CCAAT/Enhancer-binding protein delta mediates glioma stem-like cell enrichment and ATP-binding cassette transporter ABCA1 activation for temozolomide resistance in glioblastoma

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Abstract
Glioblastoma (GBM) is the most aggressive brain tumor and relapses after chemo- or radiotherapy in a short time. The anticancer drug temozolomide (TMZ) is commonly used for GBM treatment, but glioma stem-like cells (GSCs) often lead to drug resistance and therapeutic failure. To date, the mechanism of GSC formation in TMZ-treated GBM remains largely unknown. CCAAT/Enhancer-binding protein delta (CEBPD) is an inflammation-responsive transcription factor and is proposed to be oncogenic in the context of drug resistance, prompting us to clarify its role in TMZ-resistant GBM. In this study, we first found that the CEBPD protein levels in GBM patients were significantly increased and further contributed to TMZ resistance by promoting GSC formation. Accordingly, the protein levels of stemness transcription factors, namely, SRY-box transcription factor 2 (SOX2), octamer-binding transcription factor 4 (OCT4), NANOG, and ATP-binding cassette subfamily A member 1 (ABCA1), were increased in GSCs and TMZ-treated GBM cells. Increased binding of CEBPD to promoter regions was observed in GSCs, indicating the direct regulation of these GSC-related genes by CEBPD. In addition, an ABCA1 inhibitor increased the caspase 3/7 activity of TMZ-treated GSCs, suggesting that TMZ efflux is controlled by ABCA1 activity and that the expression levels of the ABCA1 gene are an indicator of the efficiency of TMZ treatment. Together, we revealed the mechanism of CEBPD-mediated GSC drug resistance and proposed ABCA1 inhibition as a potential strategy for the treatment of TMZ-resistant GBM.

Introduction
Glioblastoma (GBM) is a type of brain tumor that arises from astrocytes. There is a consensus, however, that the current treatments for GBM are ineffective. Temozolomide (TMZ) is used as a first-line therapy for GBM treatment due to its DNA-damaging effect. TMZ, a small lipophilic molecule, is an orally available mono-functional DNA alkylating agent that crosses the blood–brain barrier (BBB). Evidence indicates that cancer stem cells, especially GBM stem cells (GSCs), are generally characterized as a population of cells within the tumor bulk that participate in self-renewal, tumor initiation, and drug resistance. However, the mechanisms of GSC formation and drug resistance in TMZ-treated glioma remain largely unclear.

CCAAT/enhancer-binding protein delta (CEBPD), an inflammation-responsive transcription factor, has been recognized as an essential player in inflammatory disease and cancer progression. Previous studies have shown...
that CEBPD is involved in cell anti-apoptosis processes\textsuperscript{12}, cell migration\textsuperscript{9}, reactive oxygen species (ROS) formation\textsuperscript{10}, and cancer stemness\textsuperscript{12}. Recent evidence demonstrates a critical role for CEBPD in glioma stemness due to PDGFA expression in response to inflammatory cytokine treatment\textsuperscript{12}. However, the exact mechanism that links CEBPD to the genesis or processes of GSCs remains largely unknown. In particular, the role of CEBPD in the anti-apoptosis mechanisms of TMZ-resistant GSCs is unclear.

GSCs are involved in stemness maintenance and drug resistance due to their expression of stem cell transcription factors. Among these transcription factors, sex-determining region Y-Box (SOX2), octamer-binding transcription factor 4 (OCT4), and Nanog homeobox (NANOG) are critical components for maintaining pluripotency in embryonic stem cells (ESCs) and somatic stem cells\textsuperscript{14}. SOX2, OCT4, and NANOG are known to be highly expressed in subpopulations of GSCs, maintaining self-renewal and cellular proliferation\textsuperscript{15–17}.

