IRF1-triggered ZBP1 Transcription Mediates Cell Death of Neurons

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Abstract

Neurodegenerative disease (ND) characterized by progressive neuronal cell death is closely associated with excessive production of TNF-α in the cerebrum. However, the specific molecular mechanism linking TNF-α and neuronal cell death remains to be fully elucidated. Here, we report that TNF-α-induced expression of ZBP1 plays a central role in neuronal cell death. We further demonstrate that IRF1 activates ZBP1 expression by directly binding to a core regulatory motif in the ZBP1 promoter in murine neuronal cells but not microglial cells. Moreover, the binding of IRF1 to the ZBP1 promoter causes the increase in ZBP1 expression in two human cell lines. Importantly, the expression levels of IRF1 and ZBP1 are positively correlated in TNF-α-related neurodegenerative disease, suggesting that the TNF-α-IRF1-ZBP1 axis may be a previously unrecognized mechanism of neuronal cell death in neurodegenerative diseases. Our study expands the knowledge on the upstream regulators that induce ZBP1 transcription and provides new insight into the role of ZBP1 in neurodegenerative diseases.

Introduction

Mature neurons lack the ability to proliferate and are susceptible to aberrant cell death when exposed to acute or chronic inflammatory reactions in the cerebrum[1]. Neurodegenerative diseases (NDs) include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and some cerebral infectious diseases with symptoms of neuronal impairment, such as angiostrongyliasis caused by *Angiostrongylus cantonensis* (hereafter abbreviated as AC), a nematode exclusively parasitic to the brain of its nonpermissive hosts[2]. Neurodegenerative disease pathology conventionally progresses along with inflammation, which is responsible for the progressive impairment and death of neural cells[3]. Recently, studies have revealed that neurodegenerative disease pathogenesis is closely associated with tumour necrosis factor (TNF)-α (also termed TNF)[4], a pro-inflammatory cytokine well known to contribute to the apoptosis of neuronal cells[5]. In fact, inhibition of TNF-α is reported as a promising effective strategy to attenuate the onset of AD and PD[6–8]. However, the underlying regulatory axis bridging TNF-α and neuronal death has not been fully elucidated.

Z-DNA-binding protein1 (ZBP1, also known as DAI and DLM-1), initially considered a cytosolic nucleotide sensor of RNA and DNA viruses, plays an essential role in cell death[9–11], inflammasome activation[12] and the innate immune response[13, 14]. Accumulating evidence has demonstrated the molecular mechanism by which the increase in ZBP1 expression regulates the death of diverse cell types. For instance, ZBP1 is transcriptionally upregulated in mouse alveolar epithelial cells infected by influenza A virus (IAV), which further activates RIP3, leads to MLKL phosphorylation and eventually results in apoptosis and necroptosis of the cells in the mouse lung[15]. Moreover, the enhanced protein synthesis of ZBP1 induced by interferons (IFNs) could mediate necroptosis of fibroblast cells in a RIPK3-dependent manner[16]. In addition, high expression of ZBP1, found in virus-infected neurons, has the potential to inhibit virus replication by regulating IRG1 to affect neuronal metabolism[17]. However, the molecular function of ZBP1 in neuronal death and the upstream regulatory mechanism of the transcription of ZBP1 are unknown.

IRF1 is the first known member of the IRF family[18], and its expression can be induced in various cells upon exposure to stimuli, such as IFNs and TNF-α[19, 20]. Growing evidence has demonstrated that IRF1 functions as a regulator of apoptosis[21] and inflammasome activation (including pyroptosis)[22, 23] induced by DNA damage and TNF-α stimulation in diverse cancer cells[24]. Mechanistically, IRF1 typically activates the caspase cascade[25, 26], Fas ligand[27] and TRAIL[28], which eventually leads to cell death. In addition, IRF1 was discovered to bind to the promoter of p53 upregulated modulator of apoptosis (PUMA) and enhance PUMA transcription, resulting in the intrinsic apoptosis of gastric cancer cells[29]. Furthermore, during spinal cord injury, IRF1 expression is upregulated to mediate caspase-dependent neuronal apoptosis[30]. Given that TNF-α can enhance the expression of IRF1, which in turn regulates TNF-α-induced cell death in cancer cells, and that aberrant overexpression of IRF1 has been observed in neurons impaired due to inflammation[30], we hypothesize that there is be a direct link between IRF1 and neuronal death induced by TNF-α.

To elucidate the function of ZBP1 and its upstream factor in TNF-α-induced neuronal cell death in neurodegenerative diseases, we investigated the correlation of the expression levels of TNF-α and IRF1 with ZBP1 in multiple transcriptomic datasets of neurodegenerative diseases. To address the regulatory relationship, we measured quantitative changes in cell death and ZBP1 expression in response to TNF-α stimulation using cultured neuronal cells. We confirmed that TNF-α is able to induce high expression of ZBP1 in neuronal cells, which further triggers neuronal death. Furthermore, we identified IRF1 as an important mediator that activates ZBP1 transcription in response to TNF-α. Our results show that IRF1 binds to two regions in the ZBP1 promoter in mouse neuronal cells. Similarly, IRF1 binds to the ZBP1 promoter and activates the expression of ZBP1 in various human cells. In conclusion, our findings suggest that IRF1-activated ZBP1 transcription mediates TNF-α-induced neuronal cell death.

Materials And Methods

Collection and processing of omics data

To explore the association of TNF-α and IRF1 with ZBP1 and to evaluate the mRNA expression levels of TNF-α, IRF1 and ZBP1 in neurons of neurodegenerative diseases such as AD, PD, and HD, we downloaded all the transcriptome data of cerebrum of mouse models or patients related to these diseases from Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/). All the raw data were obtained via the GEOquery package in R language (version 3.6.0) by inputting the accession numbers of datasets and were quantile-normalized to eliminate the batch effect using the Limma package prior to the analysis. After the removal of samples with specific treatment (CD33 knockdown) that might affect the expression of the genes of interest (TNF-α, IRF1 and ZBP1) in the GSE74441 dataset (mouse model of AD), 173 samples and their corresponding raw transcriptional data profiled by the Illumina MouseRef-8 v2.0 platform (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74441) were obtained, of which 46 samples acted as controls and 127 samples had AD. Transcriptome data from a mouse model of PD and HD were acquired from GSE31458 (which includes 29 samples) with an Affymetrix Mouse Genome 430A 2.0 platform (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31458) and GSE135057 (which

includes 16 samples) with an Illumina NextSeq 500 platform (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135057). The raw microarray data from patients with AD and PD were downloaded from GSE132903 (containing 195 samples: 98 control samples and 97 AD samples) detected by the Illumina HumanHT-12 V4.0 platform (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132903) and GSE49036, which included 28 samples profiled by the Affymetrix Human Genome U133 Plus 2.0 platform (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE49036). In addition, angiostrongyliasis caused by AC infection has been reported to be characterized by an increase in TNF-α expression[31] and symptoms of neurodegenerative disease[32]. Thus, we downloaded the microarray data of this model from GEO (GSE159486). All the datasets obtained from GEO in this study are summarized in **Supplementary Table 1**.

Cell lines

In this study, seven mouse and human cell lines were employed. To confirm the effect of TNF-α on ZBP1 in neurons, HT22 cells (a mouse neuron cell line) were stimulated with the indicated concentration of TNF-α for the indicated times. To explore whether IRF1 specifically enhanced ZBP1 expression in neuronal cells in brain tissue, BV2 and N9 (mouse microglial cell lines) and U87MG and U118MG (human glioma cell lines) cells were transfected with an IRF1 overexpression vector or control vector followed by RNA quantitative analysis. In addition, 293T (human embryonic kidney cell line) and A549 (human lung cancer cell line) cells were included. The N9 cell line was cultured with Dulbecco's modified Eagle's medium/nutrient mixture F-12 medium (Gibco, USA), while all the other cell lines were grown in Dulbecco's modified Eagle's medium (Gibco, USA). All cell lines were cultured in medium containing 10% foetal bovine serum (FBS) and 100 U/ml penicillin/streptomycin under appropriate humidity and temperature.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was extracted from treated cells or mouse cerebrum with the use of TRIzol™ LS Reagent (Invitrogen, Carlsbad, CA) and quantified with NanoDrop One (Thermo Fisher Scientific, Waltham, USA), after which cDNA was synthesized *in vitro* utilizing 1 μg purified total RNA with a Revert Aid First Strand cDNA Kit (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions. RT-qPCR was carried out with a two-step method (40 cycles at 95°C for 20 s and 60°C for 30 s) using SYBR Green (TaKaRa, Dalian, China) on a LightCycler480® Real-Time PCR System (Roche Diagnostics, Reinach, Switzerland). The mean cycle threshold (Ct) value of each gene was normalized to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) value and processed by the $2^{-\Delta\Delta Ct}$ method to calculate the relative mRNA expression levels of the indicated genes. All primers for RT-qPCR are listed in **Supplementary Table 2**.

