*Research Article*

Newcastle disease virus promotes pyroptosis in medulloblastoma cells by regulating interferon-gamma-mediated guanylate-binding protein 1 expression and activating caspase-4

Pengwu Ren, MD[1#], Jiayan Yu, MD[1#], Dongxiang Wang, MS[2], Lijuan Zeng, MD[1], Xianqiang Zhang, MD[1], Xiaohe Liu, MD[1], Yongfu Cao, MD[1], Zijian Hu, MD[1], Xiaoyong Zhao, MD[1], Kongbin Yang, MD[1]

[1]Department of Neurosurgery, Key Laboratory of Biological Targeting Diagnosis, Therapy and Rehabilitation of Guangdong Higher Education Institutes, Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, [2]Department of Dermatology, The First Clinical Medical School of Guangzhou University of Chinese Medicine, Guangzhou, China.

#These authors contributed equally to this work.
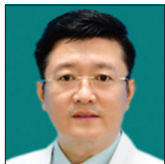
*Corresponding author:
Kongbin Yang,
Department of Neurosurgery,
Fifth Affiliated Hospital,
Guangzhou Medical University,
Guangzhou, Guangdong, China.

gzyangkongbin@163.com

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ABSTRACT

Objective: The literature has reported that Newcastle disease virus (NDV) can have inhibitory effects on various tumors. This study aims to investigate the mechanism by which NDV induces pyroptosis in medulloblastoma (MB) cells.

Material and Methods: We treated MB cell lines Daoy and D283 with NDV or recombinant interferon-gamma (IFN-γ) proteins. Guanylate-binding proteins (GBPs) were measured using real-time quantitative polymerase chain reaction. Small interfering RNA-specific targeting *GBP1* was transfected into MB cells. Apoptosis was assessed using Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nucleoside nick end labeling and flow cytometry assays. Pyroptosis-related proteins, including caspase-4, caspase-1, and gasdermin D (GSDMD), were detected using Western blotting.

Results: Bioinformatics analysis revealed that GBP family genes and interferon-related genes might be responsive to NDV stimulation in MB cells. Treatment with NDV resulted in increased IFN-γ levels and upregulated GBP expression, particularly *GBP1*. In addition, IFN-γ treatment induced *GBP1* expression and enhanced cell apoptosis. *GBP1* knockdown attenuated the decreased cell proliferation and increased cell apoptosis induced by NDV in MB cells. *GBP1* overexpression upregulated the expression of pyroptosis-related proteins, including caspase-4, caspase-1, and GSDMD, subsequently leading to inhibition of cell proliferation and an increase in cell apoptosis levels. The silencing of caspase-4 confirmed the regulatory role of *GBP1* in MB cell pyroptosis.

Conclusion: Our findings suggest that NDV elevates IFN-γ and *GBP1* expression in MB cells, potentially contributing to caspase-4-mediated pyroptosis activation.

Keywords: Newcastle disease virus (NDV), Pyroptosis, Interferon-gamma, Caspase-4

INTRODUCTION

Medulloblastoma (MB) is a highly malignant intracranial tumor, accounting for approximately 60% of all pediatric embryonal tumors. The complexity of its pathogenesis, the lack of effective treatment, and prognostic uncertainty make MB an urgent challenge to overcome.[1] As molecular biology, genetics, and immunology have advanced, our understanding of MB has deepened, and

the treatment methods have expanded. However, the high recurrence rate and low survival rate of MB remain serious problems.[2] Therefore, this study aimed to explore a new treatment for MB, specifically the Newcastle disease virus (NDV).

As a highly infectious agent of avian disease, NDV has attracted widespread attention in cancer therapy. By utilizing its selective replication and lysis of tumor cells, NDV exhibits potential anti-tumor effects.[3] Furthermore, NDV is anticipated to become a new type of biotherapy in cancer treatment, offering safer and more effective treatment options to patients with cancer. In addition, with the continuous development of genetic engineering technology, NDV can be genetically modified to enhance its anti-tumor effect and reduce side effects.[4] Meng *et al.* modified NDV to express an angiogenic inhibitor, which exerts an enhanced therapeutic efficacy in the colon cancer model.[5] However, NDV has different effects on various tumors.[6] Herein, we attempted to examine the killing effect and molecular mechanism of NDV on MB cells.

The interaction between NDV and the host immune system, especially their ability to induce interferon (IFN) production, may be one of the mechanisms by which NDV kill tumor cells.[7] Production of IFN can be induced by NDV, thereby activating the host immune system to change the tumor microenvironment.[8] As an essential antiviral protein, IFN has broad antiviral, anti-tumor, and immune regulatory functions. Meister *et al.* reported that IFN-gamma (IFN-γ)-mediated restoration of caspase-8 in MB cells might enhance apoptotic pathways relevant to chemotherapy and radiotherapy response.[9] Weng *et al.* reported that IFN-β induced MB cell apoptosis by activating extracellular signal-regulated kinase 2 and p38 mitogen-activated protein kinase signaling pathway.[10] Investigating whether NDV mediates MB cell apoptosis through IFN is worthwhile.

Guanylate-binding proteins (GBPs) are characterized by their ability to specifically bind guanine nucleotides, which regulate membrane, cytoskeleton, and cell cycle progression dynamics.[11] Abnormal expression of GBPs is closely correlated with cancer. Zhao *et al.* reported that *GBP1* expression can be a predictive biomarker for immunotherapy.[12] Moreover, *GBP1* expression is strongly provoked by IFN-γ and restrains cellular proliferation during inflammation.[13] Besides, *GBP2* is associated with poor survival and malignancy in clear-cell renal cell carcinoma.[14] Furthermore, there is a close relationship between GBP expression and viral infection.[15,16] Hence, we hypothesized that NDV may inhibit MB cells through IFN-activated GBPs. In this study, we are the first to use NDV to stimulate MB cells and explore their mechanisms.

MATERIAL AND METHODS

Database analysis

To investigate the effect of NDV on gene expression in MB cells, a sequencing dataset (GSE227791, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE227791) for the NDV-infected glioma cell line LN229 was reanalyzed with GEO2R method. The differentially expressed genes were visualized as volcano plot.