Another key protein is ATP-binding cassette subfamily A member 1 (ABCA1), which is one of the ABC transporter membrane proteins that is expressed in GSCs and contributes to the movement of a wide variety of materials, such as drugs, lipids, and metabolic products, across the plasma and intracellular membranes\textsuperscript{16,19}. Nonetheless, the molecular mechanism of action of stem cell transcription factors and ABC transporters in TMZ-treated glioma remains a critical question. In this manuscript, we focused on stem cell transcription factors and ABC transporters in TMZ-treated glioma to establish whether CEBPD affects cancer stemness and drug resistance in TMZ-treated glioma.

In this paper, we used a bioinformatic dataset to analyze relative gene expression in glioma patients, as well as caspase 3/7 activity and spheroid assays and molecular biology techniques to examine cell death, stemness features, and gene regulation. We found that CEBPD is related to the increase in glioma stemness and TMZ resistance in glioma cells. Stem cell transcription factors (SOX2, OCT4, and NANOG) and ABCA1 are responsive to regulation by CEBPD, which directly binds to the promoter regions of those genes in glioma spheroid cells and TMZ-treated glioma cells. Taken together, our results suggest CEBPD as a therapeutic target to block certain actions of TMZ-resistant glioma and glioma stemness.

Results

CEBPD is expressed at high levels in GBM patients and correlates with poor survival probability

Glioma GEO datasets showed that CEBPD messenger RNA (mRNA) levels are higher in GBM tissues than in normal brain tissues (Fig. 1A, B). Importantly, CEBPD significantly correlated with a poor prognosis of GBM (Fig. 1C). The immunohistochemical results showed that CEBPD levels are increased in GBM tissues compared with normal brain tissues (Fig. 1D).

CEBPD is upregulated in GSCs and contributes to TMZ resistance

A previous study showed that GSCs are more resistant to chemo- and radiotherapy and further contribute to GBM recurrence\textsuperscript{20}. To evaluate this phenomenon in our system, we cultured U87MG-derived spheroids to enrich GSCs since a spheroid environment can be applied to enrich cancer stem cells (CSCs)\textsuperscript{21,22} and then treated them with TMZ. As expected, caspase 3/7 activity was decreased in GSCs compared with monolayer GBM cells treated with TMZ (Fig. 2A). Notably, knockdown of CEBPD in GSCs led to increased cell death (Fig. 2B). These results suggested that CEBPD mediates TMZ resistance in GSCs.

To further examine the role of CEBPD in GSCs, a spheroid-forming assay was conducted. We found that the spheroid size of U87MG cells gradually increased on different days in GSC-enriched culture medium (Fig. 2C). Furthermore, the expression levels of stemness factors (CD133, SOX2, OCT4, and NANOG) and CEBPD were elevated in GSCs (Fig. 2D, E). We further sorted GSCs from U87MG cells based on the CD133 marker and found that the expression of CEBPD was higher in U87MG-CD133\textsuperscript{+} cells (Fig. 2F).

CEBPD regulates stemness-related genes

Stemness-related factors OCT4, SOX2, NANOG, and KLF4 are common transcriptional regulators in cancer stem cells\textsuperscript{23}. In particular, SOX2, OCT4, and NANOG can maintain the self-renewal and stemness properties of GSCs\textsuperscript{15–17}. To elucidate whether CEBPD regulates these factors, gain- and loss-of-function experiments were conducted. The spheroid size was increased in CEBPD-overexpressing GSCs compared with control GSCs (Fig. 3A). The expression levels of SOX2, OCT4, and NANOG were upregulated in CEBPD-overexpressing GSCs (Fig. 3B, C). However, the spheroid size was decreased in CEBPD-knockdown GSCs (Fig. 3D). The gene and protein expression levels of SOX2, OCT4, and NANOG were downregulated in CEBPD-knockdown GSCs (Fig. 3E, F). These results suggested that CEBPD expression was related to the stem-like properties and drug resistance of GSCs by regulating SOX2, OCT4, NANOG, and ABCA1 transcription and expression.

