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miRNA-214 Inhibits Cellular Proliferation and Migration in Glioma Cells Targeting Caspase 1 Involved in Pyroptosis

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Pyroptosis is a type of proinflammatory programmed cell death mediated by caspase 1 activity and occurs in several types of eukaryotic tumor cells, including gliomas. MicroRNAs (miRNAs), small endogenous noncoding RNAs, have been demonstrated to be advantageous in glioma therapy. However, the question of whether miRNAs regulate pyroptosis in glioma remains unknown. The current study found that caspase 1 expression was substantially increased in both glioma tissues and glioma cell lines, U87 and T98G, while miR-214 expression was significantly downregulated. Luciferase reporter assay recognized caspase 1 as a target gene of miR-214. These findings demonstrate that miR-214 could inhibit cell proliferation and migration through the regulation of pyroptosis intermediated by caspase 1 in glioma U87 and T98G cells and may suggest a novel therapeutic for the intervention of glioma.

Key words: miR-214; Pyroptosis; Caspase 1; Glioma

INTRODUCTION

Gliomas are the most common type of malignant primary brain tumors in adults. Despite comprehensive therapies including surgical resection, radiotherapy, and chemotherapy, the prognosis of patients with glioma remains poor, as the median survival period of patients suffering from malignant gliomas such as glioblastoma multiforme (GBM) is only 14 months[1]. As glioma develops, it infiltrates into adjacent normal brain tissues, making it difficult to treat. Therefore, there is a pressing need to understand the mechanisms of glioma cell proliferation and migration in order to develop more effective treatment strategies.

Pyroptosis is a type of proinflammatory programmed cell death[2]. Although it shares biochemical and morphological characteristics of necrosis and apoptosis, it remains a unique process triggered by various stimuli and leads to the release of cytokines that activate proinflammatory immune cell mediators[3]. Caspase 1, the effector protease of the inflammasome, is activated during pyroptosis and cleaves the proinflammatory cytokines interleukin-1β (IL-1β) and IL-18[4]. This proinflammatory microenvironment is favorable for tumor initiation and progression, as increased serum levels of proinflammatory ILs such as IL-1β and IL-18 have been observed in several types of cancer. However, no studies have focused on the function of pyroptosis in glioma.

MicroRNAs (miRNAs) are small endogenous noncoding RNAs approximately 21–23 nucleotides in length. They regulate gene expression primarily through interaction with the 3′-untranslated regions (3′-UTRs) of mRNAs,

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resulting in mRNA decay or translational repression[5]. Since miRNAs can have many targets, they may play an important role in tumorigenesis through regulation of proliferation[6], cell death[7], autophagy[8], or differentiation[9]. Specifically, low expression of miR-214 has been observed in several types of human malignant tumors, distinguishing it as a tumor suppressor[10–13]. Conversely, some studies have found that miR-214 was upregulated in malignant melanoma and associated with disease progression and distant metastasis[14,15]. Although previous studies have shown miR-214 dysregulation in glioma tissue[16–18], there have been few published articles involving the functional analysis and molecular network of miR-214 in glioma. Further studies are needed to decode the underlying regulatory pathways by which miRNAs participate in the proliferation and migration of glioma and to identify miRNA function to determine novel therapeutic targets for glioma.

In the current study, we observed that pyroptosis is activated in glioma tissues as well as the glioma cell lines U87 and T98G. Through computational prediction and experimental confirmation, we have shown that miR-214 directly targets the caspase 1 gene. Consequently, we investigated the role of pyroptosis in promoting the proliferation and migration of glioma cells. Additionally, we demonstrated that the level of caspase 1 mRNA is inversely correlated with miR-214 expression. Together, these results indicate that miR-214 inhibits glioma cell proliferation and migration partially through regulating pyroptosis intermediated by caspase 1. These findings facilitate the understanding of glioma pathogenesis and identify a potential therapeutic for the treatment of glioma.

MATERIALS AND METHODS

Human Tissue Samples

Six glioma tissues and the corresponding adjacent normal tissues were obtained from the Department of Neurosurgery at the First Affiliated Hospital of Harbin Medical University. Informed consent was obtained for each patient. Histological features were confirmed by a pathologist according to the WHO criteria. All tissue samples were immediately collected and stored in liquid nitrogen after resection. This study was approved by the ethical committee of the First Affiliated Hospital of Harbin Medical University, P.R. China.

Cell Line and Reagents

The glioma cell lines U87 and T98G were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Biological Industries, Beit-Haemek, Israel).

Immunofluorescence Staining

Immunofluorescence analysis was performed on the glioma tissue samples to assess the protein expression of GFAP and IL-1β. Tumor tissues were fixed and processed for paraffin sectioning by standard protocols. The samples were then blocked with 2% BSA (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 1 h and incubated with mouse monoclonal GFAP and IL-1β primary antibody. Samples were washed three times with PBS before incubation with the FITC- and TRITC-conjugated secondary antibody for 1 h at room temperature. Cell nuclei were counterstained with DAPI (300 nM in D-PBS; Thermo Fisher Scientific, Waltham, MA, USA) for 10 min. Images were obtained on an inverted fluorescence microscope (Nikon Instruments, Melville, NY, USA).

Cells were plated on coverslips in 24-well plates and allowed to reach 60% confluence. Cells were then treated with 100 μM caspase 1 inhibitor for 48 h and fixed in 4% paraformaldehyde, permeabilized with 0.05% Triton X-100 in PBS, and blocked with 10% BSA in PBS. The treated cells were incubated with primary antibodies against caspase 1 (1:200; Cell Signaling Technologies, Danvers, MA, USA) at 4°C overnight. Subsequently, cells were washed three times in PBS and incubated for 1 h with the appropriate secondary antibodies (1:1,000). Finally, the stained cells were observed with a confocal microscope.

Transfection

U87 and T98G glioma cells were transiently transfected with mimic control, miR-214 mimic, inhibitor control, miR-214 inhibitor, and miR-214 inhibitor+caspase 1 inhibitor (Ac-YVAD-CMK).

