1RN related shRNA to animal and clinical trial glioma patients by adeno-associated virus. In conclusion, our results prove that IL1RN could be a rational hub between IL1 mediate inflammation/immunity pathway and methionine deprivation induced metabolic reprogramming pathway in glioma, and demonstrate a strong rationale insight that targeting amino acid metabolism such as methionine deprivation/IL1RN related gene therapy may offer novel direction for glioma treatment.

Conflicts of interest

The authors declare no potential conflicts of interest.

CRediT authorship contribution statement

Kaikai Wang: Conceptualization, Methodology, Project administration, Writing - original draft. **Huailei Liu:** Conceptualization, Funding acquisition, Methodology, Project administration. **Jiaqi Liu:** Data curation, Investigation. **Xiaoxiong Wang:** Investigation. **Lei Teng:** Formal analysis. **Jun Zhang:** Investigation. **Yi Liu:** Validation, Visualization, Writing - review & editing. **Yizheng Yao:** Validation, Visualization, Writing - review & editing. **Jun Wang:** Formal analysis. **Yuan Qu:** Validation, Visualization, Writing - review & editing. **Xin Chen:** Funding acquisition, Writing - original draft. **Fei Peng:** Writing - original draft. **Hongbo Liu:** Formal analysis, Software. **Ning Wang:** Investigation. **Yingqiang Zhong:** Investigation. **Xu Hou:** Funding acquisition, Visualization, Writing - review & editing. **Haiping Jiang:** Investigation. **Ozal Beylerli:** Writing - original draft. **Xiang Liao:** Formal analysis, Software. **Xinjian Zhang:** Investigation. **Xu Zhang:** Investigation. **Xiangtong Zhang:** Conceptualization, Supervision. **Shiguang Zhao:** Conceptualization, Funding acquisition, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.04.004.

References

[1] J. Chen, Y. Li, T.S. Yu, R.M. McKay, D.K. Burns, S.G. Kernie, L.F. Parada, A restricted cell population propagates glioblastoma growth after chemotherapy, Nature 488 (2012) 522–526 [PubMed: 22854781].

[2] S. Lapointe, A. Perry, N.A. Butowski, Primary brain tumours in adults, Lancet 392 (2018) 432–446 [PubMed: 30060998].

[3] Z.A. Binder, A.H. Thorne, S. Bakas, E.P. Wileyto, M. Bilello, H. Akbari, S. Rathore, S.M. Ha, L. Zhang, C.J. Ferguson, S. Dahiya, W.L. Bi, D.A. Reardon, A. Idbaih, J. Felsberg, B. Hentschel, M. Weller, S.J. Bagley, J.J.D. Morrissette, M.P. Nasrallah, J. Ma, C. Zanca, A.M. Scott, L. Orellana, C. Davatzikos, F.B. Furnari, D.M. O'Rourke, Epidermal growth factor receptor extracellular domain mutations in glioblastoma present opportunities for clinical imaging and therapeutic development, Cancer Cell 34 (2018) 163–177 e167 [PubMed: 29990498].

[4] G. Germano, S. Lamba, G. Rospo, L. Barault, A. Magri, F. Maione, M. Russo, G. Crisafulli, A. Bartolini, G. Lerda, G. Siravegna, B. Mussolin, R. Frapolli, M. Montone, F. Morano, F. de Braud, N. Amirouchene-Angelozzi, S. Marsoni, M. D'Incalci, A. Orlandi, E. Giraudo, A. Sartore-Bianchi, S. Siena, F. Pietrantonio, F. Di Nicolantonio, A. Bardelli, Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth, Nature 552 (2017) 116–120 [PubMed: 29186113].

[5] C. Rubio-Patino, J.P. Bossowski, G.M. De Donatis, L. Mondragon, E. Villa, L.E. Aira, J. Chiche, R. Mhaidly, C. Lebeaupin, S. Marchetti, K. Voutetakis, A. Chatziioannou, F.A. Castelli, P. Lamourette, E. Chu-Van, F. Fenaille, T. Avril, T. Passeron, J.B. Patterson, E. Verhoeyen, B. Bailly-Maitre, E. Chevet, J.E. Ricci, Low-protein diet induces IRE1alpha-dependent anticancer immunosurveillance, Cell Metabol. 27 (2018) 828–842 e827 [PubMed: 29551590].

[6] D.R. Green, Eating the beast: dietary protein and anticancer immunity, Cell Metabol. 27 (2018) 703–705 [PubMed: 29617635].

[7] A. Viale, G.F. Draetta, Sugar? No thank you, just a deep breath of oxygen for cancer stem cells, Cell Metabol. 22 (2015) 543–545 [PubMed: 26445511].