ABCA1 positively correlates with CEBPD expression and is involved in TMZ resistance

Multidrug resistance is a serious problem that hinders the success of cancer pharmacotherapy. A common mechanism of multidrug resistance is the overexpression
of ABC efflux transporters in cancer cells\textsuperscript{18,24}. According to our previous microarray data, ABCA1 is upregulated in response to CEBPD activation in U373MG cells\textsuperscript{25}. The GEO dataset of glioma showed that the \textit{ABCA1} level was higher in GBM tissues than in normal brain tissues (Fig. 4A). Moreover, the \textit{ABCA1} expression level was highly corresponded with the CEBPD expression level in these samples (Fig. 4B). The expression level of \textit{ABCA1} is also elevated in GSCs and regulated by CEBPD (Figs. 2D, E and 3B, C, E, F). To further elucidate whether \textit{ABCA1} is involved in TMZ resistance, a caspase 3/7 activity assay was conducted following treatment with \textit{ABCA1} antagonists, DIDS or Probucol, and TMZ. The results showed that caspase 3/7 activity was increased in GSCs following co-treatment with TMZ and the \textit{ABCA1} antagonists compared with TMZ. Furthermore, the cytotoxic effects of \textit{ABCA1} antagonists were eliminated in CEBPD-knockdown spheroids (Fig. 4C). These results suggested that \textit{ABCA1} participates in CEBPD-mediated TMZ resistance in GSCs.

\textbf{SOX2, OCT4, NANOG, and ABCA1 genes are direct targets of CEBPD}

As CEBPD is a transcription factor, we next examined whether \textit{SOX2}, \textit{OCT4}, \textit{NANOG}, and \textit{ABCA1} are downstream targets of CEBPD. According to the prediction website for transcription factor binding (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3), several CEBPD binding sites were identified in the \textit{SOX2}, \textit{OCT4}, \textit{NANOG}, and \textit{ABCA1} promoter regions (Fig. 5A). The promoter activities of \textit{SOX2}, \textit{OCT4}, \textit{NANOG}, and \textit{ABCA1} were upregulated in CEBPD-overexpressing cells.
Furthermore, an in vivo DNA binding assay showed that CEBPD bound to the SOX2, OCT4, NANOG, and ABCA1 promoter regions in U87MG-derived spheroids (Fig. 5B). These results suggested that CEBPD can upregulate SOX2, OCT4, NANOG, and ABCA1 expression in GSCs.
CEBPD mediates the activation of stemness-related factors and ABCA1 in TMZ-treated GBM cells

A previous study showed the phenotypic shift in the non-GSC population to a GSC-like state in GBM after primary chemotherapy. To examine whether CEBPD is involved in acquired TMZ resistance in GBM by inducing stemness, we treated U87MG monolayer cells with TMZ and found that CEBPD was induced (Fig. 6A). Moreover, SOX2, OCT4, NANOG, and ABCA1 were also activated in TMZ-treated cells (Fig. 6A). These results suggested that TMZ could contribute to the activation of stemness and acquisition of TMZ resistance. As CEBPD was responsive to TMZ, we further examined whether CEBPD is involved in TMZ-induced stemness and acquired TMZ resistance. In CEBPD-knockdown U87MG cells, the SOX2, OCT4, NANOG, and ABCA1 transcripts were downregulated following TMZ treatment (Fig. 6B). These results showed that CEBPD contributed to TMZ-induced SOX2, OCT4, NANOG, and ABCA1 activation. In accordance with the results from spheroid cells and TMZ-treated GBM cells, we showed that CEBPD serves as a key regulator of TMZ resistance in GBM by regulating SOX2, OCT4, NANOG, KL4, and ABCA1 at the molecular and transcriptional levels.

