SCIENTIFIC Publishing Limited

Trends in Immunotherapy

https://ojs.ukscip.com/index.php/ti

Article

Targeting Notch1 for Neuroinflammatory Immunotherapy: Insights from a Neuronal Apoptosis Model

Shuyuan Jiang and **Wei Xie** *

Department of Public Health, International College, Krirk University, Bang Khen, Bangkok 10220, Thailand

* Correspondence: xiewei@tmu.edu.cn

Received: 5 March 2025; **Revised:** 8 March 2025; **Accepted:** 9 March 2025; **Published:** 7 May 2025

Abstract: The Notch1 signaling pathway is pivotal in neuroimmunomodulation and inflammation, and it significantly contributes to the development and pathogenesis of the nervous system. Consequently, targeting Notch signaling may offer a promising therapeutic approach for neurological disorders. In this investigation, we elucidated the crucial role of Notch1 signaling in neuronal apoptosis, immune regulation, and inflammatory signaling by knocking down the Notch1 gene in mouse hippocampal HT22 cells. Suppression of Notch1 resulted in a marked reduction in the expression of its downstream effector molecule Hes1, accompanied by a significant rise in apoptosis, increased levels of apoptosis-related proteins, and diminished cell viability. RNA sequencing analyses further revealed that differential expression was closely linked to apoptosis, immune-regulatory pathways, and inflammatory signaling. Apoptosis serves as a critical mechanism for eliminating abnormal cells and can impact immune response balance by modulating immune cell activation and function. Notch1 signaling can indirectly affect the neuroimmune microenvironment by regulating neuronal apoptosis. Thus, targeting the Notch1 signaling pathway not only safeguards neuronal function by inhibiting apoptosis but also modulates immune cell activation and inflammatory responses, offering a novel strategy for the immunotherapy of neurodegenerative and cerebrovascular diseases. Comprehending this mechanism provides a crucial foundation for exploring Notch1 immunotherapy for these conditions. By precisely modulating Notch1 signaling, it is anticipated that future therapies can achieve the dual benefits of neuroprotection and immunomodulation, paving the way for innovative treatments for related diseases.

Keywords: Notch1; Neuroinflammatory; Immunotherapy; Apoptosis; Neurodegenerative Diseases

1. Introduction

The Notch signaling pathway is important in immunomodulation and immunotherapy, emerging as a potential target for targeted therapies. The Notch signaling pathway influences the homeostasis of the immune system and anti-tumor immune responses by regulating the differentiation, proliferation, and function of immune cells. It has been found that Notch signaling is involved in regulating the balance between regulatory T cells (Tregs) and effector T cells, thereby affecting immune tolerance and immune responses [1, 2]. During T cell development, Notch signaling promotes the differentiation of CD4+ and CD8+ T cells by regulating the expression of the transcription factor RBP and inhibits the over-proliferation of Treg cells to maintain immune homeostasis [2]. In addition, Notch signaling exhibits a dual role in the tumour microenvironment [3, 4]. Inactivating mutations in the Notch1 gene are strongly associated with the development of acute myeloid leukaemia (AML), and restoring the activity of the Notch signaling pathway has been shown to inhibit the proliferation of leukemia cells and induce their apoptosis [3]. This discovery offers experimental support for targeting Notch signaling as a potential treatment for myeloid

leukemia.

The Notch signaling pathway not only plays an important role in immunomodulation and tumour immunotherapy, but also has potential significance in immunotherapy for neurodegenerative and neurological diseases. Notch signaling is involved in neurodegenerative diseases by regulating the proliferation, differentiation, and survival of neural stem cells (NSCs). In these conditions, Notch signaling is involved in the disease process through its regulation of neuroinflammatory responses and synaptic plasticity. It has been found that Notch signaling influences neuroinflammation and neuronal survival in Alzheimer's disease (AD) and Parkinson's disease (PD) by regulating microglia and astrocyte activity [5, 6]. In addition, Notch signaling is important in neural repair after stroke [7, 8]. Modulation of Notch signaling can inhibit the neuroinflammatory response and reduce pathological damage in neurodegenerative diseases [9]. Meanwhile, this signaling pathway can interact with other neuroprotective pathways, such as NF-κB and nerve growth factor (NGF), presenting opportunities for combination therapies [5, 10–13]. Overall, the Notch signaling pathway holds significant potential for immunotherapy in neurological disorders [6–8].

The Notch signaling pathway presents potential therapeutic targets for a variety of neurological diseases by modulating the interactions between immune cells and neural cells. In cerebral ischemia models, Notch1 signaling significantly increases the number of NSCs in the brain and promotes neuronal differentiation by regulating the expression of target genes such as Hes5 and Mash1, thereby improving the recovery of neurological function [14–18]. In addition, acute blockade of the Notch signaling pathway (e.g., with the γ-secretase inhibitor DAPT) induces neuroprotection and neuroregeneration in a neonatal stroke model [19]. In Alzheimer's disease (AD), Notch1 competes with the amyloid precursor protein (APP) for binding to γ-secretase, resulting in the downregulation of Notch signaling, which subsequently impacts neuronal survival and synaptic plasticity [8]. Inhibition of Notch signaling attenuates neuroinflammatory responses, thereby reducing pathological damage in both AD and Parkinson's disease (PD) [20–25]. In addition, Notch signaling has been closely associated with neuroinflammation-mediated cognitive dysfunction. Studies have shown that minocycline attenuates postoperative cognitive dysfunction by inhibiting neuroinflammation mediated by the Notch signaling pathway [26]. Consequently, the Notch signaling pathway plays various roles in the immunotherapy of neurological disorders. It offers potential therapeutic targets for conditions such as neurodegenerative diseases, stroke, and diseases related to neuroinflammation by influencing neural stem cell function, neuroinflammatory responses, and the survival of neurons [18, 20, 25].

The Notch signaling pathway regulates apoptosis through multiple mechanisms, and its potential as an immunotherapeutic target has been confirmed by several studies. In models of myocardial ischemia, Notch1 significantly reduces cardiomyocyte apoptosis by inhibiting Bax expression, decreasing caspase-9 and -3 activity, and upregulating Bcl-2 [27]. Within the tumor microenvironment, Notch1 inhibits p53-mediated apoptosis in HaCaT cells and enhances chemoresistance through the activation of the PI3K/Akt pathway [28]. Targeted intervention in the Notch pathway induces tumor-specific apoptosis: rhamnetin promotes apoptosis in breast cancer cells by activating caspase-3 and -9 via the miR-34a/Notch1 axis [29], while the herbal extract AB4 induces apoptosis in hepatocellular carcinoma cells by inhibiting the Notch signaling pathway, activating caspase-3 and -9, and triggering the mitochondrial apoptotic pathway [30]. These findings suggest that the Notch pathway can be a precise target to enhance the response to immunotherapy by regulating apoptosis-related molecules (caspase cascade reactions) and cross-talking key pathways.

However, to date, few studies have reported on the involvement of Notch1 in hippocampal neuronal apoptosis and neuroimmunomodulation. Furthermore, our understanding of the precise role of Notch1 and its associated molecular events as prognostic indicators of neurological injury-related diseases remains inadequate. In the current investigation, we evaluated the specific role of Notch1 in apoptosis through the application of siRNA-mediated knockdown of Notch1 via cell transfection techniques. Our results show that Notch1 has a crucial function in the apoptosis of hippocampal neurons and the sequencing results obtained following the knockdown of Notch1 demonstrated that the differentially expressed genes (DEGs) play a significant role in neuronal cellular immunity detected by the KEGG enrichment analysis. Therefore, targeting the Notch1 signaling pathway not only preserves neuronal function through suppression of neuronal apoptosis but also establishes a novel immunotherapeutic approach for neurodegenerative and cerebrovascular disorders through the regulation of immune cell activation and inflammatory responses. This establishes a basis for further exploration of the mechanisms underlying its involvement in the recovery of learning and memory, as well as its neuroprotective effects in the context of neurological disorders.

2. Materials and Methods

2.1. Cell Cultures and Model Building

The HT22 mouse hippocampal neuronal cell line was procured from the Cell Resource Centre at the Institute of Basic Medical Sciences, Peking Union Medical College, affiliated with the Chinese Academy of Medical Sciences. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA, 12800017), which was supplemented with 10% fetal bovine serum (Ever Green, CHN, 80230-6412) and penicillin/streptomycin (Gibco, USA, 15140122) at a working concentration of 100 U mL^{-1}. The incubation conditions were set at 37 °C in a controlled atmosphere containing 5% CO_2 and 21% oxygen.

This study was divided into three groups: HT22 cells that were not treated (untransfected), HT22 cells transfected with scramble siRNA (scramble), and HT22 cells transfected with siRNA targeting Notch1 (siNotch1). Cell transfection was performed using Lipofectamine™ LTX Reagent (Lipofectamine™ LTX Reagent with PLUS™ Reagent; Thermo Fisher, USA, catalog number 15338100). For specific methods, please refer to the section on cell transfection.

2.2. Cell Transfection

Cell transfection used Lipofectamine™ LTX Reagent. Cells were seeded one day prior to transfection and plated in growth medium to ensure that they reached approximately 70% confluency at the time of transfection. Diluted siRNA (chemically synthesized by Sangon Biotech, CHN) in Plus reagent and Opti-MEM™ Reduced Serum Medium (Opti-MEM; Thermo Fisher, USA, 31985062) were mixed gently. Mixed Lipofectamine® LTX with Opti-MEM, incubated for 5 minutes at room temperature. To initiate the transfection process, the siRNA should be added to a freshly prepared solution of Lipofectamine® LTX, followed by gentle mixing. This mixture should then be incubated at room temperature for a duration of 5 minutes. Concurrently, the cell culture medium should be replaced with serum-free DMEM in each well. Upon completion of the incubation period, the siRNA-Lipofectamine® LTX complex should be introduced into the wells containing the cells, ensuring a proportional distribution, then the plate should be gently agitated back and forth to facilitate mixing. Subsequently, the plates must be placed in a CO_2 incubator set at 37 °C for 4 to 6 hours, the medium can be substituted with complete medium (serum DMEM). Transgene expression can be observed within 24 to 48 hours following transfection. In instances where the siRNA is labeled with a fluorescent marker, it is crucial to limit exposure to light throughout the duration of the experiment. The volumes of reagents used in the transfection experiment are detailed in **Table 1**.