Immunoblotting analysis

Cells were cultured in cell culture plates (Corning, New York, USA), treated with TNF-α or transfected with siRNA or vectors for the indicated times and then lysed on ice in radioimmune precipitation assay (RIPA)

buffer (Thermo Fisher Scientific, USA) plus protease and phosphatase inhibitor cocktail (1:1000, Thermo Fisher Scientific, USA) for 10 min. Next, the lysates were centrifuged at 4°C and 13000 ×g for 15 min, and the supernatants were transferred into new 1.5 ml centrifuge tubes. A bicinchoninic acid (BCA) assay (Beyotime, Wuhan, China) was used to quantify the protein concentration, and 20 μg of total protein was mixed with 1× sodium dodecyl sulfate (SDS) buffer and boiled at 100°C for 5 min. Then, the denatured proteins were subjected to SDS-polyacrylamide gel electrophoresis, followed by transfer to polyvinylidene fluoride (PVDF) membranes with pre-cooling precooled transfer buffer and blocking with 5% non-fat milk. The membranes were incubated with primary antibodies at 4°C overnight and with HRP-conjugated secondary antibodies at room temperature for 2 h. Chemiluminescence was assayed with an enhanced chemiluminescence (ECL) kit (Merck Millipore, MA, USA) and a ChemiDoc Imaging System (Bio-Rad, California, USA). All the antibodies used in this study are listed in **Supplementary Table 3**.

Plasmid construction and siRNA design

The basic plasmids used in this study, namely, pcDNA3.1, pGL4.10 and pGL4.13, were purchased from Promega (WI, USA). The full-length coding sequences (CDs) of mouse Irf1 and Zbp1 and human IRF1 were obtained from the NCBI Gene database (https://www.ncbi.nlm.nih.gov/). Next, reverse transcription PCR (RT-PCR) was carried out with the use of a RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, USA) to obtain total cDNA, which was then separately cloned into the multiple cloning site (MCS) of pcDNA3.1 via a seamless cloning kit (Beyotime, Wuhan, China) to generate transient overexpression plasmids. The Zbp1 promoter sequences (2000 bp upstream of the transcription start site (TSS) of Zbp1 exon 1) of mice and humans were downloaded from the UCSC Genome browser (https://genome.ucsc.edu/cgi-bin/hgGateway). The full-length, truncated and mutant (Mut) Zbp1 mouse promoter sequences (F2000, F1714, F1341, F1029, F743, F398, wild-type (WT)-1, WT-2, WT-3, Mut-1, Mut-2, Mut-3, Mut-4, Mut-5, and Mut-6) and human ZBP1 promoter sequences (full length, WT-1, WT-2, and Mut-1) were amplified by RT-PCR and inserted into the MCS of pGL4.10. All the mutant sequences were introduced by chemical synthesis (TsingKe, Beijing, China). All siRNAs involved in this study were designed by the online siRNA-designing tool of Sigma-Aldrich (https://www.sigmaaldrich.com) and produced through chemical synthesis (GenePharma, SuZhou, China).

Cell transfection and stimulation

Cells cultured in complete medium and at 60% cell density were transfected with 2 μg transient expression plasmids or 100 pmol siRNA with the use of Lipofectamine™ 3000 Transfection Reagent (Invitrogen, Carlsbad, CA) and Opti-MEM™ I Reduced Serum Medium (Gibco, California, USA) for 48 h followed by analysis of RNA and protein. To explore the effect of Irf1 on the upregulation of Zbp1 expression and the role of Zbp1 in neuronal cell death induced by TNF-α, cells were pre-stimulated with 20 ng/ml TNF-α for 24 h and then transfected with plasmids or siRNA for 48 h prior to subsequent analysis. The siRNA oligo sequences used here were as follows: siIrf1 (5’-AGAUGGACAUUAUACCAGAUATT-3’ and 5’-UAUCUGGUAUAAUGUCCAUCUTT-3’), siIrf2 (5’-GAUCAAGAGAUCGUCACUAACTT-3’ and 5’-GUUAGUGACGAUCUCUUGAUCTT-3’), siArnt (5’-

CCAAGACUCGUUCUUCCCAAUTT-3’ and 5’-AUUGGGAAGAACGAGUCUUGGTT-3’), siMax (5’-UGCCCAACUGCAGACCAACUATT-3’ and 5’-UAGUUGGUCUGCAGUUGGGCATT-3’), siZbp1-1 (5’-GCGATTATTTGTCAGCACAATTT-3’ and 5’-ATTGTGCTGACAAATAATCGCTT-3’), siZbp1-2 (5’-GTCCAGACAGTCCACATCAAATT-3’ and 5’-TTTGATGTGGACTGTCTGGACTT-3’).

Luciferase reporter assay

To confirm whether Irf1 could directly regulate the expression of Zbp1 and identify the specific promoter region regulated by Irf1, a luciferase reporter assay was carried out as described previously[33]. The potential core regions of the Zbp1 promoter regulated by Irf1 were analysed by AnimalTFDB (http://bioinfo.life.hust.edu.cn/AnimalTFDB2/). The full-length, truncated and Mut mouse and human Zbp1 promoter sequences were separately cloned into the pGL4.10 vector (without promoter sequence) as a reporter vector, and pGL4.13 (an SV40-firefly luciferase reporter vector) served as a positive control. Briefly, HEK293T cells were seeded into 24-well plates overnight and then co-transfected by Lipofectamine^{TM} 3000 reagent with 1 μg of overexpression plasmid (mouse or human Irf1 overexpression vector, pcDNA3.1 empty control vector), 400 ng of luciferase reporter plasmid (pGL4.10, pGL4.13 or pGL4.10 carrying WT or Mut fragments of mouse or human Zbp1 promoter sequence) and 5 ng of internal control vector (Renilla luciferase vector pRL-TK) for 36 h. Then, the cells were harvested, and luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer’s instructions. The luminescence intensities were detected by Tecan Spark^{TM} 10 M, and firefly luciferase activity was normalized to Renilla luciferase activity.

Chromatin immunoprecipitation (ChIP)

To confirm the direct binding of Irf1 to the Zbp1 promoter region, ChIP was performed as reported previously[33]. Briefly, neuronal cells (HT22) transiently co-transfected with the Irf1 expression vector or pcDNA3.1 empty vector for 36 h were cross-linked with formaldehyde (1%) for 10 min, and then the cross-linking was terminated by glycine at 4°C for 5 min. Then, the cells were washed twice with PBS plus protease and phosphatase inhibitor cocktail (1:1000, Thermo Fisher Scientific, USA), collected with a cell scraper, sonicated (50 W peak power, 35 W duty power, 800 cycles, 10 min) on ice in SDS lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris-HCl-pH 8.1) to generate DNA fragments (200-500 bp), and the debris was removed by centrifugation at 4°C and 15000 ×g for 10 min. Next, the protein concentration of the supernatant was quantified by a BCA kit (Beyotime, Wuhan, China) and 400 μg of proteins were mixed with 5 μg anti-HA-tag antibody to detect HA-tagged Irf1 (Cell Signaling Technology, Danvers, USA) or equal amount of rabbit immunoglobulin G (IgG, Beyotime, Wuhan, China) as a negative control. The DNA fragments were immunoprecipitated with agarose-conjugated protein G beads (Roche Diagnostics, Reinach, Switzerland) and purified by spin columns (Qiagen, Hilden, Germany). The samples were then subjected to ChIP-PCR and ChIP-qPCR with a specific primer (Supplementary Table S2) targeting the -19 to -206 genomic DNA sequence upstream of the Zbp1 promoter, and the PCR product (188 base pairs) was analysed by 2% agarose gel electrophoresis.

Flow cytometry analysis

Flow cytometry analysis was performed to explore the effect of Zbp1 on Tnf-α-induced neuronal cell death. Neuronal cells (HT22) were pre-stimulated with 20 ng/ml Tnf-α for 24 h followed by transfection with Zbp1-overexpressing plasmid or two independent siRNAs against Zbp1 for 48 h, and then the cells were washed twice with PBS, stained with Annexin V-FITC at 4°C for 30 min and propidium iodide (PI) at room temperature for 5 min, followed by flow cytometry analysis on a CytoFLEX flow cytometer (Beckman Coulter, Atlanta, USA). All procedures were performed according to the instructions of the eBioscience^{TM} Annexin V Apoptosis Detection Kit (Invitrogen, USA).

Gene set enrichment analysis (GSEA)

To explore the relationship between the ZBP1 expression level and TNF-α-induced cell death in the cerebrum, we conducted GSEA of the transcriptional data of human AD (GSE132903). Briefly, all the AD samples were regrouped by ZBP1 transcript level, and samples at the more than 90% quantile were designated ZBP1_high, those with less than 10% quantile were designated ZBP1_low, and the remaining samples were designated ZBP1_medium. Next, the transcriptome data of 40 AD samples, consisting of 20 samples with high ZBP1 expression and 20 samples with low ZBP1 expression, were selected for GSEA with the use of GSEA software (https://www.gsea-msigdb.org/gsea/index.jsp) according to the manufacturer's instructions.

Statistical analysis

Pearson or Spearman correlation coefficients based on a linear regression model were calculated in R language (version 3.6.0, Missouri, USA) to analyse the association between two genes. All the data are presented as the means ± standard deviation (SD) of three independent experiments, and two-tailed unpaired Student's *t* tests were performed in GraphPad Prism 7.00 (San Diego, CA, USA) to assess significant differences between two groups in order to consider a *p* value less than 0.05 as a significant difference.

Results

Tnf-α induced the expression of Zbp1 in neuronal cells

Neurodegenerative disease characterized by the progressive and irreversible impairment or death of neurons is closely associated with excessive production of TNF-α, a pro-inflammatory cytokine that induces cell death[34] in the cerebrum[4]. A recent study suggested that ZBP1 expression is increased in neural cells in neuroinflammatory disease[17]. Additionally, TNF-α was shown to induce the expression of ZBP1 in other types of cells, including immune cells and lung alveolar epithelial cells, leading to cell death[15]. To determine whether ZBP1 functions as a pivotal regulator in neural cell death induced by TNF-α in neurodegenerative disease, we examined the correlation between the expression levels of TNF-α and ZBP1 by calculating Pearson and Spearman correlation coefficients using available public

transcriptome datasets from cerebral cells of neurodegenerative disease mouse models or human patients. The results showed a clear positive association between the transcriptional levels of Tnf-α and Zbp1 in the cerebral cells of mouse models of AD and PD (both $p \leq 0.01$, Fig. 1a, b). Similarly, in human PD patients, a positive correlation between TNF-α and ZBP1 expression was observed in cerebral cells ($r = 0.45$ and $p < 0.05$, Fig. 1c). Moreover, we observed that the mRNA expression of TNF-α was significantly upregulated in AD mice ($p < 0.001$, Fig. 1e).