Discussion

CSCs contribute to tumor initiation, drug resistance, and tumor relapse and are associated with tumor progression. While there is evidence that suggests that small subpopulations of CSCs exist in the tumor bulk, the exact molecular mechanism of CSC formation in the context of anticancer drug treatment remains largely unclear. Previous studies have shown that GBM leads to poor prognosis for patients due to the presence of glioma.
CEBPD activation can regulate PDGFA transcription, which promotes GSC formation in response to inflammatory cytokine treatment. Whether CEBPD can directly regulate stemness-related factors to form CSCs remains unknown.

Our results show that CEBPD can directly bind to the SOX2, OCT4, NANO, and ABC1 promoter regions to promote the properties of cancer stemness and drug resistance. Interestingly, some studies suggest that ABC1 is involved in drug resistance and upregulated in side population cells. We also found that treatment with ABC1 antagonists, such as DIDS and Probucol, can increase GSC death following TMZ treatment, suggesting that ABC1 plays a key role in preventing cell death. TMZ treatment can increase ABC1 transcription and expression through CEBPD activation. CEBPD could be activated by TMZ stimulation due to induction of stress. Previous studies showed that TMZ treatment can increase ROS imbalance and genotoxic stress, suggesting that TMZ-induced CEBPD expression may promote drug resistance by stimulating ROS stress. However, how these upstream mechanisms affect CEBPD remains to be elucidated in the future.

The findings here show that CEBPD can regulate SOX2, OCT4, and NANO expression. It will be interesting to determine whether SOX2, OCT4, and NANO can directly regulate ABC1 expression. ABC1 may be regulated by two possible mechanisms: (a) stemness-related factors (SOX2, OCT4, and NANO) may regulate ABC1 expression through promoter regulation or microRNA regulation and (b) CEBPD may directly interact with stemness factors to bind to CEBPD, SOX2, OCT4, and NANO binding sites in the ABC1 promoter to regulate ABC1 expression. The detailed mechanism needs to be further clarified.

In summary, we have found that this novel role of CEBPD may represent a clinical target for treating TMZ-resistant glioma. We have also found that CEBPD can facilitate GSC formation and drug resistance by binding to the SOX2, OCT4, NANO, and ABC1 promoter regions. ABC1 antagonists may act as therapeutic agents to inhibit GSC-induced drug resistance (Fig. 7). Thus, targeting CEBPDs or ABC1s may be a beneficial therapeutic approach, in combination with TMZ or anticancer drugs, to attenuate glioma progression.

Materials and methods

Materials

The TRizol™ RNA extraction reagent, Lipofectamine® 2000 transfection reagent, Lipofectamine® RNAiMAX transfection reagent, Opti-MEM medium, Dulbecco’s modified Eagle’s medium (DMEM), B-27™ Supplement (50X) (17504044), and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Waltham, MA,
USA). PrimeScript™ RT reagent kit was purchased from TaKaRa (Kusatsu, Shiga, Japan). SensiFAST™ SYBR was purchased from Bioline (Taunton, MA, USA). A luciferase assay system and the Caspase-Glo® 3/7 assay was purchased from Promega (Madison, WI). Human EGF (GFH26) was purchased from Cell Guidance Systems (Cambridge, UK) and Human FGF-basic (100-18B) was purchased from PeproTech (Rocky Hill, NJ, USA). 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid disodium salt hydrate (DIDS) (D3514), Probucol, Temolozomide (SI-T2577), and antibody against α-Tubulin (T6199) were purchased from Sigma (St. Louis, MO, USA). An antibody against CEBPD (SC-636) and poly-(2-hydroxyethyl methacrylate) (sc-253284) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against p84 (GTX70220), SOX2 (GTX101507), NANOG (GTX100863), and ABCA1 (GTX27360) were purchased from GeneTex (Irvine, CA, USA). Antibodies against OCT4 (#2750) were purchased from Cell Signaling Technology (Danvers, MA, USA). An antibody against HA was purchased from Covance (Barkeley, CA). An antibody against CEBPD for immunostaining was purchased from Abcam (ab65081, Cambridge, MA, USA). All oligonucleotides were synthesized by PURIGO biotechnology (Taipei, Taiwan). Expression plasmid pcDNA3/HAI and pEGFP-C1 were a gift of Dr. Hsin-Fang Yang-Yen (Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan).