Table 1. The volumes of reagents of transfection experiment.

Component	Opti-MEM® Medium	Lipofectamine® LTX Reagent	Opti-MEM® Medium	RNA (100 pmoL)	PLUS™ Reagent
6-well	95 μL	5 μL	93.3 μL	5 μL	1.7 μL
24-well	23.5 μL	1.5 μL	23.9 μL	0.75 μL	0.34 μL
96-well	4.7 μL	0.3 μL	0.3 μL	0.1 μL	4.6 μL

2.3. Bioinformatic Analysis (RNA-Sequencing)

To verify the correlation of Notch1 with other signaling pathways and cellular functions, we performed transcriptome sequencing on HT22 cells with Notch1 knockdown. The construction and sequencing of the cDNA library were conducted by Meiji Biotech Ltd., located in Shanghai, China. Raw reads obtained from the Illumina HiSeq sequencing platform were processed using Cutadapt software. We normalized the raw expression data using transcripts per million (TPM) and the RESM model and analyzed the consistency and comparability of expression distributions across samples. HTSeq-count was employed to calculate the read counts. Differentially expressed genes were identified utilizing the R package DESeq2, with a fold change threshold set at ≥2 or ≤0.5, alongside a significance criterion of $P < 0.05$.

To identify genes that exhibit differential expression and to conduct an analysis of functional enrichment, we utilized Gene Ontology (GO) and Reactome FIViz. Functional enrichment analyses for both GO and Reactome were conducted using Goatools (https://github.com/tanghaibao/Goatools) and the Reactome database (https://reactome.org/). Correlation data were calculated using the 'corrplot' function, and the resulting graphs were

generated with the 'ggplot2' package in R software.

2.4. Cell Viability Assays

In this investigation, the viability of cells was assessed through the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenol)-2-(4-sulfophenyl)-2H-tetrazolium) assay, utilizing the CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (MTS; Promega, USA, G3580). HT22 cells were plated in 96-well plates at a density of 2 × 10^3 cells per well one day prior to the experiment and were subsequently cultured for a period of 24 hours. After this incubation period, the cells were transfected with 20 μL of MTS solution 24 hours post-transfection. Each plate included a control well containing only the complete medium and MTS solution (void of cells), with readings taken at intervals of 30 minutes. The procedures adhered to the protocols established by Zhao et al. [31]. All experiments were conducted in triplicate.

2.5. qRT-PCR

Total RNA was extracted using a highly efficient guanidine-phenol-chloroform method, commonly referred to as the TRIzol extraction method, utilizing TRIzol™ Reagent (Invitrogen, USA, 15596018CN). This method is based on the principle of phase separation and effectively extracts high-quality RNA from cell or tissue samples. After extraction, the NanoDrop 2000 spectrophotometer was used to evaluate the concentration and purity of the RNA and with the A260/280 ratio ranging from 1.8 to 2.1, indicating that the extracted RNA was of good quality and suitable for subsequent experiments. RNA reverse transcription employed PrimeScript™ RT Reagent Kit (Perfect Real Time) (Takara, JPN, RR047B). The primer information for the genes studied is detailed in **Table 2**. In quantitative real-time PCR experiments, the ABI 7900 Real-Time PCR system (Invitrogen, USA) was used to carry out the reaction, and the specific experimental steps were as follows: First, initial denaturation was performed at 95 °C for 10 minutes to ensure complete separation of the DNA strands. This was followed by 40 cycles of PCR amplification, each cycle involving 30 seconds at 95 °C (denaturation), 60 seconds at 60 °C (annealing), followed by a 2-minute extension at 60 °C to yield ample cDNA. To analyze the relative expression levels of mRNA, the 2-ΔΔCT analysis method was utilized [32], with β-actin utilized as a reference gene for internal normalization purposes to correct for potential variability in the experiments and to guarantee the precision and dependability of the findings. All experiments were performed in triplicate to improve the reliability and reproducibility of the results.

Table 2. Primers sequence for qRT-PCR.

Gene Name	Forward (5′ - 3′)	Backward (5′ - 3′)
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Notch1	CACCCATGACCACTACCCAGTT	CCTCGGACCAATCAGAGATGTT
Hes1	ACACCGGACAAACCAAAGAC	ATGCCGGGAGCTATCTTTCT

2.6. Cell Apoptosis Detection

We used flow cytometry (FACS Canto II; BD, USA) and the Annexin V-FITC Assay Kit (BD, USA, BD559763) as per our previous studies [33, 34]. HT22 cells were subjected to digestion, followed by two washes with cold PBS, and were subsequently resuspended in cold 1X binding buffer at a concentration of 1 × 10^6 cells per milliliter.

Subsequently, aliquots of cell suspensions, precisely 100 μL in volume, were transferred into designated flow cytometry tubes. To each tube, 5 μL of FITC Annexin V conjugate and 5 μL of propidium iodide (PI) solution were meticulously introduced. This dual-staining approach, leveraging the distinct properties of Annexin V and PI, allows for a comprehensive assessment of cellular viability and the discrimination of apoptotic and necrotic cell populations via flow cytometric analysis. The mix underwent vortexing and a 15-minute dark incubation at ambient conditions. Thereafter, 400 μL of 1X Binding Buffer was added to the mixture to resuspend the cells. The analysis of apoptotic cells was conducted via flow cytometry within one hour of preparation.

2.7. Cell Cycle Detection

The present study utilized a flow cytometer (FACS Canto II; BD, USA) to conduct an analysis of the cell cycle. To maintain experimental consistency, the cells were synchronized by substituting the complete medium with serum-

free medium, thereby inducing a state of starvation for a duration of 12 hours. When cells reached 60%–70% confluence before transfection. Following a 48-hour period post-transfection, the cells were subjected to trypsin digestion, after which HT22 cells were washed twice with cold phosphate-buffered saline (PBS) and subsequently resuspended in cold PBS. The resuspension was achieved through the gradual addition of ice-cold anhydrous ethanol to reach a final concentration of 75%, and the cells were then fixed at 4 °C overnight. In the course of our experimental procedures, samples comprising approximately 1 × 10^6 cells were subjected to resuspension in a total volume of 0.5 mL of BD Pharmingen™ PI/RNase Staining Buffer, a reagent provided by BD Biosciences, USA (Product Code: 550825). This buffer is specifically formulated to facilitate the effective staining of cellular components, allowing for subsequent analysis of cellular viability and integrity. To ensure optimal staining conditions, the samples were incubated in a dark environment for a duration of 15 minutes at room temperature. The resulting cell cycle data were processed using FlowJo v10.0.7 software.

2.8. Western Blot Analysis

As outlined in our earlier research [33], at the end of the indicated incubation period, cells were carefully harvested through gentle scraping to minimise cell damage and ensure optimal protein integrity. To remove residual medium and non-adherent debris, the harvested cells were subsequently washed twice in succession with ice-cold phosphate-buffered saline (PBS). Protein extraction was then performed using RIPA lysis buffer (Beyotime, P0013B), a commercially available buffer optimised to effectively lyse cells and solubilise a wide range of proteins. To further facilitate protein release and disruption of nucleic acid complexes, cell lysates were subjected to controlled sonication on ice. This involves the application of short pulses of high-frequency sound waves, which mechanically disrupt the cellular structure and improve the accessibility of proteins in the lysate. The concentrations of protein were assessed utilizing a Protein Quantification Kit (BCA Assay, Thermo, 23227), then with mixed protease inhibitors (NuPAGE™ Antioxidant; Invitrogen, NP0005). Equal amounts of protein samples (20 μg each) were subjected to separation via SDS/PAGE at a current of 30 mA for 2.5 hours. Subsequently, after a series of preparations in accordance with established laboratory protocols, the gel after electrophoresis was transferred to the nitrocellulose membrane by flattening the gel with an acetate membrane, as required for transferring the proteins to the nitrocellulose membrane (Roche, Switzerland, catalog number 3010040001), followed by a blocking step using 5% nonfat dry milk for a duration of 1 hour. The membrane was then incubated overnight at 4 °C with anti-Hes1 (Cell Signalling Technology, 11988s), anti-Notch1 (Cell Signalling Technology, 4380S), anti-β-Actin (Santa Cruz Biotechnology, 47778), anti-cleaved caspase-3 (Cell Signalling Technology, 9661s), and anti-spectrin (Sigma-Aldrich, MAB1622) antibodies. Following this, the secondary antibody was incubated for a duration of 1.5 hours at room temperature. Subsequently, the identification of protein signals utilizing advanced chemiluminescence reagents (ECL, Thermo, 34580) with the Tanon 5200 automatic chemiluminescence imaging analysis system (Tanon, Shanghai, China). The quantification of protein expression levels was achieved through the analysis of gray values by ImageJ software, with β-Actin employed as the internal control.

2.9. Statistical Analyses

The statistical analyses were performed using GraphPad Prism 9 software, and employed the statistical method of one-way analysis of variance (ANOVA) in conjunction with Tukey's HSD post-hoc test, which is suitable for comparisons between independent samples across multiple groups and effectively controls the accumulation of Type I errors that can arise from multiple comparisons. When ANOVA indicated a significant difference between groups ($p < 0.05$), Tukey's HSD test was utilized for further analysis. This method is based on the Studentized Range Distribution (SRD), and confidence intervals for inter-group comparisons were established by calculating the threshold for the least significant difference (HSD). This allows for the accurate identification of groups with statistically significant differences while maintaining the familywise error rate at a predetermined α level. This combination ensures statistical validity and rigor in correcting for multiple comparisons. Data were obtained from at least three separate experiments and are shown as mean ± standard error of the mean (SEM). A p-value of less than 0.05 was deemed indicative of statistical significance.

