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Interferon-gamma inhibits the neuronal differentiation of neural progenitor cells by inhibiting the expression of Neurogenin2 via the JAK/STAT1 pathway

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ABSTRACT

Interferon-gamma (IFN-γ) is one of the critical cytokines released by host immune cells upon infection. Despite the important role(s) of IFN-γ in host immune responses, there has been no *in vivo* study regarding the effects of IFN-γ on brain development, and the results from many *in vitro* studies are controversial. In this study, the effects of IFN-γ on embryonic neurogenesis were investigated. Treatment of E14.5 mouse neural progenitor cells (NPCs) with IFN-γ resulted in a decrease in the percentage of TuJ1-positive immature neurons but an increase in the percentage of Nestin-positive NPCs. Similar results were obtained *in vivo*. Treatment of NPCs with a JAK inhibitor or the knockdown of STAT1 expression abrogated the IFN-γ-mediated inhibition of neurogenesis. Interestingly, the expression of one of proneural genes, Neurogenin2 (*Neurog2*) was dramatically inhibited upon IFN-γ treatment, and cells overexpressing Neurog2 did not respond to IFN-γ. Taken together, our results demonstrate that IFN-γ inhibits neuronal differentiation of NPCs by negatively regulating the expression of *Neurog2* via the JAK/STAT1 pathway. Our findings may provide an insight into the role of IFN-γ in the development of embryonic brain.

1. Introduction

Interferon-gamma (IFN-γ) is one of the inflammatory cytokines expressed in the fetal brain following an inflammatory stimulus [1]. It can be expressed from embryonic day (E) 7 in mice and week 21 of pregnancy in humans [2,3]. The binding of IFN-γ to its receptor results in the phosphorylation of Janus tyrosine kinase (JAK) 1 and JAK2 and the subsequent phosphorylation of signal transducer and activator of transcription (STAT) 1. Phosphorylated STAT1 then forms a homodimer and translocates into the nucleus [4]. STAT1, acting as a transcription factor, can positively or negatively regulate downstream target genes, depending on the presence of other co-factors [5].

The effects of IFN-γ on neurogenesis have been investigated *in vitro* using recombinant IFN-γ in various types of cells. However, depending on the cell type and the concentration of IFN-γ, different results have been reported. In experiments involving murine adult neural stem cells, a human neuroblastoma cell line, a neonatal neural progenitor cell line (C17.2), and E15–E16 neurons, treatment with IFN-γ resulted in increased neurogenesis [6–9]. However, in an experiment involving cells from the subventricular zone of postnatal day 2 mouse brains, IFN-γ had the opposite effect [10]. Furthermore, it was also reported that a high level of IFN-γ resulted in irregular cell types double-positive for glial fibrillary acid protein (GFAP) and TuJ1 when E14 neural stem/precursor cells were used [11]. Not only are the effects of IFN-γ on neurogenesis controversial, but the downstream mechanism of this cytokine is barely elucidated.

In this study, we investigated the role of IFN-γ in embryonic neurogenesis, both *in vitro* and *in vivo*. It was found that IFN-γ effectively inhibits neurogenesis, and this inhibition was dependent on the JAK/STAT1 signaling pathway in primary neural progenitor cells (NPCs). An *in vivo* study using E14.5 embryonic brains also showed a similar result. Interestingly, IFN-γ treatment specifically decreased the RNA level of Neurogenin2 (Neurog2) among other proneural genes. To our knowledge, this is the first study reporting a role of IFN-γ in the regulation of proneural gene expression in differentiating NPCs.

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2. Materials and methods

2.1. Plasmid constructs

The murine IFN-γ and Neurog2 were amplified from cDNAs isolated from the PMA/Ionomycin stimulated mouse splenocytes, and adult murine hippocampal tissue, respectively using primers listed in S1 Table. These sequences were cloned into pGEM®-T Easy (Promega), and verified sequences were cloned into the MS-IRES-eGFP vector [12].

The shRNA sequences (S2 Table) targeting mouse STAT1 or luciferase were cloned into pSIREN-DsRed (Clontech) vector, which was manipulated to contain HA tag.

2.2. MTT assay

NPCs were differentiated with various concentrations of IFN-γ for 2 days. Cell viability was analyzed using MTT assay kit (Roche) according to the manufacturer's protocol.

2.3. Quantitative RT-PCR

Total RNA was isolated using RNAiso Plus (Takara)1ug of each RNA was used to synthesize cDNAs using Reverse Transcriptase XL (AMV) (Takara). Quantitative PCR was performed using SYBR Premix Ex Taq (Takara) and the primers listed in S3 Table.

2.4. Others

All other experiments were performed as previously described [13]. All primary and secondary antibodies were listed in S4 Table. Animal procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University.

2.5. Statistical analysis

All values are presented as mean ± standard deviation from triplicate measurements. Differences between values were determined using GraphPad Prism software (Version 5, GraphPad Software). Statistical tests used in each experiment are indicated in each figure legend.

3. Results

3.1. Effects of IFN-γ on the neuronal differentiation of NPCs

To investigate the effects of IFN-γ on neuronal differentiation, primary NPCs were treated with different concentrations of recombinant IFN-γ. Primary NPCs were prepared from E14.5 mouse embryos and cultured in NPC proliferation medium. After 2 days, NPCs were differentiated with DMEM containing 2% FBS in the presence of 5–50 ng/mL IFN-γ for another 2 days. Differentiated cells were stained for specific markers of immature neurons (TuJ1), NPCs (Nestin), and astrocytes (GFAP). 45 ± 5.2% of total cells were TuJ1-positive immature neurons, while 35 ± 5.3% of them were Nestin-positive NPCs. Only a small proportion of cells (15 ± 1.2%) were GFAP-positive astrocytes. Upon treatment with 50 ng/mL IFN-γ, the percentage of TuJ1-positive cells was decreased to 17 ± 2.0%, while the percentage of Nestin-positive cells was increased to 72 ± 1.2%. There was no significant difference in the percentage of GFAP-positive astrocytes (Fig. 1A,B). These results strongly suggest an inhibitory role of IFN-γ in the neuronal differentiation of NPCs.