Cell culture

Two MB cell lines – Daoy (Cat: HTB-186) and D283 (Cat: HTB-185) cells – were obtained from the American Type Culture Collection. Daoy cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Cat: E600003, Sangon Biotech, Shanghai, China), whereas D283 cells were cultured in the DMEM/F12 medium (Cat: PM150312B, Pricella, Wuhan, China). Cell mycoplasma and STR testing was performed to ensure the availability of cell lines. Both media were supplemented with 10% fetal bovine serum (Cat: A5256701, GIBCO BRL, Grand Island, NY, USA) and 1% penicillin/streptomycin (Cat: B540734, Sangon Biotech). Cells were cultured at 37°C and 5% carbon dioxide (CO_2) in a Thermo Scientific™ BB 150 CO_2 incubator (Thermo Fisher Scientific, Waltham, MA, USA).

Cell transfection

To intervene in the expression of *GBP1* in cancer cells, the coding sequence of *GBP1* was cloned and recombined into the pcDNA3.1 plasmid (Cat: V79520, Invitrogen, Carlsbad, CA, USA). The small interfering RNA specifically targeting *GBP1* (siRNA: 5'- UUUUUGACUGGUACCUUUCUU -3') and caspase-4 (siRNA: 5'- UGUUAAAAAAGGUUUGAAGAA -3') was synthesized using (Genechem Co., Ltd., Shanghai, China). The siRNA and the pcDNA3.1 plasmid were transfected into Daoy or D283 cells according to the instructions on the Lipofectamine 2000 kit (Cat: 11668500, Invitrogen).

Cell treatment

The NDV (D90 strain) was provided by the Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Harbin, China). To explore the inhibitory effect of NDVs, the MB cells grown to 80% density were inoculated with NDVs (1 MOI) at 37°C. After 1 h of incubation, the supernatant was removed to clean unattached viruses. Subsequently, the cells were washed thrice and cultured in a maintenance medium. As the NDV may inhibit MB cells by promoting IFN secretion, we stimulated MB cells with IFN-γ recombinant protein (100 ng/L, for 48 h, Cat: IF002, Merck Millipore, Billerica, MA, USA).

Cell activity detection

We seeded MB cells (1×10^4) in a 96-well plate containing 100 µL of maintenance medium in each hole to measure cell activity. After grouping processing, we removed the culture medium from each well and added 100 µL of pre-configured cell counting kit-8 solution (Cat: C0038, Beyotime Biotechnology, Shanghai, China). After incubation in the cultivation chamber for 1 h, the plate was transfected to a microplate reader (Thermo Fisher Scientific) to detect the optical density at 450 nm wavelength.

Apoptosis detection

Apoptosis was measured using Terminal deoxynucleotidyl transferase -mediated deoxyuridine triphosphate nucleoside nick end labeling (TUNEL, Cat: C1090, Beyotime Biotechnology). The fixed cells (1×10^6) in a 6-well plate were rapidly treated with TritonX-100 for 8 min. After washing, we added 500 µL of the pre-prepared TUNEL reaction mixture to each well and incubated them for 1 h in a dark and humid box at 37°C. The cell nucleus was stained with 3, 3'-diaminobenzidine, and the stained cells (red) were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Furthermore, apoptosis was detected using a flow cytometry assay with an Annexin V-FITC/PI detection kit (Cat: C1065M, Beyotime Biotechnology). After the cells were digested with trypsin, we added 195 µL of Annexin V-FITC binding buffer and gently resuspended the cells (2.5×10^5). We added 5 µL Annexin V- Fluorescein Isothiocyanate solution and 10 µL propidium iodide staining solution and incubated at 20 ~ 25°C for 15 min in the dark. The fluorescence signals were quantified using a BD FACSCantoII flow cytometer (BD Biosciences, San Jose, CA, USA).

Reverse transcription polymerase chain reaction (PCR)

Total RNA was extracted using TRIzol (Cat: 15596018CN, Invitrogen), and its concentration was measured using a NanoDrop microspectrophotometer (Thermo Fisher Scientific). A total of 1 µg of RNA was used as a template for reverse transcription by using the BeyRT II cDNA First Chain Synthesis Kit (Cat: D7168M, Beyotime Biotechnology). Real-time quantitative PCR (qPCR) was performed using SYBRGreen qPCR Mix (Cat: D7510, Beyotime Biotechnology) in an ABI 7500 system (Applied Biosystems, Carlsbad, CA, USA). Primers targeting *GBP1*, *GBP2*, *GBP3*, *GBP4,* and *GBP5* were synthesized using Sangon Biotech (Shanghai, China). Table S1 shows the sequence. The gene 3-phosphate dehydrogenase (*GAPDH*) was utilized as an internal reference. The reaction process includes 94°C for 2 min, 94°C for 20 s, 58°C for 20 s, 72°C for 20 s, and 40 cycles. The $2^{-\Delta\Delta Ct}$ method was used to quantify gene expression levels.

Western blotting method

Total protein was collected using radioimmunoprecipitation assay lysis buffer (Cat: R0010, Solarbio, Beijing, China) and quantified by a bicinchoninic acid protein assay kit (Cat: P0011, Beyotime Biotechnology). Protein samples were separated on an 8% or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel using electrophoresis and transferred to a polyvinylidene fluoride membrane. After incubation with a 5% skim milk powder solution for 1 h, the membrane was incubated with primary antibodies, anti-*GBP1* (ab252832, 1:1000 diluted, Abcam, Cambridge, MA, USA), caspase-4 (ab238124, 1:1000 diluted), caspase-1 (ab179515, 1:1000 diluted), GSDMD-N (ab215203, 1:1000 diluted), and anti-GAPDH (ab8245, 1:5000 diluted), followed by horseradish peroxidase-labeled immunoglobin G secondary antibody (ab6721, 1:10000 diluted). After washing and color reaction with enhanced chemiluminescence solution (Cat: PE0010, Solarbio), the target protein appeared as visible bands on the membrane and transfer it to x-ray film (Cat: FF082, Beyotime Biotechnology).