3. Results

3.1. Notch1 Was Significantly Knocked Down after Transfection of siNotch1 in HT22 Cells

The siNotch1 fragment was labeled with FAM, exhibiting green fluorescence after the transfection of cells. The results were observed using a fluorescence inverted microscope 24 hours after transfection (**Figure 1a**). The mRNA expression level of Notch1 was assessed via real-time PCR 24 hours after cell transfection (**Figure 1b**). Additionally, a semi-quantitative analysis of Notch1 was conducted using Western blot experimental technique (**Figure 1c**) and ImageJ (**$P < 0.01$) (**Figure 1d**), and the experimental results indicated that Notch1 was significantly knocked down in HT22 cells at both the protein and mRNA levels. At the same time, we measured the mRNA (**$P < 0.01$) (**Figure 1e**) and protein levels (**$P < 0.01$) (**Figure 1f**) of Hes1, which is a key gene that functions downstream of the Notch signaling pathway. We observed a significant reduction in mRNA levels (**$P < 0.01$) and protein levels (*$P < 0.05$) of Hes1.

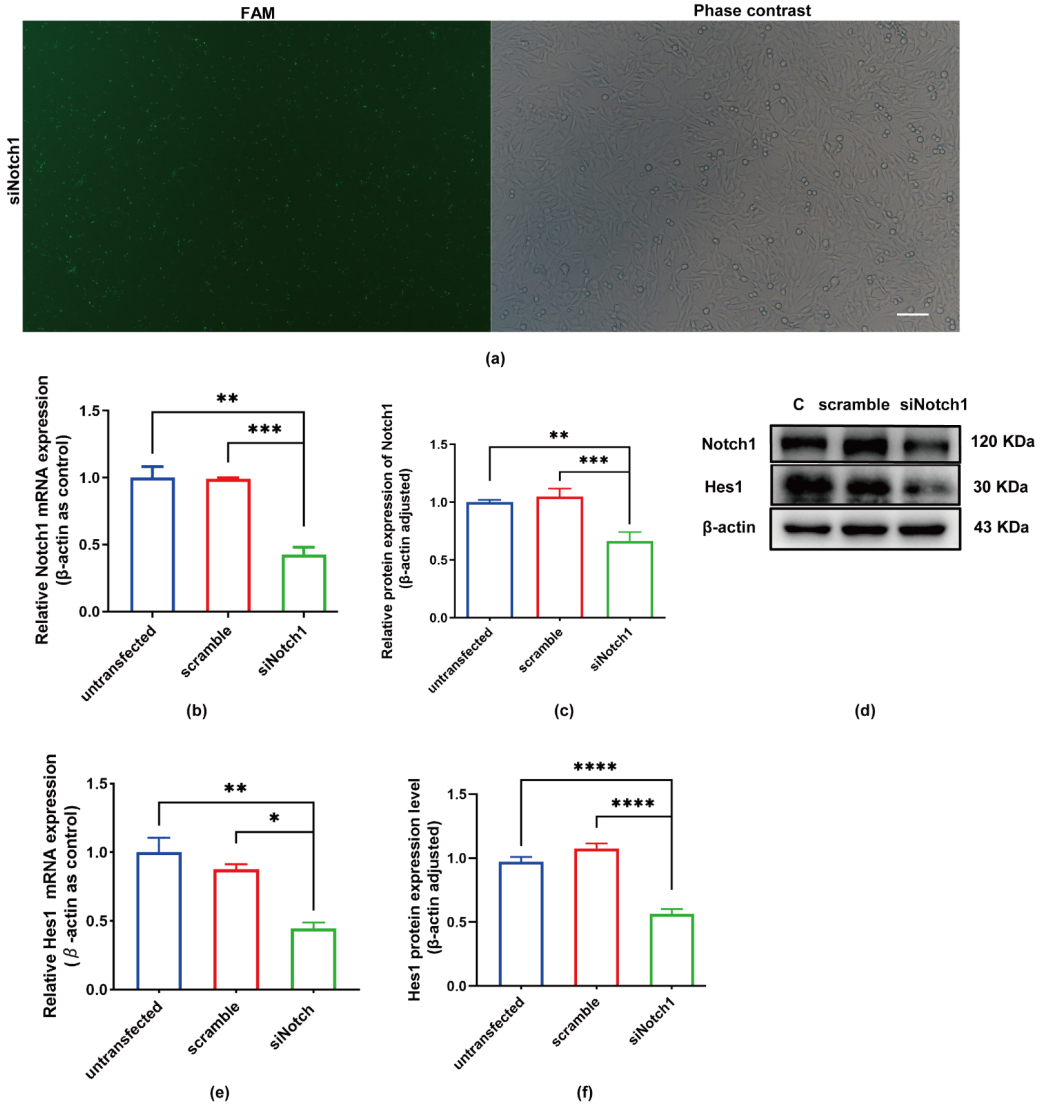

Figure 1. The Notch signaling pathway was significantly affected after transfection of siNotch1 in HT22 cells. (**a**) The transfection of siNotch1 into HT22 cells was observed by fluorescence inverted microscope. (**b**) Notch1 mRNA expression level was determined by real-time polymerase chain reaction after transfection of siNotch1, n = 3. (**c**) Semi-quantitative analysis of Notch1 protein, β-actin as inner reference, n = 6. (**d**) The protein level was measured using a Western blot assay. (**e**) Hes1 mRNA expression levels were detected by real-time polymerase chain reaction with β-actin as the reference gene, n = 3. (**f**) The level of Hes1 protein was detected by Western blot assay and semi-quantitative analysis, β-actin as inner reference, n = 6.

Note: Data are mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ****, $P < 0.0001$; Scale bar, 100 μm.

3.2. Notch1 is Closely Associated with the Apoptosis of Mouse Hippocampal Neurons

To further investigate the effects of Notch1 knockdown on HT22 cells, the groups of untransfected, scrambled, and siNotch1 were subjected to RNA sequencing to compare differentially expressed genes (DEGs). The normalization process used to construct the RESM model (**Figure 2a**) demonstrated that the visualization of mRNA expression distribution in each group was homogeneous. A Venn diagram (**Figure 2b**) illustrated the DEGs between the untransfected and scrambled groups, as well as between the scrambled and siNotch1 groups; three of the four DEGs identified were predicted genes (**Figure 2c**) that could not be subsequently enriched. Compared to the untransfected group, the scrambled group exhibited 23 upregulated, and 20 downregulated differentially expressed genes **(**criteria: $P < 0.01$, fold change > 2, **Figure 2d**). The Gene Ontology (GO) analysis conducted on all differentially expressed genes indicated the absence of any statistically significant differences (**Figure 2e**). Based on these results, we will focus on the DEGs between the scrambled and siNotch1 groups.

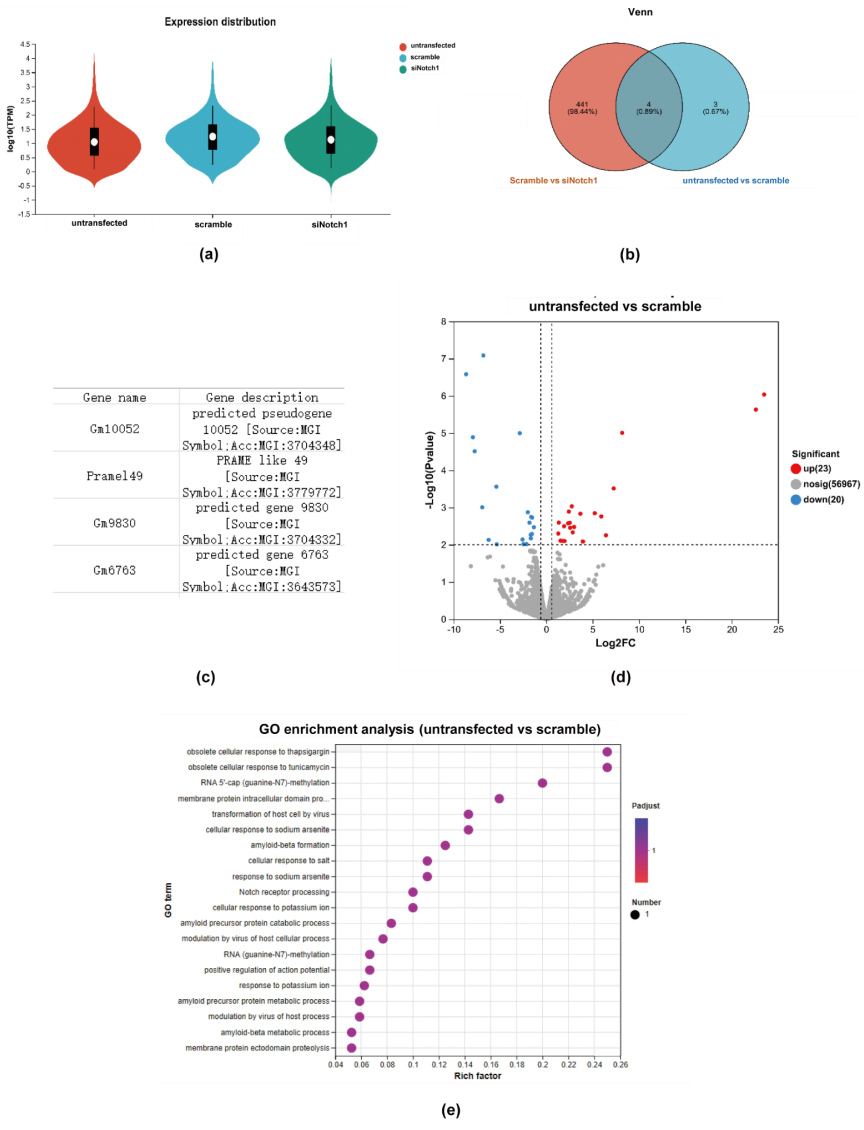

Figure 2. RNA sequencing. (**a**) Visualisation of the distribution of mRNA expression in each group is homogeneous. (**b**) Venn diagram showing differentially expressed genes (DEGs) between groups. (**c**) Three of the DEGs were predicted genes. (**d**) A volcano plot illustrating the 23 upregulated and 20 downregulated DEGs (criteria: $P < 0.01$, fold change > 2 or <0.5) in scrambled group HT22 cells compared to those from untransfected cells. (**e**) Gene Ontology (GO) analysis of the DEGs reveals no significant difference (untransfected vs. scrambled).