To further investigate the regulatory relationship between TNF-α and ZBP1, we analysed the published data of a neurodegenerative disease model induced by AC. AC is a nematode exclusively parasitizing the cerebrum and causing symptoms of neurodegenerative disease accompanied by excessive production of Tnf-α (Fig. 1d). Published data on AC infection in a mouse model indicated that the mRNA level of Zbp1 was elevated along with an increase in Tnf-α expression after AC infection but decreased when the mice were treated with a Tnf-α inhibitor ($r = 0.81$ and $p < 0.001$, Fig. 1d). These results suggest that Tnf-α likely induced Zbp1 production in neuronal cells.

Next, we tested whether Tnf-α could induce Zbp1 expression in neurons. To this end, we treated HT22 cells (a mouse hippocampal neuronal cell line) with 10 ng/ml Tnf-α for the indicated hours and then measured the mRNA level of Zbp1 by RT-qPCR. As expected, the level of Zbp1 mRNA gradually increased under Tnf-α stimulation in a time-dependent manner (Fig. 1f). Similarly, the Zbp1 protein level also increased in HT22 cells under the same treatment (Fig. 1h). Moreover, the increase in Zbp1 mRNA and protein expression was dependent on the dosage of Tnf-α, as a higher concentration of Tnf-α led to a higher expression level of Zbp1 (Fig. 1g and 1i). Together, these results confirm that Tnf-α directly induces Zbp1 expression in neuronal cells andsuggest that the Tnf-α-Zbp1 axis may function in neuronal cell death in neurodegenerative disease.

Zbp1 participates in neuronal cell death induced by Tnf-α

To investigate the role of Zbp1 in Tnf-α-induced neuronal cell death, we examined the effects of Zbp1 siRNA knockdown and overexpression. First, we confirmed the knockdown efficiency of the two siRNA constructs (Fig. 2a) and the high expression generated by the overexpression construct (Fig. 2b). We then performed flow cytometry analysis with PE (fluorescent dye for dead cells) and Annexin V (fluorescent dye for apoptotic cells) in HT22 cells (with or without Tnf-α pre-stimulation) transfected with Zbp1 siRNAs, Zbp1 overexpression plasmid or negative control vectors. As expected, TNF-α treatment dramatically induced cell death (20% vs 5%, Fig. 2c-d**).** Interestingly, in the cells treated with TNF-α, knockdown of Zbp1 suppressed cell death to a level comparable to that of the untreated TNF-α cells ($p < 0.0001$, Fig. 2c **bottom and** Fig. 2d). In contrast, Zbp1 overexpression induced more cell death in both TNF-α-treated and untreated cells (Fig. 2e **left, f**). These results demonstrate that Zbp1 plays a central role in Tnf-α-induced neuronal cell death.

As reported previously, cell death includes apoptosis, necroptosis, pyroptosis, ferroptosis and so on[35–37]. In this study, we found that Zbp1 strongly promoted apoptosis (Fig. 2c, e **right**) and partly enhanced necroptosis (Fig. 2e **left**) in HT22 cells. To confirm these observations, we measured the protein levels of

additional molecular markers of apoptosis (Caspase-3 and cleaved Caspase-3 (Cl. Caspase-3)) and necroptosis (Rip3 and phosphorylated Rip3 (pRip3)) in Tnf-α pre-stimulated HT22 cells with subsequent overexpression or knockdown of Zbp1. We found that downregulation of Zbp1 expression significantly reduced the protein level of Cl. Caspase-3 and pRip3 (Fig. 2g), while upregulation of Zbp1 expression produced the opposite results (Fig. 2h). Neither of these conditions had an effect on total Caspase-3 or Rip3 (Fig. 2g, h). In agreement with the results, genes with dysregulated expression in human AD patients with increased ZBP1 expression were also enriched in apoptosis signalling pathways, including core genes such as TNFRSF1A, TNFRSF10B, TNFRSF10C and CASP3 (Fig. 2i). Taken together, these data demonstrated that Zbp1 mediates neuronal cell death induced by Tnf-α by activating apoptotic genes.

Irf1 acts as a transcription factor (TF) positively regulating Zbp1 expression

Next, we aimed to identify the upstream regulator(s) that activate Zbp1 expression in response to TNF-α. We first scanned the Zbp1 promoter sequence (2000 bp upstream of the Zbp1 TSS) for potential TF binding sites using the Consite and AnimalTFDB databases. We focused on the 11 potential TFs identified by both databases (Fig. 3a) and evaluated their expression levels using our transcriptome data from the mouse neurodegenerative disease model (Fig. 3b). Four TFs showed consistent changes in response to AC infection, with three of them becoming upregulated and 1 downregulated.

To determine whether these factors are also induced by TNF-α, we treated HT22 cells with Tnf-α (20 ng/ml) and performed RT-qPCR analysis to measure the mRNA levels of the four TFs. We found that Tnf-α substantially enhanced the mRNA expression of Irf1 (Fig. 3c) and Irf2 (Fig. 3d) and suppressed the expression of Max (Fig. 3e). However, the effect on Arnt expression was not significant (**Supplementary Fig. 1**). The RT-qPCR results were consistent with the transcriptome data (Fig. 3b). We then transiently knocked down the 4 TFs via siRNA transfection (Fig. 3f) and explored whether these treatments could enhance or suppress Zbp1 expression induced by Tnf-α. We found that the downregulation of the expression of Irf1 ($p < 0.05$, Fig. 3h) but not the other 3 TFs (Fig. 3g) obviously inhibited Zbp1 transcription regardless of Tnf-α pre-stimulation. Thus, Irf1 is a promising candidate TF that activates Zbp1.

Irf1 activates Zbp1 by binding to the Zbp1 promoter

Our next question was how Irf1 regulates the transcription of Zbp1. To this end, we first scanned the promoter of Zbp1 for potential Irf1-binding motifs by using AnimalTFDB and identified six motifs. We then generated six truncation fragments that together covered the full-length Zbp1 promoter (F2000, F1714, F1341, F1029, F743 and F396) and cloned them into the luciferase reporter construct pGL4.10 (Fig. 4a). While co-transfection of the Irf1 expression vector increased reporter expression driven by all six fragments, the F2000 fragment showed the highest activity (all $p < 0.01$, Fig. 4b). Of note, compared with the negative control (pGL4.10), even the shortest fragment, F398, enhanced reporter expression by 20-fold ($p < 0.0001$, Fig. 4b). These data suggested that the core Irf1 binding motifs in the Zbp1 promoter are located in the range of -2000 to -1341 (WT-1), -1341 to -1029 (WT-2) and − 398 to the TSS (WT-3) of the

Zbp1 promoter (Fig. 4c). To further identify the core motifs regulated by Irf1, we mutated WT-1, WT-2 and WT-3 to Mut-1, Mut-2 and Mut-3 by deleting motifs 1, 2 and 3 together and motifs 4, 5 and 6 together (Fig. 4c). We then generated reporter constructs that contained these WT and Mut fragments and tested their response to Irf1 overexpression in 293T cells. While all three WT reporters were activated by IRF1 compared with the control reporter, the WT-3 reporter showed the strongest response, with an increase of approximately 4700-fold (Fig. 4d). Remarkably, IRF1 was unable to elevate the expression of the Mut-2 and Mut-3 reporters, indicating that the motifs in the WT-2 and WT-3 fragments are important for IRF1 regulation.

Given that the WT-3 fragment showed strong induction of reporter activity by IRF1, we next studied in further detail this region to determine which motif is critical for IRF1 regulation by generating additional luciferase constructs, namely, Mut-4, Mut-5 and Mut-6, with individual deletions of motif 4, motif 5 and motif 6 in the WT-3 fragment, as shown by the diagram (Fig. 4e). Consistent with previous results, IRF1 increased the activity of WT-3 by nearly 5000-fold. Although the activities of the Mut-4 and Mut-6 reporters were significantly reduced (Fig. 4f), the Mut-5 reporter that completely lacked motif 5 lost its response to IRF1 (Fig. 4f). These data suggest that IRF1 activates Zbp1 by directly binding several core motifs in the Zbp1 promoter and that its binding to motif 5 is the most critical.

Irf1 directly activates Zbp1 transcription in neuronal cells

Given that Irf1 could activate the transcription of Zbp1 in reporter assays, we next explored whether Irf1 could directly bind to the core Irf1-responsive region (WT-3, -398 to 0 bp) of the Zbp1 promoter in neuronal cells. To this end, we performed ChIP assays using an HA antibody and IgG control from a mouse neuronal cell line (HT22) expressing ectopic HA-Irf1 and a control cell line and analysed IRF1 enrichment by qPCR. The PCR primers covered 206 to -19 bp of the Zbp1 promoter, as shown by the schematic (Fig. 5a **top**). Agarose gel electrophoresis (AGE) analysis of the PCR products (188 bp) showed that there was no enrichment by IgG immunoprecipitation at either the Zbp1 or GAPDH promoter. In contrast, the HA antibody significantly enriched the Zbp1 promoter but not the GAPDH promoter (Fig. 5a **bottom**). We further confirmed this result by qPCR ($p < 0.001$, Fig. 5b). This finding demonstrates that Irf1 directly binds to the Zbp1 promoter in neuronal cells.