To test whether IFN-γ has an effect on NPCs in a proliferative status, isolated NPCs were grown in NPC proliferation medium with 50 ng/mL IFN-γ for 2 days and differentiated in the absence of IFN-γ. There were no statistically significant differences in the percentages of different cell types between the control and IFN-γ-treated cells (Fig. 1C,D). This indicates that IFN-γ may have effects only on NPCs that have started differentiation and not on actively proliferating NPCs.

To investigate whether the decreased number of TuJ1-positive cells was due to increased cell death, NPCs were differentiated with 5–50 ng/mL IFN-γ for 2 days and subjected to the MTT and TUNEL assays. As shown in Fig. 1E, no statistically significant difference was found in the number of viable cells between the control and IFN-γ-treated groups. Moreover, similar numbers of TUNEL-positive cells were observed in the control and IFN-γ-treated groups (Fig. 1F,G). These data demonstrate that IFN-γ does not have cellular toxicity and does not have an effect on apoptotic cell death.

3.2. Effects of IFN-γ on the neuronal differentiation of NPCs in vivo

To confirm the inhibitory effect of IFN-γ on neuronal differentiation *in vivo*, a retroviral vector, expressing IFN-γ and enhanced green fluorescent protein (eGFP) from a bicistronic message, was injected into the ventricles of E9.5 embryonic brains using the ultrasound image-guided gene delivery technique [14]. The activity of exogenous IFN-γ was confirmed in NPCs by measuring the phosphorylation status of STAT1 (Fig. S1). When NPCs in the ventricular zone (VZ) are transduced with such retroviral vectors, the neurons produced from the NPCs are supposed to migrate away from the VZ during neurogenesis. The brains were analyzed at E14.5, when neurogenesis reaches its peak level [15]. When embryonic brains were injected with a control vector expressing only eGFP, 64 ± 13% of eGFP-positive cells were localized in the TuJ1-stained region. In contrast, when the vector expressing both IFN-γ and eGFP was delivered to embryonic brains, transduced cells were predominantly found in the VZ (Fig. 2A,B). Only approximately 24% of cells were positive for TuJ1, which was significantly lower than that of the control ($P = 0.0287$). These data suggest that IFN-γ indeed suppresses neuronal differentiation during brain development.

3.3. Involvement of the JAK/STAT1 pathway in the IFN-γ-mediated inhibition of neurogenesis

To understand the downstream mechanism of the inhibitory role of IFN-γ in embryonic neurogenesis, the involvement of the JAK/STAT1 pathway was analyzed as it is the main signaling route activated by IFN-γ. Primary E14.5 NPCs were differentiated with DMEM containing 2% FBS and treated with IFN-γ alone or in combination with 1 μM JAK1/2 inhibitor (Ruxolitinib) for 2 days. The proportion of TuJ1-positive cells was reduced from 60 ± 3.1% to 26 ± 5.6% upon IFN-γ treatment and was restored to the control level when Ruxolitinib was added concurrently. Conversely, the proportion of Nestin-positive cells increased from 36 ± 4.8% to 56 ± 2.9% after IFN-γ treatment but remained similar to the control level after treatment with both IFN-γ and Ruxolitinib (41 ± 11% and 34 ± 8.1%, respectively). There was no significant difference in the number of GFAP-positive astrocytes between the control and IFN-γ-treated groups (Fig. 3A,B). The activity of Ruxolitinib was confirmed by examining the phosphorylation level of STAT1 by western blotting. Upon IFN-γ treatment, the STAT1 tyrosine residue was phosphorylated as expected, but phosphorylation was totally inhibited when 1 μM Ruxolitinib was added (Fig. S2).

As JAK1/2 is involved in the activation of not only STAT1 but also other signaling pathways such as STAT3 and MAPK pathways [16–18], shRNA targeting STAT1 was employed to confirm the role of STAT1 and rule out the effects of other factors on neurogenesis. Retroviral vectors expressing three different shRNA sequences against STAT1, together with HA-tagged DsRed, were constructed.