To further investigate the biological functions of DEGs between the scrambled and siNotch1 groups, we conducted heatmap cluster analysis (**Figure 3a**) and volcano plots (V-panel) (**Figure 3b**). Compared to the scrambled group, the siNotch1 group exhibited a total of 857 DEGs, of which 469 (in red) were upregulated and 388 (in blue) were downregulated (criteria: $P < 0.01$, fold change > 2). GO analysis indicated that the knockdown of Notch1 was closely associated with apoptosis-related signaling pathways (**Figure 3c**). To provide additional clarity regarding the functions of the DEGs, we employed Reactome analysis, which suggested that the enriched signaling pathways are linked to apoptosis, programmed cell death, execution of apoptosis, apoptotic cleavage of cellular proteins, and apoptotic factor-mediated response (**Figure 3d**). The Notch pathway significantly influences programmed cell death in murine hippocampal neurons. At the same time, we also performed KEGG enrichment analysis on DEGs (**Figure 3e**), in which the results not only showed that Notch signalling was closely related to apoptosis, but also to TNF, C-type lectin receptor, and NF-kappa B signaling pathways, which are involved in important physiological processes such as innate and adaptive immunity, immune defense, and immune stabilisation, and are closely related to stress responses and inflammation. This result suggests that the Notch signaling pathway, a highly conserved and intricately regulated intercellular communication mechanism, assumes a pivotal and multifaceted role in cellular immunity within the nervous system. This pathway consists of a series of well-coordinated molecular events that are fundamental for maintaining immune homeostasis in neural tissues.

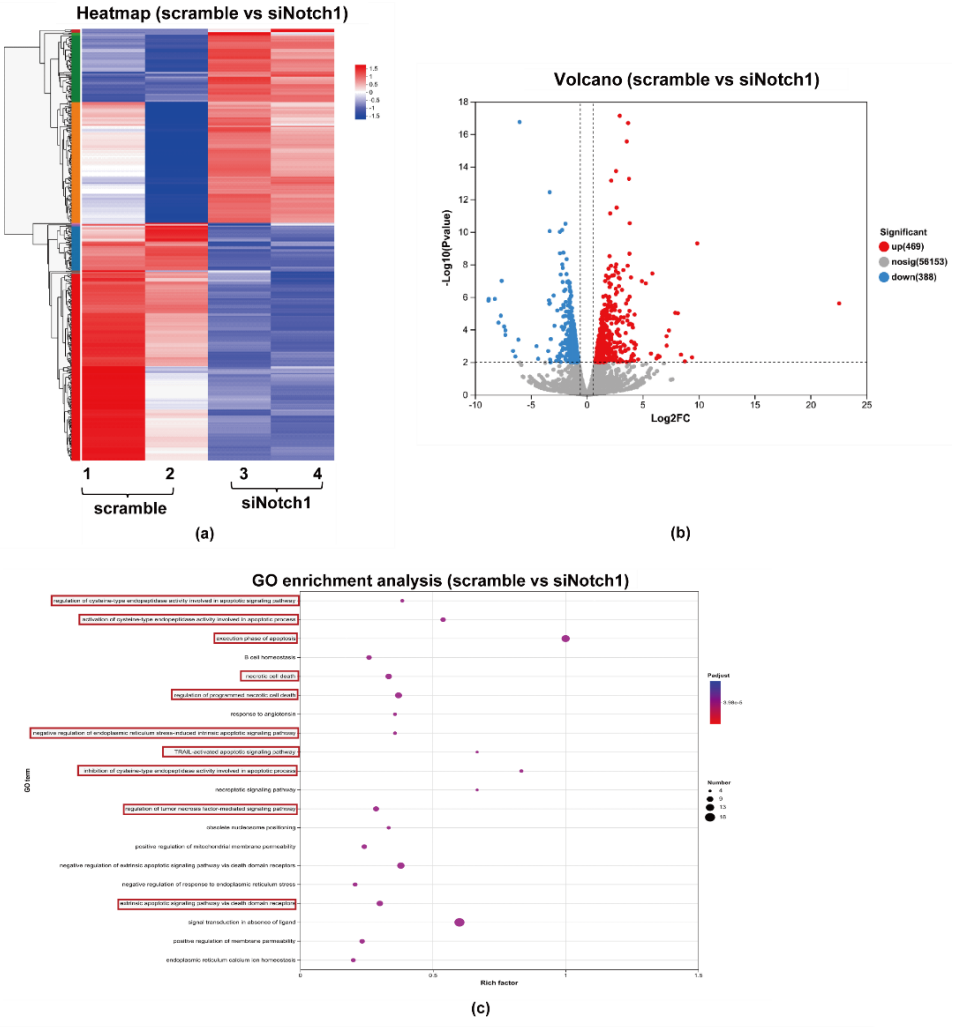

Figure 3. *Cont.*

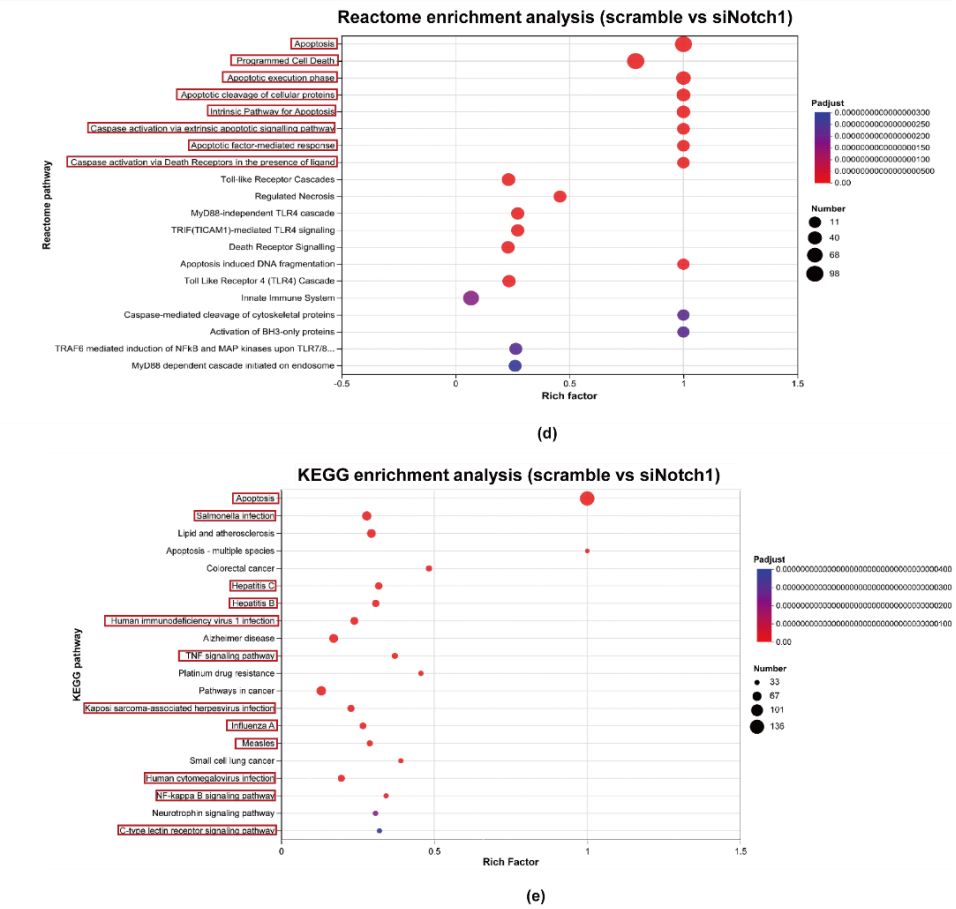

Figure 3. RNA sequencing identifies knockdown of Notch1 involved in regulation of apoptosis. (**a**) Heatmap showing DEGs identified by RNA sequencing between scrambled and siNotch1 HT22 cells. (**b**) A volcano plot illustrating the 469 upregulated and 388 downregulated differentially expressed genes (scrambled vs. siNotch1). (**c**) GO analysis. (**d**) Reactome analysis of DEGs showing the enriched signalling pathways were related to apoptosis. (**e**) KEGG analysis showing the enriched signalling pathways were related to apoptosis, immunity and inflammation.

3.3. Effect of Knockdown of Notch1 on HT22 Cell Cycle and Cell Viability

Our earlier findings indicated that Notch1 overexpression elevated the fraction of cells in the S phase and enhanced cell proliferation [32]. In order to clarify the function of the Notch1 gene in the process of apoptosis, we conducted an analysis of the cell cycle distribution in HT22 cells using the BD Canto II Flow Cytometer (**Figure 4a**). The results showed that G1-phase cells, although increased, were not significantly different (**Figure 4b** and **Figure 4c**), and S-phase cells were significantly decreased, again not statistically significant (**Figure 4d**). Cell proliferation was also examined in this experiment, and cell viability was assessed using the MTS assay. Following the knockdown of Notch1, the viability of HT22 cells was markedly reduced (***, $P < 0.001$; ****, $P < 0.0001$) (**Figure 4e**). This observation aligns with our earlier findings [32], indicating that Notch1 is instrumental in facilitating cell proliferation.

3.4. Knockdown of Notch1 Contributes to HT22 Cell Apoptosis

To verify the effect of Notch1 knockdown on apoptosis in HT22 cells, we detected apoptosis using flow cytometry (**Figure 5a**). The results indicated that the number of apoptotic cells in the siNotch1 group significantly increased compared to the scrambled group (*$P < 0.05$) (**Figure 5b**). Additionally, we analyzed the protein expression of spectrin, caspase-3 and cleaved caspase-3 through Western blotting to further confirm the occurrence of apoptosis and cell death following Notch1 knockdown in HT22 cells (**Figure 5c**). Normal HT22 cells exhibited a high level of spectrin, with spectrin cleavage producing 145/150 kDa (reflects cell necrosis and excitotoxic neu-

ronal death) and 120 kDa (reacts to the death of apoptotic cells) [35]. The results (**Figure 5c–f**) demonstrated that Notch1 knockdown led to a significant increase in 120 kDa (**$P < 0.01$) (**Figure 5d**), 145 kDa (* $P < 0.05$, ** $P < 0.01$) (**Figure 5e**), and 150 kDa (****$P < 0.0001$, *** $P < 0.001$) protein expressions compared to both the untransfected and scrambled groups (**Figure 5f**). Therefore, Notch1 knockdown promotes both cell necrosis and apoptosis.