After confirming that Irf1 binds to the promoter of Zbp1, we next asked whether this binding led to enhanced expression of Zbp1 in neurons. We conducted RT-qPCR analysis of HT22 cells transfected with an Irf1-expressing plasmid and a control vector. The results indicated that ectopic expression of Irf1 strongly increased the mRNA level of Zbp1 20-fold ($p < 0.01$, Fig. 5c). HT22 cells with Tnf-α pre-stimulation and Irf1 overexpression displayed higher levels of Zbp1 mRNA than Tnf-α pre-stimulated cells without Irf1 overexpression ($p < 0.05$, Fig. 5c). Consistent with the increase in Zbp1 mRNA levels, the protein level of Zbp1 was also elevated in neuronal cells overexpressing Irf1 with or without Tnf-α pre-stimulation (Fig. 5d). In contrast, Irf1 knockdown decreased Zbp1 protein levels in cells without Tnf-α pre-stimulation. In response to TNF-α treatment, Zbp1 protein levels increased. However, the increase was suppressed by Irf1 siRNAs (Fig. 5e). These data confirm that Irf1 increases the mRNA and protein levels of Zbp1 in neuronal cells.

Next, to explore whether the Irf1-mediated increase in Zbp1 was specific to neuronal cells in the mouse cerebrum, we transfected two mouse microglial cell lines (BV2 and N9) with the Irf1 overexpression plasmid or the negative control vector prior to RT-qPCR analysis. In contrast to the upregulation of Zbp1 expression by Irf1 in HT22 cells ($p < 0.001$, Fig. 5f **left**), there was no significant increase in Zbp1 mRNA levels after Irf1 transfection (Fig. 5f **middle and right**), suggesting that Irf1 activates the expression of Zbp1 specifically in mouse neuronal cells.

We also examined the correlation of the transcript levels of Irf1 and Zbp1 in cerebral cells from mouse models of neurodegenerative diseases, including AD, PD and HD. We discovered that the expression of Irf1 was strongly associated with that of Zbp1 in AD ($r = 0.35$, $p < 0.001$, Fig. 5g), PD ($r = 0.57$, $p = 0.001$, Fig. 5h) and HD ($r = 0.61$, $p = 0.01$, Fig. 5i). This observation strengthened our finding that Irf1 positively regulates neuronal Zbp1 transcription in Tnf-α-related neurodegenerative disease.

IRF1 directly activates ZBP1 transcription in human cells

Since our results show that Tnf-α-induced Irf1 activates Zbp1 transcription in mouse neuronal cells and that there is a positive correlation between TNF-α and ZBP1 transcription in cerebral cells from human PD patients, we next explored the relationship between human IRF1 and ZBP1 using 293T cells. The mouse and human IRF1 protein amino acid (AA) sequences share more than 85% identity (**Supplementary material 4**), indicating that IRF1 is a highly conserved TF. Furthermore, the DNA sequences that spanned 2000 bp upstream of the Zbp1 TSS showed 44% similarity between the mouse and human genomes (**Supplementary material 5**). These comparisons suggest that human IRF1 likely regulates ZBP1 transcription in the same manner as its mouse counterpart. Next, we scanned the ZBP1 promoter for potential IRF1 binding sites and identified three motifs that reside within − 2000 to -1771 bp (motif 1, WT-1) and − 274 to 0 bp (motif 2 and motif 3, WT-2) in the ZBP1 promoter (Fig. 6a). We generated a human IRF1 overexpression plasmid and luciferase reporters driven by the human ZBP1 promoter (2000 bp upstream of the TSS of ZBP1) and two truncations that covered WT-1 and WT-2. We first confirmed elevated IRF1 expression in 293T cells transfected with the IRF1 construct (Fig. 6b). We then tested the reporter activities. IRF1 significantly increased the expression of the full-length, WT-1 and WT-2 reporters 4-fold ($p < 0.0001$, Fig. 6c), 15-fold ($p < 0.001$, Fig. 6d) and 2-fold ($p < 0.01$, Fig. 6d), respectively. This suggests that motif 1 in the WT-1 fragment may be the most critical motif for IRF1 binding and regulation. To verify this, we generated a Mut version of the reporter from WT-1, namely, Mut-1, that has a deletion of motif 1 and tested its response to IRF1. Expression of the Mut-1 reporter was significantly lower than that of the WT-1 reporter, confirming that motif 1 was the main IRF1-binding site in the human ZBP1 promoter.

Next, we directly assessed the expression of ZBP1 mRNA in response to IRF1 overexpression. In 293T cells transfected with the IRF1 overexpression plasmid, ectopic IRF1 expression significantly increased the level of ZBP1 transcripts 160-fold compared to the control ($p < 0.0001$, Fig. 6f). Moreover, TNF-α pre-treatment further increased the level of ZBP1 mRNA in the presence of high IRF1 expression (530-fold, $p < 0.0001$, Fig. 6f). Together, these results confirm that the TNF-α-IRF1-ZBP1 axis is present in human cells.

We then tested this regulatory axis in more cell lines. We used two human neural progenitor cell lines (U87MG and U118MG) but did not detect activation of ZBP1 by IRF1 (data not shown here). Moreover, it was previously shown that TNF-α together with IFN-γ can activate the expression of ZBP1 in lung-derived cells[15]. We tested the A549 cell line, a human lung-derived carcinoma cell line, to investigate whether IRF1 regulates ZBP1 transcription. Similar to the result from 293T cells, IRF1 activated ZBP1 expression with or without treatment with TNF-α, confirming the presence of the TNF-α-IRF1-ZBP1 axis in another human cell type.

Finally, we searched for a potential link between the TNF-α-IRF1-ZBP1 axis and neurodegenerative diseases. We found that IRF1 and ZBP1 levels were positively correlated in cerebral cells from PD ($r = 0.37$, $p = 0.05$, Fig. 6h) and AD ($r = 0.37$, $p < 0.001$, Fig. 6i) patients. Compared with the expression levels of ZBP1 and IRF1 in the samples with no neurodegenerative disease, the expression levels of IRF1 (Fig. 6j) and ZBP1 (Fig. 6k) significantly increased in AD patients.

Altogether, the results above demonstrated that IRF1 directly activates ZBP1 transcription in human cells and that the TNF-α-IRF1-ZBP1 axis may play a critical role in neurodegenerative disease.

Discussion

The molecular mechanisms that cause neuronal cell death in neurodegenerative disease remain largely unknown. In this study, we identified ZBP1 and IRF1 as two critical mediators in the process of TNF-α-induced neuronal cell death. We found that the expression of ZBP1 is positively correlated with TNF-α and IRF1 expression in cerebral cells from patients with neurodegenerative disease. We demonstrated that TNF-α could activate the expression of ZBP1, which further induces neuronal cell death. By combining bioinformatic and experimental analyses, we determined that IRF1 is a critical TF that induces the transcription of ZBP1 through direct binding to core IRF1 binding motifs in the ZBP1 promoter.

The pathology of neurodegenerative diseases involves hyperproduction of amyloid-β (Aβ) and tau proteins[38–40]. In recent years, accumulating evidence has revealed that inflammation related to TNF-α in the cerebrum is the primary cause of the development and progression of neurodegenerative disease[4, 6, 8]. Increased expression of TNF-α, a potent pro-inflammatory cytokine, is responsible for apoptosis, necroptosis and multiple types of cell death in diverse cell types via distinct molecular pathways[41]. A recent study also showed that the aberrant expression of microglial TNF-α drives neuronal apoptosis in the brain[5]. However, whether TNF-α directly induces neuronal cell death and what pathway mediates neuronal cell death under TNF-α stimulation are unclear. In the present study, we provided direct evidence supporting that TNF-α directly induced neuronal cell death. Considering that TNF-α inhibitors show favourable outcomes in neurodegenerative disease, such as inhibiting synaptic deficits and postponing the development of AD[42, 6] and ZBP1-mediated TNF-α-induced cerebral cell death, it would be useful to investigate whether antagonizing ZBP1 to block TNF-α-induced neuronal cell death has a better beneficial therapeutic effect on neurodegenerative disease.

As a cytosolic DNA sensor, ZBP1 (also called DAI) possesses numerous biological functions in the immune response and cell death[43]. The critical roles of ZBP1 principally depend on its transcriptional regulation and post-transcriptional modification. To date, many investigations have painted a detailed picture of the latter. For instance, upon exposure to stimuli such as B-DNA, the ZBP1 protein monomer dimerizes and is phosphorylated at two sites, serine 352 and 353, followed by enhanced binding to TBK1[44], which leads to the formation of the ZBP1-IRF3-TBK1 complex and regulation of immune-related gene expression; after infection by IAV, K17 and K43 in the Zα region of ZBP1 are ubiquitinated, which results in the assembly of a complex contributing to cell death[45]. However, a comprehensive understanding of the molecular basis of the transcriptional regulation of ZBP1 has remained elusive. Although IFNs and TNF induce the transcription of ZBP1[16, 46, 15], little is known about how ZBP1 is transcriptionally regulated. In this context, we systematically investigated this gap in our knowledge and found that IRF1 acted as the direct TF inducing ZBP1 transcription by binding to the − 102 to -184 bp region of the murine neuronal ZBP1 promoter. Furthermore, the direct regulatory effect of IRF1 on ZBP1 in human cells was confirmed.