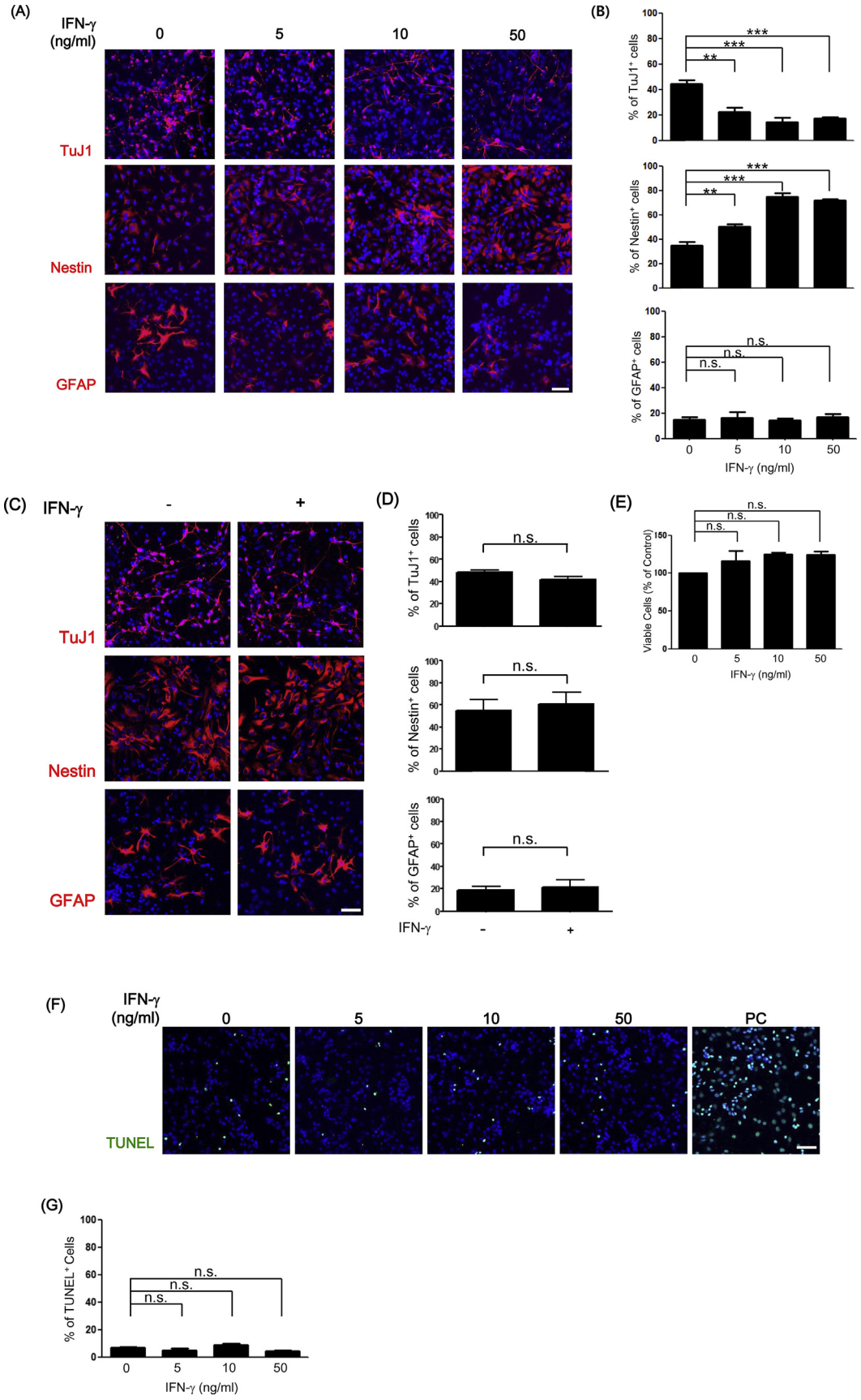

Fig. 1. IFN-γ decreases the neuronal differentiation of primary NPCs. (A, B) Primary NPCs were induced to differentiate in the presence of different concentrations of IFN-γ for 2 days and then immunostained for different cell markers (TuJ1, Nestin, and GFAP). (C, D) NPCs were grown in proliferation medium in the presence of 50 ng/mL IFN-γ for 2 days and induced to differentiate in the absence of IFN-γ. After 2 days, immunostaining was done for TuJ1, Nestin, and GFAP. (E) NPCs were differentiated with various concentrations of IFN-γ for 2 days and subjected to the MTT assay. (F, G) NPCs were differentiated with different concentrations of IFN-γ for 2 days, and then the TUNEL assay was performed. DNase-treated cells were used as a positive control (PC). DNA was labeled with Hoechst 33258 (blue). Scale bar, 50 μm. The statistical significance of differences between two groups was analyzed using the unpaired *t* test. Other differences in values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant.