[8] J. Font-Burgada, B. Sun, M. Karin, Obesity and cancer: the oil that feeds the flame, Cell Metabol. 23 (2016) 48–62 [PubMed: 26771116].

[9] O.D. Maddocks, C.F. Labuschagne, P.D. Adams, K.H. Vousden, Serine metabolism supports the methionine cycle and DNA/RNA methylation through De Novo ATP synthesis in cancer cells, Mol. Cell 61 (2016) 210–221 [PubMed: 26774282].

[10] K. Palanichamy, K. Thirumoorthy, S. Kanji, N. Gordon, R. Singh, J.R. Jacob, N. Sebastian, K.T. Litzenberg, D. Patel, E. Bassett, B. Ramasubramanian, T. Lautenschlaeger, S.M. Fischer, A. Ray-Chaudhury, A. Chakravarti, Methionine and Kynurenine activate oncogenic kinases in glioblastoma, and methionine deprivation compromises proliferation, Clin. Cancer Res. 22 (2016) 3513–3523 [PubMed: 26936918].

[11] O.A. Kharenko, R.G. Patel, S.D. Brown, C. Calosing, A. White, D. Lakshminarasimhan, R.K. Suto, B.C. Duffy, D.B. Kitchen, K.G. McLure, H.C. Hansen, E.H. van der Horst, P.R. Young, Design and characterization of novel covalent bromodomain and extra-terminal domain (BET) inhibitors targeting a methionine, J. Med. Chem. 61 (2018) 8202–8211 [PubMed: 30165024].

[12] F.L. Zhang, J.L. Cao, H.Y. Xie, R. Sun, L.F. Yang, Z.M. Shao, D.Q. Li, Cancer-associated MORC2-mutant M276I regulates an hnRNPM-mediated CD44 splicing switch to promote invasion and metastasis in triple-negative breast cancer, Cancer Res. 78 (2018) 5780–5792 [PubMed: 30093560].

[13] H. Liu, W. Zhang, K. Wang, X. Wang, F. Yin, C. Li, C. Wang, B. Zhao, C. Zhong, J. Zhang, F. Peng, Y. Bi, C. Shen, X. Hou, D. Zhang, Y. Liu, J. Ai, S. Zhao, Methionine and cystine double deprivation stress suppresses glioma proliferation via inducing ROS/autophagy, Toxicol. Lett. 232 (2015) 349–355 [PubMed: 25448282].

[14] C.A. Dinarello, A. Simon, J.W. van der Meer, Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases, Nat. Rev. Drug Discov. 11 (2012) 633–652 [PubMed: 22850787].

[15] S.C. Lin, Y.C. Lo, H. Wu, Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling, Nature 465 (2010) 885–890 [PubMed: 20485341].

[16] I. Aksentijevich, S.L. Masters, P.J. Ferguson, P. Dancey, J. Frenkel, A. van Royen-Kerkhoff, R. Laxer, U. Tedgard, E.W. Cowen, T.H. Pham, M. Booty, J.D. Estes, N.G. Sandler, N. Plass, D.L. Stone, M.L. Turner, S. Hill, J.A. Butman, R. Schneider, P. Babyn, H.I. El-Shanti, E. Pope, K. Barron, X. Bing, A. Laurence, C.C. Lee, D. Chapelle, G.I. Clarke, K. Ohson, M. Nicholson, M. Gadina, B. Yang, B.D. Korman, P.K. Gregersen, P.M. van Hagen, A.E. Hak, M. Huizing, P. Rahman, D.C. Douek, E.F. Remmers, D.L. Kastner, R. Goldbach-Mansky, An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist, N. Engl. J. Med. 360 (2009) 2426–2437 [PubMed: 19494218].

[17] S. Ceeraz, S.K. Eszterhas, P.A. Sergent, D.A. Armstrong, A. Ashare, T. Broughton, L. Wang, D. Pechenick, C.M. Burns, R.J. Noelle, M.P. Vincenti, R.A. Fava, VISTA deficiency attenuates antibody-induced arthritis and alters macrophage gene expression in response to simulated immune complexes, Arthritis Res. Ther. 19 (2017) 270 [PubMed: 29216931].

[18] C. Grond-Ginsbach, M. Hummel, T. Wiest, S. Horstmann, K. Pfleger, M. Hergenhahn, M. Hollstein, U. Mansmann, A.J. Grau, S. Wagner, Gene expression in human peripheral blood mononuclear cells upon acute ischemic stroke, J. Neurol. 255 (2008) 723–731 [PubMed: 18465111].