Immunofluorescence

GBP1 expression in MB cells after stimulation with NDV was measured using an immunofluorescence assay. The cells were collected and fixed with 4% paraformaldehyde in phosphate-buffered saline for 15 min and washed thrice with phosphate-buffered saline with Tween 20. After permeabilization with triton X-100 and blocking with 5% bovine serum albumin, cells were incubated with anti-*GBP1* antibody (ab252832, 1:500 diluted, and Abcam), followed by washing and incubation with secondary antibodies (ab130798, Abcam). To stain the cell nucleus, the 4',6-diamidino-2-phenylindole (DAPI, Cat: C1002, Beyotime Biotechnology) was added and incubated in the dark for 15 minutes. The fluorescence signal was observed under a fluorescence microscope (Olympus). Six images with a random field of view were obtained and used for quantitative analysis of *GBP1* expression. The images were treated with Image-Pro Plus 6.0 software (Media Cybernetics, MD, USA), and the optical density value of the positive signal was determined and considered as the relative expression level of *GBP1*.

Enzyme-linked immunosorbent assay (ELISA)

The release of lactate dehydrogenase (LDH) can reflect cellular damage, while interleukin (IL)-18 and IL-1β are inflammatory factors associated with caspase-1 protein during cell pyroptosis. Hence, the LDH, IFN-α, IFN-γ, IL-18, and IL-1β concentrations in MB cell culture supernatant were detected using ELISA. The process was performed

according to the instructions on the LDH, IFN-α, IFN-γ, IL-18, or IL-1β ELISA detection kit (Cat: BC0685, SEKH-0045, SEKH-0046, SEKH-0028, SEKH-0002, Solarbio).

Statistic analysis

All values are expressed as the mean ± standard error of the mean. Statistical analysis was performed with the unpaired *t*-test for two groups or one-way analysis of variance (GraphPad Prism software, Version 8.0.1.244; La Jolla, CA, USA) followed by a post-Tukey test for multiple groups with all data points showing a normal distribution. All experiments were independently repeated 3 times. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of GBP family in NDV-infected MB

A sequencing dataset (GSE227791) for the NDV-infected glioma cell line LN229 discovered many differentially expressed genes. Figure 1a displays that many GBP family genes (*GBP1*, *GBP3*, *GBP4*, and *GBP5*) and IFN-related genes (*IRF1*, *IFIT3*, *IFIT5*, *ISG20*, *IFNB1*, *IFNL1*, *IFNL2*, *IFNL3*, and *IFNA7*) were significantly upregulated after NDV infection (log2 [fold change] >2). Considering the role of IFN in viral infections and the relationship between GBP family genes and IFN activation pathways, we detected the expression of GBP family genes and two IFN-related genes (*IFNB1* and *IFNA7*) in NDV-infected MB cells. After infection, the cell activity was significantly reduced at 48 h [Figure 1b]. The level of IFN-α and IFN-γ in the supernatant was significantly increased in the NDV group [Figure 1c]. The mRNA expressions of *IFNB1*, *IFNA7*, *GBP1*, *GBP4*, and *GBP5* were significantly increased in Daoy and D283 cells after NDV infection, while *GBP2* and *GBP3* did not differ significantly in D283 cells [Figure 1d and e]. The increased expression level of *GBP1* protein was further expressed using Western blotting and immunofluorescence assay [Figure 1f and g]. Thus, NDV-induced decreases in cell activity may be associated with IFN-mediated pathways and abnormal expression of GBP family genes.

IFN-γ stimulation activated *GBP1* expression

To investigate the effect of IFN-mediated pathways on *GBP1* expression, Daoy and D283 cells were stimulated with recombinant IFN-γ proteins. Results found that the cell activity was significantly inhibited by IFN-γ stimulation at 48 h [Figure 2a]. Moreover, TUNEL staining and flow cytometry assay revealed that IFN-γ stimulation significantly promoted the MB cells' apoptosis [Figure 2b and c]. Notably, mRNA and protein expression levels of *GBP1* were increased in IFN-γ-stimulated cells [Figure 2d-f]. Furthermore,

pyroptosis-related proteins, including caspase-4, GSDMD-N, and cleaved caspase-1, were significantly upregulated by IFN-γ stimulation [Figure 2e]. Therefore, it can be inferred that IFN-γ stimulation activated *GBP1* expression and the MB cell's pyroptosis.

GBP1 knockdown reduced cytotoxicity of NDV to MB cells

To verify whether *GBP1* is involved in NDV inhibition of MB cells, *GBP1* knockdown was performed in NDV-infected MB cells. After 24 h of transfection, the mRNA level of *GBP1* was significantly downregulated [Figure 3a]. The decreased cell activity induced by NDV was partially reversed in silencing *GBP1* group [Figure 3b]. The increased expression of caspase-4, GSDMD-N, and cleaved caspase-1 induced by NDV was partially reduced by *GBP1* knockdown [Figure 3c], and the apoptosis rate [Figure 3d-3f]. In addition, LDH, IL-18, and IL-1β levels in the supernatant were significantly decreased by *GBP1* knockdown [Figure 3g]. These results suggested that *GBP1* was involved in the inhibitory action of NDV on MB cells.

GBP1 overexpression promoted the pyroptosis of MB cells

To investigate the function of *GBP1*, its overexpression was performed in MB cells [Figure 4a]. *GBP1* overexpression significantly decreased the cell activity of MB cells [Figure 4b] and significantly increased the protein levels of caspase-4, GSDMD-N, and cleaved caspase-1 [Figure 4c]. Moreover, it promoted MB cell apoptosis [Figure 4d and e]. The LDH, IL-18, and IL-1β levels in the supernatant significantly increased *GBP1* overexpression [Figure 4f]. We hypothesized that *GBP1* may function as a cancer suppressor gene in MB cells by inducing pyroptosis.