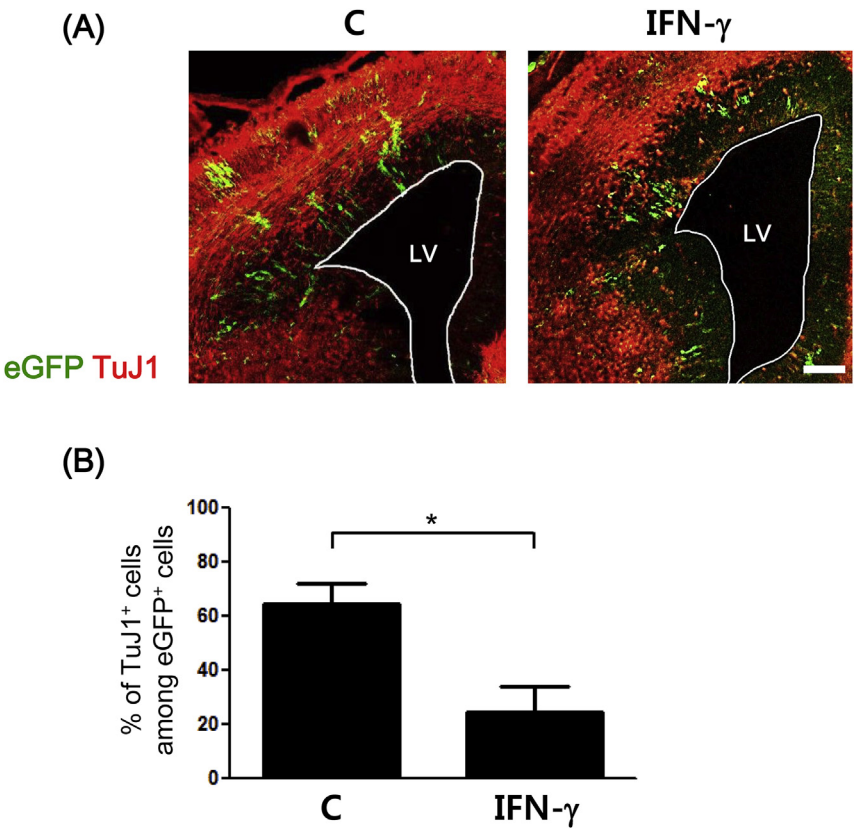

Fig. 2. IFN-γ inhibits neuronal differentiation *in vivo*. (A,B) Retroviral vectors expressing IFN-γ and eGFP were injected into E9.5 embryonic brains (n = 3/group) and then the brains were immunostained with antibodies to eGFP and TuJ1 at E14.5. Scale bar, 100 μm. The statistical significance of differences was analyzed using the unpaired *t* test. *P < 0.05. LV, lateral ventricle; C, control.

STAT1 shRNA sequence #2, which showed the strongest inhibition of STAT1 expression (approximately 67.2%), was chosen for subsequent experiments (Fig. 3C).

NPCs were transduced with the retroviral vector expressing STAT1 shRNA and, 2 days later, induced to differentiate in the presence of IFN-γ for 2 days. The percentages of HA-positive cells expressing different cell markers were calculated. Among NPCs transduced with a control vector, the percentage of TuJ1-positive cells was reduced from 51 ± 25% to 10 ± 8.4% upon IFN-γ treatment. When cells were transduced with the vector expressing STAT1 shRNA, however, there was no statistically significant difference between the control and IFN-γ-treated groups (44 ± 7.9% and 40 ± 10%, respectively). The percentage of Nestin-positive cells was increased approximately 2.5-fold upon IFN-γ treatment, while such an increase was not observed in cells expressing STAT1 shRNA. Neither IFN-γ treatment nor the knockdown of STAT1 expression affected the number of GFAP-positive cells (Fig. 3D,E). These results clearly show that the inhibition of neurogenesis by IFN-γ is dependent on the JAK/STAT1 signaling pathway.

3.4. Effects of IFN-γ on the expression of Neurogenin2

It was tested whether IFN-γ had an effect on the expression of the basic helix-loop-helix (bHLH) proneural genes as they are key players in the neuronal differentiation of NPCs [19]. E14.5 NPCs were treated with IFN-γ and differentiated with DMEM containing 2% FBS. Total RNA was collected every 3 h up to 12 h, and the RNA levels of *Neurog2*, *Mash1* and *Math1* were analyzed by qRT-PCR. While the expression levels of *Mash1* and *Math1* were similar between the control and IFN-γ-treated groups, there was a remarkable change in the case of *Neurog2*. The RNA level of *Neurog2* was induced more than 10-fold upon differentiation, while this induction was almost completely inhibited in the IFN-γ-treated group (Fig. 4A).

To determine whether an increased level of *Neurog2* expression can generate actual biological effects, the expression level of *Tbr2*, one of the downstream targets of Neurog2, was measured [20]. The RNA level of *Tbr2* was also increased in a time-dependent manner upon differentiation, while its upregulation was reduced upon treatment with IFN-γ, correlating with the level of *Neurog2* expression.

To investigate whether Neurog2 is indeed the key downstream target of IFN-γ, NPCs were transduced with a retroviral vector expressing Neurog2 and eGFP from a bicistronic message for 2 days and then differentiated with or without 50 ng/mL IFN-γ for another 2 days. IFN-γ treatment did not affect the level of eGFP expression when NPCs were transduced with a control vector expressing eGFP only, suggesting that IFN-γ did not have an influence on the function of viral long terminal repeats (data not shown). The expression of exogenous Neurog2 was confirmed in NIH/3T3 cells by western blotting (Fig. S3). As expected, among cells transduced with the control vector, IFN-γ treatment reduced the number of TuJ1-positive cells by nearly half while increasing the number of Nestin-positive cells more than 2-fold. However, almost all cells overexpressing Neurog2 had differentiated to TuJ1-positive neurons, and IFN-γ treatment showed no inhibitory effect (92 ± 14% and 94 ± 5.2%, respectively). There was no statistically significant difference in the percentage of GFAP-positive cells between the control and IFN-γ-treated groups (Fig. 4B,C). These results reveal that the inhibition of *Neurog2* expression is necessary for the IFN-γ-