IRF1, acting as a TF, was reported to bind the promoter of PUMA and enhance PUMA transcription, resulting in the intrinsic apoptosis of gastric cancer cells[29]. Similarly, our data confirmed that IRF1 transcription could be induced by TNF-α, and IRF1 acted as the key TF to activate ZBP1 transcription in mouse neuronal cells and human cells. Interestingly, we also found that an increase in IRF1 could induce the transcription of ZBP1 with or without TNF-α pre-stimulation, suggesting that the IRF1-ZBP1 axis might be a universal feature of diverse pathological contexts in addition to TNF-α accumulation. In agreement with our findings, a previous study discovered that knocking out IRF1 markedly inhibited the transcription of ZBP1 in a mouse model of influenza virus infection[22]. However, the study did not show whether IRF1 directly acted as the TF of ZBP1 and how IRF1 induced ZBP1 transcription. Since the primary cause of neurodegenerative disease is the overproduction of TNF-α[4], the TNF-α-IRF1-ZBP1 axis might be ubiquitous in neurodegenerative diseases, which awaits further investigation. Due to the limitation of the blood-brain barrier of the nervous system and mouse models, in this study, we mainly used *in vitro* assays and bioinformatics analysis of omics data in mouse and human neurodegenerative disease samples to provide the molecular data for future studies of genetically modified animals or mouse models of neurodegenerative diseases.

In summary, we have demonstrated a vicious axis of IRF1-ZBP1 in neurons that might account for pathological neuronal cell death. Our findings also indicate that the positive regulatory effect of the TF IRF1 on ZBP1 is exclusive to mouse neuronal cells (absent in glial cells) and is present in human cells, expanding our knowledge about the direct regulatory mechanism inducing ZBP1 transcription and providing new insight into the role of ZBP1 in neurodegenerative disease.

Declarations

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Authors' contributions This project was designed and conceived by LZY, LZY and ZHL wrote the article. Experiments were performed by ZHL. JL download and analyzed all the public data. LYT, CYX, WH prepared figures and tables. YL, DP and MW participated in study design and coordination. All authors read and approved the final paper.

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Data availability The data that support the findings of this study are available upon request from the corresponding author, Dr. Zhiyue Lv.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Code Availability Not applicable.

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Figures

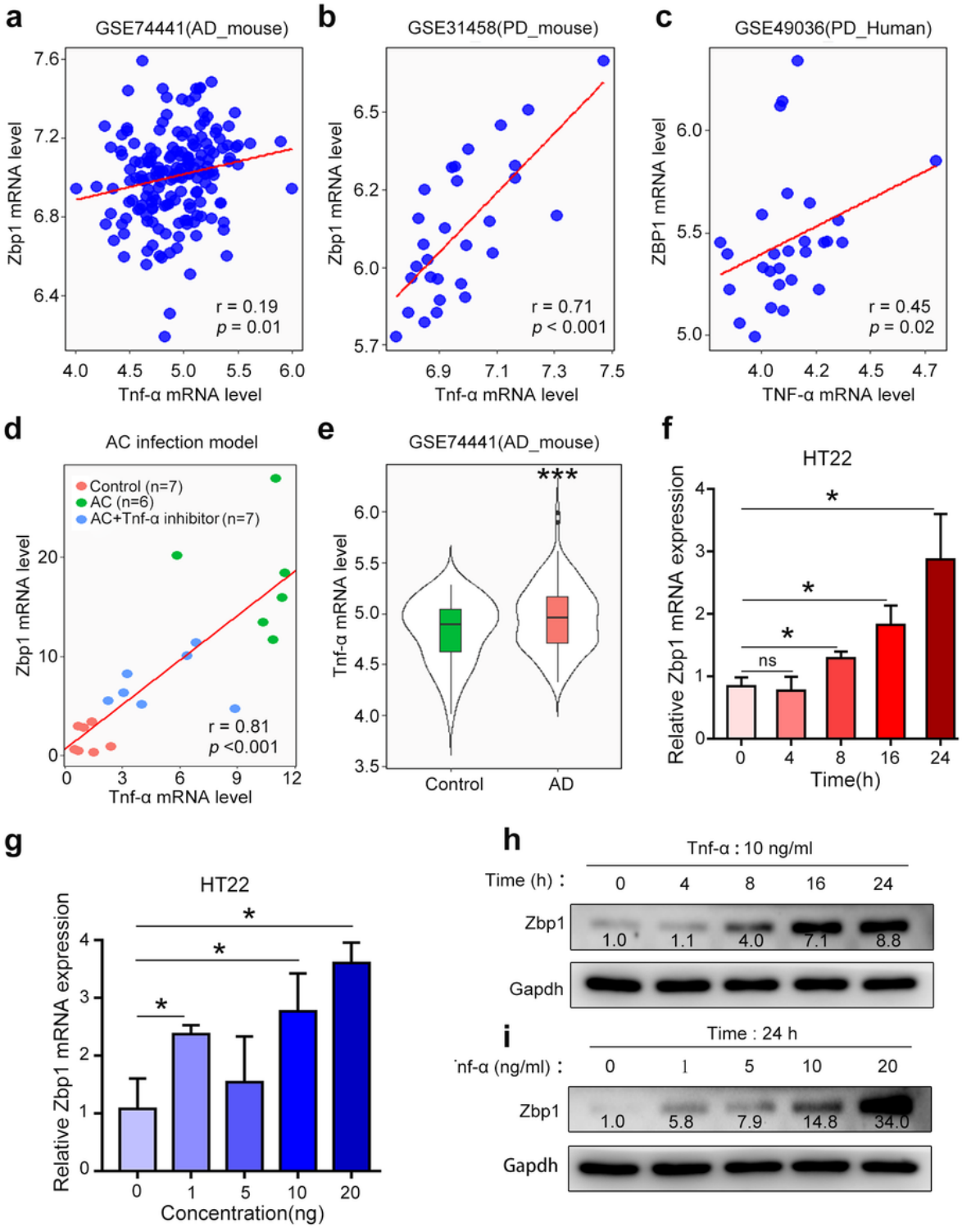

Figure 1

Tnf-α strongly induced the expression of Zbp1 in neurons. a-d The expression of Tnf-α was significantly positively associated with Zbp1 expression in neurodegenerative diseases evaluated by Pearson correlation analysis of public datasets of AD (a), PD (b, c) and meningoencephalitis caused by AC infection (d). Each dot in a-d represents a tissue sample obtained from the GEO database. e Tnf-α expression was dramatically increased in some neurodegenerative diseases, including AD (GSE74441). f Levels of Zbp1 mRNA and protein in neurons (HT22) stimulated with 10 ng/ml Tnf-α increased at the indicated hours, as shown by RT-qPCR (f) and immunoblotting analysis (h). HT22 cells were cultured in complete medium plus Tnf-α at the indicated concentration gradient for 24 hours, and then total RNA and protein were extracted and subjected to RT-qPCR (g) and immunoblotting (i) for RNA and protein analyses. The data in the bar graph represent the mean ± standard deviation (SD) of three experimental repeats (hereinafter the same). *$p < 0.05$ (unpaired two-tailed student’s t test). Abbreviations: AD, Alzheimer's disease; PD, Parkinson's disease; AC, Angiostrongylus cantonensis.