[19] T.S. Worst, V. Reiner, U. Gabriel, C. Weiss, P. Erben, T. Martini, C. Bolenz, IL1RN and KRT13 expression in bladder cancer: association with pathologic characteristics and smoking status, 2014, Adv Urol (2014) 184602 [PubMed: 25114677].

[20] G.A. Rocha, J.B. Guerra, A.M. Rocha, I.E. Saraiva, D.A. da Silva, C.A. de Oliveira, D.M. Queiroz, IL1RN polymorphic gene and cagA-positive status independently increase the risk of noncardia gastric carcinoma, Int. J. Cancer 115 (2005) 678–683 [PubMed: 15704154].

[21] Z. Hu, M. Shao, Y. Chen, J. Zhou, J. Qian, L. Xu, H. Ma, X. Wang, Y. Xu, D. Lu, H. Shen, Allele 2 of the interleukin-1 receptor antagonist gene (IL1RN*2) is associated with a decreased risk of primary lung cancer, Cancer Lett. 236 (2006) 269–275 [PubMed: 16019127].

[22] H.S. Venkatesh, L.T. Tam, P.J. Woo, J. Lennon, S. Nagaraja, S.M. Gillespie, J. Ni, D.Y. Duveau, P.J. Morris, J.J. Zhao, C.J. Thomas, M. Monje, Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma, Nature 549 (2017) 533–537 [PubMed: 28959975].

[23] X. Wang, C. Shen, Z. Liu, F. Peng, X. Chen, G. Yang, D. Zhang, Z. Yin, J. Ma, Z. Zheng, B. Zhao, H. Liu, L. Wang, J. Wu, D. Han, K. Wang, C. Zhong, X. Hou, W. Zhao, M. Shu, X. Wang, S. Zhao, Nitazoxanide, an antiprotozoal drug, inhibits late-stage autophagy and promotes ING1-induced cell cycle arrest in glioblastoma, Cell Death Dis. 9 (2018) 1032 [PubMed: 30302016].

[24] T. Huang, C.K. Kim, A.A. Alvarez, R.P. Pangeni, X. Wan, X. Song, T. Shi, Y. Yang, N. Sastry, C.M. Horbinski, S. Lu, R. Stupp, J.A. Kessler, R. Nishikawa, I. Nakano, E.P. Sulman, X. Lu, C.D. James, X.M. Yin, B. Hu, S.Y. Cheng, MST4 phosphorylation of ATG4B regulates autophagic activity, tumorigenicity, and radioresistance in glioblastoma, Cancer Cell 32 (2017) 840–855 e848 [PubMed: 29232556].

[25] C.J. Sherr, D. Beach, G.I. Shapiro, Targeting CDK4 and CDK6: from discovery to therapy, Cancer Discov. 6 (2016) 353–367 [PubMed: 26658964].