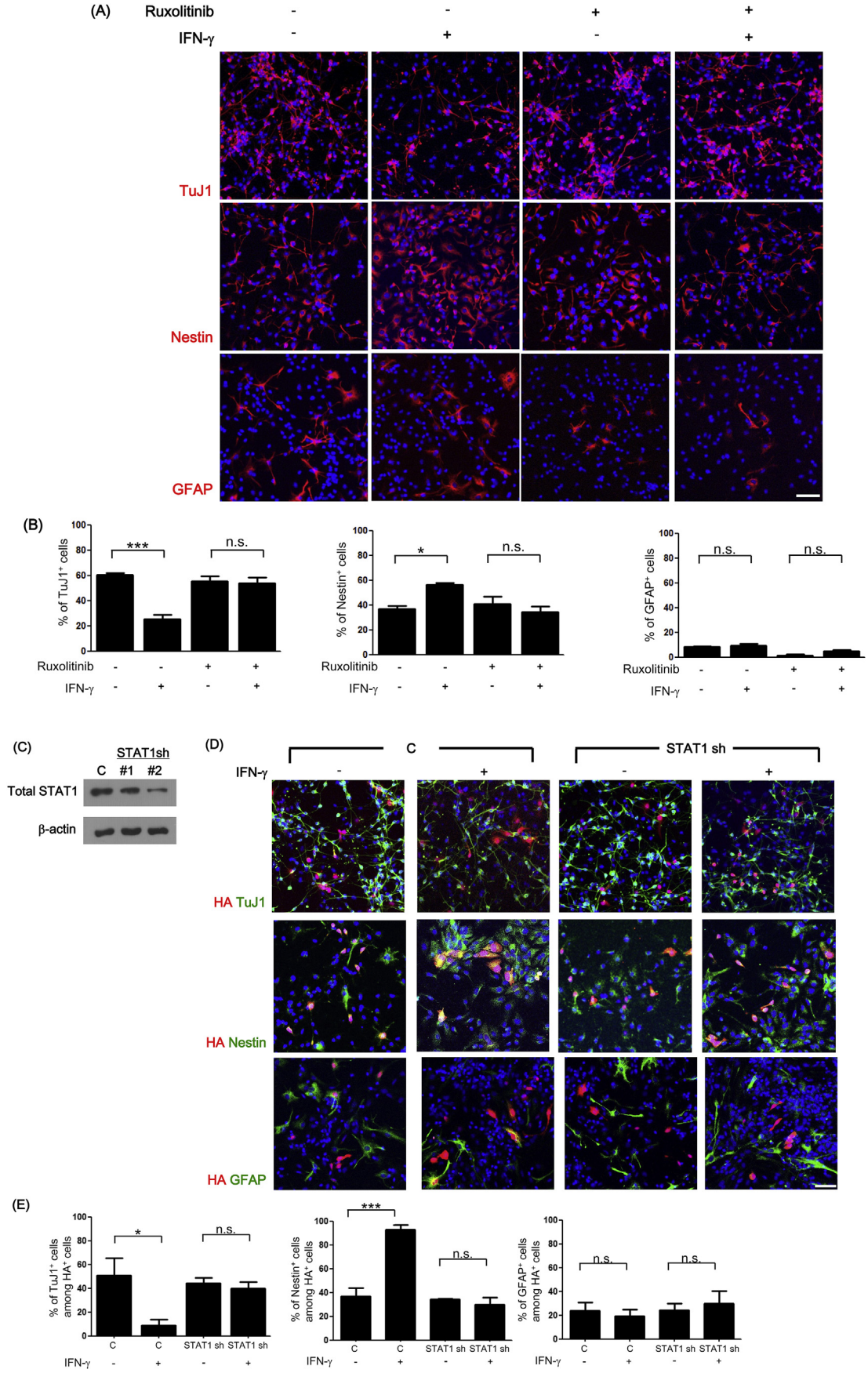

Fig. 3. IFN-γ-mediated inhibition of neurogenesis depends on the JAK/STAT1 pathway. (A, B) NPCs were induced to differentiate in the presence of 50 ng/mL IFN-γ with or without 1 μM Ruxolitinib for 2 days and then immunostained for TuJ1, Nestin, and GFAP. (C) NIH/3T3 cells were transduced with the same titer of retroviral vectors expressing control or STAT1 shRNA. After 2 days, proteins were prepared and subjected to western blotting. (D, E) NPCs were transduced with retroviral vectors expressing control or STAT1 shRNA together with HA-tagged DsRed. After 2 days, cells were differentiated with or without IFN-γ and then co-immunostained for HA and different cell markers (TuJ1, Nestin, and GFAP). DNA was labeled with Hoechst 33258 (blue). Scale bar, 50 μm. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. *$P < 0.05$; ***$P < 0.001$; n.s., not significant. C, control.