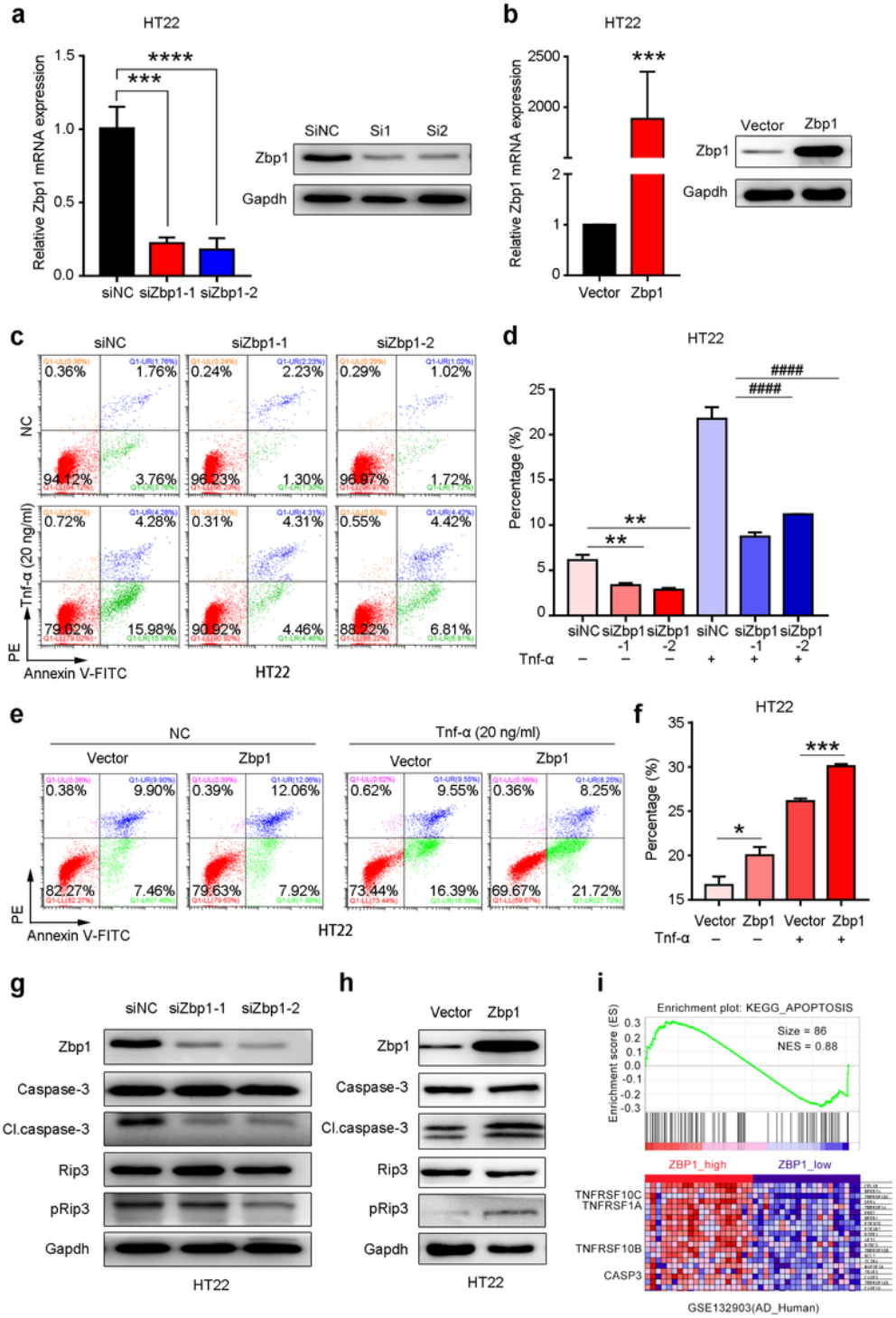

Figure 2

Zbp1 mediated Tnf-α-induced neuronal cell death. a The knockdown efficiency of two independent siRNAs against Zbp1 was confirmed by RT-qPCR. b Transient transfection of HT22 cells with the Zbp1 overexpression plasmid for 48 hours successfully generated high levels of Zbp1, as shown by RT-qPCR. c, d HT22 cells with or without pre-stimulation with 20 ng/ml TNF-α for 24 hours were transfected with Zbp1 siRNAs or negative control siRNA for 48 hours followed by flow cytometry analysis to determine the

percentage of dead cells (c). The data above (c) were analysed by unpaired two-tailed Student's t test and visualized via bar graph (d). e, f HT22 cells pre-stimulated as described above (c) were transfected with the Irf1 overexpression plasmid or pcDNA3.1 empty vector as a negative control, after which flow cytometry (e) and bar graph analysis (f) were performed to compare the cell death of HT22 cells between the negative control and Irf1 overexpression groups. g, h The key proteins involved in cell death (including Cl. caspase-3 and pRIP3) in HT22 cells pre-treated with Tnf-α (20 ng/ml) followed by transfection with Zbp1 siRNAs (g) or overexpression plasmids (h) were detected by immunoblotting. i Gene set enrichment analysis (GSEA) revealed that the core genes of the apoptosis signalling pathway were enriched by high ZBP1 expression. Data represent the mean ± SD (n=3); *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; ####p < 0.0001. Abbreviations: NC, negative control; Cl. caspase-3, cleaved caspase-3.

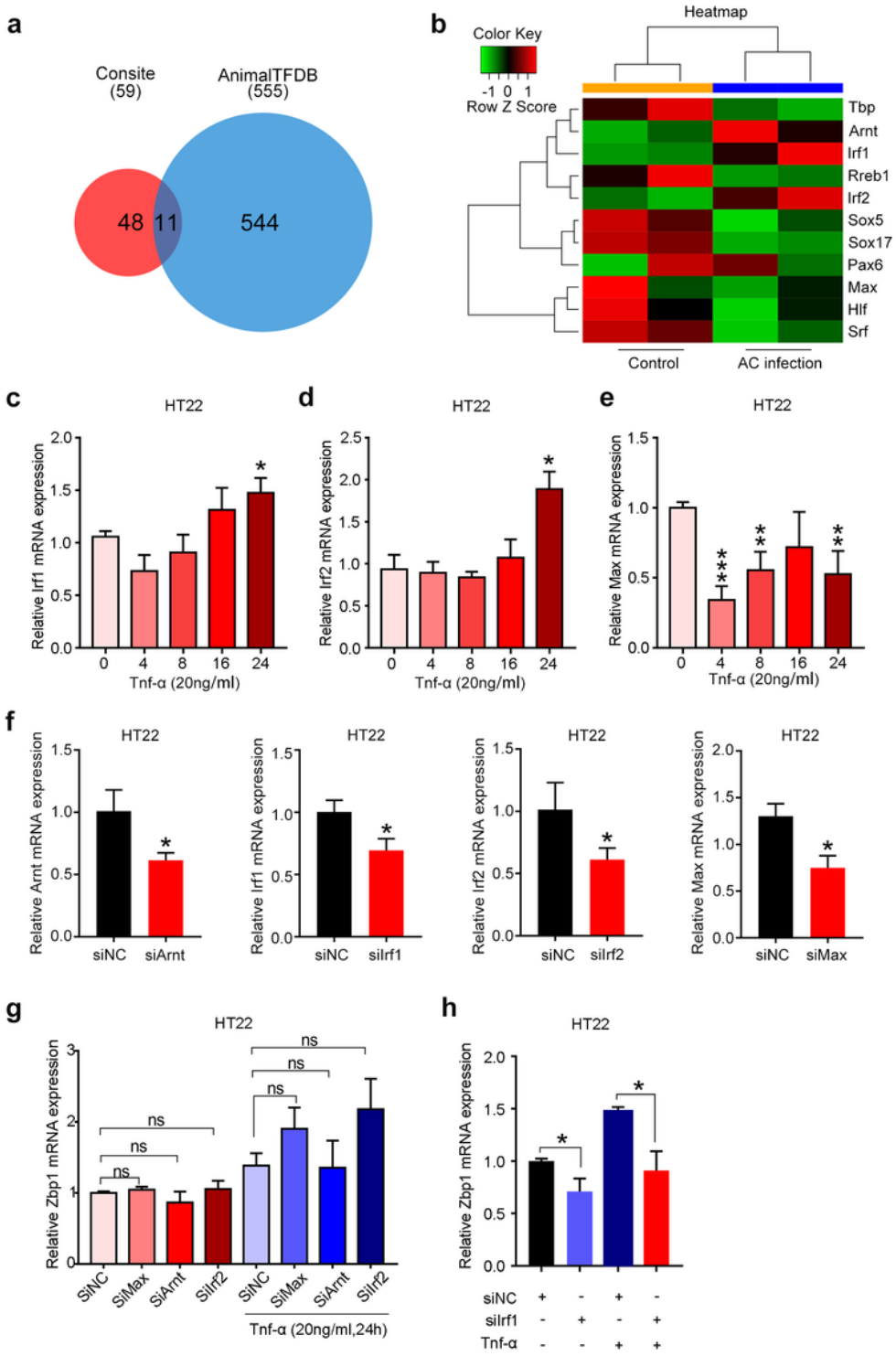

Figure 3

Irf1 acted as a transcription factor (TF) of Zbp1. a The candidate TFs likely regulating the expression of Zbp1 were predicted by two online tools (Consite and AnimalTFDB), and 11 TFs were identified, as displayed by a Venn diagram. b Heatmap showing the differential expression of these 11 TFs in mouse brain tissues between the AC infection and control groups. c-e RT-qPCR was used to evaluate the effect of stimulation with Tnf-α (20 ng/ml) for the indicated hours on the expression of the three TFs described

above. f The efficiency of siRNAs against Arnt, Irf1, Irf2 and Max was confirmed by RT-qPCR after 24 hours of treatment with the siRNAs. g HT22 cells were transfected with siRNAs against Arnt, Irf2 and Max alone or after Tnf-α (20 ng/ml) pre-stimulation for 24 hours, and at 48 hours post transfection, the mRNA level of Zbp1 was determined via RT-qPCR. h Irf1 knockdown significantly reversed the transcriptional upregulation of Zbp1 induced by Tnf-α (20 ng/ml) stimulation. Data represent the mean ± SD (n=3); *p < 0.05, **p < 0.01, ***p < 0.001. Abbreviations: ns, not statistically significant; NC, negative control; AC, Angiostrongylus cantonensis.

