Annals of Anatomy 221 (2019) 148–155

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

RESEARCH ARTICLE

Spatiotemporal expression of NDRG2 in the human fetal brain

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ARTICLE INFO

Article history:
Received 22 May 2018
Received in revised form 6 August 2018
Accepted 26 September 2018

Keywords:
NDRG2
Human embryo brain
Development
Expression
Embryogenesis

ABSTRACT

N-myc downstream-regulated gene 2 (NDRG2) has been implicated in the development of central nervous system and brain diseases such as brain tumors, ischemic stroke and neurodegenerative disorders. However, it remains unclear that the spatiotemporal distribution of NDRG2 in the human fetal brain. In this study, we examined the expression pattern of NDRG2 in different regions of human fetal brain at 16–28 gestational weeks (GWs) by using RT-PCR, western blot and immunohistochemistry. Firstly, RT-PCR revealed that mRNA of *NDRG2* was detected in the human brain regions of fetuses at 16–28 GWs such as medulla oblongata (MdO), mesencephalon (MeE), cerebellum (Cbl), frontal lobe (Fr), ventricular (VZ)/subventricular zone (SVZ) and hippocampus (hip), and the expressions of *NDRG2* mRNA in these human fetal brain regions were increased with gestational maturation. Furthermore, western blot and immunohistochemistry results revealed that at 28 GWs, the expression of NDRG2 protein was restricted to the MdO's olivary nucleus, MeE's aqueduct, cerebellar internal granular layers, cerebral cortex of the Fr, VZ/SVZ of lateral ventricle, and hippocampal dentate gyrus, and highest expression in the VZ/SVZ, and lowest in the MeE. Finally, double immunohistochemistry results showed that NDRG2 in the MdO, Cbl and VZ/SV at 28 GWS was mainly expressed in neurons (NeuN positive cells), and in some astrocytes (GFAP positive cells). Taken together, these results suggest that NDRG2 is mainly expressed in human fetal neurons of various brain regions during development, which may be involved in neuronal growth and maturation.

1. Introduction

The N-myc downstream-regulated gene (NDRG) family of proteins consists of 4 members, NDRG1-4 (Pourquie, 2011). NDRG proteins are characterized by NDR- and α/β hydrolase-fold regions, show high homology among the 4 proteins and share 57–65% amino acid identity. Human NDRG2 was first cloned from a cDNA library of normal human brain by subtractive hybridization in our laboratory (GenBank, Accession No. AF159092). The *NDRG2* gene is located at chromosome 14q11.2, and encodes six types of isoforms, and the full-length of human NDRG2 protein consists of 371 amino acid residues (about 40 kDa) (Boulkroun et al., 2002). The bioinformatic analysis reveals that NDRG2 protein contains an acyl carrier protein-like domain, and several phosphorylation sites (Thr330, Ser332, Thr348, T330, T334 etc.), which can be phosphorylated by certain kinases such as Akt, serum- and glucocorticoid-induced kinase 1 (Burchfield et al., 2004; Chen et al., 2007; Murray et al., 2004).

NDRG2 is widely and highly expressed in the most embryonic and adult tissues, especially in highly differentiated tissues, such as brain, heart and muscles (Choi et al., 2008; Hou et al., 2009; Hu et al., 2006a, 2006b; Lin et al., 2015; Mitchelmore et al., 2004). In the most mammalian nervous system, NDRG2 is widely and highly expressed in brain regions (Hu et al., 2006a; Lin et al., 2015; Liu et al., 2012). In the embryonic mouse brain, mRNA of *NDRG2* is gradually increased during the development of brain regions such

Abbreviations: NDRG2, N-myc downstream-regulated gene 2; GWs, gestational weeks; RT-PCR, reverse transcriptase-polymerase chain reaction; MdO, medulla oblongata; MeE, mesencephalon; Cbl, cerebellum; Fr, frontal lobe; VZ, ventricular; SVZ, subventricular zone; AD, Alzheimer's disease; CNS, central nervous system; GAPDH, D-glyceraldehyde-3-phosphate dehydrogenase; IGL, internal granular layer; PCL, purkinje cell layer.

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https://doi.org/10.1016/j.aanat.2018.09.010
0940-9602/

as telencephalon, diencephalon, mesencephalon, cortical ventricular zone (Hu et al., 2006a; Liu et al., 2012). In the adult human brain, northern blots show that NDRG2 mRNA is detected in the cerebral cortex, striatum, cerebellum, brainstem and spinal cord (Mitchelmore et al., 2004). NDRG2 protein is found to be predominately and selectively expressed in glial cells, especially astrocytes, but not in the neurons or microglia (Okuda et al., 2008). The studies of NDRG2 expression pattern in several mammalian brains, including human, marmoset, rat, mouse and tree shrew have shown that NDRG2 is a new extensive and specific maker of mature and non-reactive astrocytes (Flugge et al., 2014). Nowadays, accumulating studies have shown that NDRG2 has the diverse functions associated with cell proliferation, differentiation, transmembrane transport and stress responses (Lin et al., 2015; Melotte et al., 2010; Yao et al., 2008). In the central nervous systems, NDRG2 is involved in neuronal differentiation, neurogenesis, and astrocytic proliferation and activation under physiological conditions (Deng et al., 2018; Lin et al., 2015; Ma et al., 2017; Matschke et al., 2015). Recent studies also have shown that NDRG2 is associated with nervous system diseases (Ichikawa et al., 2015; Li et al., 2017), such as brain tumors (Hu et al., 2016; Li et al., 2012, 2011), ischemic stroke (Gao et al., 2018; Ma et al., 2017; Sun et al., 2011; Takarada-Iemata et al., 2018; Wang et al., 2013) and neurodegenerative disorders (Le et al., 2018; Rong et al., 2017).