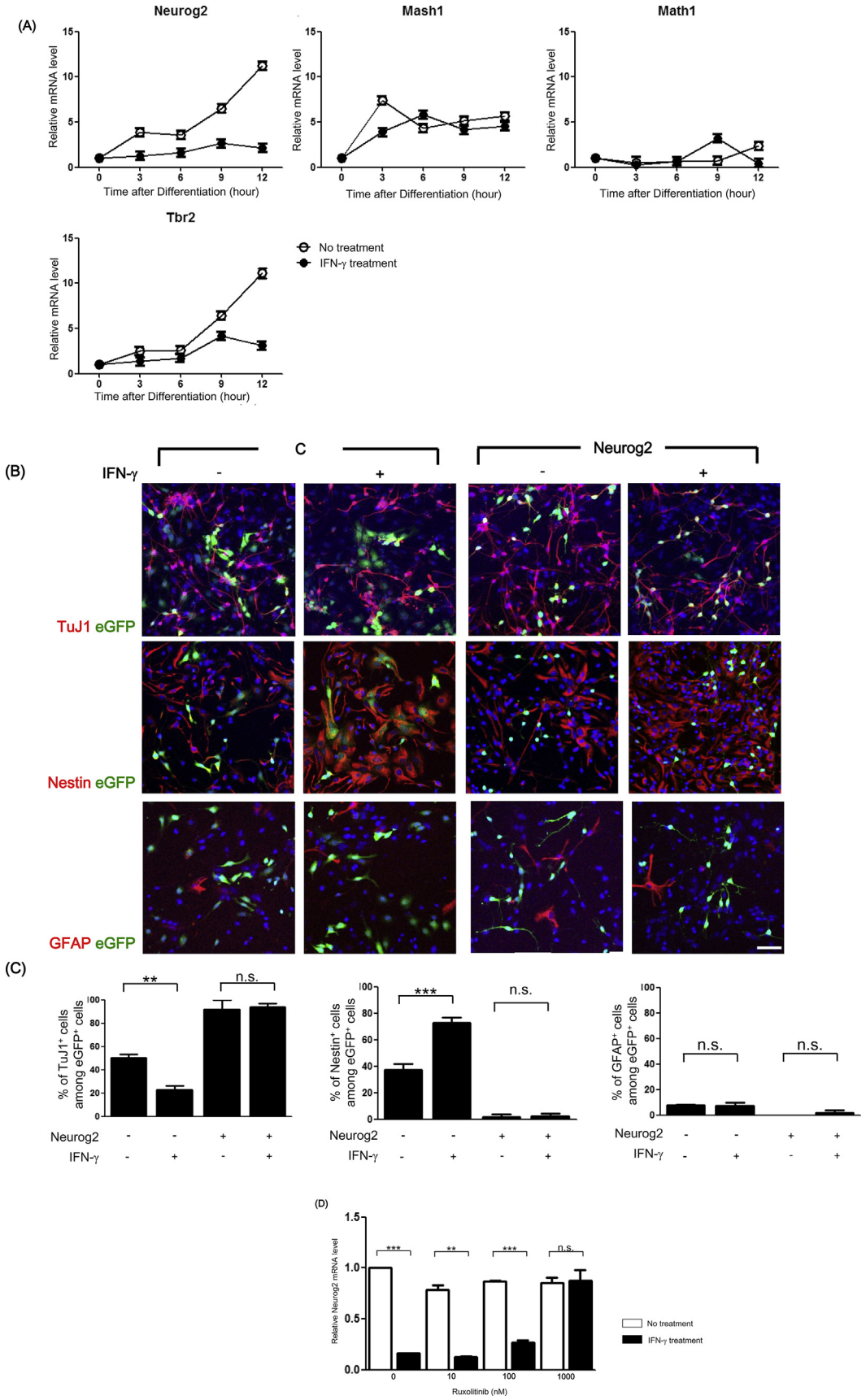

Fig. 4. IFN-γ negatively regulates the expression of *Neurog2* mRNA. (A) Total RNA was isolated from differentiated NPCs treated with or without 50 ng/mL IFN-γ every 3 h for 12 h. The levels of *Neurog2*, *Math1*, *Mash1*, and *Tbr2* mRNA were measured by qRT-PCR. Differences between the values of two groups were analyzed by two-way ANOVA (Source of Variation = IFN-γ treatment). P values for *Neurog2*, *Math1*, *Mash1*, and *Tbr2* are <0.0001, 0.3012, 0.3357, and 0.0001, respectively. (B, C) NPCs were transduced with a retroviral vector expressing Neurog2 and eGFP for 2 days. Cells were then differentiated with or without IFN-γ for another 2 days and subjected to co-immunostaining for eGFP and TuJ1, Nestin, or GFAP. DNA was labeled with Hoechst 33258 (blue). Scale bar, 50 μm. (D) NPCs were differentiated in the presence of different concentrations of Ruxolitinib with or without 50 ng/ml IFN-γ. After 12 h, RNA was isolated and the level of *Neurog2* mRNA was measured by qRT-PCR. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. **P < 0.01; ***P < 0.001; n.s., not significant.

mediated inhibition of neurogenesis.

Since the effects of IFN-γ on neurogenesis depend on the JAK/STAT1 pathway, it was tested whether inhibition of the JAK/STAT1 pathway would affect the IFN-γ-mediated downregulation of *Neurog2* expression. NPCs were treated with IFN-γ in the presence of 10 nM to 1 μM Ruxolitinib and differentiated for 12 h. Total RNA was isolated, and the level of *Neurog2* expression was determined by qRT-PCR (Fig. 4D). IFN-γ treatment reduced the expression of *Neurog2* by approximately 84%, but the expression level was restored to that of the control at 1 μM Ruxolitinib. Taken together, these data indicate that IFN-γ inhibits neurogenesis by inhibiting *Neurog2* expression through the JAK/STAT1 pathway.

4. Discussion

The role of IFN-γ in neurogenesis has been controversial. Here, we demonstrated that IFN-γ negatively regulates neuronal differentiation both *in vitro* and *in vivo*. Our data are in contrast with the results of several previous studies. For example, a study involving C17.2 cell line has showed that IFN-γ induces neuronal differentiation through activation of the JNK pathway [8]. In the study of Walter et al., treatment of proliferating E14 neural stem/precursor cells with 1,000 U/mL IFN-γ (equivalent to 1 mg/mL) resulted in atypical gene expression and cell functions through regulation of the sonic hedgehog pathway [21]. None of the above observations were made in our study. The different findings of these studies compared with our study may be due to the various types of cells and the different concentrations of IFN-γ used, or they may be due to differences in other experimental conditions such as the time of NPC isolation and the availability of cofactors of the JAK/STAT1 pathway.