Silencing caspase-4 weakened the inhibitory effect of NDV on MB cells

To validate whether NDV activates the pyroptosis of MB cells, we silenced caspase-4 expression with siRNA in NDV-treated cells. Results of qPCR showed that the mRNA level of caspase-4 was significantly decreased after transfection [Figure 5a]. The deceased cell activity induced by NDV was partially reversed in the silencing caspase-4 group [Figure 5b]. Besides, the increased expression of GSDMD-N and cleaved caspase-1 induced by NDV was partially reduced by *GBP1* knockdown [Figure 5c], and the apoptosis rate [Figure 5d-5f]. Furthermore, LDH, IL-18, and IL-1β levels in the supernatant were significantly decreased by caspase-4 knockdown [Figure 5g]. Results suggested that caspase-4-mediated pyroptosis was involved in the NDV-induced cancer cell-killing.
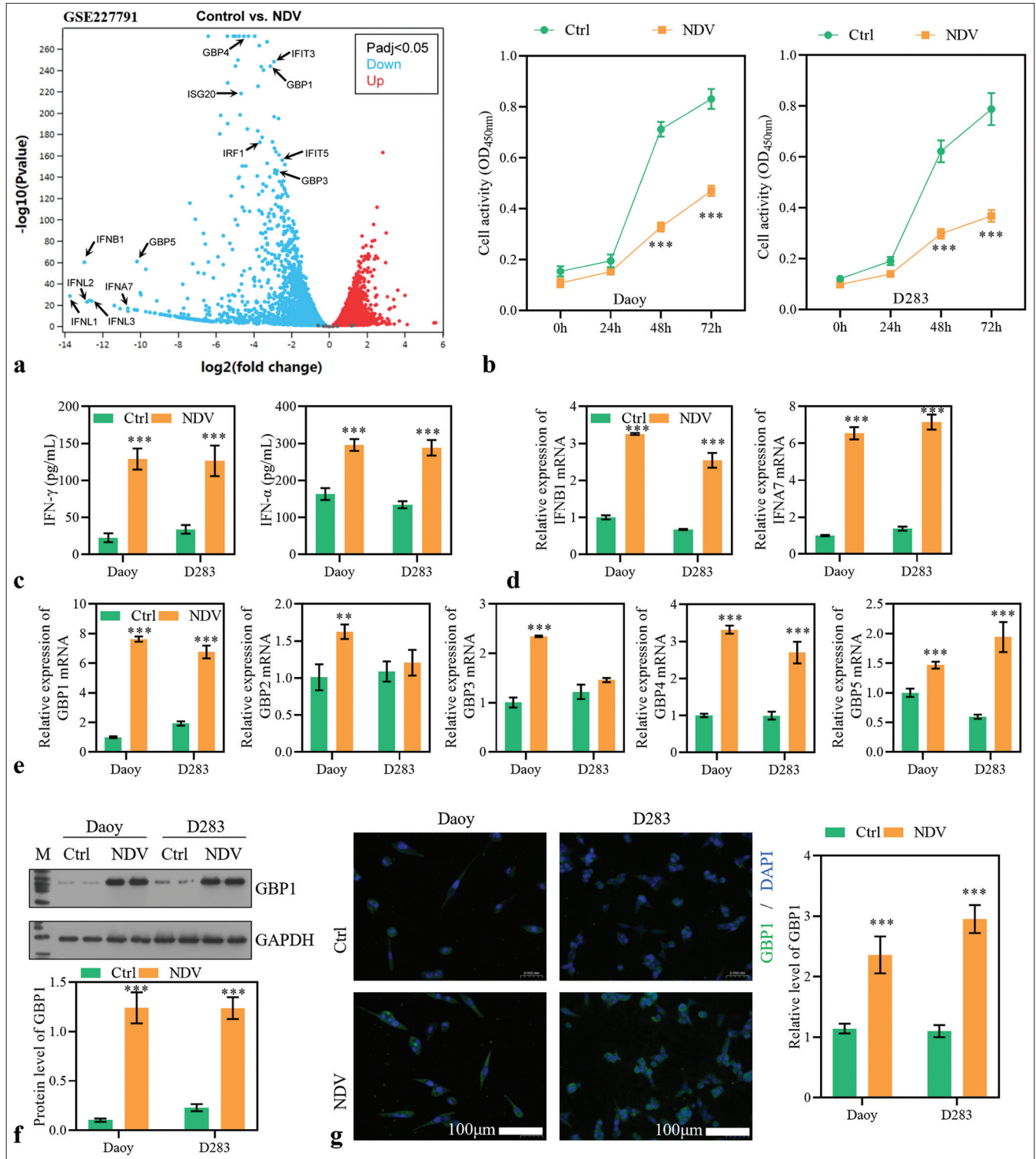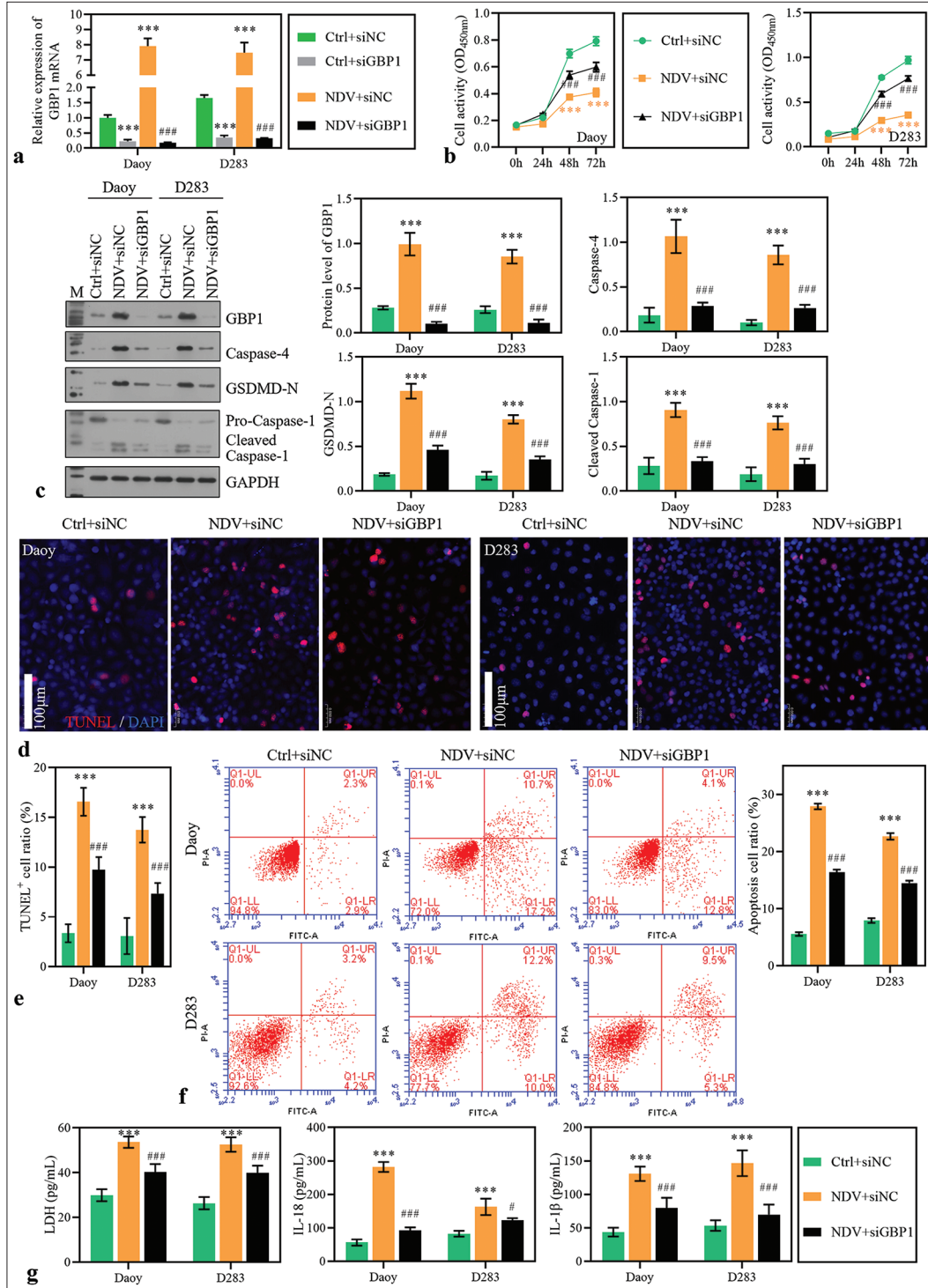
Figure 1: Newcastle disease virus (NDV) infection changed the expression of guanylate-binding protein (GBPs) in medullobl astoma (MB) cells. (a) A sequencing dataset (GSE227791) for NDV rLa