**Fig. 5** CEBP-D can directly bind to the SOX2, OCT4, NANO-G, and ABCA1 promoter regions to regulate gene expression. **A** Schematic representation of reporter constructs with the SOX2, OCT4, NANO-G, and ABCA1 promoters. The approximate locations of the putative CEBP-D-binding motifs are indicated by ovals. **B** U87MG cells were co-transfected with pcDNA3-HA or pcDNA3-HA/CEBP-D and SOX2, OCT4, NANO-G, and ABCA1 reporter vectors and then examined by reporter assay. **C** CEBP-D binds to the SOX2, OCT4, NANO-G, and ABCA1 promoters in vivo. Chromatin from U87MG monolayer or spheroid cells were isolated, and ChIP assays were performed with the indicated antibodies. The precipitated DNA was amplified by PCR using specific primers. The summary data are presented as the mean ± SEM; the numbers in the bars represent the sample sizes; Student’s t-test; **p < 0.01, ***p < 0.001.
Cell culture

Human GBM cell line U87MG was maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 100 μg/ml streptomycin, and 100 μg/ml penicillin. Inducible knockdown LacZ U87MG cells and inducible knockdown CEBPD U87MG cells were maintained in above medium with 500 μM IPTG.

Spheroid formation assay

U87MG cells were seeded and cultured in DMEM/F12 medium. The medium was supplemented with B27, 20 ng/ml EGF and 10 ng/ml bFGF. The dishes were coated with poly-(2-hydroxyethyl methacrylate). After 3 days and 6 days, the sizes of spheroids were measured at 100X magnification under a microscope and analyzed by ImageJ software.

Reverse transcription polymerase chain reaction (RT-qPCR)

After cells were exposed to simulation, the total RNA was harvested and extracted in TRIzol™. The isolated RNA was subjected to reverse transcription reaction with PrimeScript™ for cDNA synthesis. The oligonucleotide primers used in the qPCR analysis were as follows:

- **GAPDH specfic primer (F):** 5′-CCACCCCAGAAGACTGATGGAT-3′ and **GAPDH specfic primer (R):** 5′-TTCAGCTCAGGGATGACCTT-3′;
- **human CEBPD specfic primer (F):** 5′-GCCATGTACGACGACGAGAG-3′ and **CEBPD specfic primer (R):** 5′-TGTGATTGCTGTTGAGGTC-3′;
- **human CD133 specfic primer (F):** 5′-CCAAGTTCTACCTCATGTTTGG-3′ and **CD133 specfic primer (R):** 5′-ACCAACAGGGAGATTGCAAAGC-3′;
- **human SOX2 specfic primer (F):** 5′-CACAACTCGGAGATCAGCAA-3′ and **SOX2 specfic primer (R):** 5′-CTCCGGGAAGCGTGTACTTA-3′;
- **human OCT4 specfic primer (F):** 5′-GGAAGGTATTCAGCCAAACGACCA-3′ and **OCT4 specfic primer (R):** 5′-CTCCTCGGCTGTGTATCTTA-3′;
- **human NANOG specfic primer (F):** 5′-ACCAGAACTGTGTTCTCTTCCACC-3′ and **NANOG specfic primer (R):** 5′-CCATTGCTATTCTTCGGCCAGTG-3′;
- **human KLF4 specfic primer (F):** 5′-CCCAATTCCTCCCTCTCTC-3′ and **KLF4 specfic primer (R):** 5′-AAGTTCAGCTGGGGTGACT-3′;
- **human ABCA1 specfic primer (F):** 5′-AACAGTTTGTGGCCCTTTTG-3′ and **ABCA1 specfic primer (R):** 5′-AAGTTCCAGGCTGGGGTAC-3′.