Real-Time PCR Analysis

Caspase 1, IL-18, IL-1β mRNA, and miR-214 transcripts were measured using real-time PCR. Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized using a reverse transcriptase kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Real-time PCR was carried out using the SYBR Green PCR Master Mix Kit (Applied Biosystems) and performed on the 7500 FAST Real-Time PCR System (Applied Biosystems). GAPDH was used as an internal control. The following primers were used in the study: caspase 1, 5′-ACACGTCTTGCCCTCATTATC T-3′ (forward) and 5′-ATAACCTTGGGCTTGTCTTTC A-3′ (reverse); GAPDH, 5′-ATCACTGCCACCCAGAA GAC3′ (forward) and 5′-TTTCTAGACGGCAGGTCAG G-3′ (reverse); IL-18, 5′-ACAACCGCAGTAATACGGA GCA-3′ (forward) and 5′-TGTGCTCTGCTTGAGAGG

TGCT-3′ (reverse); IL-1β, 5′-CCCTGCACTGGAGAGT GTGG-3′ (forward) and 5′-TGTGCTCTGCTTGAGAG GTGCT-3′ (reverse); and miR-214, 5′-ATAGAATTCTT TCTCCCTTTCCCCTTACTCTCC-3′ (forward) and 5′-CCAGGATCCTTTCATAGGCACCACTCACTTTAC-3′ (reverse).

MTT Assay

Cell proliferation was determined using a methylthiazoletetrazolium (MTT) assay according to the manufacturer's instructions (Sigma-Aldrich). Cells were seeded onto a 96-well plate and treated with the mimic control, miR-214 mimic, inhibitor control, miR-214 inhibitor, and miR-214 inhibitor + caspase 1 inhibitor (Ac-YVAD-CMK). The medium was then removed, and cells were washed with PBS followed by incubation with MTT (0.5 mg/ml) for 2 h at 37°C. The MTT reagent was then replaced with dimethyl sulfoxide (100 µl per well) and mixed on a shaker at room temperature for 10 min to dissolve the formazan crystals. Absorbance was determined at 550 nm using a microplate reader.

Wound Healing Assay

U87 and T98G cells were incubated in a six-well culture plate to achieve 90% confluence, and the cell monolayer was scratched in a straight line with a pipette tip. In order to remove the debris and smooth the edge of the scratch, culture medium was removed, and wells were washed with medium three times. After 48 h, the width of the open area was measured. The distance of wound closure was used to estimate migration ability.

Western Blot

Western blot was performed as described previously. The following primary antibodies were used: anti-caspase 1 antibody (1:1,000) and anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

TUNEL Staining

Cells were plated onto coverslips inside culture plates, and In Situ Cell Death Kits (TUNEL fluorescence FITC kit; Roche, Indianapolis, IN, USA) were used to detect DNA fragmentation of individual cells according to the manufacturer's instructions. The nuclei were stained with DAPI. TUNEL staining was then assessed by fluorescence microscopy. Nuclei that were double labeled with DAPI and TUNEL were considered positive.

Luciferase Reporter Assay

The putative binding site of miRNA-214 in caspase 1 3′-UTR was retrieved from TargetScan and identified as the caspase 1 3′-UTR fragment containing wild- or mutant-type miRNA-214-binding sequence. Cells were cultured in 96-well plates and cotransfected with 100 nM miRNA inhibitors and 1 µg of reporter plasmids using Lipofectamine 2000 (Invitrogen). Cells were harvested at 72 h after transfection, and luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Luciferase activity was normalized to *Renilla* luciferase activity.

ELISA Assay

Caspase 1 response to miR-214 was measured in vitro by IL-1β and IL-18 ELISA assay. U87 and T98G cells were plated onto a 96-well plate. Forty-eight hours after transfection, cell culture supernatants were harvested, and the presence of IL-1β and IL-18 was determined by ELISA as per the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Statistical Analysis

The data were presented as mean ± SEM. Statistical analysis was performed using Student's *t*-test or one-way ANOVA test, where $p < 0.05$ was considered significant.

RESULTS

Pyroptosis Is Activated in Glioma Tissues

Immunofluorescence analysis of the brain tissue revealed colocalization of proinflammatory cytokine IL-1β with the glioma cell marker GFAP (Fig. 1a). Simultaneously, expression of caspase 1 was remarkably increased at both the mRNA and protein levels in the tumor group (Fig. 1b and c), indicating that pyroptosis was activated in glioma tissues.

miR-214 Targets Caspase 1

Computational analysis predicated a conserved binding site for miR-214 in the 3′-UTR of the caspase 1 gene (Fig. 2a). To verify that miR-214 directly targets caspase 1, a luciferase construct carrying the caspase 1 3′-UTR was constructed. Cotransfection of the miR-214 mimic caused a sharp decrease in luciferase activity compared with transfection of mimic control. However, the miR-214 mimic failed to affect the luciferase activity elicited by a construct carrying the caspase 1 3′-UTR with a mutant miR-214-binding site (Fig. 2b). In addition, we confirmed that the miRNA-214 mRNA expression was significantly downregulated in the brain tissues of glioma patients compared to normal brain tissues (Fig. 2c). The protein levels of caspase 1 in the mimic control, miR-214 mimic, inhibitor control, and miR-214 inhibitor groups (U87 and T98G cell lines) were significantly downregulated following transfection with miR-214 mimics into the U87 and T98G cell lines, while being notably upregulated subsequent to transfection with the miR-214 inhibitor (Fig. 2d). The relative mRNA levels of caspase 1 in the miR-214 mimic, mimic control, miR-214 inhibitor, and inhibitor control groups