Many downstream targets of IFN-γ are known, but they have not been extensively studied in the context of embryonic neuronal differentiation. According to our data, one of the final target genes of IFN-γ seems to be *Neurog2*. Neurog2 is an essential factor in the initiation of the neuronal differentiation of NPCs and is sufficient enough to generate neurons from mouse embryonic stem cells [22–25]. Although Neurog2 is a key factor in the determination of neural cell types and its expression pattern is tightly regulated, only a few factors are known to regulate its expression [26–28]. In our study, IFN-γ specifically suppressed the RNA level of *Neurog2* among many proneural genes. IFN-γ treatment inhibited the upregulation of *Neurog2* expression in NPCs throughout the differentiation period, and the overexpression of *Neurog2* completely abrogated the inhibitory effect of IFN-γ on neurogenesis. Because STAT1 can regulate gene expression in various ways, there are many possible mechanisms by which it might control the level of *Neurog2* mRNA. Although the exact IFN-γ activated sequence (GAS) element (TTCNNNGAA) is not present in the promoter of *Neurog2*, STAT1 may bind to similar sequences, as it is known to be permissive for mismatches [29] or to unique DNA sequences by interacting with other proteins. STAT1 may also bind to an enhancer region or induce the expression of one or more factors that might in turn regulate *Neurog2* expression. Further investigation is required to unravel the precise mechanism of how the JAK/STAT1 pathway controls the expression level of *Neurog2* mRNA.

Several recent studies have suggested that the cause of neurological disorders in infants that survive from congenital infection may be the inflammatory response, rather than the pathogen itself [30]. Lipopolysaccharide or polyinosinic:polycytidylic acid (synthetic double-stranded RNA) injections administered to rat or mouse dams have resulted in sensorimotor gating dysfunction, increased anxiety, impairment of social interactions, and other abnormal behaviors in newborns, showing that an inflammatory response is sufficient to cause behavioral abnormalities associated with brain dysfunction [31–33]. IFN-γ is one of the cytokines known to be released in the fetal brain during infection [1,3,34]. As Neurog2 is known to generate glutamatergic neurons while suppressing the generation of GABAergic neurons, the downregulation of *Neurog2* expression due to IFN-γ would lead to an abnormal ratio of glutamatergic to GABAergic neurons in the mature brain. An imbalance of glutamate and GABA has been reported to be one of the main causes of various neurological diseases including autism, Rett syndrome, schizophrenia, and mood disorders [35–37]. In this regard, it is interesting to note that the glutamate to GABA ratio was reduced while the level of IFN-γ was increased in the plasma of autistic patients compared with healthy subjects, suggesting a link between IFN-γ and brain abnormalities [35]. Although it is not yet clear what the consequences of the IFN-γ-mediated dysregulated neuronal differentiation during embryonic development are, our findings may provide an interesting starting point for understanding the role(s) of IFN-γ during brain development and ultimately neurodevelopmental disorders caused by congenital infections.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.08.104.

Transparency document

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References

[1] M.J. Bell, J.M. Hallenbeck, V. Gallo, Determining the fetal inflammatory response in an experimental model of intrauterine inflammation in rats, Pediatr. Res. 56 (2004) 541–546.
[2] D.P. Barlow, B.J. Randle, D.C. Burke, Interferon synthesis in the early post-implantation mouse embryo, Differ. Res. Biol. Divers. 27 (1984) 229–235.
[3] J. Raymond, M.H. Poissonnier, P.H. Thulliez, F. Forestier, F. Daffos, P. Lebon, Presence of gamma interferon in human acute and congenital toxoplasmosis, J. Clin. Microbiol. 28 (1990) 1434–1437.
[4] J.E. Darnell Jr., I.M. Kerr, G.R. Stark, Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins, Science 264 (1994) 1415–1421.
[5] C.V. Ramana, M. Chatterjee-Kishore, H. Nguyen, G.R. Stark, Complex roles of Stat1 in regulating gene expression, Oncogene 19 (2000) 2619–2627.
[6] G. Wong, Y. Goldshmit, A.M. Turnley, Interferon-gamma but not TNF alpha promotes neuronal differentiation and neurite outgrowth of murine adult neural stem cells, Exp. Neurol. 187 (2004) 171–177.
[7] J.H. Song, C.X. Wang, D.K. Song, P. Wang, A. Shuaib, C. Hao, Interferon gamma induces neurite outgrowth by up-regulation of p35 neuron-specific cyclin-dependent kinase 5 activator via activation of ERK1/2 pathway, J. Biol. Chem. 280 (2005) 12896–12901.
[8] S.J. Kim, T.G. Son, K. Kim, H.R. Park, M.P. Mattson, J. Lee, Interferon-gamma promotes differentiation of neural progenitor cells via the JNK pathway, Neurochem. Res. 32 (2007) 1399–1406.
[9] M.E. Barish, N.B. Mansdorf, S.S. Raissdana, Gamma-interferon promotes differentiation of cultured cortical and hippocampal neurons, Dev. Biol. 144 (1991) 412–423.
[10] L. Li, T.L. Walker, Y. Zhang, E.W. Mackay, P.F. Bartlett, Endogenous interferon gamma directly regulates neural precursors in the non-inflammatory brain, J. Neurosci. 30 (2010) 9038–9050.
[11] J. Walter, S.D. Honsek, S. Illes, J.M. Wellen, H.P. Hartung, C.R. Rose, M. Dihne,