Plasmid transfection

Cells were re-plated 24 h before transfection at an optimal density in 2 ml of fresh culture medium in a 6-well plastic
dish. They were then transfected with plasmids by Lipofectamine® 2000 transfection reagent according to the manufacturer’s instructions. The total amount of DNA for each experiment was matched with the empty vector. The Opti-MEM media were changed to culture medium after 6 h, incubated for 15 h and harvested for further analysis.

**Plasmid construction and reporter assay**

The pcDNA3/HA/CEBPDP (HA/CD) was constructed previously. The CEBPD fragment was digested with BamHI and XbaI from HA/CD and inserted into BamHI- and XbaI-digested pEGFP-C1 to produce GFP-tagged CEBPD (GFP/CD). The 5′ promoter regions of NANOG, OCT4, SOX2, and ABCA1 genes were obtained by PCR with U87MG genomic DNA. The PCR products were individually cloned into pGL-3 basic vector. The primers with U87MG genomic DNA. The PCR products were incubated for 15 h and harvested for further analysis. Opti-MEM media were changed to culture medium after 6 h, each experiment was matched with the empty vector. The manufacturer.

**Small interfering RNA (siRNA) assay**

The virus was produced from Phoenix Ampho cells co-transduced with pMD2.G and psPAX2 vectors and the plKO.1-shRNA expression vectors using Mirus Bio transfection reagent according to the manufacturer’s instructions. The total amount of DNA for each experiment was matched with the empty vector. The Opti-MEM media were changed to culture medium after 6 h, incubated for 15 h and harvested for further analysis. Opti-MEM media were changed to culture medium after 6 h, each experiment was matched with the empty vector. The manufacturer.

**Western blotting assay**

This assay was carried out as described previously. Briefly, cells were lysed in modified radioimmune precipitation assay buffer. Following lysis, the lysates were resolved on a SDS polyacrylamide gel, transferred to a polyvinylidene difluoride membrane by an electroblot apparatus. Membranes were incubated with primary antibodies overnight at 4 °C and secondary antibodies at RT for 1 h. Proteins were detected by an enhanced chemiluminescence western blot system from Pierce (Rockford, IL, USA) and visualized by an autoradiographic film.

**Chromatin immunoprecipitation (ChIP) assay**

The ChIP assay was carried out as described previously. The cross-linked protein/DNA lysates were immunoprecipitated with specific antibodies recognizing CEBPD, or control rabbit immunoglobulin G (IgG) at 4 °C for 12–16 h. After reversal of the crosslinking between proteins and genomic DNA, the precipitated DNA was amplified by PCR with primers related to the specific regions on the genomic locus of target genes. The primers included SOX2 (F): 5′-TTACCCCTCGCAAAAAAGT-3′; SOX2 (R): 5′-ACGACCACCATCTGAGAT GTACGGATAATGCAGACATT-3′; shCEBPDP (shCD): 5′-CCGGCAAGCTTCTCAACAGCAATC CGAGATTTGCTTGGAAGGAGGATCGCCTTTTTT-3′. The expression plasmids and shRNAs were purchased from the National RNAi Core Facility located at the Genomic Research Center of Institute of Molecular Biology, Academia Sinica, Taiwan.

**Gene expression omnibus (GEO) database**

The GEO databases used in this study are Lee data set (GSE4536), Sun data set (GSE4290), Bredel data set (GSE2223), Murat data set (GSE7696), and Shai data set. These databases were used to access gene expression levels in normal and glioma tissues.

**Immunohistochemistry (IHC) analysis**

The tissue arrays were purchased from Biomax (GL481, Rockville, MD, USA). The staining was performed using a Ventana BenchMark XT automated stainer (Ventana, Tucson, AZ, USA) with CEBPD primary antibody.
Statistical analysis
Results are shown as mean ± SEM. All statistical analysis was conducted using the Prism GraphPad software. Student’s t-test or one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test was used. The correlation analysis was determined by the Pearson correlation test. All experiments were repeated three times. Statistically significant differences were indicated by ***p < 0.001, **p < 0.01, *p < 0.05.