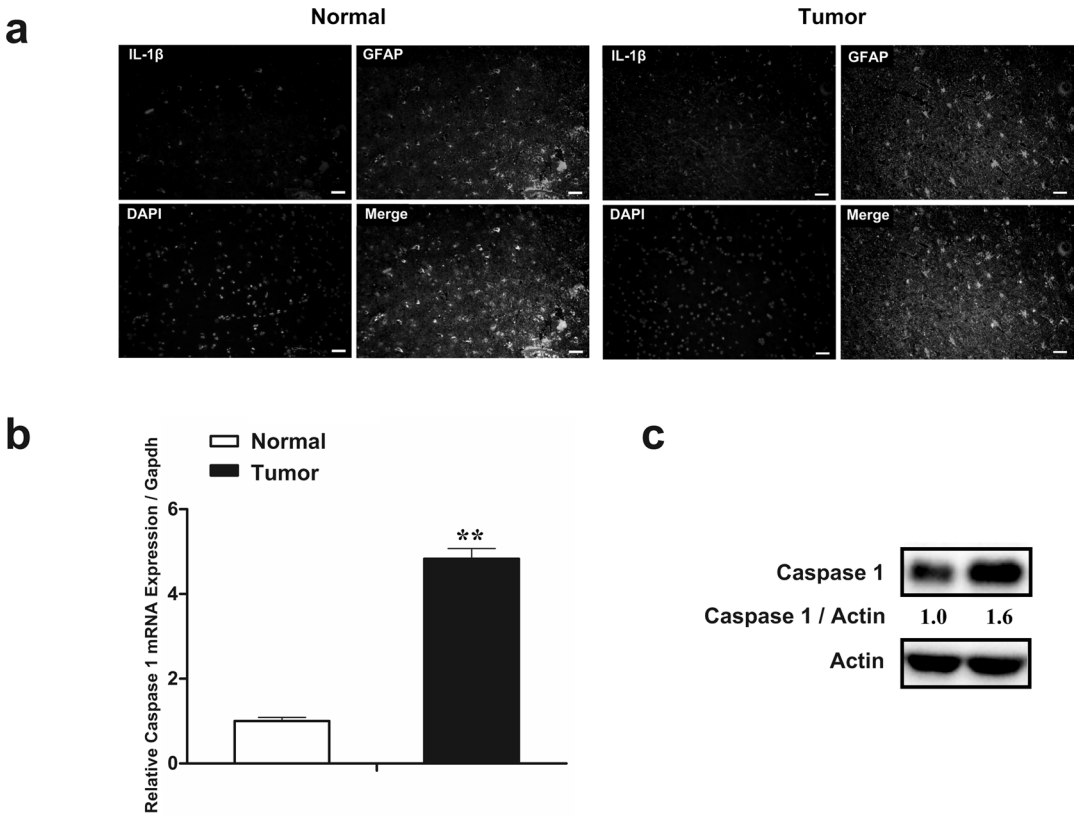

Figure 1. Pyroptosis was activated in glioma tissues. (a) IL-1β and GFAP immunofluorescence staining of brain tissues in the normal and tumor groups. Relative mRNA (b) and protein levels (c) of caspase 1 in the normal and tumor groups. Scale bars: 20 μm. $^{**}p<0.01$.

(U87 cell line) showed an expression trend similar to the protein (Fig. 2e). Immunofluorescence staining also demonstrated that caspase 1 expression was decreased remarkably in the miR-214 mimic groups (U87 and T98G cell lines), while caspase 1 expression was increased remarkably in the miR-214 inhibitor groups (U87 and T98G cell lines) (Fig. 2f and g).

miR-214 Inhibits Cell Proliferation and Migration

To confirm the effect of miR-214 on tumor cellular processes, we transfected U87 and T98G cells. Cell proliferation was investigated using the MTT assay. Cells transfected with the miR-214 mimic exhibited significant growth suppression compared to those transfected with the mimic control (Fig. 3a and b). Furthermore, suppression was eradicated by miR-214 inhibitor transfection. Moreover, the migration ability of the transfected cells was tested using the wound healing assay at 48 h. This demonstrated that cells transfected with the miR-214 mimics exhibited retarded mobility in comparison with those transfected with the mimic control (Fig. 3c and d). Moreover, this phenomenon was reversed when transfected with the miR-214 inhibitor.

miR-214 Regulates Cell Pyroptosis

We used TUNEL staining to determine the potential role of miR-214 in the cell death of U87 and T98G cells. Cells transfected with the miR-214 mimic exacerbated cell death, which was reversed by the miR-214 inhibitor (Fig. 4a and b). Moreover, we evaluated the effects of miR-214 on the mRNA transcript levels of IL-18 and IL-1β, which were the downstream targets of caspase 1, using real-time PCR assay. Cells transfected with the miR-214 mimic were found to significantly decrease the mRNA expression of IL-18 and IL-1β, which was recovered after transfection with the miR-214 inhibitor (Fig. 4c and d).

miR-214 Mediates Cell Proliferation and Migration Through Caspase 1

We attempted to clarify the possible mechanisms through which miR-214 regulates cell proliferation and migration in U87 (Fig. 5) and T98G (Fig. 6) cells. We found that cells transfected with the miR-214 inhibitor exhibited significantly increased proliferation and mobility and that these phenomena were reversed by transfection with the miR-214 inhibitor + caspase 1 inhibitor