Sota-GFP strain infected glioma cell line LN229 discovered many differentially expressed genes. (b) Daoy and D283 cells were treated with NDV. Cell activity was measured with cell counting kit-8. (c) Interferon (IFN)-gamma and IFN-α levels in the supernatant. (d) The expressions of IFNB1 and IFNA7 were detected using reverse transcription polymerase chain reaction (RT-PCR) in MB cells 48 h after virus infection. (e) The expressions of *GBP1, GBP2, GBP3, GBP4,* and *GBP5* were detected using RT-PCR. (f and g) The protein level of *GBP1* was assessed using Western blotting method and immunofluorescence assay. (IFNL1~3: interferon lambda 1~3, IFNB1: interferon beta 1, IFNA7: interferon alpha 7, GBP1~5: guanylate binding protein 1~5, IFIT3, 5: interferon induced protein with tetratricopeptide repeats 3, 5, IRF1: interferon regulatory factor 1, ISG20: interferon stimulated exonuclease gene 20, Ctrl: Control, NDV: Newcastle disease virus. **$P < 0.01$, ***$P < 0.001$).
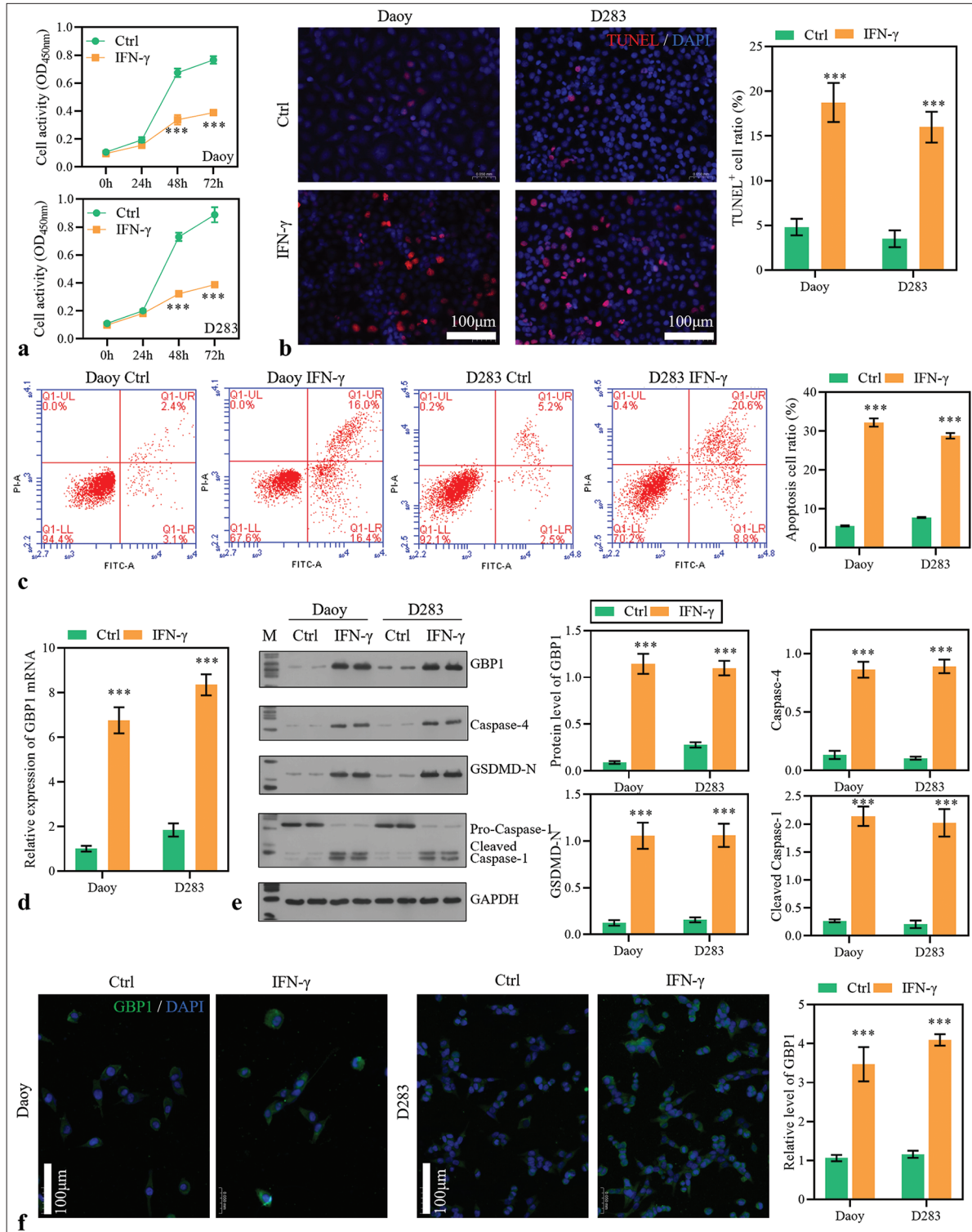