Although the expression patterns and functions of NDRG2 in the central nervous system have been extensively studied, it remains unclear that the spatiotemporal distribution of NDRG2 in the human fetal brain. Therefore, the aim of the present study was to provide a more comprehensive and detailed analysis of NDRG2 expression in cells and tissues of human fetal brain during development.

2. Materials and methods

2.1. Embryo collection and tissue preparation

The experimental protocols were approved by the Ethics Committee of the Fourth Military Medical University (NO. 20040310). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Institutional review board approval and informed consent from all parents/guardians were obtained. We prospectively collected aborted fetuses at gestational ages of 16–28 weeks (n = 10 in each group, 16, 20, 24, 28 weeks group), from January 2013 to January 2015. Misoprostol was used to induce labor and terminate pregnancy in the second or third trimester in women who wished to have an abortion. The brain of each fetus was removed within one hour after labor induction and divided into two parts. Immediately after excision, half of the brain was stored in liquid nitrogen for 10 min and transferred to a −70° C freezer for RNA and DNA extraction. The remaining brain tissues were dissected according to various anatomical locations and fixed in fresh 4% paraformaldehyde-phosphate-buffered saline (PBS) (pH 7.4) before dehydration through graded ethanol. The samples were then paraffin embedded and serially sectioned into 4 μm thick slices.

2.2. RT-PCR

Total RNA was extracted from whole embryo and tissue samples using TRIzol Reagent (GIBCO BRL) according to the manufacturer's instructions. Briefly, 100 mg of brain tissue were suspended in 1 ml TRIzol and homogenized. To determine the purity of RNA, the UV spectrophotometer was used to detect the light density at 260 nm and 280 nm wavelengths, and the ratio of A260/A280 was calculated and the RNA sample between the ratio 1.8–2.0 was selected. To identify the integrity of the RNA, the total RNA was subjected to 1.2% agarose gel electrophoresis. It has been selected good integrity RNA samples that the electrophoretic results showed clear 28 s and 18 s RNA bands, the ratio of their brightness was about 2:1, and there were no trailing and heterogeneity bands.

Total RNA was reverse-transcribed by using a ThemoScript™ RT-PCR system (Invitrogen Life Technologies). Primers were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). *NDRG2* primers were: 5′-CCTTGACCTTTA ACCCGTGATT-3′ (forward) and 5′-CCGATTCCTCTACAGTTTATTG-3′ (reverse), the product fragment was 670 bp. *GAPDH* primers were: 5′-AGGTCCACCACTGAC ACGTT-3′ (forward) and 5′-GCCTCAAGATCATCAGCAAT-3′ (reverse), the product fragment was 310 bp. Primers were designed with Primer Express Software. The reverse transcription reaction was set up according to Promega's reverse transcription system protocol. A total of 1 μl total RNA was added to a 25 μl reaction system and denatured at 94 °C for 5 min. Then, 0.5 μl Taq DNA polymerase was added to the mixture, which was submitted to PCR. A standard thermal cycle profile was used as follows: 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. The number of cycles was determined for each amplicon as required to reach the mid-logarithmic phase of amplification. A total of 30 cycles were used for RT-PCR. The final cycle was extended for 7 min at 72 °C.

In order to confirm that the product of PCR was our target gene (*NDRG2*), we verified the PCR amplification DNA sequence by 1.15% agarose gel electrophoresis (Supplementary Fig. 1), stained with ethidium bromide, and photographed on a Kodak Digital Science1D imaging system (Akribis Scientific Ltd, USA). *GAPDH* was used for normalization. According to analyze the gray level of electrophoresis band, the ratio of *NDRG2* band gray value to *GAPDH* band gray value was used to represent *NDRG2* mRNA abundance value. The difference analysis was made by one-way ANOVA, and statistical analysis was conducted to compare the difference.

2.3. Western blot analysis

Total tissue specimen proteins were extracted using a Tissue Protein Extraction kit (Thermo Scientific, USA). Total proteins in the lysate were quantified by the pierce BCA Protein Assay Kit (Pierce, Rockford, Illinois, USA). Equal amounts of proteins were resolved by 10% SDS-polyacrylamide gel electrophoresis and transferred onto Hybond enhanced chemiluminescence nitrocellulose membranes (Amersham Bio-sciences, Little Chalfont, Buckinghamshire, UK). The membranes were blocked with 5% non-fat dry milk in TBST for 1 h at room temperature, washed three times with TBST and then incubated at 4 °C overnight with Anti-NDRG2 rabbit monoclonal (1:1000; ab174850, abcam) or anti-GADPH rabbit polyclonal (1:2000, ab37168, abcam). After being washed three times with TBST, the membranes were incubated with the appropriate secondary antibodies (Cell Signaling Technology, USA) for 1 h at room temperature and immuno-reactive protein bands were visualized using ECL. FluorChem Software was used to analysis the gray image of immunoblots. The ratio of NDRG2 band gray value to GAPDH band gray value was used to statistical analysis. The difference analysis was made by one-way ANOVA, and statistical analysis was conducted to compare the difference to MeE group.