Acknowledgements
This work was supported by the grants MOST 107-2320-B-038-038 and MOST 108-2320-B-038-062-MY2 from the Ministry of Science and Technology, Taiwan. This work was also supported by the “TMU Research Center of Cancer Translational Medicine” from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan. Support was also provided by the National Institute on Drug Abuse, National Institutes of Health.

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S.-M.W., W.-C.L., H.-Y.L., and Y.-L.C. performed the experiments and analyzed data. S.-M.W. and C.-Y.K. wrote the manuscript and organized all the figures. J. M.W. performed the investigation, methodology, project administration, writing review, and editing.

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.

Received: 26 August 2020 Revised: 3 December 2020 Accepted: 23 December 2020
Published online: 12 January 2021

References
1. Louis, D. N. et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114, 97–109 (2007).
2. Zhang, J., Stevens, M. F. & Bradshaw, T. D. Temozolomide mechanisms of action, repair and resistance. Curr. Mol Pharmacol 5, 102–114 (2012).
3. Kanawami, T., Bedwell, I., Kondo, Y., Kondo, S. & Germain, J. M. Inhibition of DNA repair for sensitizing resistant glioma cells to temozolomide. J. Neurosurg 99, 1047–1052 (2003).
4. Charles, N. A., Holland, E. C., Gilbertson, R., Glass, R. & Kettenmann, H. The brain tumor microenvironment. Glia 59, 1169–1180 (2011).
5. Soltysova, A., Altanerova, V. & Altaner, C. Cancer stem cells. Neoplasma 52, 435–440 (2005).
6. Chang, K. Y. et al. Specificity protein 1-modulated superoxide dismutase 2 enhances temozolomide resistance in glioblastoma, which is independent of O6-(methylguanine-DNA methyltransferase. Redox Biol 13, 655–664 (2017).
7. Ko, C. Y., Lin, C. H., Chuang, J. Y., Chang, W. C. & Hsu, T. I. MDM2 degrades deacetylated nucleolin through ubiquitination to promote glioma stem-like cell enrichment for chemotherapeutic resistance. Mol. Neurobiol 55, 3211–3223 (2018).
8. Lai, H. Y. et al. CCAAT/enhancer-binding protein delta promotes intracellular lipid accumulation in M1 macrophages of vascular lesions. Cardiovasc. Res 113, 1376–1388 (2017).
9. Wang, S. M. et al. Astrocytic CCAAT/enhancer-binding protein delta contributes to glial scar formation and repairs functional recovery after spinal cord injury. Mol. Neurobiol 53, 5912–5927 (2016).
10. Wang, S. M. et al. Astrocytic CCAAT/enhancer-binding protein delta contributes to reactive oxygen species formation in neuroinflammation. Redox Biol 16, 104–112 (2018).
11. Wang, W. J. et al. Inhibition of the EGF/STAT3/CEBPD axis reverses cisplatin cross-resistance with pazlitaxel in the urothelial carcinoma of the urinary bladder. Clin. Cancer Res 23, 503–513 (2017).
12. Wang, S. M. et al. CCAAT/enhancer-binding protein delta regulates the stemness of glioma stem-like cells through activating PDGFA expression upon inflammatory stimulation. J. Neuroinflammation 16, 146 (2019).
13. Wang, S. M. et al. Increase of zinc finger protein 179 in response to CCAAT/enhancer binding protein delta conferring an ant apoptotic effect in astrocytes of Alzheimer’s disease. Mol. Neurobiol 51, 370–382 (2015).
14. Boye, L. A. et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122, 947–956 (2005).
15. Niu, C. S. et al. Expression of NANOG in human gliomas and its relationship with undifferentiated glioma cells. Oncol. Res 26, 593–601 (2011).