Figure 2: Stimulation with interferon-gamma (IFN-γ) activated guanylate-binding protein (GBP)1 expression and pyroptosis. (a) Medulloblastoma (MB) cells were stimulated with IFN-γ recombinant protein (100 ng/L), and cell activity was measured with cell counting kit-8. (b and c) The apoptosis of MB cells 48 h after virus infection was measured by TdT-mediated dUTP nick end labeling and flow cytometry assay. (d) *GBP1* expression was detected using reverse transcription polymerase chain reaction. (e) The protein levels of *GBP1*, caspase-4, gasdermin D (GSDMD)-N, and caspase-1 were detected using Western blotting method. (f) The protein level of *GBP1* detected using immunofluorescence assay. ***P < 0.001.
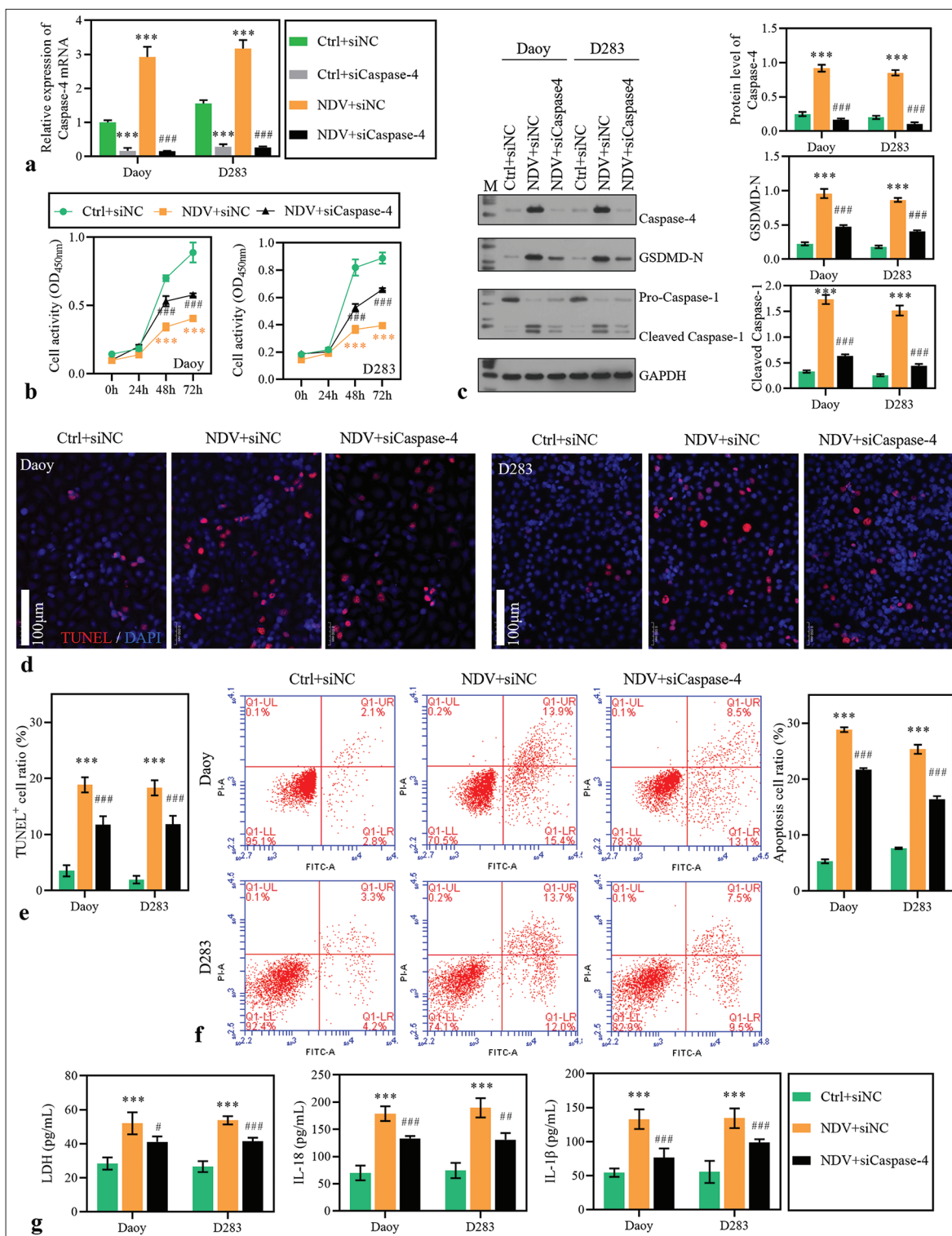
Figure 3: Guanylate-binding protein (GBP)1 knockdown alleviated Newcastle disease virus (NDV)-induced pyroptosis. Daoy and D283 cells were transfected with small interfering RNA targeting *GBP1* and treated with NDV. (a) *GBP1* expression was detected using reverse transcription polymerase chain reaction in medulloblastoma (MB) cells 48 h after treatment. (b) Cell activity was measured with cell counting kit-8. (c) The protein levels