2.4. Immunohistochemistry

Immunohistochemical staining was performed on paraffin-embedded brain slices using the Streptavidin-Biotin peroxidase complex (SABC) method (SABC kit, Boster Ltd, China) with DAB hydrogen peroxide as the chromogen. Tissue sections were

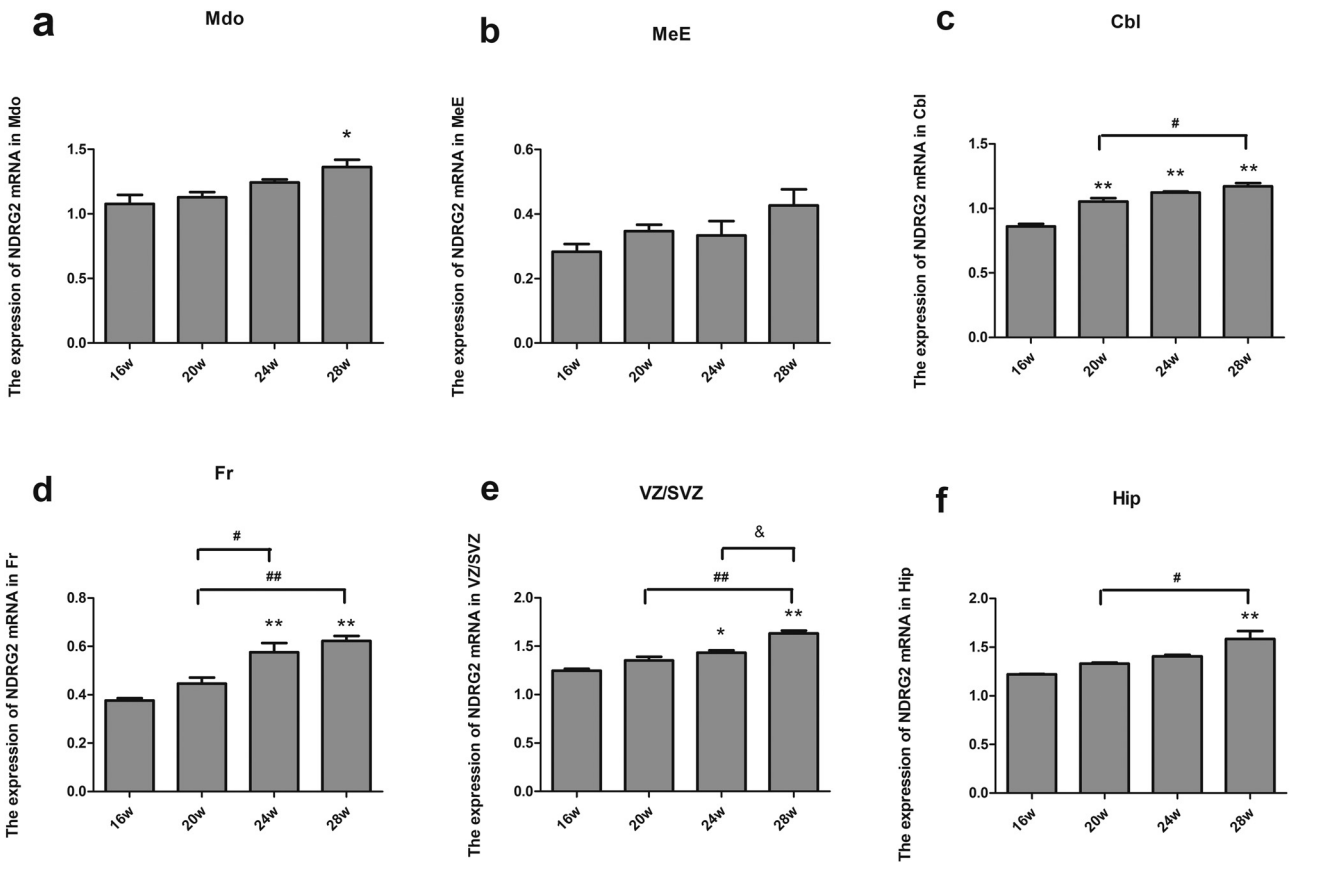

Fig. 1. RT-PCR analysis of spatiotemporal expression of *NDRG2* mRNA in human fetal brain regions at 16–28 GWs. (a–f) Semi-quantitative RT-PCR analysis of *NDRG2* mRNA expression in the MdO (a), MeE (b), Cbl (c), Fr (d), VZ/SVZ (e), and Hip (f) of 16–28-week-old embryos, respectively. D-Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. MdO, medulla oblongata; MeE, mesencephalon; Cbl, cerebellum; Fr, frontal lobe; VZ, ventricular; SVZ, subventricular zone; Hip, Hippocampus. Data were mean ± SEM (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, compared to 16 w group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to 20 w group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ compared to 24 w group. One-way ANOVA and Bonferroni multiple comparisons test.

deparaffinized in xylene and dehydrated with a series of decreasing ethanol concentrations before blocking endogenous peroxidase activity with 0.5% H_2O_2 in methanol for 10 min. Nonspecific binding was blocked with 10% normal goat serum in PBS for 1 h at room temperature. Subsequently, the sections were incubated with the anti-NDRG2 antibody (1:100, ab174850, abcam) in PBS at 4 °C for overnight in a moist box. This was followed by incubation with biotinylated goat anti-rabbit immunoglobin G (IgG; 1:400; Sigma-Aldrich Biotechnology, St Louis, Missouri, USA) for 1 h at room temperature, and detection using a streptavidin–peroxidase complex. Brown color indicative of peroxidase activity was developed by using 0.1% 3,3-diaminobenzidine (Sigma) in PBS containing 0.05% H_2O_2 for 5 min at room temperature. Sections were then counterstained with hematoxylin, dehydrated in a graded series of ethanol, cleared in xylene, and mounted with Neutral Balsam (PTS11773891, sinopharm).