16. Du, Z. et al. Oct4 is expressed in human gliomas and promotes colony formation in glioma cells. Glioma 57, 724–733 (2009).
17. Gangemi, R. M. et al. SOX2 silencing in glioblastoma tumor-initiating cells causes stop of proliferation and loss of tumorigenicity. Stem Cells 27, 40–48 (2009).
18. Dean, M., Rhedtik, A. & Allkims, R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 11, 1156–1166 (2001).
19. Fletcher, J. I., Haber, M., Henderson, M. J. & Norris, M. D. ABC transporters in cancer: more than just drug efflux pumps. Nat. Rev. Cancer 10, 147–156 (2010).
20. Auffinger, B., Spencer, D., Pytel, P., Ahmed, A. U. & Lesniah, M. S. The role of glioma stem cells in chemotherapy resistance and glioblastoma multiforme recurrence. Expert Rev. Neurother. 15, 741–752 (2015).
21. Hsu, C. C. et al. Suberylanid hydroxide acid represses glioma stem-like cells. J. Biomed. Sci. 23, 81–92 (2016).
22. Yu, S. C. et al. Isolation and characterization of cancer stem cells from a human glioblastoma cell line U87. Cancer Lett. 265, 124–134 (2008).
23. Hadjimichael, C. et al. Common stemness regulators of embryonic and cancer stem cells. World J. Stem Cells 7, 1150–1184 (2015).
24. Bachmeier, B. E. et al. Overexpression of the ATP binding cassette gene ABCA1 determines resistance to Curcumin in M14 melanoma cells. Mol. Cancer 8, 147–156 (2009).
25. Ko, C. Y. et al. CCAAT/enhancer binding protein delta (CEBPD) elevating PTX3 expression inhibits macrophage-mediated phagocytosis of dying neuron cells. Neurobiol. Aging 33, 422 e411–422 e425 (2012).
26. Auffinger, B. et al. Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy. Cell Death Differ 21, 1119–1131 (2014).
27. Ahmed, A. U., Auffinger, B. & Lesniah, M. S. Understanding glioma stem cells: rationale, clinical relevance and therapeutic strategies. Expert Rev. Neurother. 13, 545–555 (2013).
28. Chu, Y. Y. et al. Bortezomib-induced miRNAs directly epigenetic silencing of locus genes and trigger apoptosis in leukemia. Cell Death Dis 8, e3167–e3177 (2017).
29. Tsai, H. H. et al. Metformin promotes apoptosis in hepatocellular carcinoma through the CEBPD-induced autophagy pathway. Oncotarget 8, 13832–13845 (2017).
30. Iwasaki, H. et al. Down-regulation of lipids transporter ABCA1 increases the cytotoxicity of nitidine. *Cancer Chemother. Pharmacol.* **66**, 953–959 (2010).

31. Sun, D. X., Liao, G. J., Liu, K. G. & Jian, H. Endosialin-expressing bone sarcoma stem-like cells are highly tumor-initiating and invasive. *Mol. Med. Rep.* **12**, 5665–5670 (2015).

32. Oliva, C. R. et al. Acquisition of temozolomide chemo-resistance in gliomas leads to remodeling of mitochondrial electron transport chain. *J. Biol. Chem.* **285**, 39759–39767 (2010).

33. Wang, J. M. et al. Functional role of NF-IL6beta and its sumoylation and acetylation modifications in promoter activation of cyclooxygenase 2 gene. *Nucleic Acids Res.* **34**, 217–231 (2006).

34. Wang, J. M., Tseng, J. T. & Chang, W. C. Induction of human NF-IL6beta by epidermal growth factor is mediated through the p38 signaling pathway and cAMP response element-binding protein activation in A431 cells. *Mol. Biol. Cell* **16**, 3365–3376 (2005).

35. Shai, R. et al. Gene expression profiling identifies molecular subtypes of gliomas. *Oncogene* **22**, 4918–4923 (2003).