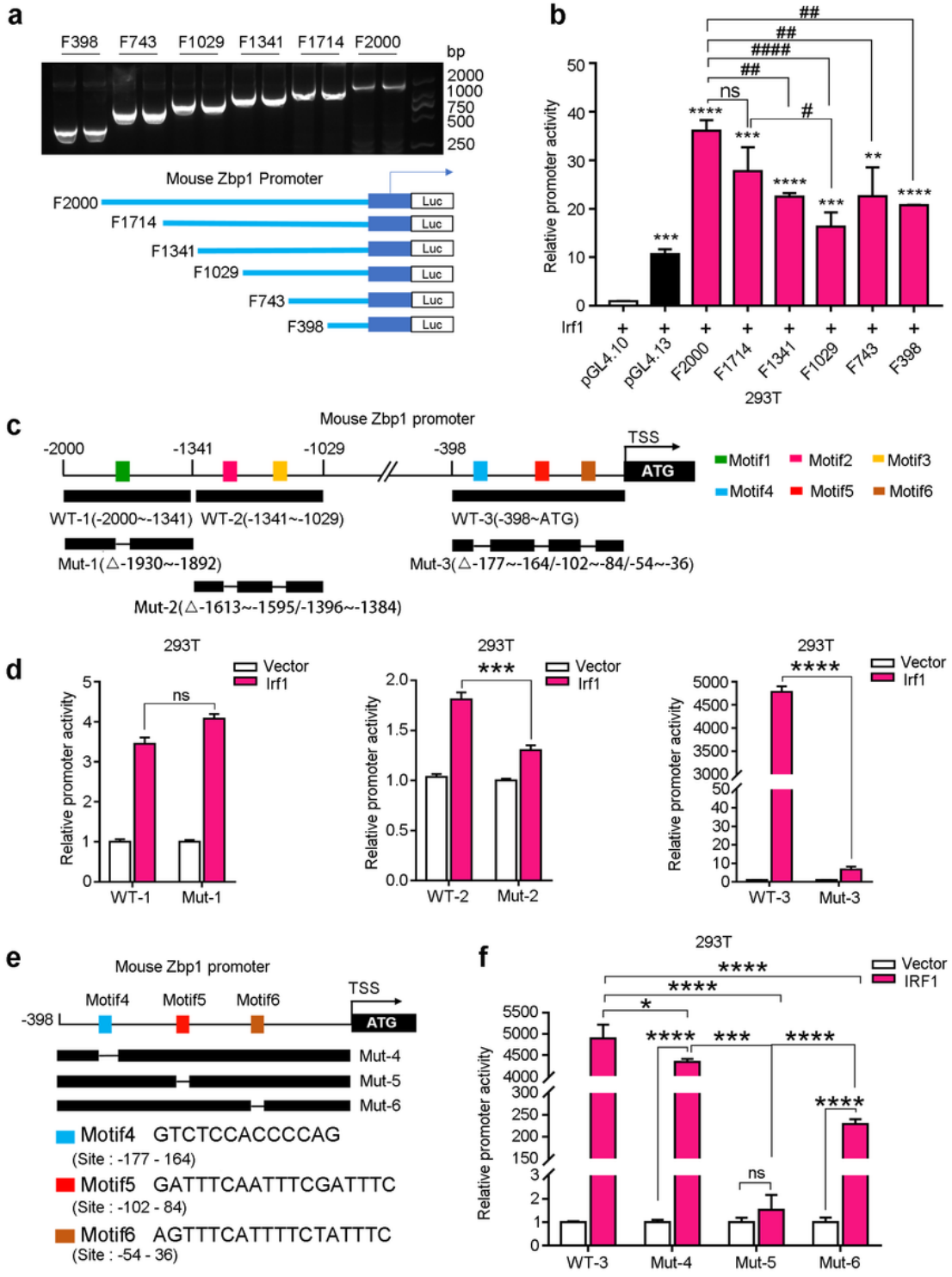

Figure 4

Irf1 activated Zbp1 transcription by enhancing the activity of the Zbp1 promoter. a The mouse Zbp1 promoter (2000 bp upstream of the transcription start site (TSS) of Zbp1) was truncated into six fragments and then cloned into the pGL4.10 vector, as shown by agarose gel electrophoresis (AGE). b The transcriptional activity of the different fragments of the Zbp1 promoter under Irf1 overexpression was evaluated by luciferase reporter assay in 293T cells transfected with the indicated plasmids. pGL4.10 and pGL4.13 served as negative and positive controls, respectively. c Schematics of the mouse Zbp1 promoter showing the six motifs that were predicted to be bound by Irf1. d The transcriptional activity of three wild-type (WT) and corresponding mutant (Mut) promoters containing the six motifs was tested by luciferase reporter assay in 293T cells. e Schematic representation of the mouse Zbp1 genomic regions showing that Irf1 had three binding sites in the Zbp1 promoter (-398 to the TSS of Zbp1). f 293T cells were co-transfected with the pcDNA3.1 empty vector as a negative control, Irf1-overexpressing plasmid, or WT or Mut Zbp1 promoter plasmids for 36 hours, and then a luciferase reporter assay was carried out to explore the core regions regulated by Irf1 in the mouse Zbp1 promoter. Bars show the mean ± SD; n = 3 replicates; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001. Abbreviations: TSS, transcription start site; WT, wild type; Mut, mutant; ns, not significant.

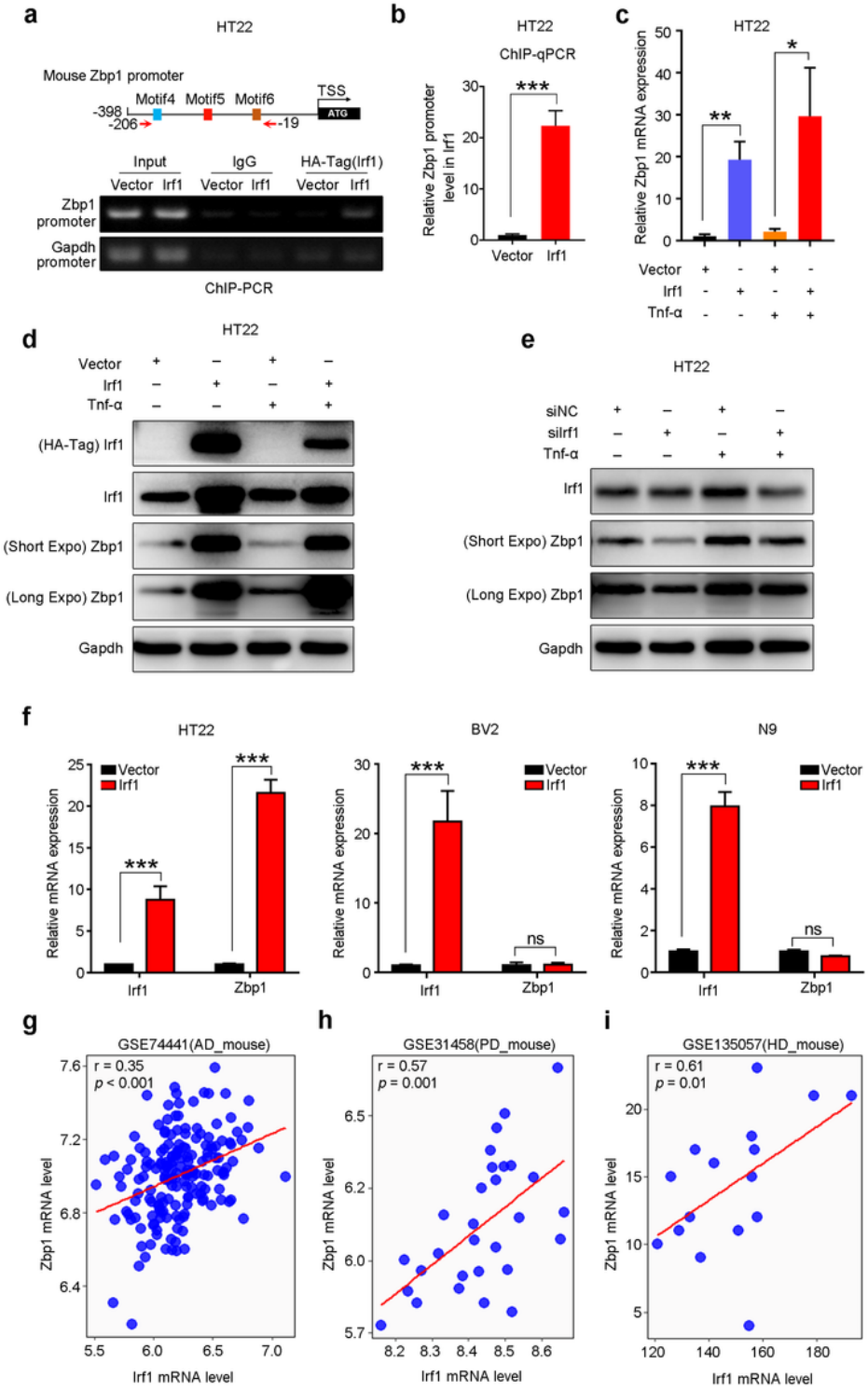

Figure 5

Irf1 directly bound to the Zbp1 promoter and positively regulated the expression of Zbp1 in neuronal cells. a Schematic diagram of mouse Zbp1 genomic regions (-398 to the TSS of Zbp1) displaying the location of three motifs (predicted by AnimalTFDB) most likely bound by mouse Irf1 and showing the amplification sites (red arrows) of primers used for ChIP-PCR/qPCR (upper panel). ChIP was conducted with antibody against Irf1 or immunoglobulin G (IgG) (as a negative control), and the binding of Irf1 to

the Zbp1 promoter was confirmed using PCR with the immunoprecipitated DNA fragments followed by ACE (lower panel). b The DNA fragments pulled down by the anti-Irf1 antibody and anti-IgG antibody were subjected to RT-qPCR, showing that Irf1 bound to the Zbp1 promoter. c Bar graph showing the elevated transcript level of Zbp1 in the presence of Irf1 overexpression with or without Tnf-α (20 ng/ml) stimulation. d, e The protein level of Zbp1 was increased in the presence of Irf1 overexpression (d) but reduced when Irf1 was knocked down (e) with or without pre-stimulation with Tnf-α (20 ng/ml) for 24 hours. f Neuronal cells (HT22) overexpressing Irf1 expressed higher levels of Zbp1 mRNA than control cells. Microglial cells (BV2 and N9) transfected with the Irf1-overexpressing plasmid failed to produce more Zbp1 than the negative control cells. g-i Pearson correlation analysis revealed a highly positive relationship between Irf1 and Zbp1 in the cerebrum of mouse models of neurodegenerative diseases, including AD (g), PD (h) and HD (i). Data show the mean ± SD of triplicate experiments; *p < 0.05, **p < 0.01, ***p < 0.001. Abbreviations: TSS, transcription start site; ns, not significant; AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's disease.