2.5. Immunofluorescence assay

For double immunofluorescence, paraffin embedded embryo brain tissue sections (4 μm) were deparaffinized in xylene and ethanol. The endogenous peroxidase activity was blocked by incubating 3% hydrogen peroxide diluted in methanol for 20 min at room temperature. Slides were treated with proteinase K at room temperature for 15 min. Nonspecific antibody-binding sites were first blocked with 1% bovine serum albumin (BSA) in PBS (1% BSA-PBS); then, slides were incubated with anti-NDRG2 mouse monoclonal (1:150, Abnova Corporation, Taipei, Taiwan) and anti-GFAP rabbit polyclonal (1:100, Sigma) or anti-NeuN rabbit monoclonal (1:300, ab177487, abcam) primary antibodies in 1% BSA-PBS. Antibody binding was detected with the following secondary antibodies: fluorescein isothiocyanate-conjugated goat anti-mouse IgG (1:160; Santa Cruz Biotechnology) and tetramethyl rhodamine isothiocyanate-conjugated goat anti-rabbit IgG (1:300; Santa Cruz Biotechnology). The sections were mounted in glycerol and analyzed on a fluorescence microscope and an FV3000 laser-scanning microscope, respectively (Olympus, Tokyo, Japan). All experimental slides were assessed by a histopathologist before scoring.

2.6. Statistical analysis

Data were expressed as the means ± sd, Statistical analysis was conducted with GraphPad Prism software 5.0 software. One-way ANOVA followed by the Bonferroni multiple comparisons test was used for statistical comparisons among experimental groups, with a value of $P < 0.05$ being considered statistically significant.

3. Results

3.1. NDRG2 *mRNA was detected in the human fetal brain regions and increased with gestational maturation*

To examine the spatial-temporal expression of *NDRG2* mRNA in human fetal brain, total RNA of various human fetal brain regions at 16–28 GWs were collected including medulla oblongata (MdO), mesencephalon (MeE), cerebellum (Cbl), frontal lobe (Fr), ven-

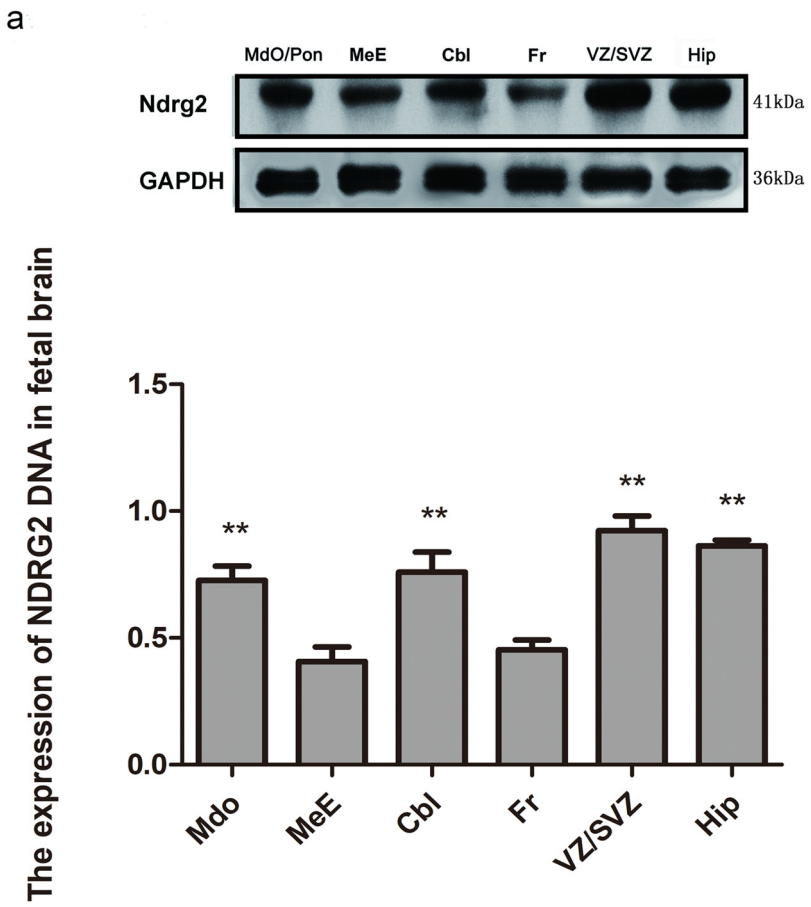

Fig. 2. Western blot analysis of NDRG2 protein in human fetal brain regions at 28 GWs. (a) Representative Western blot showing NDRG2 immunoreactive bands in the MdO/Pon, MeE, Cbl, Fr, VZ/SVZ, and Hip tissues, respectively. The GAPDH protein was used as a loading control. (b) Quantitative analysis of the relative amounts of the NDRG2 protein as shown in (a). MdO, medulla oblongata; MeE, mesencephalon; Cbl, cerebellum; Fr, frontal lobe; VZ, ventricular; SVZ, subventricular zone; Hip, Hippocampus. Data were mean ± SEM (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, compared to MeE group. The original blots for panel were presented in Supplementary Fig. 1. One-way ANOVA and Bonferroni multiple comparisons test.