of *GBP1*, caspase-4, gasdermin D (GSDMD)-N, and caspase-1 were detected using Western blotting method. (d-f) The apoptosis of MB cells 48 h after treatment was measured using TdT-mediated dUTP nick end labeling and flow cytometry assay. (g) Lactate dehydrogenase, interleukin (IL)-18, and IL-1β levels in the supernatant. ***$P < 0.001$, versus Ctrl + siNC group; #$P < 0.05$, ###$P < 0.001$, versus NDV + siNC group.
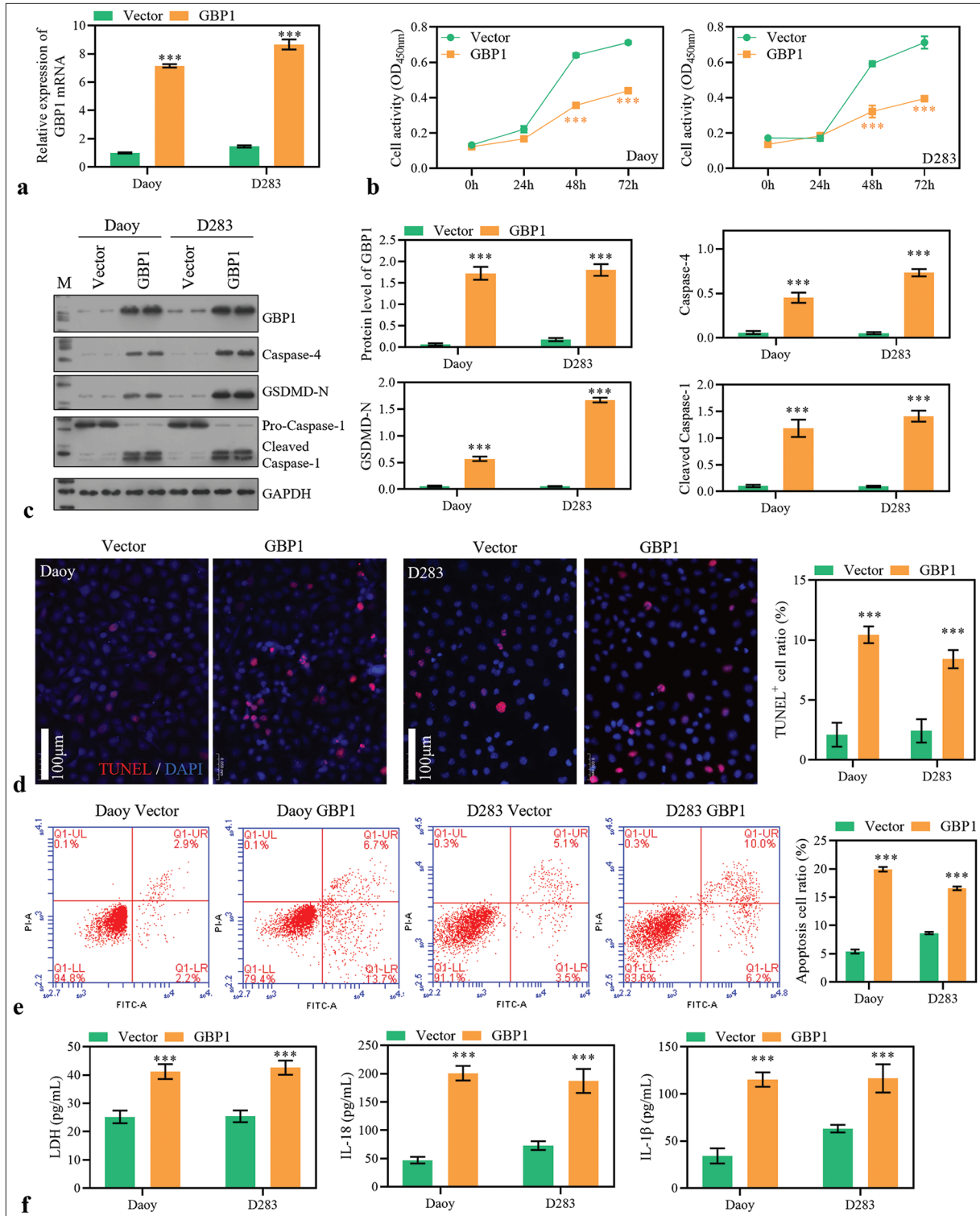
Figure 4: Guanylate-binding protein (GBP)1 overexpression promotes medulloblastoma (MB) cell pyroptosis. (a) *GBP1* expression was detected using reverse transcription polymerase chain reaction in MB cells 48 h after transfection. (b) Cell activity was measured with cell counting kit-8. (c) The protein level of *GBP1*, caspase-4, GSDMD-N, and caspase-1 was detected using Western blotting method. (d and e) The apoptosis of MB cells 48 h after transfection was measured using TdT-mediated dUTP nick end labeling and flow cytometry assay. (f) Lactate dehydrogenase, interleukin (IL)-18, and IL-1β levels in the supernatant. ***$P < 0.001$.