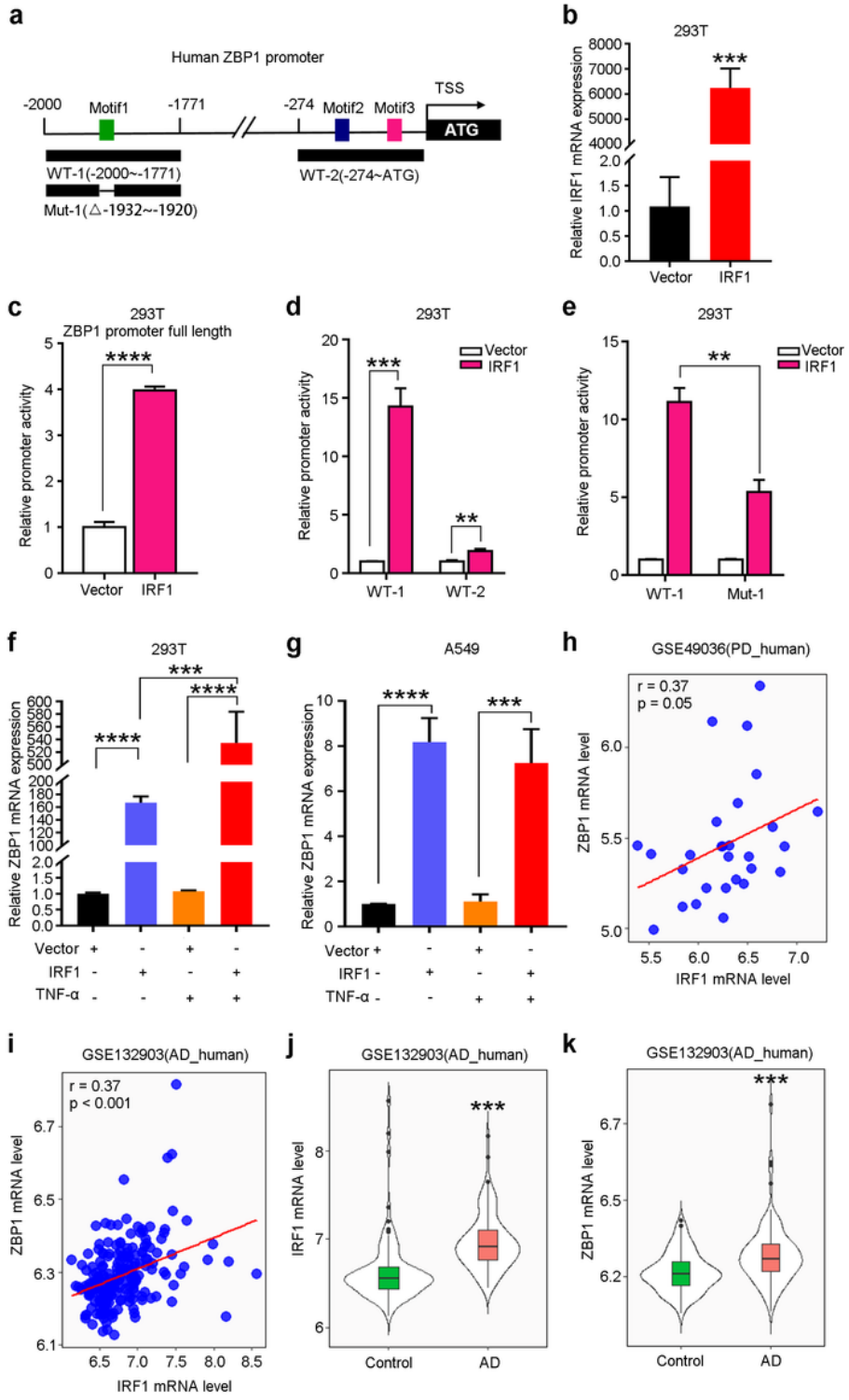

Figure 6

IRF1 transcriptionally activated ZBP1 by increasing the activity of the ZBP1 promoter in human cells. a Diagram of the human ZBP1 promoter depicting the locus of three possible binding motifs of the TF IRF1 in the ZBP1 promoter (-2000 bp to the TSS of ZBP1), showing two truncated ZBP1 promoters (WT-1 and WT-2) and a mutant ZBP1 promoter (Mut-1). b The human IRF1 overexpression plasmid was successfully used in 293T cells, as shown by RT-qPCR. c The activity of the full-length ZBP1 promoter under IRF1

overexpression was measured with a luciferase reporter assay, showing that IRF1 significantly induced the activity of the ZBP1 promoter. d 293T cells were co-transfected with the IRF1 overexpression plasmid, luciferase reporter plasmid (WT-1 or WT-2 cloned into pGL4.10) and Renilla luciferase plasmid for 36 hours followed by determination of luminescence intensity. e The activity of the WT-1 and Mut-1 reporters of the ZBP1 promoter with or without IRF1 overexpression was compared by reporter assay. f Human IRF1 dramatically enhanced the mRNA level of ZBP1 in the presence or absence of Tnf-α (20 ng/ml) (pre-stimulation with Tnf-α for 24 hours prior to IRF1 transfection) in 293T cells. g The same treatment as described above (f) was conducted in A549 cells (a cell line from human lung carcinoma with a relatively high level of ZBP1), and the expression of ZBP1 was investigated by RT-qPCR. h, i A positive relationship between the transcript levels of IRF1 and ZBP1 was observed in neural cells from patients with PD (h) and AD (i). j, k IRF1 and ZBP1 expression levels were markedly increased in neural tissues of patients with AD compared with those of normal patients. Bars represent the mean ± SD (n=3); **p < 0.01, ***p < 0.001. Abbreviations: AD, Alzheimer's disease; PD, Parkinson's disease; WT, wild type; Mut, mutant.

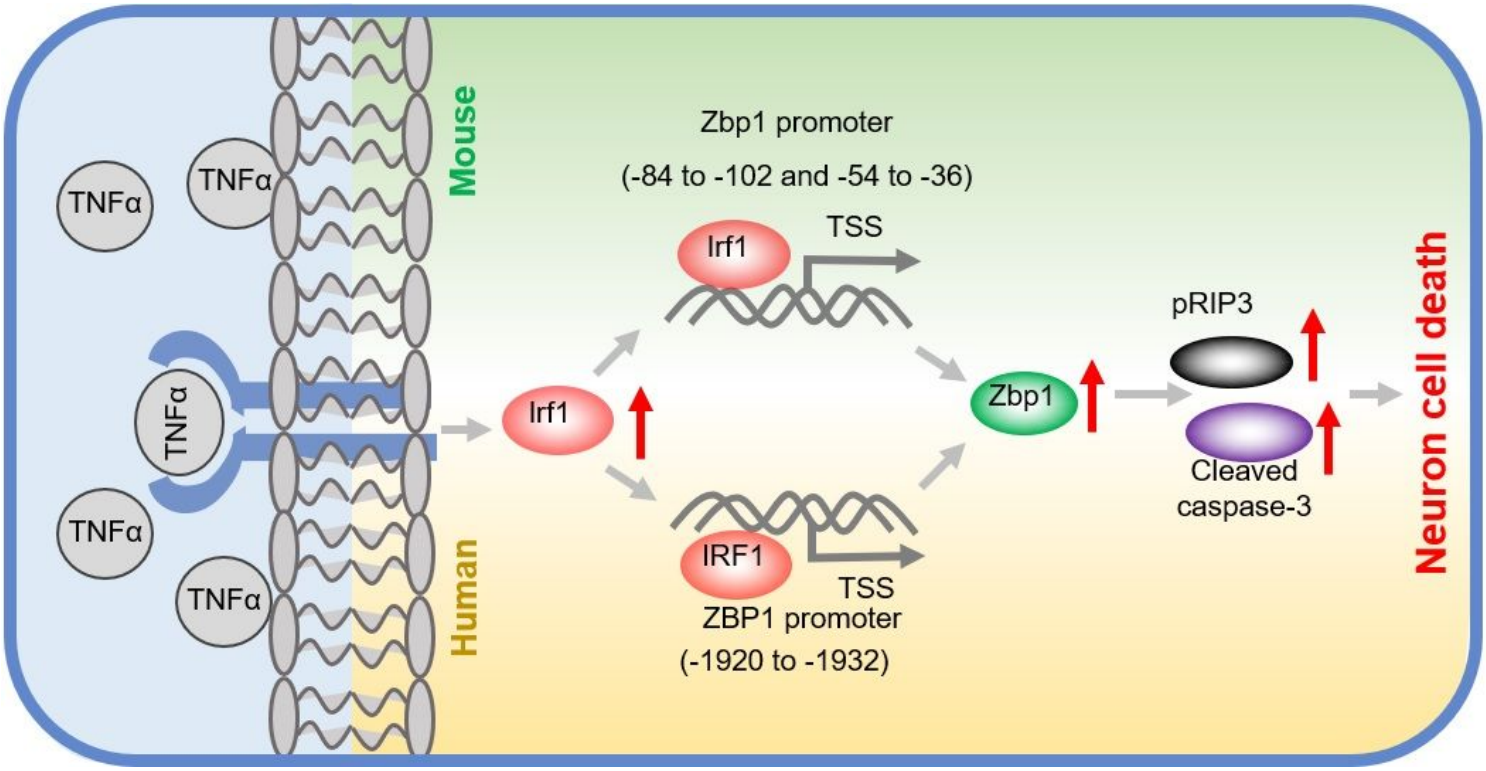

Figure 7

Working model of the study findings. A vicious axis of IRF1-ZBP1 is present in neurons exposed to excessive TNF-α, leading to the activation of Caspase-3 and RIP3 and ultimately to neuronal cell death.

Supplementary Files

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- Supplementalmaterials.docx