tricular (VZ)/subventricular zone (SVZ) and hippocampus (hipp), and RT-PCR was performed as described above. The product of PCR was verified by 1.15% agarose gel electrophoresis. The resulting bands had the expected sizes for *NDRG2* (670 bp) and *GAPDH* (310 bp) (Supplementary Fig. 1). *NDRG2* mRNA expression levels was represented by *NDRG2* mRNA abundance value (NDRG2 band gray value/GAPDH band gray value). As shown in Fig. 1, *NDRG2* mRNA was detected human fetal brain regions of MdO, MeE, Cbl, Fr, VZ/SVZ and Hip at 16–28 GWs embryos brain. Interestingly, *NDRG2* mRNA expression levels in the above regions were increased with age (Fig. 1a–f). The relative expression levels of *NDRG2* mRNA in the MdO of different ages were 1.08 ± 0.07, 1.13 ± 0.04, 1.24 ± 0.02 and 1.36 ± 0.05, respectively (Fig. 1a); in the MeE, expression levels of 0.28 ± 0.02, 0.35 ± 0.02, 0.33 ± 0.04 and 0.43 ± 0.05, respectively (Fig. 1b) were obtained. The relative expression levels of *NDRG2* mRNA in the Cbl of different ages were 0.89 ± 0.02, 1.05 ± 0.03, 1.12 ± 0.01 and 1.17 ± 0.02, respectively (Fig. 1c); in the Fr expression levels of 0.38 ± 0.01, 0.45 ± 0.02, 0.58 ± 0.04 and 0.62 ± 0.02, respectively (Fig. 1d) were obtained. The relative expression levels of *NDRG2* mRNA in the VZ/SVZ of 16–28-week-old embryos were 1.25 ± 0.02, 1.35 ± 0.04, 1.43 ± 0.02, and 1.63 ± 0.03, respectively (Fig. 1e). The relative expression levels of *NDRG2* mRNA in the Hip of different ages were 1.22 ± 0.01, 1.33 ± 0.01, 1.41 ± 0.01, and 1.58 ± 0.08, respectively (Fig. 1f). The relative expression levels of *NDRG2* mRNA of 28 week-old were 1.36 ± 0.05, 0.43 ± 0.05, 1.17 ± 0.02, 0.62 ± 0.02, 1.63 ± 0.03 and 1.58 ± 0.08 in the MdO, MeE, Cbl, Fr, VZ/SVZ and Hip, respectively (Fig. 1a–f). The highest NDRG2 *mRNA* expression was highest in the VZ/SVZ and weakest in the MeE. These results suggest that *NDRG2* mRNA was expressed in the human fetal brain regions and increased with gestational maturation.

3.2. Spatial-temporal expression patterns of NDRG2 protein in the human fetal brain regions

To further examine the spatial expression of NDRG2 protein level in human fetal brain regions, total proteins of various human fetal brain regions were collected at 28 GWs and western blot was performed. As shown in Fig. 2, immunoblot showed a band corresponding to the predicted molecular weight of NDRG2 (approximately 40 kDa) in lysates from whole human fetal brain as well as in the brain regions, including MdO, MeE, Cbl, Fr, VZ/SVZ and Hip (Fig. 2a, Supplementary Fig. 2). Consistent with RT-PCR results, the expression level of NDRG2 protein in the VZ/SVZ was higher, and slightly lower in the MeE, compared with the remaining regions (Fig. 2b). These results strongly suggest that NDRG2 protein was highly expressed in the human fetal brain regions.

To further examine the spatial NDRG2 localization in the central nervous system (CNS) of human embryos brain at different gestational ages, immunohistochemistry was performed with an anti-NDRG2 antibody. As shown in Fig. 3, NDRG2 displayed the different expression level in the human fetal brain regions of 16–28 weeks. At 16 weeks, the immuno-positive signals of NDRG2 was only in the MdO, Cbl, VZ/SVZ (Supplementary Fig. 3). In the human