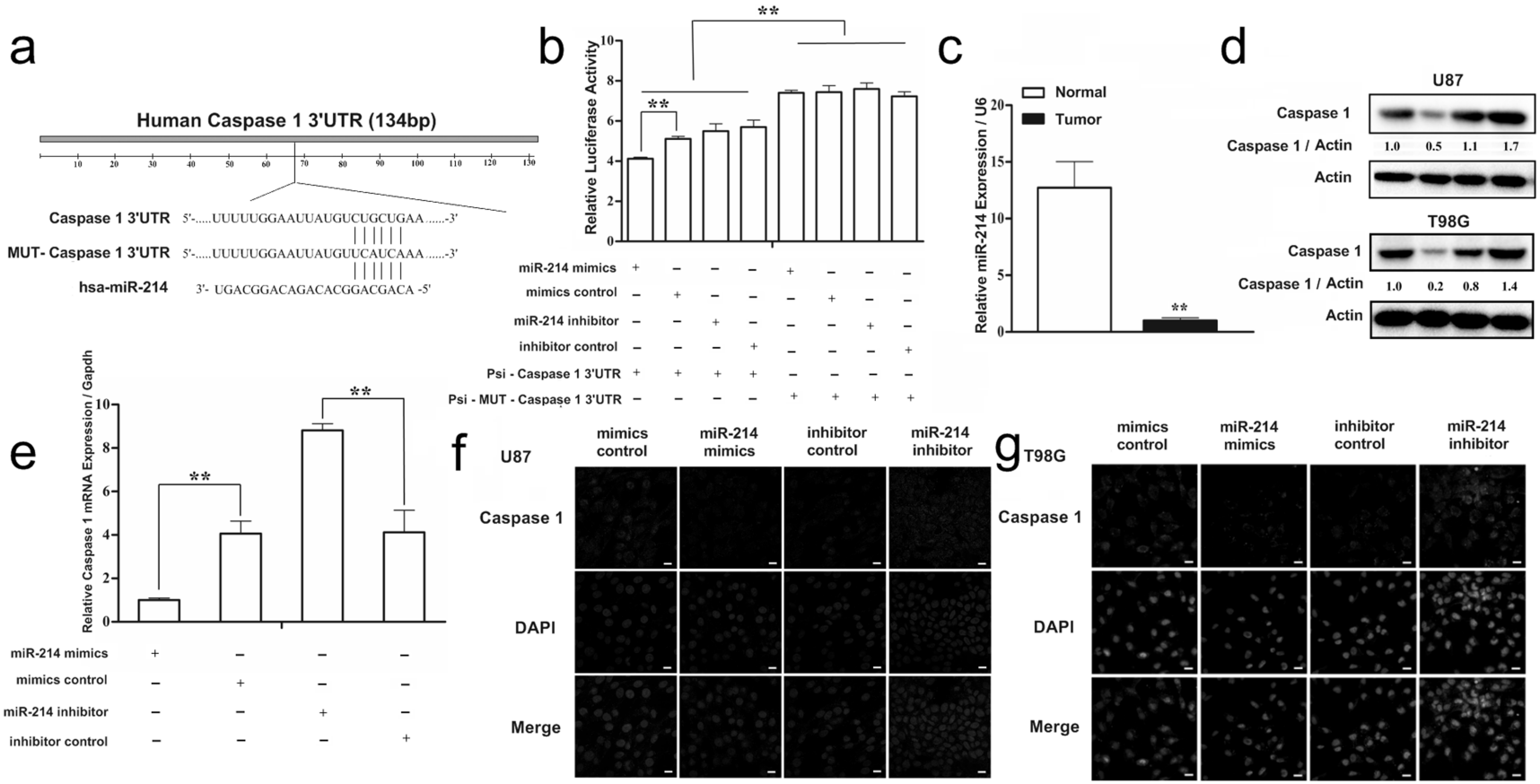

Figure 2. miR-214 directly targets caspase 1. (a) The pattern diagram shows the combination sequence of hsa-miR-214 with the 3′-UTR of caspase 1. (b) Luciferase activities assay the modulation of miR-214 on caspase 1 3′-UTR. (c) A real-time PCR analysis of the miR-214 level in the normal brain tissues and glioma tissues. (d) The protein levels of caspase 1 in the mimic control, miR-214 mimic, inhibitor control, and miR-214 inhibitor groups (U87 and T98G cell lines). (e) The relative mRNA levels of caspase 1 in the miR-214 mimic, mimic control, miR-214 inhibitor, and inhibitor control. (f) Immunofluorescence images showing the expression of caspase 1 in the mimic control, miR-214 mimic, inhibitor control, and miR-214 inhibitor groups (U87 cell line). Scale bars: 20 μm. (g) Immunofluorescence images showing the expression of caspase 1 in the mimic control, miR-214 mimic, inhibitor control, and miR-214 inhibitor groups (T98G cell line). Scale bars: 20 μm. $^{**}p<0.01$.

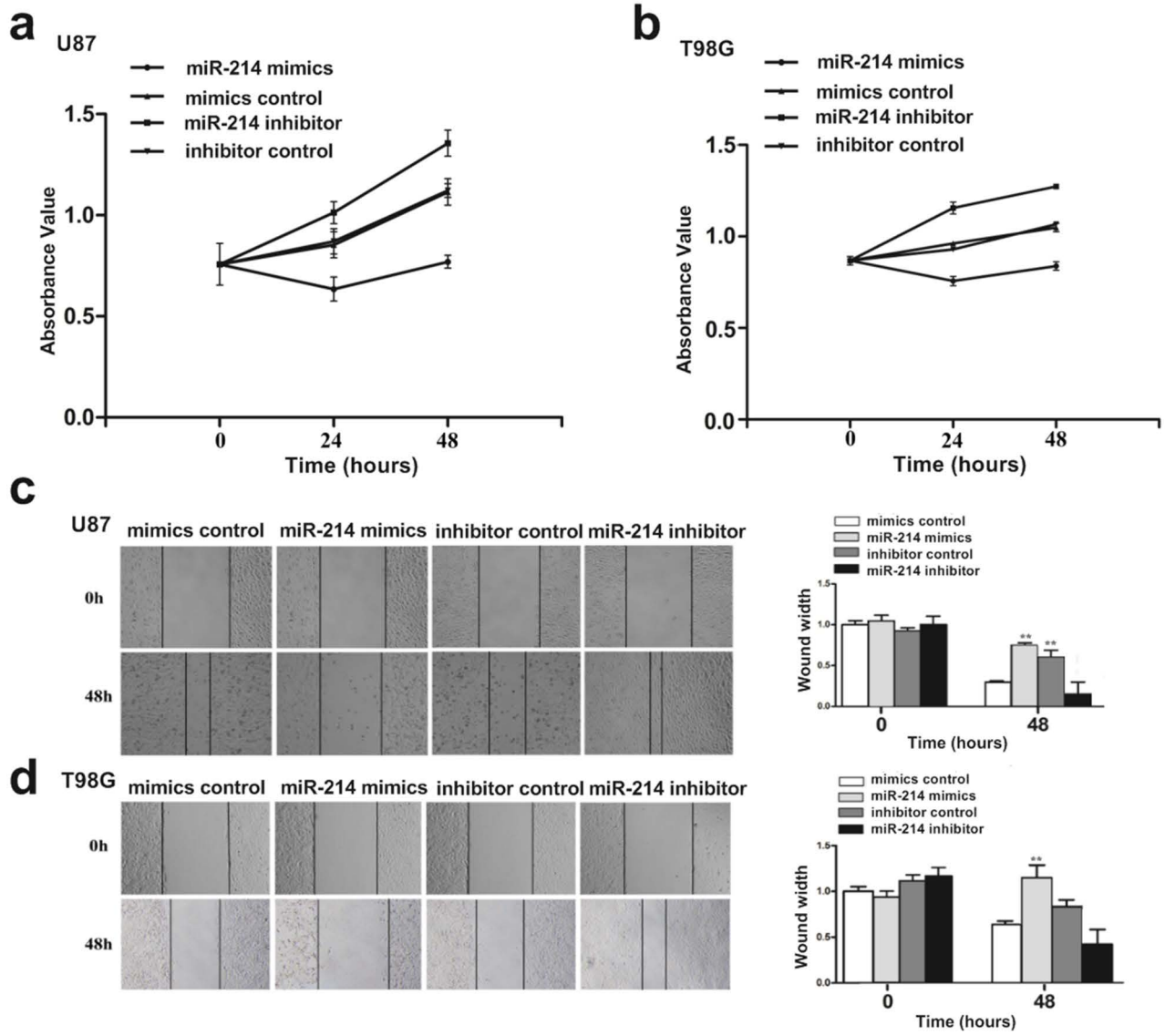

Figure 3. miR-214 affects glioma cell proliferation and migration. (a) The MTT assay in the U87 cell line. (b) The MTT assay in the T98G cell line. (c) The wound healing assay in the U87 cell line. (d) The wound healing assay in the T98G cell line. $^{**}p<0.01$.