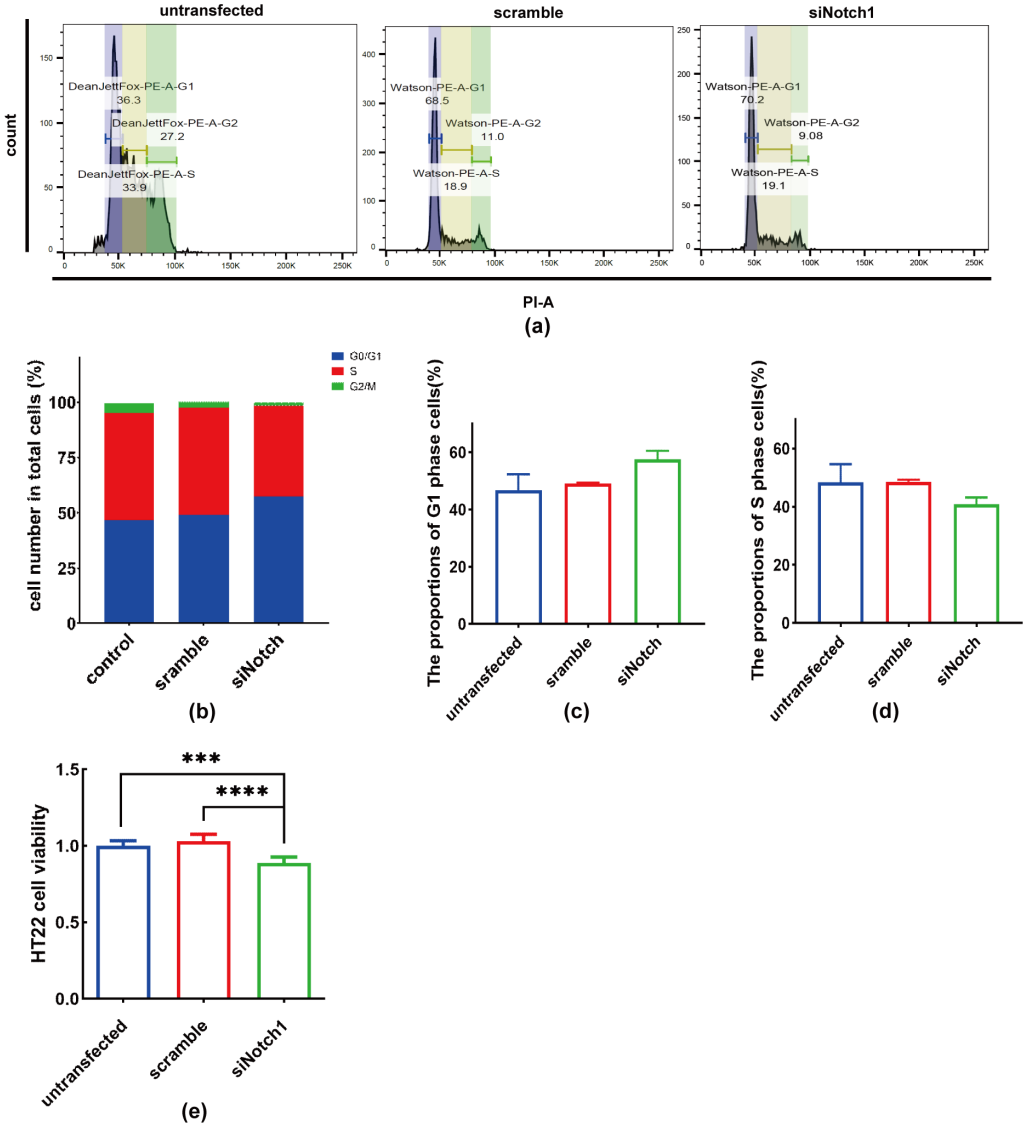

Figure 4. Effect of knockdown of Notch1 on HT22 cell cycle and cell viability. (**a**) The cell cycle was detected by flow cytometry and data analysis was performed with FlowJo v10.0.7 program. (**b**) Cell cycle distribution result. (**c**) The cell cycle of knockdown Notch1 was increased in G1 phase compared with scrambled, n = 3. (**d**) The cell cycle of knockdown Notch1 was decreased in S phase compared with scrambled, n = 3. (**e**) Knockdown of Notch1 reduced the viability of HT22 cells, n = 6.

Note: Data are mean ± SEM, *, $P < 0.05$; ****, $P < 0.0001$.

Subsequently, we assessed the expression levels of caspase-3 (**Figure 5c**). The caspase family, also known as cysteine aspartate-specific proteases, plays a crucial role in the regulation of apoptosis through mitochondrial and death receptor pathways. Caspase-3, a prominent member of this family, is widely acknowledged as a key protease in the apoptotic process and is often designated as a death protease. It is regarded as the ultimate executor of apoptosis and is significantly implicated in the onset and progression of various pathological conditions, including tumors [36], ischemia [37], and reperfusion injury [38, 39]. Cleaved caspase-3 represents the active fragment generated during the activation of this protease, and its expression level indicates the activity and extent

of protease-mediated apoptosis. Our study demonstrated a significant increase in levels of caspase-3 (**Figure 5g**), cleaved caspase-3 (****$P < 0.0001$) (**Figure 5h**) in siNotch1 cells compared to the untransfected and scrambled groups. The findings indicate that Notch1 knockdown enhances the expression levels of caspase-3 and its cleaved form, cleaved caspase-3, thereby promoting apoptosis and neuronal injury in mouse hippocampal neurons.

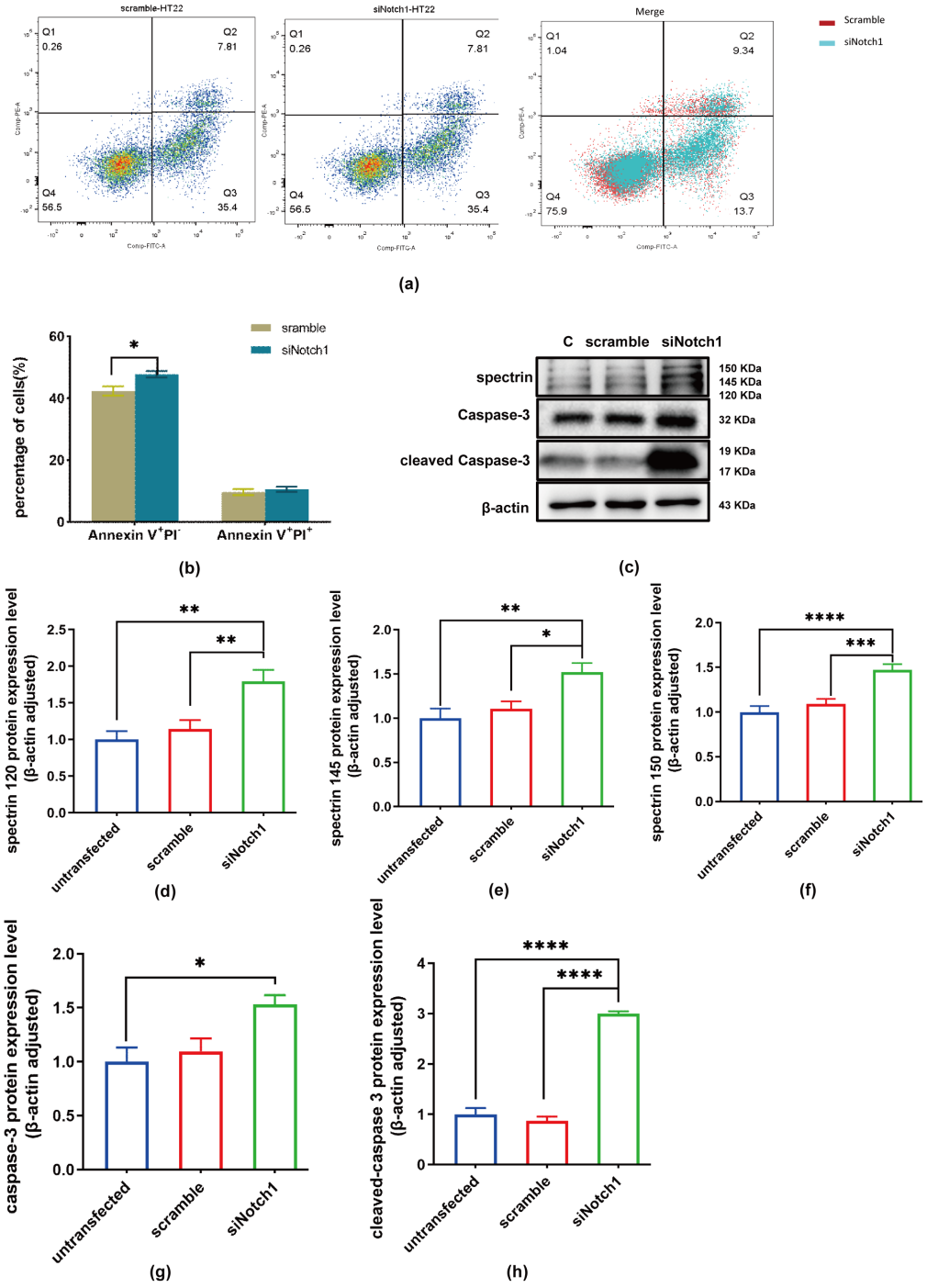

Figure 5. Knockdown of Notch1 contributes to HT22 cell apoptosis. (**a**) Flow cytometry results of HT22 cells apoptosis, n = 3. (**b**) Apoptosis data statistics. Apoptosis levels in siNotch1 cells have increased. (**c**) Results from the Western blot analysis. Semi-quantitative analysis of changes in the protein levels of 120 kDa (**d**), 145 kDa (**e**), 150 kDa (**f**) of spectrin, and caspase-3 (**g**), cleaved caspase-3 (**h**), n = 6.