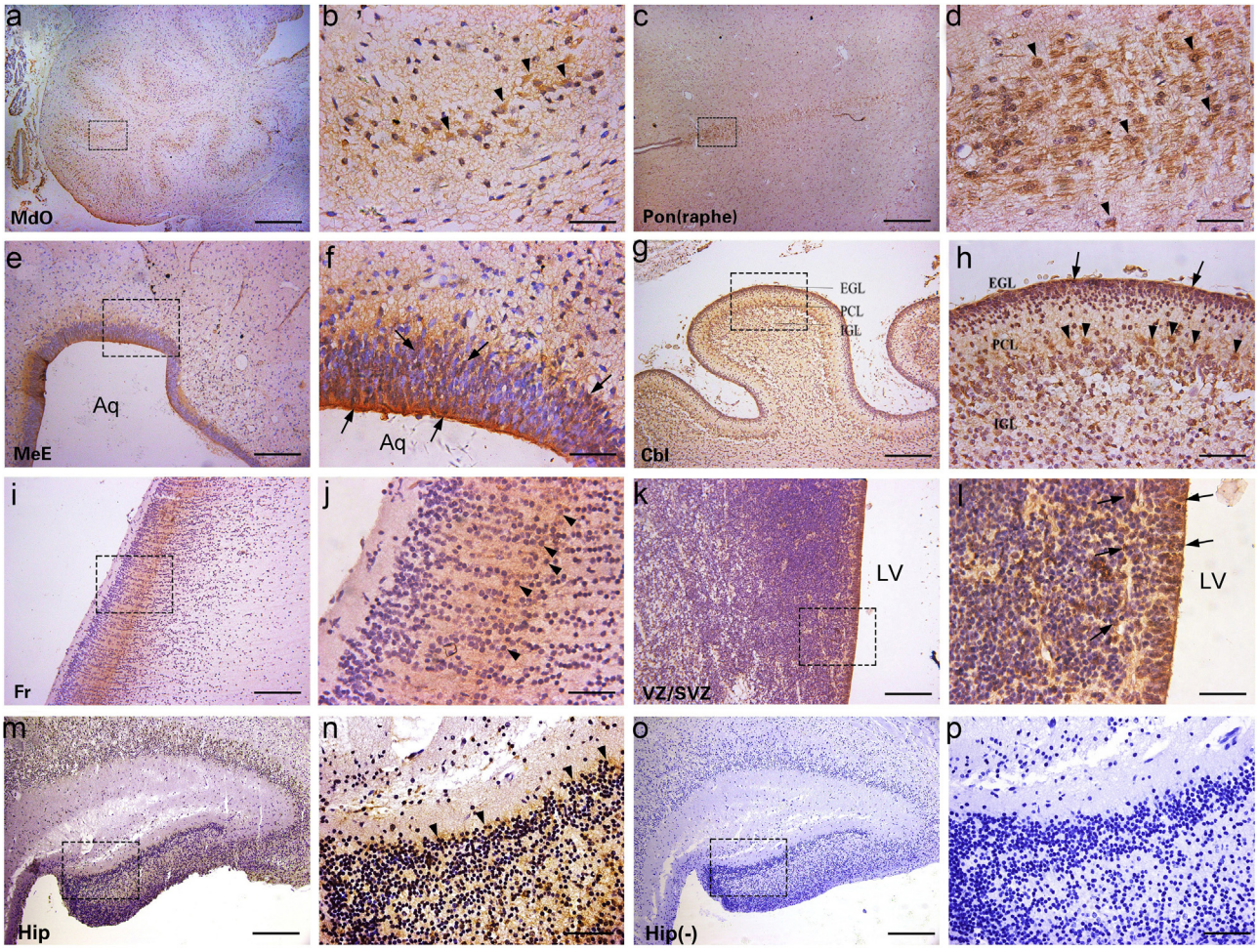

Fig. 3. Immunohistochemical analysis of NDRG2 expression in human fetal brain regions at 28 GWs. Images of selected regions in (a, c, e, g, i, k, m, o), were shown at higher magnification as (b, d, f, h, j, l, n, p), respectively. NDRG2 immunostaining signals were mostly detected in the olivary nucleus (arrowheads) of the MdO (a and b), pontine raphe nucleus (arrowheads) (c and d), MeE aqueduct (arrows) (e and f), external (arrows) and internal (arrowheads) granular layers of the Cbl (g and h), cerebellar cortex (arrowheads) of the Fr (i and j), VZ/SVZ (k and l, arrows) of the lateral ventricle, and dentate gyrus (arrowheads) of the Hip (m and n). A negative control was setup by omitting the primary antibody (o and p). MdO, medulla oblongata; MeE, mesencephalon; Cbl, cerebellum; Fr, frontal lobe; VZ, ventricular; SVZ, subventricular zone; Hip, Hippocampus. (a, c) Bars = 400 μm. (e, g i, k, m, o) Bars = 200 μm. (b, d, f, h, j, l, n, p) Bars = 50 μm.

fetal brain at 20–24 week, the immuno-positive signals of NDRG2 in the positive brain region was gradually enhanced (Supplementary Fig. 4). At 28 weeks, NDRG2 immunostaining signals were detected in the MdO (Fig. 3a and b), Pon (Fig. 3c and d), MeE (Fig. 3e and f), the external and internal granular layers of the Cbl (Fig. 3g and h), Fr (Fig. 3i and j), VZ/SVZ (Fig. 3k and l) and Hip (Fig. 3m and n; compare to the Hip, Fig. 3o and p), respectively. It was stronger in the Cbl, VZ/SVZ, Hip and MdO, mainly located in the cytoplasmic. In the early stage of fetal cerebellum at 16 week, there was a little NDRG2 protein positive product expression. With the increase of gestational age from 20 to 24 weeks, the cerebellar granular layer was stratified, enhanced brown positive product expression from the outer granular layer to the inner granular layer. In the olivary nucleus of the medulla oblongata of a human fetal brain, the expression level of NDRG2 was increased with gestational age from 16 to 24 weeks, which was similar to the expression pattern of the cerebellar granular layer (Supplementary Fig. 3). Taken together, these results suggest that the expression level of NDRG2 protein is increased with age in the human fetal brain regions, which is consistent with the expression level of *NDRG2* mRNA.