(Figs. 5a and e, and 6a and e). Immunofluorescence staining demonstrated increased caspase 1 expression in the miR-214 inhibitor group, which was corrected by transfection with the miR-214 inhibitor + caspase 1 inhibitor (Figs. 5b and 6b). Protein levels of caspase 1 followed similar patterns (Figs. 5d and 6d). Consistently, TUNEL staining further confirmed that cell death was obviously alleviated when cells were transfected with the miR-214 inhibitor + caspase 1 inhibitor compared with transfection with the miR-214 inhibitor (Figs. 5c and 6c). Considering the aforementioned, it is likely that miRNA-214 inhibits glioma cell proliferation and migration through regulating pyroptosis intermediated by caspase 1.

DISCUSSION

In the present study, we demonstrated that pyroptosis mediated by caspase 1 is activated in glioma tissues and T98G and U87 cells. Additionally, caspase 1 was identified as a direct target of miR-214, and it was demonstrated that caspase 1 mRNA and protein expression are negatively regulated by miR-214 in T98G and U87 cells. Furthermore, inhibition of miR-214 suppressed glioma cell proliferation and migration, while downregulation of caspase 1 reversed this suppressive effect. Based on these data, it can be suggested that miRNA-214 inhibits cell proliferation and migration through regulating pyroptosis intermediated by caspase 1.

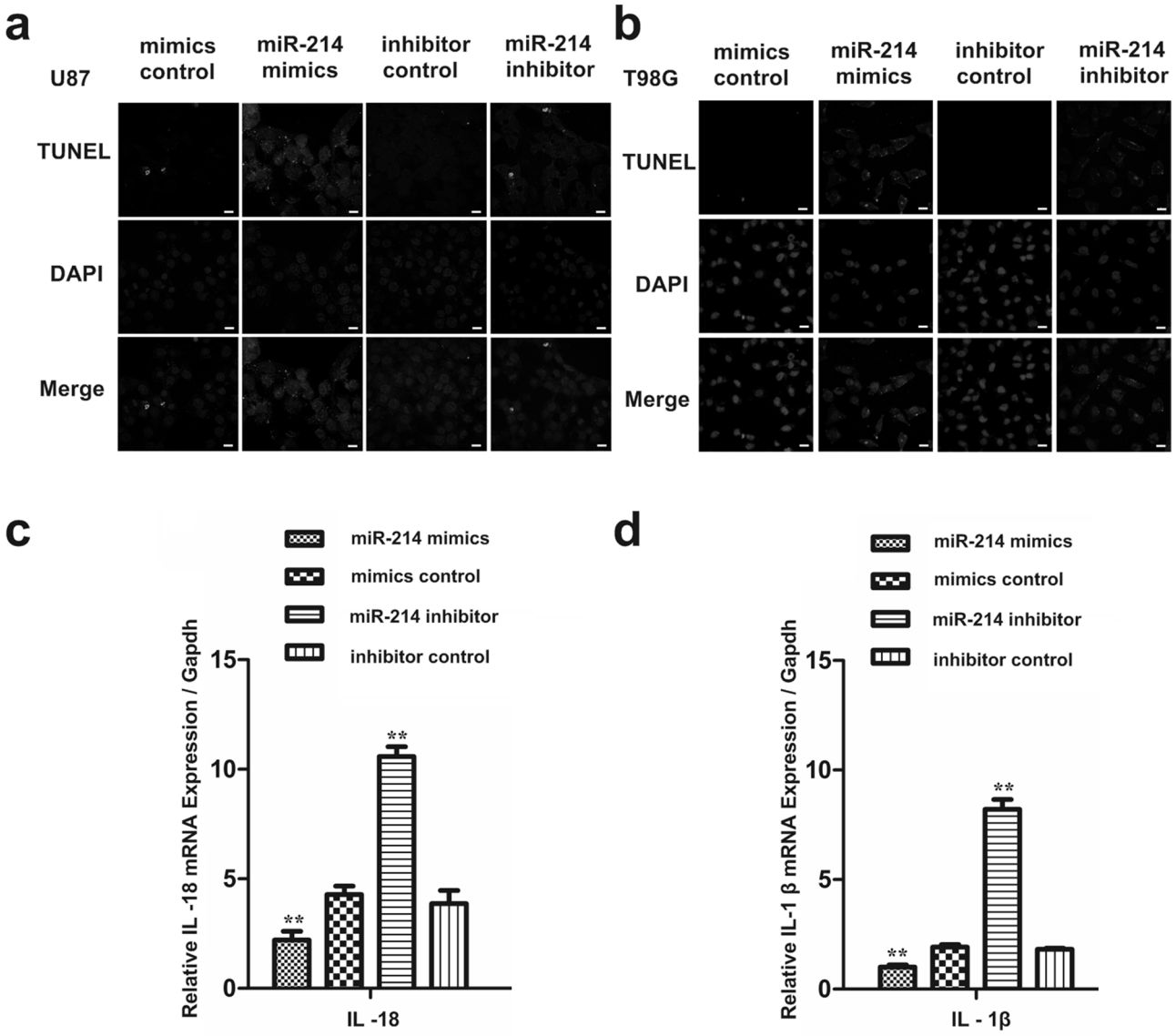

Figure 4. Effect of miR-214 on glioma cell apoptosis and IL-18 and IL-1β expression. (a) TUNEL staining showing cell death in the mimic control, miR-214 mimic, inhibitor control, and miR-214 inhibitor groups (U87 cell line). (b) TUNEL staining showing cell death in the mimic control, miR-214 mimic, inhibitor control, and miR-214 inhibitor groups (T98G cell line). (c) The relative mRNA levels of IL-18 in the miR-214 mimic, mimic control, miR-214 inhibitor, and inhibitor control groups. (d) The relative mRNA levels of IL-1β in the miR-214 mimic, mimic control, miR-214 inhibitor, and inhibitor control groups. Scale bars: 20 μm. $^{**}p<0.01$.