[26] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36

cancers in 185 countries, Ca - Cancer J. Clin. 6 (2018) 394–424 [PubMed: 30207593].
[27] Q.T. Ostrom, H. Gittleman, P. Liao, T. Vecchione-Koval, Y. Wolinsky, C. Kruchko, J.S. Barnholtz-Sloan, CBTRUS Statistical Report: primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014, Neuro Oncol. 19 (2017) v1-v88 [PubMed: 29117289].
[28] F. Lu, Y. Chen, C. Zhao, H. Wang, D. He, L. Xu, J. Wang, X. He, Y. Deng, E.E. Lu, X. Liu, R. Verma, H. Bu, R. Drissi, M. Fouladi, A.O. Stemmer-Rachamimov, D. Burns, M. Xin, J.B. Rubin, E.M. Bahassi, P. Canoll, E.C. Holland, Q.R. Lu, Olig2-Dependent reciprocal shift in PDGF and EGF receptor signaling regulates tumor phenotype and mitotic growth in malignant glioma, Cancer Cell 29 (2016) 669–683 [PubMed: 27165742].
[29] M.S. Pavlyukov, H. Yu, S. Bastola, M. Minata, V.O. Shender, Y. Lee, S. Zhang, J. Wang, S. Komarova, J. Wang, S. Yamaguchi, H.A. Alsheikh, J. Shi, D. Chen, A. Mohyeldin, S.H. Kim, Y.J. Shin, K. Anufrieva, E.G. Evtushenko, N.V. Antipova, G.P. Arapidi, V. Govorun, N.B. Pestov, M.I. Shakhparonov, L.J. Lee, D.H. Nam, I. Nakano, Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors, Cancer Cell 34 (2018) 119–135 e110 [PubMed: 29937354].
[30] X. Zhong, S. Tian, X. Zhang, X. Diao, F. Dong, J. Yang, Z. Li, L. Sun, L. Wang, X. He, G. Wu, X. Hu, L. Wang, L. Song, H. Zhang, X. Pan, A. Li, P. Gao, CUE domain-containing protein 2 promotes the Warburg effect and tumorigenesis, EMBO Rep. 18 (2017) 809–825 [PubMed: 28325773].
[31] Y. Zhang, G. Yu, H. Chu, X. Wang, L. Xiong, G. Cai, R. Liu, H. Gao, B. Tao, W. Li, G. Li, J. Liang, W. Yang, Macrophage-associated PGK1 phosphorylation promotes aerobic glycolysis and tumorigenesis, Mol. Cell 71 (2018) 201–215 e207 [PubMed: 30029001].
[32] L. Li, Y. Liang, L. Kang, Y. Liu, S. Gao, S. Chen, Y. Li, W. You, Q. Dong, T. Hong, Z. Yan, S. Jin, T. Wang, W. Zhao, H. Mai, J. Huang, X. Han, Q. Ji, Q. Song, C. Yang, S. Zhao, X. Xu, Q. Ye, Transcriptional regulation of the Warburg effect in cancer by SIX1, Cancer Cell 33 (2018) 368–385 e367 [PubMed: 29455928].
[33] T. Wang, Q. Yu, J. Li, B. Hu, Q. Zhao, C. Ma, W. Huang, L. Zhuo, H. Fang, L. Liao, Y. Eugene Chin, Y. Jiang, O-GlcNAcylation of fumarase maintains tumour growth under glucose deficiency, Nat. Cell Biol. 19 (2017) 833–843 [PubMed: 28628081].
[34] E. Strekalova, D. Malin, D.M. Good, V.L. Cryns, Methionine deprivation induces a targetable vulnerability in triple-negative breast cancer cells by enhancing TRAIL receptor-2 expression, Clin. Cancer Res. 21 (2015) 2780–2791 [PubMed: 25724522].
[35] A. Ludin, L.I. Zon, Cancer immunotherapy: the dark side of PD-1 receptor inhibition, Nature 552 (2017) 41–42 [PubMed: 29143822].
[36] A.J. Grossberg, E.G. Vichaya, D.L. Christian, J.M. Molkentine, D.W. Vermeer, P.S. Gross, P.D. Vermeer, J.H. Lee, R. Dantzer, Tumor-associated fatigue in cancer patients develops independently of IL1 signaling, Cancer Res. 78 (2018) 695–705 [PubMed: 29217760].
[37] M. Tada, A.C. Diserens, I. Desbaillets, R. Jaufeerally, M.F. Hamou, N. de Tribolet, Production of interleukin-1 receptor antagonist by human glioblastoma cells in vitro and in vivo, J. Neuroimmunol. 50 (1994) 187–194 [PubMed: 8120140].
[38] E. Oelmann, A. Kraemer, H. Serve, B. Reufi, D. Oberberg, S. Patt, H. Herbst, H. Stein, E. Thiel, W.E. Berdel, Autocrine interleukin-1 receptor antagonist can support malignant growth of glioblastoma by blocking growth-inhibiting autocrine loop of interleukin-1, Int. J. Cancer 71 (1997) 1066–1076 [PubMed: 9185713].
[39] M.A. Shevtsov, B.P. Nikolaev, L.Y. Yakovleva, A.V. Dobrodumov, A.V. Zhakhov, A.L. Mikhrina, E. Pitkin, M.A. Parr, V.I. Rolich, A.S. Simbircev, A.M. Ischenko, Recombinant interleukin-1 receptor antagonist conjugated to superparamagnetic iron oxide nanoparticles for theranostic targeting of experimental glioblastoma, Neoplasia 17 (2015) 32–42 [PubMed: 25622897].
[40] W. Sauerbrei, S.E. Taube, L.M. McShane, M.M. Cavenagh, D.G. Altman, Reporting recommendations for tumor marker prognostic studies (REMARK): an abridged explanation and elaboration, J. Natl. Cancer Inst. 110 (2018) 803–811 [PubMed: 29873743].
[41] B. Chen, Y. Li, B. Yu, Z. Zhang, B. Brommer, P.R. Williams, Y. Liu, S.V. Hegarty, S. Zhou, J. Zhu, H. Guo, Y. Lu, Y. Zhang, X. Gu, Z. He, Reactivation of dormant relay pathways in injured spinal cord by KCC2 manipulations, Cell 174 (2018) 1599 [PubMed: 30193115].
[42] L. Song, T. Llanga, L.M. Conatser, V. Zaric, B.C. Gilger, M.L. Hirsch, Serotype survey of AAV gene delivery via subconjunctival injection in mice, Gene Ther. 25 (2018) 402–414 [PubMed: 30072815].