3.3. *NDRG2 was mainly expressed in neurons and astrocytes in the human fetal brain regions*

To further examine the cellular localization of NDRG2 in the CNS of human embryos, double immunofluorescence was performed for NDRG2 and NeuN (a marker of neurons) or GFAP (a marker of astrocytes) of 28-week-old human embryonic brains. As shown in Fig. 4, NDRG2 was expressed in neurons (NeuN positive cells) and astrocytes (GFAP positive cells) of the MdO, Cbl and VZ/SVZ. The co-expressed areas of NDRG2 and NeuN were mainly in the olivary nucleus of the medulla oblongata (Fig. 4a–c); the PCL (Purkinje cell layer) and ICL (internal granular layer) region of the cerebellum, a few in the EGL- external germinal layer of the cerebellum (Fig. 4d–f); and the VZ/SVZ region of the ventricles (Fig. 4g–i). The co-expressed areas of NDRG2 and GFAP were mainly in the olivary nucleus of the medulla oblongata (Fig. 4j–l); the PCL (Purkinje cell layer) and ICL (internal granular layer) region of the cerebellum (Fig. 4m–o); and a few in the VZ/SVZ region of the ventricles (Fig. 4p–r). These results suggest that NDRG2 is mainly expressed in neurons and astrocytes in the human fetal brain regions.