Pyroptosis is inflammatory cell death mediated by caspase 1. It was first described by Zychlinsky and colleagues in 1992, who observed that rapid lysis of macrophages occurred after infection with *Shigella flexneri*[19]. Pyroptosis can be triggered by various pathological stimuli such as cancer, microbial infection, heart attack, or stroke[20]. Inflammosomes, large procaspase 1-activating multiprotein oligomers, are necessary for induction of pyroptosis and activation of the proinflammatory cytokines IL-1β and IL-18. Increased inflammation caused by inflammasome formation creates a local environment that is favorable for tumorigenesis. Furthermore, mounting evidence indicates that chronic inflammation plays an important role in carcinogenesis and tumor progression[21,22] by supplying important molecules to the tumor microenvironment, including growth factors and survival factors, which sustain proliferative signaling and favor angiogenesis, invasion, and metastasis[23]. Additionally, factors

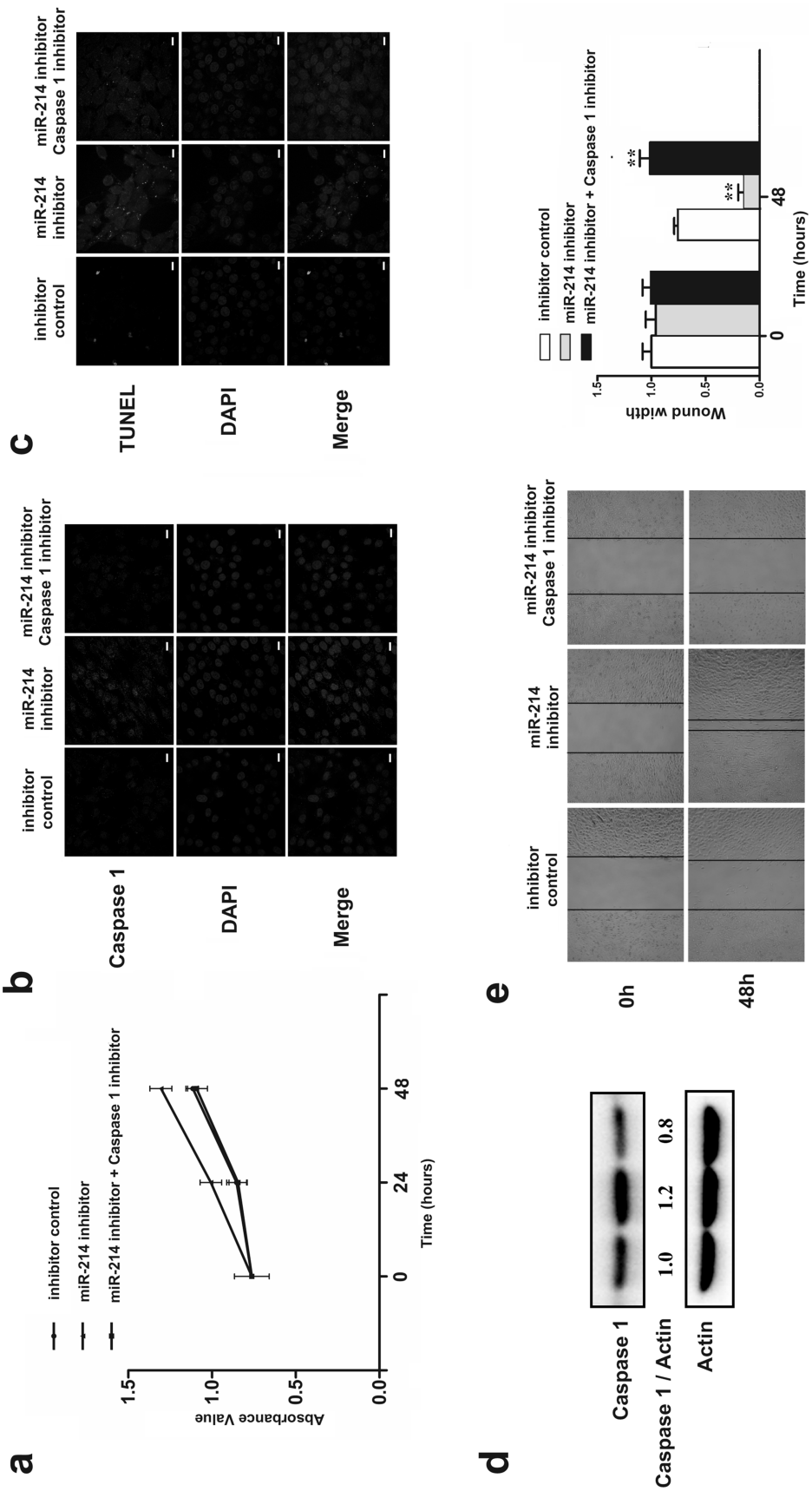

Figure 5. miR-214 regulates U87 glioma cell through caspase 1. (a) The MTT assay. (b) Immunofluorescence images showing the expression of caspase 1. (c) TUNEL staining showing cell death. (d) The protein levels of caspase 1. (e) The wound healing assay in the inhibitor control, miR-214 inhibitor, and miR-214 inhibitor+caspase 1 inhibitor groups of the U87 cell line. Scale bars: 20 μm. $^{**}p<0.01$.