Note: Data are mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

4. Discussion

The Notch1 signaling pathway is central to neuroimmunomodulation and [10, 21] regulation [22, 40], influencing the progression of neuroinflammatory and neurodegenerative diseases through its dual control of neuronal apoptosis and immune responses, thereby emerging as a critical target for immunotherapy [41, 42]. Experimental findings confirm that Notch signaling directly regulates neuronal apoptosis, which acts as both a core immunomodulatory mechanism and a significant endpoint in inflammatory processes. Moreover, Notch signaling plays a crucial role in the immune system, not only in the development and maintenance of hematopoietic stem cells [43], but also in the lineage differentiation of T and B cells within central and peripheral lymphoid organs [44], as well as in T-cell activation [45] and functional modulation [46]. In addition, Notch signaling can have a dual effect on tumor immunity by modulating the prevalence and functional capabilities of diverse immune cell types, encompassing myeloid-derived suppressor cells (MDSC), tumour-associated macrophages (TAMs), dendritic cells (DCs) and T cells [47]. During the early stages of brain development, the initial proliferation of neurons exceeds the number of neurons that are eventually integrated into mature neural circuits, and large numbers of excess neurons are systematically removed by apoptosis [48]. In neurological disorders including Alzheimer's disease, Parkinson's disease, stroke, traumatic brain injury, and amyotrophic lateral sclerosis, the process of apoptosis induces an elevated accumulation of inflammatory molecules within the nervous system, and this cascade of reactions triggers the activation of neuroglial cells and exacerbates neuronal damage through sustained inflammatory signaling [49–51]. In this study, RNA sequencing was conducted following the knockdown of Notch1 in mouse hippocampal neuronal cells (HT22) using liposome-transfected siRNA, and the results indicated that the differentially expressed genes were closely associated with apoptosis, as well as inflammation, immune regulation, and other related pathways. Defects in the Notch1 gene are associated with the promotion of apoptosis, which adversely affects neurological function and is involved in pathways related to immunomodulation. This may represent a potential mechanism underlying neurodegenerative and cerebrovascular diseases, ultimately resulting in neurological impairment and deficits in learning and memory.

We used RNAi technology to demonstrate the role of Notch1 in apoptosis, neuroinflammation and immune regulation. Our flow cytometry assay revealed that the knockdown of Notch1 effectively induced apoptosis. Furthermore, we noted a significant increase in the levels of the essential apoptotic proteins caspase-3 and cleaved caspase-3, as well as αII spectrin (120/145/150). The results indicate that siNotch1 effectively induces apoptosis in mouse hippocampal neurons. Inhibitors of miR-9 have been demonstrated to restrain neuronal apoptosis through the activation of Notch signaling pathways, thereby enhancing neuronal morphology and enhancing neural function in a rat model of depression [52]. In a notable study investigating the impact of Ubiquitin-like modifier-activating enzyme 6 (UBA6) on neuronal health, researchers found that silencing or knockdown of UBA6 in rat models significantly intensified apoptosis and cerebral injury following middle cerebral artery occlusion (MCAO). This experimental approach established a direct link between the functional presence of UBA6 and neuronal survival during ischemic conditions. The underlying mechanism implicated in this exacerbation involved a marked inhibition of the Notch1 signaling pathway, which is known for its critical role in regulating cellular differentiation, survival, and apoptosis. Specifically, the Notch1 pathway typically functions to promote cell survival and mitigate apoptotic processes within the brain's neural population [53]. This inhibition of the Notch1 signaling pathway resulted in increased neuronal apoptosis, which is consistent with our findings. In addition, our experimental results indicate that the Notch1 signaling pathway can inhibit apoptosis by regulating caspase-3 activity, and its mechanism has been validated in various cell and disease models. For instance, in pathogen infection models, Ehrlichia-induced activation of the Notch signaling pathway can block host cell apoptosis by stabilizing XIAP (X-linked inhibitor of apoptosis protein) and inhibiting caspase-3 activation [54]; and in cancer therapeutic studies, the extract of Polycladia crinita and its selenium nanopreparation significantly increased the activation level of cleaved caspase-3 through the downregulation of the Notch1 signaling pathway, which, in turn, induced apoptosis in solid cancer cells. Furthermore, in a model of colitis, the activation of the Notch1 signaling pathway reduces apoptosis in intestinal epithelial cells, and this effect is associated with the inhibition of caspase-3 cleavage [55]. Thus, the NOTCH signaling pathway can exert anti-apoptotic effects by directly or indirectly regulating the activation state of caspase-3. Research in this area offers potential targets for the treatment of related diseases.

At the same time, the results of the cell cycle analysis in this experiment indicated that the G1 phase of siNOTCH1

cells was increased, while the S phase was decreased, cell viability was significantly reduced, and cell proliferation was blocked in the G1 phase, suggesting that knocking down Notch1 inhibited the proliferation of HT22 cells. This finding aligns with our prior research [32], that the 5-Aza induced Notch1 signaling pathway promotes adult neurogenesis in the hippocampal dentate gyrus, and in vitro experiments using the immortalized mouse hippocampal neuron neuroblastoma cell line HT22 demonstrated that overexpression of Notch1 increased cell viability and the proportion of S-phase cells, thereby promoting cell proliferation. It has been reported that neurogenesis is inhibited in Notch +/– mice [56], and that inhibition of Notch1 activity leads to a decrease in the generation of new neurons in the hippocampus, ultimately resulting in the depletion of hippocampal NSC [57]. All this indicates that Notch1 may have a significant function in the inhibition of proliferation and the promotion of apoptosis in hippocampal neurons. Apoptosis is a non-inflammatory cell death that is important in normal physiology and disease. Physiological apoptosis is a suicidal behavior of the organism in order to better adapt to the survival environment; whereas apoptosis stimulated by pathological factors causes deleterious damage to the organism. Apoptosis is a crucial component of normal development and homeostasis within an organism, serving as an essential mechanism for immune-inflammatory clearance. When neurons are exposed to environmental stress, particularly in pathological conditions, they produce and secrete cytokines that activate glial cells, thereby influencing the function of immune cells and promoting their immune response. In our study, we found that the molecular basis of the Notch1 signaling pathway dictates a wide range of regulatory functions across various cell types. This pathway is not only involved in apoptosis but also plays a crucial role in immune and inflammatory regulation, which may be a series of self-protective responses generated by neurons in reaction to environmental stress.

The Notch1 signaling pathway plays a critical role in neuroinflammation by regulating the tumor necrosis factor (TNF) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways. Our RNA sequencing results revealed that the knockdown of Notch1 significantly enriched differentially expressed genes (DEGs) within the TNF and NF-κB pathways (**Figure 3e**). TNF, a key mediator of neuroinflammation, is predominantly secreted by activated microglia and astrocytes. It amplifies neuroinflammation by activating the NF-κB pathway, which promotes the release of pro-inflammatory cytokines (e.g., IL-1β, IL-6) and exacerbates neuronal damage [10, 11]. In models of Alzheimer's disease (AD), TNF overexpression induces A1-type astrocyte activation, further facilitating β-amyloid (Aβ) deposition and neuroinflammation [58]. Our study suggests that the knockdown of Notch1 may inhibit the expression of the downstream target Hes1 (**Figure 1e** and **Figure 1f**), which regulates the TNF/NF-κB pathway and influences the release of pro-inflammatory factors (**Figure 5c–h**). This mechanism has also been observed in Parkinson's disease (PD) and multiple sclerosis (MS), where neuroinflammation induces neuronal apoptosis and glial scarring through the TNF/NF-κB pathway [10, 11, 13]. Furthermore, the synergy between Notch1 and NF-κB may involve epigenetic regulation (e.g., chromatin remodeling or DNA methylation) to influence the transcriptional activity of inflammation-related genes [10, 11], highlighting the central role of the Notch1-TNF/NF-κB axis in neurodegenerative diseases. In summary, Notch1 exerts a regulatory effect on neuroinflammation by inhibiting the overactivation of TNF and NF-κB signaling pathways, thereby providing a molecular basis for Notch1-targeted immunotherapy strategies.

The Notch1 signaling pathway demonstrates multifaceted roles in neuroimmune regulation, offering a potential therapeutic target for neurodegenerative and cerebrovascular diseases. Research indicates that Notch1 modulates neuroinflammatory processes by regulating the polarization of microglia and macrophages. For example, the synergistic interaction of Notch1 with the GSK-3β and NF-κB/p65 pathways dynamically modulates the synthesis of pro-inflammatory mediators (e.g., IL-6, CXCL1) in microglial cells, whereas interventions targeting Notch1 promotes microglial cell polarisation towards an anti-inflammatory phenotype (M2) and ameliorate neuroinflammation and synaptic dysfunction in temporal lobe epilepsy [59, 60]. Rhodiola rosea glycosides promote endogenous neuroregeneration following cerebral ischemia/reperfusion through activation of the Notch1 signaling pathway in concert with neurotrophic factors (e.g., BDNF, NGF), while also inhibiting microglia-mediated neuroinflammation [61]. Similarly, Psoralea cinnamomi granules inhibit microglial activation and reduce the release of pro-inflammatory factors (e.g., TNF-α, IL-1β) by modulating Notch1-dependent signaling, thereby attenuating neuronal apoptosis [62]. In a cerebral hemorrhage model, minocycline regulates CD4+ T-cell differentiation through the Notch1 signaling pathway, inhibits Th1/Th17 cell-driven pro-inflammatory responses, and promotes regulatory T-cell (Treg) expansion, thereby alleviating white matter damage [63]. These findings 66highlight the bidirectional plasticity of the Notch1 signaling pathway: its activation exerts neuroprotective effects in specific cell types (e.g., T

cells, microglia) or microenvironments (e.g., ischemia, hemorrhage) by inhibiting NF-κB, modulating immune cell differentiation, or inducing conversion to an anti-inflammatory phenotype. Furthermore, activation of the Notch1 receptor by ligands such as DLL1, DLL4, and Jagged1 influences downstream genes like Hes1 and Hey1, which are crucial for T cell development, differentiation, and functionality [64]. Additionally, this pathway plays a significant role in antitumor immunity by curbing the production of regulatory T cells (Tregs) and boosting the activity of effector T cells [65]. The Notch1 signaling pathway regulates inflammatory responses and immune cell activity through a complex mechanism of neuroimmune regulation, and its activation can inhibit the release of inflammatory factors and overactivation of immune cells under specific pathological conditions, providing a potential therapeutic target for neurodegenerative and cerebrovascular diseases.