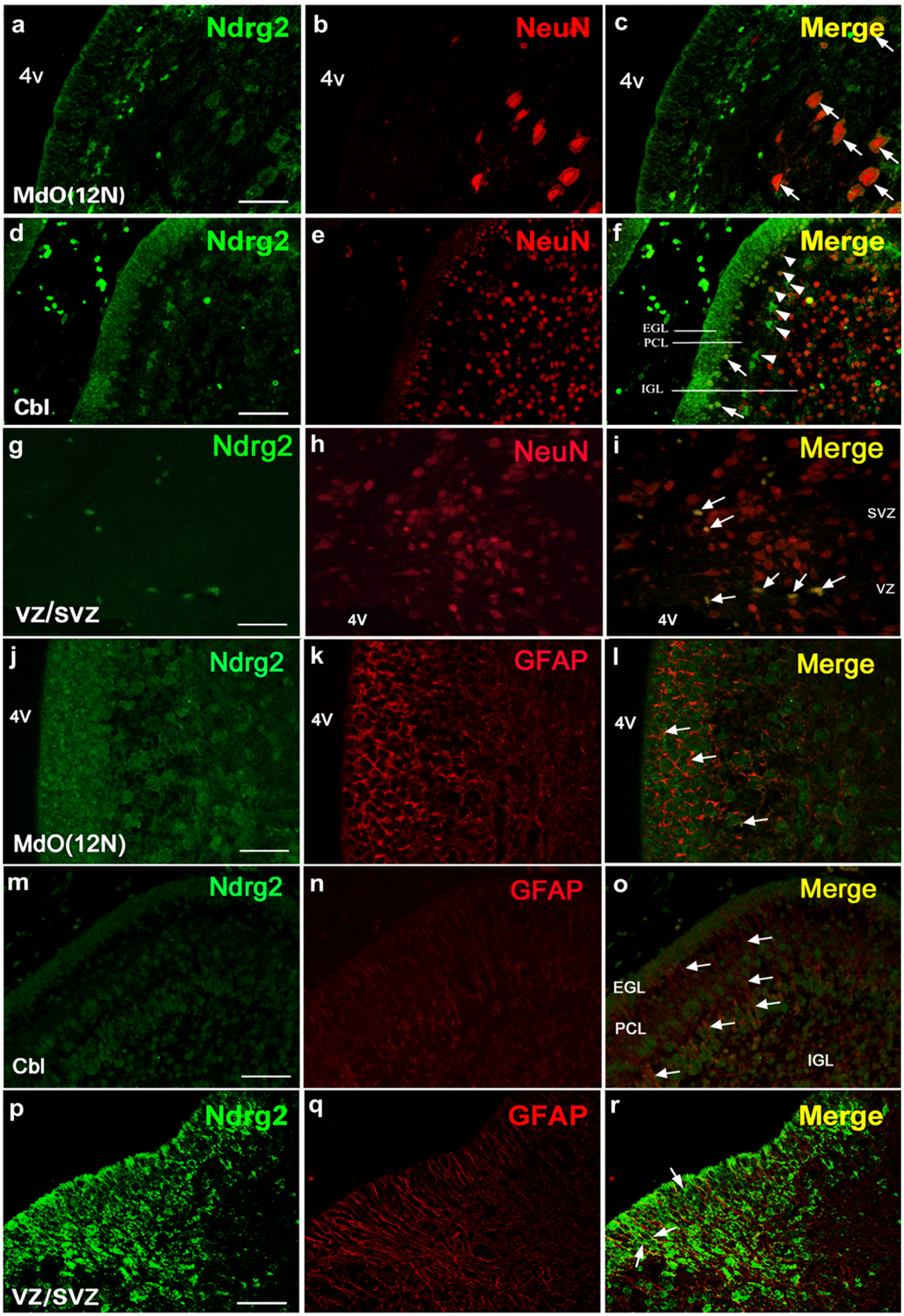

Fig. 4. Double immunofluorescence analysis of NDRG2 and NeuN or GFAP in the indicated human fetal brain regions at 28 GWs. Tissue sections were stained with anti-NDRG2 (green) and anti-NeuN or GFAP (red) antibodies. (a–i) NDRG2 expression was detected in neurons of the MdO, Cbl and VZ/SVZ. (a–c) NDRG2 expression was detected in larger neurons of the olivary nucleus (arrows) of the MdO. (d–f) NDRG2 expression was detected in neurons of the IGL (arrows) and PCL (arrowheads) of the Cbl, A few was detected in the EGL-external germinal layer. (g–i) NDRG2 expression was detected in neurons (arrowheads) of the VZ/SVZ. (j–r) NDRG2 expression was detected in astrocytes (arrows) of the MdO (j–l), Cbl (m–o) and VZ/SVZ (p–r). MdO, medulla oblongata; MeE, mesencephalon; Cbl, cerebellum; Fr, frontal lobe; VZ, ventricular; SVZ, subventricular zone; IGL, internal granular layer; PCL, Purkinje cell layer. Bars = 50 μm.

4. Discussion

The NDRG family of proteins consists of 4 members, NDRG1-4, which all appear to play the fundamental roles in diverse cell processes such as proliferation, differentiation, and stress response (Lin et al., 2015). The NDRG proteins are well conserved through evolution, and recent studies have shown that they are not only involved in development, immunity, and endocrine signaling, but also associated with neurological and electrophysiological diseases (Kokame et al., 1996; Lin et al., 2015). However, their exact function remains unclear and requires further exploration.

A recent study has explored NDRG2 is expressed in normal human tissues (Deng et al., 2003; Okuda et al., 2004) by immunohistochemistry, and found that the protein may be involved in maintaining a variety of cell types (Deng et al., 2001). Mitchelmore et al. (2004) demonstrated that the NDRG2 gene is associated with disease pathogenesis of several diseases involving the human brain, including glioblastoma, depression, and Alzheimer's disease. Further, Choi et al. assessed NDRG2 expression and regulation during dendritic cell differentiation (Malette et al., 2003). The latter study showed that NDRG2 is expressed in human monocyte-derived dendritic cells, with expression differentially regulated by maturation-inducing stimuli. Recent studies also suggested that the NDRG2 protein is widely expressed throughout the adult mouse brain, particularly in the midbrain, cerebellum, and bridge. Okuda et al. showed that *NDRG2* mRNA is expressed in the VZ throughout the CNS during mouse brain embryonic development (Choi et al., 2003; Malette et al., 2003); however, gene expression in the brain at different gestational ages was not explored. A few studies have evaluated NDRG2 protein expression in human embryonic brain tissues.

This study assessed NDRG2 protein expression in the embryonic human tissues. To the best of our knowledge, this is the first study to describe the localization of the NDRG2 protein in embryonic brain tissues. We detected the expression of NDRG2 in human fetal brain at different time points by RT-PCR, western blot at mRNA and protein levels. Our results showed that the expression of NDRG2 was spatially diverse. The expression of NDRG2 is widespread in the functional areas of human fetal brain, especially in the VZ/SVZ where the neurogenesis occurs. NDRG2 may be expressed in neural precursor cells and astrocytes in humans at embryonic stages. We found that NDRG2 expression was dynamic during human embryo development, and generally low in early stages of development, while markedly increasing in later stages. NDRG2 expression was increased with gestational age; it was higher in the VZ/SVZ. These data suggest that this protein may be involved in the developmental processes related to cell proliferation and differentiation. The immunohistochemical and immunofluorescence results still showed that the expression of NDRG2 was located in the Purkinje cell layer and the inner granular layer of cerebellum, and the olivary nucleus of medulla oblongata. Granulosa cells in cerebellum are mainly excitatory neurons of glutamate (Llinas et al., 2004), and there are many motor neurons in the anterior horn of the medulla oblongata (Fitzpatrick, 2001). These findings suggest that NDRG2 may be associated with motor neuron development and neuronal growth.

The current data contribute to the understanding of possible functional roles of NDRG2 and how its expression may be regulated in human brain development. However, additional studies are required to clarify the specific function of NDRG 2 in neurodevelopment.

5. Conclusion

Overall, we assessed NDRG2 expression levels in brain sample slices human embryos at different gestational ages by RT-PCR, western blot and immunohistochemical methods. NDRG2 was expressed in various brain regions, including the VZ, SVZ, mesencephalon, frontal lobe, cerebellum, medulla oblongata and hippocampus. Importantly, this study described the spatiotemporal expression of the NDRG2 protein in human embryonic brain tissues for the first time. These results suggest that NDRG2 expression is dynamic during human embryogenesis. These findings suggest that NDRG2 may play important roles in growth and maturation of the CNS.

Acknowledgments

The authors thank Ms. Dong-Yun Feng and Mr. Yi-Ling Zhao for technical assistance. The study was supported by grants from the Outstanding Leaders Training Program of Pudong Health Bureau of Shanghai (No. PWR12015-06), the Program of Pudong Hospital (No. 2015YJ-04) and the Social Development Plan Foundation of Shan Xi (No. 2011K12-33).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aanat.2018.09.010.

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