A new role for interferon gamma in neural stem/precursor cell dysregulation, Mol. Neurodegener. 6 (2011) 18.
[12] J. Jang, K. Yoon, D.W. Hwang, D.S. Lee, S. Kim, A retroviral vector suitable for ultrasound image-guided gene delivery to mouse brain, Gene Ther. 19 (2012) 396–403.
[13] J. Ahn, J. Jang, J. Choi, J. Lee, S.H. Oh, J. Lee, K. Yoon, S. Kim, GSK3b, But Not GSK3a, inhibits the neuronal differentiation of neural progenitor cells as a downstream target of mammalian target of Rapamycin complex1, Stem Cells Dev. 23 (2014) 1121–1133.
[14] N. Gaiano, J.D. Kohtz, D.H. Turnbull, G. Fishell, A method for rapid gain-of-function studies in the mouse embryonic nervous system, Nat. Neurosci. 2 (1999) 812–819.
[15] S.A. Bayer, J. Altman, Development of the endopiriform nucleus and the claustrum in the rat brain, Neuroscience 45 (1991) 391–412.
[16] A. Takaoka, N. Tanaka, Y. Mitani, T. Miyazaki, H. Fujii, M. Sato, P. Kovarik, T. Decker, J. Schlessinger, T. Taniguchi, Protein tyrosine kinase Pyk2 mediates the Jak-dependent activation of MAPK and Stat1 in IFN-gamma, but not IFN-alpha, signaling, Embo J. 18 (1999) 2480–2488.
[17] C.V. Ramana, M.P. Gil, R.D. Schreiber, G.R. Stark, Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling, Trends Immunol. 23 (2002) 96–101.
[18] J.N. Ihle, I.M. Kerr, Jaks and Stats in signaling by the cytokine receptor superfamily, Trends Genet. TIG 11 (1995) 69–74.
[19] R. Kageyama, S. Nakanishi, Helix-loop-helix factors in growth and differentiation of the vertebrate nervous system, Curr. Opin. Genet. Dev. 7 (1997) 659–665.
[20] R.F. Hevner, From radial glia to pyramidal-projection neuron: transcription factor cascades in cerebral cortex development, Mol. Neurobiol. 33 (2006) 33–50.
[21] J. Walter, H.P. Hartung, M. Dihne, Interferon gamma and sonic hedgehog signaling are required to dysregulate murine neural stem/precursor cells, PloS one 7 (2012) e43338.
[22] C. Fode, Q. Ma, S. Casarosa, S.L. Ang, D.J. Anderson, F. Guillemot, A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons, Genes Dev. 14 (2000) 67–80.
[23] C. Schuurmans, O. Armant, M. Nieto, J.M. Stenman, O. Britz, N. Klenin, C. Brown, L.M. Langevin, J. Seibt, H. Tang, J.M. Cunningham, R. Dyck, C. Walsh, K. Campbell, F. Polleux, F. Guillemot, Sequential phases of cortical specification involve neurogenin-dependent and -independent pathways, Embo J. 23 (2004) 2892–2902.
[24] C. Kovach, R. Dixit, S. Li, P. Mattar, G. Wilkinson, G.E. Elsen, D.M. Kurrasch, R.F. Hevner, C. Schuurmans, Neurog2 simultaneously activates and represses alternative gene expression programs in the developing neocortex, Cereb. Cortex 23 (2013) 1884–1900.
[25] E.C. Thoma, E. Wischmeyer, N. Offen, K. Maurus, A.L. Siren, M. Schartl, T.U. Wagner, Ectopic expression of neurogenin 2 alone is sufficient to induce differentiation of embryonic stem cells into mature neurons, PloS one 7 (2012) e38651.
[26] H. Shimojo, T. Ohtsuka, R. Kageyama, Oscillations in notch signaling regulate maintenance of neural progenitors, Neuron 58 (2008) 52–64.
[27] H. Nakazaki, A.C. Reddy, B.L. Mania-Farnell, Y.W. Shen, S. Ichi, C. McCabe, D. George, D.G. McLone, T. Tomita, C.S. Mayanil, Key basic helix-loop-helix transcription factor genes Hes1 and Ngn2 are regulated by Pax3 during mouse embryonic development, Dev. Biol. 316 (2008) 510–523.
[28] R. Scardigli, N. Baumer, P. Gruss, F. Guillemot, I. Le Roux, Direct and concentration-dependent regulation of the proneural gene neurogenin2 by Pax6, Development 130 (2003) 3269–3281.
[29] G.B. Ehret, P. Reichenbach, U. Schindler, C.M. Horvath, S. Fritz, M. Nabholz, P. Bucher, DNA binding specificity of different STAT proteins. Comparison of in vitro specificity with natural target sites, J. Biol. Chem. 276 (2001) 6675–6688.
[30] L.M. Shi, N. Tu, P.H. Patterson, Maternal influenza infection is likely to alter fetal brain development indirectly: the virus is not detected in the fetus, Int. J. Dev. Neurosci. 23 (2005) 299–305.
[31] J. Borrell, J.M. Vela, A. Arevalo-Martin, E. Molina-Holgado, C. Guaza, Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia, neuropsychopharmacology, Official Publ. Am. Coll. Neuropsychopharmacol. 26 (2002) 204–215.
[32] L. Zuckerman, I. Weiner, Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring, J. Psychiatr. Res. 39 (2005) 311–323.
[33] N.V. Malkova, C.Z. Yu, E.Y. Hsiao, M.J. Moore, P.H. Patterson, Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism, Brain Behav. Immun. 26 (2012) 607–616.
[34] S. Offenbacher, E.L. Riche, S.P. Barros, Y.A. Bobetsis, D. Lin, J.D. Beck, Effects of maternal campylobacter rectus infection on murine placenta, fetal and neonatal survival, and brain development, J. Periodontol. 76 (2005) 2133–2143.
[35] A. El-Ansary, L. Al-Ayadhi, GABAergic/glutamatergic imbalance relative to excessive neuroinflammation in autism spectrum disorders, J. Neuroinflamm. 11 (2014) 189.
[36] V.S. Dani, Q. Chang, A. Maffei, G.G. Turrigiano, R. Jaenisch, S.B. Nelson, Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 12560–12565.
[37] M. Thompson, C.S. Weickert, E. Wyatt, M.J. Webster, Decreased glutamic acid decarboxylase(67) mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders, J. Psychiatr. Res. 43 (2009) 970–977.