5. Conclusions

Notch signaling is widely regarded as an evolutionarily conserved pathway that plays a crucial role in the exchange of information from the cell membrane to the nucleus. This intricate signaling mechanism not only underlies many biological processes such as cell differentiation, immune regulation, and apoptosis, but also plays an important role in neuroimmune signaling. The study revealed the important role of the Notch signaling pathway in neuronal apoptosis, as well as in immune regulation and inflammation within the mouse hippocampus, and this pathway may serve as a potential target for immunotherapy of neurological diseases. A schematic figure about the Notch1-apoptosis-immunity pathway is shown in **Figure 6**. Apoptosis is an important means of immune-inflammatory clearance, and when neurons are exposed to environmental stresses, especially under pathological conditions, they produce and secrete cytokines that activate glial cells, thereby affecting the function of immune cells and facilitating their immune response. Notch signaling can influence the course of neuroinflammation and neurodegenerative diseases by modulating the interactions between immune cells and neuronal cells. The centrality of the Notch1 signaling pathway in neuroinflammation and immune regulation makes it a potential target for immunotherapy of neurological diseases. Based on this, the development of Notch1 pathway-specific agonists (e.g., small molecule compounds or active ingredients from traditional Chinese medicines) or co-targeting strategies to inhibit neuroinflammation, promote neuronal survival, and restore neurological function, thereby opening up new directions for the treatment of stroke, Alzheimer's disease, multiple sclerosis, and other disorders, could open up new pathways for the precision treatment of neuroimmune-related diseases.

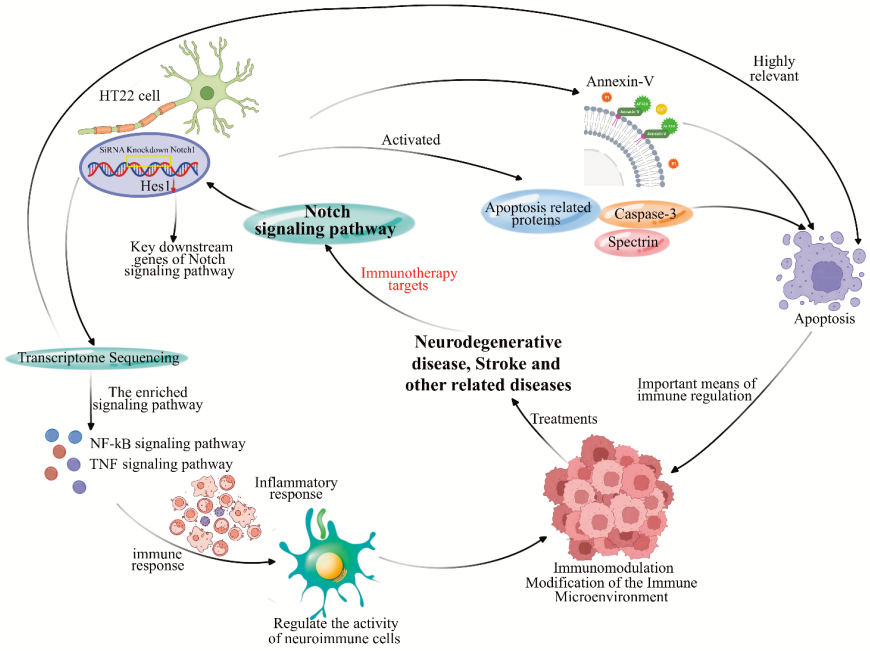

Figure 6. Notch1-Apoptosis-Immunity pathway schematic.

In the present study, our in vitro model based on a mouse hippocampal neuronal cell line (HT22 cells) has certain limitations. The in vitro culture system cannot replicate the dynamic interactions of neural-immune units, and there are species-specific differences in the downstream molecules of Notch1 signaling between mice and humans. Therefore, our subsequent study aims to evaluate the mechanisms of interaction between the Notch1 signaling pathway/apoptosis/immunity pathway using transgenic mouse models, human-derived neurons, and human iPSC-differentiated neurological organoid models to contribute to the clinical application of immunotherapy.

Author Contributions

Conceptualization, funding acquisition, W.X.; methodology, validation, data curation, writing—original draft preparation, writing—review and editing, S.J. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by support from the National Natural Science Foundation of China (82071479 and 31860307); Inner Mongolia Autonomous Region Natural Science Foundation (2020MS08006); Baotou Medical College Scientific Research and Development Fund Project (Zhenchuang Program) (BYJJ-ZCJH 202502).

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data and materials have been made available.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Bigas, A.; Espinosa, L. Hematopoietic stem cells: to be or Notch to be. *Blood* **2012**, *119*, 3226–3235. [CrossRef]
2. Koch, U.; Lehal, R.; Radtke, F. Stem cells living with a Notch. *Development* **2013**, *140*, 689–704. [CrossRef]
3. Klinakis, A.; Lobry, C.; Abdel-Wahab, O.; et al. A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. *Nature* **2011**, *473*, 230–233. [CrossRef]
4. Wang, M.; Yu, F.; Zhang, Y.; et al. Novel insights into Notch signaling in tumor immunity: potential targets for cancer immunotherapy. *Front. Immunol.* **2024**, *15*, 1352484. [CrossRef]
5. Alberi, L.; Liu, S.; Wang, Y.; et al. Activity-induced Notch signaling in neurons requires Arc/Arg3.1 and is essential for synaptic plasticity in hippocampal networks. *Neuron* **2011**, *69*, 437–444. [CrossRef]
6. Ables, J.L.; Breunig, J.J.; Eisch, A.J.; et al. Not(ch) just development: Notch signalling in the adult brain. *Nat. Rev. Neurosci.* **2011**, *12*, 269–283. [CrossRef]
7. Wang, Y.; Chan, S.L.; Miele, L.; et al. Involvement of Notch signaling in hippocampal synaptic plasticity. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9458–9462. [CrossRef]
8. Ding, X.F.; Gao, X.; Ding, X.C.; et al. Postnatal dysregulation of Notch signal disrupts dendrite development of adult-born neurons in the hippocampus and contributes to memory impairment. *Sci. Rep.* **2016**, *6*, 25780. [CrossRef]
9. Louvi, A.; Artavanis-Tsakonas, S. Notch and disease: a growing field. *Semin. Cell Dev. Biol.* **2012**, *23*, 473–480. [CrossRef]
10. Cohen, J.; Mathew, A.; Dourvetakis, K.D.; et al. Recent Research Trends in Neuroinflammatory and Neurodegenerative Disorders. *Cells* **2024**, *13*. [CrossRef]

11. Alsbrook, D.L.; Di Napoli, M.; Bhatia, K.; et al. Neuroinflammation in Acute Ischemic and Hemorrhagic Stroke. *Curr. Neurol. Neurosci. Rep.* **2023**, *23*, 407–431. [CrossRef]
12. Tater, P.; Pandey, S. Post-stroke Movement Disorders: Clinical Spectrum, Pathogenesis, and Management. *Neurol. India* **2021**, *69*, 272–283. [CrossRef]
13. Pajares, M.; A, I.R.; Manda, G.; et al. Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. *Cells* **2020**, *9*. [CrossRef]
14. Androutsellis-Theotokis, A.; Leker, R.R.; Soldner, F.; et al. Notch signalling regulates stem cell numbers in vitro and in vivo. *Nature* **2006**, *442*, 823–826. [CrossRef]
15. Sun, F.; Mao, X.; Xie, L.; et al. Notch1 signaling modulates neuronal progenitor activity in the subventricular zone in response to aging and focal ischemia. *Aging Cell* **2013**, *12*, 978–987. [CrossRef]
16. Chen, M.; Lu, T.J.; Chen, X.J.; et al. Differential roles of NMDA receptor subtypes in ischemic neuronal cell death and ischemic tolerance. *Stroke* **2008**, *39*, 3042–3048. [CrossRef]
17. Kawai, T.; Takagi, N.; Nakahara, M.; et al. Changes in the expression of Hes5 and Mash1 mRNA in the adult rat dentate gyrus after transient forebrain ischemia. *Neurosci. Lett.* **2005**, *380*, 17–20. [CrossRef]
18. Wang, L.; Chopp, M.; Zhang, R.L.; et al. The Notch pathway mediates expansion of a progenitor pool and neuronal differentiation in adult neural progenitor cells after stroke. *Neuroscience* **2009**, *158*, 1356–1363. [CrossRef]
19. Li, Z.; Wang, J.; Zhao, C.; et al. Acute Blockage of Notch Signaling by DAPT Induces Neuroprotection and Neurogenesis in the Neonatal Rat Brain After Stroke. *Transl. Stroke Res.* **2016**, *7*, 132–140. [CrossRef]
20. Arumugam, T.V.; Baik, S.H.; Balaganapathy, P.; et al. Notch signaling and neuronal death in stroke. *Prog. Neurobiol.* **2018**, *165–167*, 103–116. [CrossRef]
21. Yao, Y.Y.; Li, R.; Guo, Y.J.; et al. Gastrodin Attenuates Lipopolysaccharide-Induced Inflammatory Response and Migration via the Notch-1 Signaling Pathway in Activated Microglia. *Neuromol. Med.* **2022**, *24*, 139–154. [CrossRef]
22. Zhang, Y.H.; Wang, T.; Li, Y.F.; et al. Roles of the Notch signaling pathway and microglia in autism. *Behav. Brain Res.* **2023**, *437*, 114131. [CrossRef]
23. Wu, F.; Zuo, H.J.; Ren, X.Q.; et al. Gastrodin Regulates the Notch-1 Signal Pathway via Renin-Angiotensin System in Activated Microglia. *Neuromol. Med.* **2023**, *25*, 40–52. [CrossRef]
24. Bassil, R.; Orent, W.; Elyaman, W. Notch signaling and T-helper cells in EAE/MS. *Clin. Dev. Immunol.* **2013**, *2013*, 570731. [CrossRef]
25. Bassil, R.; Zhu, B.; Lahoud, Y.; et al. Notch ligand delta-like 4 blockade alleviates experimental autoimmune encephalomyelitis by promoting regulatory T cell development. *J. Immunol.* **2011**, *187*, 2322–2328. [CrossRef]
26. Liang, J.; Han, S.; Ye, C.; et al. Minocycline Attenuates Sevoflurane-Induced Postoperative Cognitive Dysfunction in Aged Mice by Suppressing Hippocampal Apoptosis and the Notch Signaling Pathway-Mediated Neuroinflammation. *Brain Sci.* **2023**, *13*. [CrossRef]
27. Yu, B.; Song, B. Notch 1 signalling inhibits cardiomyocyte apoptosis in ischaemic postconditioning. *Heart Lung Circul.* **2014**, *23*, 152–158. [CrossRef]
28. Nair, P.; Somasundaram, K.; Krishna, S. Activated Notch1 inhibits p53-induced apoptosis and sustains transformation by human papillomavirus type 16 E6 and E7 oncogenes through a PI3K-PKB/Akt-dependent pathway. *J. Virol.* **2003**, *77*, 7106–7112. [CrossRef]
29. Lan, L.; Wang, Y.; Pan, Z.; et al. Rhamnetin induces apoptosis in human breast cancer cells via the miR-34a/Notch-1 signaling pathway. *Oncol. Lett.* **2019**, *17*, 676–682. [CrossRef]
30. Xiang, Z.; Miao, Q.; Zhang, J.; et al. AB4 inhibits Notch signaling and promotes cancer cell apoptosis in liver cancer. *Oncol. Rep.* **2021**, *45*. [CrossRef]
31. Zhao, Z.; Lu, R.; Zhang, B.; et al. Differentiation of HT22 neurons induces expression of NMDA receptor that mediates homocysteine cytotoxicity. *Neurol. Res.* **2012**, *34*, 38–43. [CrossRef]
32. Chang, Z.; Xu, W.; Jiang, S.; et al. Effects of 5-Aza on neurogenesis contribute to learning and memory in the mouse hippocampus. *Biomed. Pharmacother. = Biomed. pharmacother.* **2022**, *154*, 113623. [CrossRef]
33. Tian, X.L.; Jiang, S.Y.; Zhang, X.L.; et al. Potassium bisperoxo (1,10-phenanthroline) oxovanadate suppresses proliferation of hippocampal neuronal cell lines by increasing DNA methyltransferases. *Neural Regen. Res.* **2019**, *14*, 826–833. [CrossRef]
34. Liu, N.; Zhang, X.L.; Jiang, S.Y.; et al. Neuroprotective mechanisms of DNA methyltransferase in a mouse hippocampal neuronal cell line after hypoxic preconditioning. *Neural Regen. Res.* **2020**, *15*, 2362–2368. [CrossRef]