Figure 5: Caspase-4 knockdown blocked Newcastle disease virus (NDV)-induced pyroptosis. Daoy and D283 cells were transfected with small interfering RNA targeting caspase-4 and treated with NDV. (a) Caspase-4 expression was detected using reverse transcription polymerase chain reaction in medulloblastoma (MB) cells 48 h after treatment. (b) Cell activity was measured with cell counting kit-8. (c) The protein levels of guanylate-binding protein 1, caspase-4, GSDMD-N, and caspase-1 were detected using Western blotting method. (d-f) The apoptosis of MB cells 48 h after treatment was measured using TdT-mediated dUTP nick end labeling and flow cytometry assay. (g) Lactate dehydrogenase, interleukin (IL)-18, and IL-1β levels in the supernatant. ***$P < 0.001$, versus Ctrl + siNC group; ##$P < 0.01$, ###$P < 0.001$, versus NDV + siNC group.

DISCUSSION

The application prospects of NDV in the treatment of tumors are extensive and full of challenges.[17] Yuan *et al.* reported that NDV activates methylation-related enzymes to reprogram m6A methylation in infected cells.[18] Therefore, the mechanism by which NDV kills tumor cells may involve multiple pathways. By reanalyzing sequencing data, we found that IFN-γ-induced GBPs may be an important pathway in response to NDV infection. The increase in IFN-γ level may be related to the immune response activated by NDV. Furthermore, NDV activates NK cells in pancreatic cancer and prevents cancer recurrence by promoting adaptive immunity.[19] However, our *in vitro* experiment revealed that the increase in IFN-γ levels is independent of immune cells; after being stimulated by NDV, MB cells spontaneously upregulated IFNB1 and IFNA7 gene expression levels. We hypothesized that this may be because MB cell lines are very primitive and infinite cells, allowing them to retain some of the characteristics of stem cells. Zhu *et al.* also reported that herpes simplex virus-1 has the potential to stimulate IFN-γ secretion in glioblastoma cells rather than T-cell secretion.[20] There are controversies on the role of IFN-γ in MB cells. Lin *et al.* reported that IFN-γ in the perinatal period can induce MB by the Sonic Hedgehog signaling pathway.[21] In addition, Zhou *et al.* reported that IFN-γ promotes immune escape in gliomas by activating the epidermal growth factor receptor pathway.[22] However, Hotfilder *et al.* reported that IFN-γ increased IL-6 production in human glioblastoma cell lines, which induces immune eradication of glioma cells.[23]

Herein, results showed that NDV induced the expression level of *GBP1* in MB cells. Ma *et al.* reported that *GBP1* is upregulated by treatment with IFN-γ and infectious bronchitis virus (IBV) in chicken macrophage HD11 cells.[24] However, they found that *GBP1* was pivotal in anti-IBV activity by degrading the IBV-N protein through the autophagy pathway. Our results suggested that high expression of *GBP1* promotes caspase-4-mediated pyroptosis in MB cells. We recommend that the death effect induced by *GBP1* targets the virus and the host. Some scholars believe that *GBP1* may act as a double-edged sword in cancer. Furthermore, *GBP1* can inhibit cancer cell proliferation and enhance their chemotherapy resistance. The previous studies reported that IFN-γ-induced *GBP1* inhibits mammary tumor growth *in vivo*.[25-27] Other previous studies reported that *GBP1* plays a promoting role in glioblastoma,[28] lung adenocarcinoma,[29] and prostate cancer.[30] Tailor *et al.* reported that *GBP1* overexpression enhanced the paclitaxel resistance of ovarian cancer through boosted cellular proteasomal activity.[31]

Unlike the controversy surrounding the role of *GBP1* in tumors, *GBP1* can activate cell pyroptosis, which regulates various disease progressions. Wandel *et al.* reported that *GBP1* acts as a pattern recognition receptor that directly connects to lipopolysaccharide.[32] Johns and Galam reported that *GBP1* induced inflammatory caspase-4 and triggered the proinflammatory cytokine release.[33] Our results demonstrated that the non-classical pathway, caspase-4 mediated pyroptosis, can be activated by *GBP1*. Dickinson *et al.* reported that supplementation with recombinant polymerized *GBP1* or *GBP2* can enhance lipopolysaccharides-induced caspase-4 activation.[34] Fisch *et al.* previously reported that *GBP1* plays a crucial role in cell death by regulating pyroptosis and apoptosis.[35] Recently, they reported that the viral protein TgIST can increase *GBP1* activity by depleting PIM1.[36] Furthermore, PIM1 can modify *GBP1* and cause its inactivation. In MB cells, we found that *GBP1* transcription was activated by NDV-induced IFN-γ stimulation. Notably, our results showed that the classical pathway of pyroptosis, caspase-1, was induced in the NDV infection group.[37] Because caspase-1 and caspase-4 protein levels changed after NDV stimulation, we chose to detect the Annexin V protein signal. In addition, our results displayed that the concentrations of LDH, IL-18, and IL-1β in MB cell culture supernatant were increased by NDV treatment, which proved that NDV induced cell damage and pyroptosis in MB cells. However, there is no evidence to suggest a correlation between NDV and caspase-1 activation. The accumulation of reactive oxygen species can activate caspase-1,[38] which may be a follow-up reaction to the caspase-4 pathway.

Although clinical trials utilizing NDV are limited, the NDV-induced activation of the immune system plays a significant role in oncolysis and the maintenance of long-lasting anti-tumor effects.[39] There are limitations to our study. First, cell apoptosis, rather than cell pyroptosis, was detected. Cell apoptosis primarily relies on exogenous cytokine stimulation and endogenous mitochondrial pathways. Cell apoptosis and pyroptosis are similar in their reliance on activating caspase family proteins.[40] Second, there is a lack of *in vivo* animal experiments to confirm the relevant results. Modifying NDV and conducting *in vivo* animal experiments may promote the clinical application of NDV in MB. In addition, with the promotion and application of multi-omics technology and analysis,[41] the mechanism of NDV inhibiting tumor will be clearer.

SUMMARY

We propose that NDV promotes IFN-γ and *GBP1* expression and induces cell apoptosis in MB cells. With the continuous innovation of genetic modification technology, NDV treatment for tumors will become increasingly precise. In addition, IFN-γ might activate the caspase-4-mediated pyroptosis in MB cells by regulating *GBP1* expression. Furthermore, GBP family proteins may be essential targets in future cancer treatment.

AVAILABILITY OF DATA AND MATERIALS

The datasets used in this study are available from the corresponding author on reasonable request.

ABBREVIATIONS

NDV - Newcastle disease virus
MB - medulloblastoma
GBPs - Guanylate-binding proteins
IFN-γ - interferon-gamma
IFN-α - interferon- alpha
IL-18 - interleukin-18
IL-1β - interleukin-1beita
GSDMD - gasdermin D
DMEM - Dulbecco's modified Eagle medium
LDH - lactate dehydrogenase

AUTHOR CONTRIBUTIONS

KBY and PWR: Concepted and designed the research; JYY, XHL and YFC: Acquired the data; LJZ, XYZ and JYY: Analyzed and interpreted data; PWR and XQZ: Drafted the manuscript; DXW and ZJH: Revised manuscript for important intellectual content. All authors contributed to this present work. All authors read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve direct research with human or animal samples and therefore does not require ethics committee approval.

FUNDING

CONFLICT OF INTEREST

The authors declare no conflict of interest.

EDITORIAL/PEER REVIEW

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded for reviewers and vice versa) through an automatic online system.

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