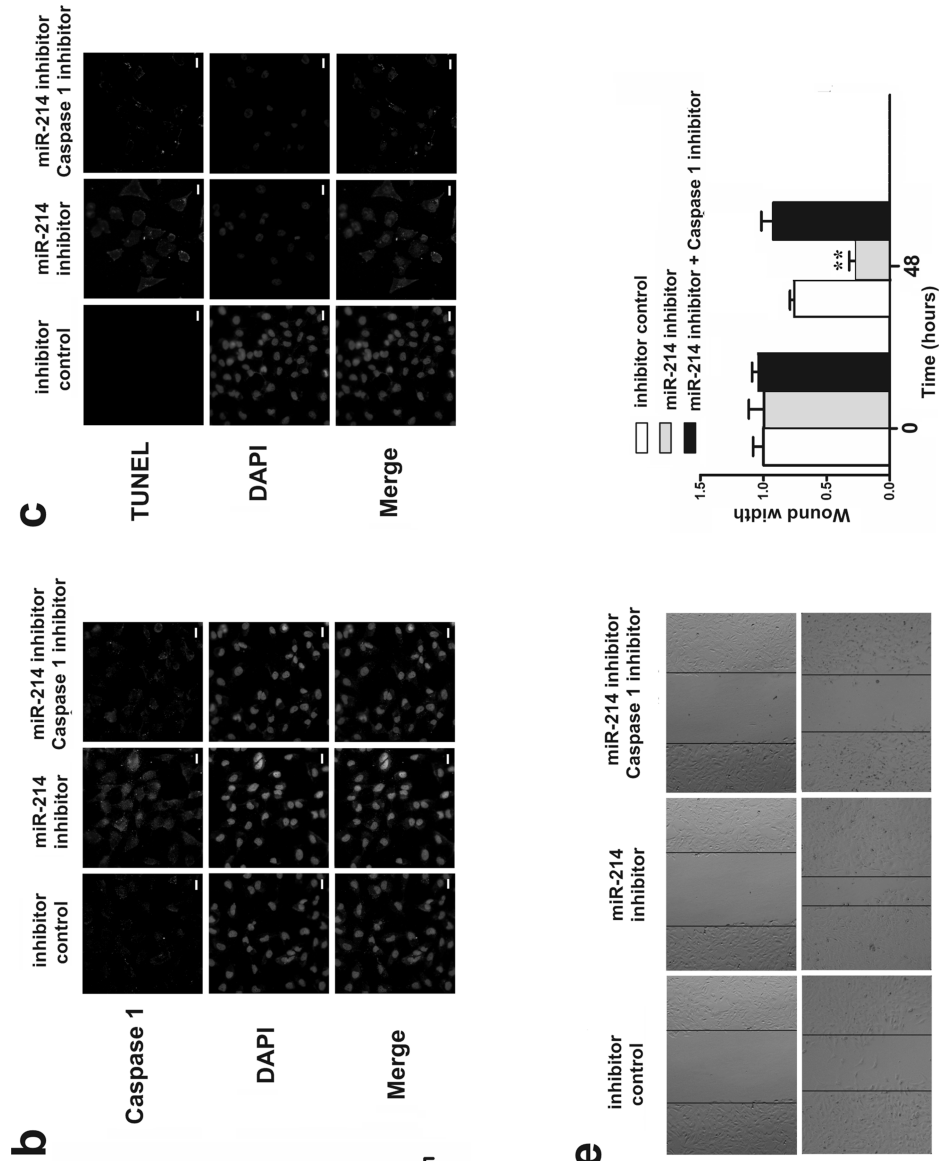

Figure 6. miR-214 regulates the T98G glioma cell through caspase 1. (a) The MTT assay. (b) Immunofluorescence images showing the expression of caspase 1. (c) TUNEL staining showing cell death. (d) The protein levels of caspase 1. (e) The wound healing assay in the inhibitor control, miR-214 inhibitor, and miR-214 inhibitor+caspase 1 inhibitor groups of the T98G cell line. Scale bars: 20 μm. $^{**}p<0.01$.

released by inflammatory cells may lead directly or indirectly to marked suppression of the immune response, which has a potential role in tumor eradication. Moreover, inflammation manifestations are observed at the earliest stages of tumor progression and are capable of nurturing insipient neoplasias into fully developed cancers.

Several inflammatory cytokines have been implicated in the mediation of different steps in the pathway leading to carcinogenesis. Caspase 1 activates pro-IL-1β or pro-IL-18 by proteolytic cleavage, which in turn promotes inflammation and regulates immune responses. IL-1β is an important proinflammatory cytokine that is released upon activation of inflammasomes, which have been associated with carcinogenesis and the maintenance of a tumorigenic microenvironment[24,25], and it is known to contribute to tumor proliferation, angiogenesis, and local invasion[26]. In humans, an elevated level of IL-18 is correlated with various types of cancer including ovarian carcinoma, breast cancer, and melanoma[27–29]. In breast cancer tissue or serum, higher IL-1β levels were correlated with more aggressive disease progression and poor outcome[30]. Recent studies investigated IL-1β in melanomas and found that biopsies constitutively express activated caspase 1 and secrete IL-1β leading to tumor progression[31] and that elevated levels of IL-1β influence recruitment of immune-suppressor cells to further reduce antitumor immune surveillance[32]. Recent studies also highlight the importance of the tumor microenvironment in glioma progression[33,34]. Thus, there is a possible strong interplay between pyroptosis and glioma progression.

miRNAs have been recognized either as tumor suppressors or as oncogenes[35]. Recently, downregulation of miR-214 has been observed in various cancers, including colon cancer, bladder cancer, and colorectal cancer. However, it has been reported that miR-214 is upregulated in lung adenocarcinoma, breast cancer, nasopharyngeal carcinoma, and melanoma. Therefore, previous studies suggest that the role of miR-214 in human cancers may be tumor type specific, and the main target genes of miR-214 may be responsible for its diverse function.

Results showed that miR-214 was strongly downregulated in glioma tissues, conforming with previous studies. In the present study, the expression of caspase 1 in glioma tissues was found to be upregulated in comparison with normal tissues, which is the first observation of caspase 1 in glioma cells to our knowledge. Further results suggest that pyroptosis mediated by caspase 1 is involved in the regulation of glioma cell proliferation and migration. Although the current study verified caspase 1 as a direct target of miR-214, other targets of miR-214 also occur, and further investigation is required.

In summary, this study has demonstrated that pyroptosis occurs in the pathogenesis of glioma and identifies caspase 1 as a potential target for the clinical treatment of gliomas. Additionally, these findings provide insight into the regulatory role of caspase 1 activation. Future studies are needed to define the specific pathways and factors that induce pyroptosis during the pathogenesis and development of glioma in vivo.