35. Lu, F.; Zhu, J.; Guo, S.; et al. Upregulation of cholesterol 24-hydroxylase following hypoxia-ischemia in neonatal mouse brain. *Pediatr. Res.* **2018**, *83*, 1218–1227. [CrossRef]
36. Eskandari, E.; Eaves, C.J. Paradoxical roles of caspase-3 in regulating cell survival, proliferation, and tumorigenesis. *J. Cell Biol.* **2022**, *221*. [CrossRef]
37. Zhu, L.; Chen, Y.; Ding, W.; et al. Caspase-3/Treg and PI3K/AKT/mTOR pathway is involved in Liver Ischemia Reperfusion Injury (IRI) protection by everolimus. *Transpl. Immunol.* **2022**, *71*, 101541. [CrossRef]
38. Zapata-Lopera, Y.M.; Trejo-Tapia, G.; Cano-Europa, E.; et al. Neuroprotective effect of *Bouvardia ternifolia* (Cav.) Schltdl via inhibition of TLR4/NF-κB, caspase-3/Bax/Bcl-2 pathways in ischemia/reperfusion injury in rats. *Front. Pharmacol.* **2024**, *15*, 1471542. [CrossRef]
39. Fan, W.; Dai, Y.; Xu, H.; et al. Caspase-3 modulates regenerative response after stroke. *Stem Cells* **2014**, *32*, 473–486. [CrossRef]
40. Mora, P.; Chapouly, C. Astrogliosis in multiple sclerosis and neuro-inflammation: what role for the notch pathway? *Front. Immunol.* **2023**, *14*, 1254586. [CrossRef]
41. Mochizuki, K.; He, S.; Zhang, Y. Notch and inflammatory T-cell response: new developments and challenges. *Immunotherapy* **2011**, *3*, 1353–1366. [CrossRef]
42. Zhang, Y.; Wang, T.; Wu, S.; et al. Notch signaling pathway: a new target for neuropathic pain therapy. *J. Headache Pain* **2023**, *24*, 87. [CrossRef]
43. Yashiro-Ohtani, Y.; Ohtani, T.; Pear, W.S. Notch regulation of early thymocyte development. *Semin. Immunol.* **2010**, *22*, 261–269. [CrossRef]
44. Tanigaki, K.; Honjo, T. Regulation of lymphocyte development by Notch signaling. *Nature Immunol.* **2007**, *8*, 451–456. [CrossRef]
45. Eagar, T.N.; Tang, Q.; Wolfe, M.; et al. Notch 1 signaling regulates peripheral T cell activation. *Immunity* **2004**, *20*, 407–415. [CrossRef]
46. Ostroukhova, M.; Qi, Z.; Oriss, T.B.; et al. Treg-mediated immunosuppression involves activation of the Notch-HES1 axis by membrane-bound TGF-beta. *J. Clin. Investig.* **2006**, *116*, 996-1004. [CrossRef]
47. Li, X.; Yan, X.; Wang, Y.; et al. The Notch signaling pathway: a potential target for cancer immunotherapy. *J. Hematol. Oncol.* **2023**, *16*, 45. [CrossRef]
48. Blanquie, O.; Kilb, W.; Sinning, A.; et al. Homeostatic interplay between electrical activity and neuronal apoptosis in the developing neocortex. *Neuroscience* **2017**, *358*, 190–200. [CrossRef]
49. Bredesen, D.E. Genetic control of neural cell apoptosis. *Perspect. Dev. Neurobiol.* **1996**, *3*, 101–109.
50. Passeri, E.; Elkhoury, K. Alzheimer's Disease: Treatment Strategies and Their Limitations. *Brain Sci.* **2022**, *23*. [CrossRef]
51. Dhapola, R.; Hota, S.S.; Sarma, P.; et al. Recent advances in molecular pathways and therapeutic implications targeting neuroinflammation for Alzheimer's disease. *Inflammopharmacology* **2021**, *29*, 1669–1681. [CrossRef]
52. Xiao, P.; Zhang, X.; Li, Y.; et al. miR-9 inhibition of neuronal apoptosis and expression levels of apoptosis genes Bcl-2 and Bax in depression model rats through Notch pathway. *Exp. Ther. Med.* **2020**, *19*, 551–556. [CrossRef]
53. Chen, Z.; Liu, J.; Chen, Q.; et al. Down-regulation of UBA6 exacerbates brain injury by inhibiting the activation of Notch signaling pathway to promote cerebral cell apoptosis in rat acute cerebral infarction model. *Mol. Cell. Probes* **2020**, *53*, 101612. [CrossRef]
54. Patterson, L.L.; Byerly, C.D.; Solomon, R.; et al. Ehrlichia Notch signaling induction promotes XIAP stability and inhibits apoptosis. *Infect. Immunity* **2023**, *91*, e0000223. [CrossRef]
55. Wu, H.; Chen, Q.Y.; Wang, W.Z.; et al. Compound sophorae decoction enhances intestinal barrier function of dextran sodium sulfate induced colitis via regulating notch signaling pathway in mice. *Biomed. Pharmacother. = Biomed. Pharmacother.* **2021**, *133*, 110937. [CrossRef]
56. Zhang, K.; Zhao, T.; Huang, X.; Notch1 mediates postnatal neurogenesis in hippocampus enhanced by intermittent hypoxia. *Neurobiol. Dis.* **2014**, *64*, 66–78. [CrossRef]
57. Ables, J.L.; Decarolis, N.A.; Johnson, M.A.; et al. Notch1 is required for maintenance of the reservoir of adult hippocampal stem cells. *J. Neurosci. off. J. Soc. Neurosci.* **2010**, *30*, 10484–10492. [CrossRef]
58. Goetzl, E.J.; Schwartz, J.B.; Abner, E.L.; et al. High complement levels in astrocyte-derived exosomes of Alzheimer disease. *Ann. Neurol.* **2018**, *83*, 544–552. [CrossRef]
59. Cao, Q.; Karthikeyan, A.; Dheen, S.T.; et al. Production of proinflammatory mediators in activated microglia is synergistically regulated by Notch-1, glycogen synthase kinase (GSK-3β) and NF-κB/p65 signalling. *PLoS ONE* **2017**, *12*, e0186764. [CrossRef]

60. Deng, X.L.; Feng, L.; Wang, Z.X.; et al. The Runx1/Notch1 Signaling Pathway Participates in M1/M2 Microglia Polarization in a Mouse Model of Temporal Lobe Epilepsy and in BV-2 Cells. *Neurochem. Res.* **2020**, *45*, 2204–2216. [CrossRef]
61. Zheng, J.; Zhang, J.; Han, J.; et al. The effect of salidroside in promoting endogenous neural regeneration after cerebral ischemia/reperfusion involves notch signaling pathway and neurotrophic factors. *BMC Complement. Med. Ther.* **2024**, *24*, 293. [CrossRef]
62. Li, X.; Huang, H.; Li, Y.; et al. Gualou Guizhi Granule inhibits microglia-mediated neuroinflammation to protect against neuronal apoptosis in vitro and in vivo. *Front. Immunol.* **2024**, *15*, 1527986. [CrossRef]
63. Yang, H.; Gao, X.; Xiao, W.; et al. Minocycline Alleviates White Matter Injury following Intracerebral Hemorrhage by Regulating CD4+ T Cell Differentiation via Notch1 Signaling Pathway. *Oxid. Med. Cell. Longev.* **2022**, *2022*, 3435267. [CrossRef]
64. Radtke, F.; Wilson, A.; Mancini, S.J.; et al. Notch regulation of lymphocyte development and function. *Nat. Immunol.* **2004**, *5*, 247–253. [CrossRef]
65. Amsen, D.; Blander, J.M.; Lee, G.R.; et al. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* **2004**, *117*, 515–526. [CrossRef]