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REFERENCES

1. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med. 2008;359:492–507.
2. Coll NS, Epple P, Dangl JL. Programmed cell death in the plant immune system. Cell Death Differ. 2011;18:1247–56.
3. Xu J, Jiang Y, Wang J, Shi X, Liu Q, Liu Z, Liu Y, Scott MJ, Xiao G, Li S, Fan L, Billiar TR, Wilson MA, Fan J. Macrophage endocytosis of high-mobility group BOX1 triggers pyroptosis. Cell Death Differ. 2014;21:1229–39.
4. Cieniewicz B, Dong Q, Li G, Forrest JC, Mounce BC, Tarakanova VL, van der Velden A, Krug LT. Murine Gammaherpesvirus 68 pathogenesis is independent of caspase-1 and caspase-11 in mice and impairs interleukin-1beta production upon extrinsic stimulation in culture. J Virol. 2015;89:6562–74.
5. Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. Cell 2008;132:9–14.
6. Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 2003;113:25–36.
7. Chen CH, Guo M, Hay BA. Identifying microRNA regulators of cell death in Drosophila. Methods Mol Biol. 2006; 342:229–40.
8. Fu LL, Wen X, Bao JK, Liu B. MicroRNA-modulated autophagic signaling networks in cancer. Int J Biochem Cell Biol. 2012;44:733–6.
9. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004;303: 83–6.
10. Long LM, He BF, Huang GQ, Guo YH, Liu YS, Huo JR. MicroRNA-214 functions as a tumor suppressor in human colon cancer via the suppression of ADP-ribosylation factor-like protein 2. Oncol Lett. 2015;9:645–50.
11. Chen DL, Wang ZQ, Zeng ZL, Wu WJ, Zhang DS, Luo HY, Wang F, Qiu MZ, Wang DS, Ren C, Wang FH, Chiao LJ, Pelicano H, Huang P, Li YH, Xu RH. Identification of microRNA-214 as a negative regulator of colorectal cancer liver metastasis by way of regulation of fibroblast growth factor receptor 1 expression. Hepatology 2014;60: 598–609.
12. Wang YW, Shi DB, Chen X, Gao C, Gao P. Clinicopathological significance of microRNA-214 in gastric cancer and its effect on cell biological behavior. PLoS One 2014;9:e91307.

13. Yang Z, Chen S, Luan X, Li Y, Liu M, Li X, Liu T, Tang H. MicroRNA-214 is aberrantly expressed in cervical cancers and inhibits the growth of HeLa cells. IUBMB Life 2009;61:1075–82.
14. Penna E, Orso F, Cimino D, Vercellino I, Grassi E, Quaglino E, Turco E, Taverna D. miR-214 coordinates melanoma progression by upregulating ALCAM through TFAP2 and miR-148b downmodulation. Cancer Res. 2013;73:4098–111.
15. Penna E, Orso F, Cimino D, Tenaglia E, Lembo A, Quaglino E, Poliseno L, Haimovi A, Osella-Abate S, De Pittà C, Pinatel E, Stadler MB, Provero P, Bernengo MG, Osman I, Taverna D. MicroRNA-214 contributes to melanoma tumor progression through suppression of TFAP2C. EMBO J. 2011;30:1990–2007.
16. Tang SL, Gao YL, Chen XB. MicroRNA-214 targets PCBP2 to suppress the proliferation and growth of glioma cells. Int J Clin Exp Pathol. 2015;8:12571–6.
17. Wang S, Jiao B, Geng S, Ma S, Liang Z, Lu S. Combined aberrant expression of microRNA-214 and UBC9 is an independent unfavorable prognostic factor for patients with gliomas. Med Oncol. 2014;31:767.
18. Zhao Z, Tan X, Zhao A, Zhu L, Yin B, Yuan J, Qiang B, Peng X. microRNA-214-mediated UBC9 expression in glioma. BMB Rep. 2012;45:641–6.
19. Zychlinsky A, Prevost MC, Sansonetti PJ. Shigella flexneri induces apoptosis in infected macrophages. Nature 1992;358:167–9.
20. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: Host cell death and inflammation. Nat Rev Microbiol. 2009;7: 99–109.
21. Carmi Y, Dotan S, Rider P, Kaplanov I, White MR, Baron R, Abutbul S, Huszar M, Dinarello CA, Apte RN, Voronov E. The role of IL-1beta in the early tumor cell-induced angiogenic response. J Immunol. 2013;190:3500–9.
22. Dinarello CA. Why not treat human cancer with interleukin-1 blockade. Cancer Metastasis Rev. 2010;29:317–29.
23. Ben-Baruch A. Inflammation-associated immune suppression in cancer: The roles played by cytokines, chemokines and additional mediators. Semin Cancer Biol. 2006;16: 38–52.
24. Lamkanfi M, Dixit VM. Mechanisms and functions of inflammasomes. Cell 2014;157:1013–22.
25. Zitvogel L, Kepp O, Galluzzi L, Kroemer G. Inflammasomes in carcinogenesis and anticancer immune responses. Nat Immunol. 2012;13:343–51.
26. Apte RN, Krelin Y, Song X, Dotan S, Recih E, Elkabets M, Carmi Y, Dvorkin T, White RM, Gayvoronsky L, Segal S, Voronov E. Effects of micro-environment and malignant cell-derived interleukin-1 in carcinogenesis, tumor invasiveness and tumor-host interactions. Eur J Cancer 2006;42:751–9.
27. Orengo AM, Fabbi M, Miglietta L, Andreani C, Bruzzone M, Puppo A, Cristoroni P, Centurioni MG, Gualco M, Salvi S, Boccardo S, Truini M, Piazza T, Canevari S, Mezzanzanica D, Ferrini S. Interleukin (IL)-18, a biomarker of human ovarian carcinoma, is predominantly released as biologically inactive precursor. Int J Cancer 2011;129:1116–25.
28. Gunel N, Coskun U, Sancak B, Hasdemir O, Sare M, Bayram O, Celenkoglu G, Ozkan S. Prognostic value of serum IL-18 and nitric oxide activity in breast cancer patients at operable stage. Am J Clin Oncol. 2003;26:416–21.
29. Drexler SK, Yazdi AS. Complex roles of inflammasomes in carcinogenesis. Cancer J. 2013;19:468–72.
30. Goldberg J, Schwertfeger KL. Proinflammatory cytokines in breast cancer: Mechanisms of action and potential targets for therapeutics. Curr Drug Targets 2010;11:1133–46.
31. Okamoto M, Liu W, Luo Y, Tanaka A, Cai X, Norris DA, Dinarello CA, Fujita M. Constitutively active inflammasome in human melanoma cells mediating autoinflammation via caspase-1 processing and secretion of interleukin-1beta. J Biol Chem. 2010;285:6477–88.
32. Dunn JH, Ellis LZ, Fujita M. Inflammasomes as molecular mediators of inflammation and cancer: Potential role in melanoma. Cancer Lett. 2012;314:24–33.
33. Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H. The brain tumor microenvironment. Glia. 2012;60:502–14.
34. Cooper LA, Gutman DA, Chisolm C, Appin C, Kong J, Rong Y, Kurc T, Van Meir EG, Saltz JH, Moreno CS, Brat DJ. The tumor microenvironment strongly impacts master transcriptional regulators and gene expression class of glioblastoma. Am J Pathol. 2012;180:2108–19.
35. Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. MicroRNAs in cancer management. Lancet Oncol. 2012;13: e249–58.