Int J Clin Exp Pathol 2018;11(4):1956-1964
www.ijcep.com /ISSN:1936-2625/IJCEP0075063

Original Article

Neuritin 1 expression in human normal tissues and its association with various human cancers

Hongchang Dong[1*], Xing Luo[1*], Yuqin Niu[2*], Na Yu[1], Rui Gao[1], Haiyan Wang[1,3], Li Yang[1], Jin Huang[1]

[1]Department of Biochemistry, The Key Laboratory of Xinjiang Endemic & Ethnic Diseases, School of Medicine, Shihezi University, Shihezi 832002, Xinjiang, China; [2]Medical Center of The Affiliated Hospital of Shihezi University, Shihezi, Xinjiang, China; [3]Hangzhou Normal University, Hangzhou, Zhejiang, China. [*]Equal contributors.

Received February 26, 2018; Accepted March 28, 2018; Epub April 1, 2018; Published April 15, 2018

Abstract: Objective(s): Neuritin (Nrn1) is a glycophosphatidylinositol-linked protein that can be induced by neural activity in the central nervous system. However, its expression outside the nervous system and association with human cancers is unclear. This study investigated the expression of Nrn1 in human tissues as well as its association with human cancers. Materials and methods: Nrn1 gene expression in human adult tissues was evaluated with the Clontech Multiple Tissue cDNA panel. Nrn1 protein in various tissues was detected by immunohistochemistry. Signal v.4.0 and TMHMM v.2.0 software were used to identify the signal peptide and transmembrane helix of Nrn1. The subcellular localization of Nrn1 in cultured SH-SY5Y cells was assessed by immunocytochemistry and western blotting. The expression of Nrn1 in human cancers were assessed using the online tools GEPIA. Results: Nrn1 mRNA was expressed in various tissues, compared to mRNA levels in the brain tissues, expression was high in the placenta, lungs, skeletal muscle, thymus, pancreas, liver and the heart tissues; lower levels were detected in the small intestine, ovary, spleen, and testes, but there was no detectable expression in the kidneys, colon, prostate or leukocytes. In SY5Y cells, Nrn1 was colocalized with caveolin 1 at the plasma membrane. Nrn1 was downregulated in Bladder Urothelial Carcinoma (BLCA); Breast invasive carcinoma (BRCA); Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC); Colon adenocarcinoma (COAD); Glioblastoma multiforme (GBM); Kidney Chromophobe (KIHC); Kidney renal papillary cell carcinoma (KIRP); Lower Grade GLioma (LGG); Rectum adenocarcinoma (READ); Uterine Corpus Endometrial Carcinoma (UCEC); Lung adenocarcinoma (LUA), Ovarian serous cystadenocarcinoma (OV) and upregulated in Lymphoid Neoplasm Diffuse Large B-cell Lymphoma (DLBC). A combination of the overall survival analysis of the 12 kinds of human tumors with Nrn1 downregulation revealed that patients with high levels of Nrn1 present a long term survival. But there is no significant effect on DLBC patients' survival. Conclusion: Nrn1 is expressed in various human tissues including the nervous system, specifically in the lipid rafts of cell membranes. We also provided the strong evidence that Nrn1 is associated with 13 kinds of human cancers and could function as biomarkers and therapeutic targets for these cancers.

Keywords: Nrn1, expression profile, lipid raft, human cancers, biomarker

Introduction

Neuritin (Nrn1), also known as the candidate plasticity gene (CPG) 15, was identified in a screen of novel genes involved in activity-dependent synaptic plasticity in the neocortex [1, 2]. Nrn1 is involved in neural development, synaptic plasticity, and synapse maturation [3, 4]. In addition, it has been shown to promote retinal ganglion cell survival and axonal regeneration following optic nerve crush injury [5] and attenuate cognitive impairment in a mouse model of Alzheimer's disease [6]. Nrn1 promotes neurite outgrowth, dendritic growth, neuronal migration, and synapse maturation in neurons of the visual cortex; it also regulates synaptic plasticity, apoptosis of peripheral neurons and spinal axon regeneration and promotes recovery following cerebral ischemia. Recombinant Nrn was shown to enhance the structural and functional recovery of injured sciatic nerves [7], inhibit nerve cell apoptosis, and promote neurite regeneration and recovery of motor function after spinal cord injury in rats [8].

Nrn1 mRNA and proteins were detected in the *Xenopus* spinal cord during development [9],

and they have also been detected in retinal ganglion cells of the retina and axonal tracts [10], as well as in primary cultured Schwann cells [11]. Nrn has limited expression and function in the nervous system. On the other hand, it is expressed in muscle satellite cells [12] and has been implicated in liver maturation and regeneration [13]. However, Nrn1 expression has mainly been characterized in animals. In fact, Nrn1 expression is highly conserved among species [14, 15]. Information on the normal expression patterns of the nervous systems of Nrn1 in adult tissues is limited, and information on its subcellular localization obtained by immunohistochemistry [16] is controversial. In this study, we investigated the expression of Nrn1 in brains and in other human tissues in addition to its subcellular localization. We found that Nrn1 was expressed in multiple tissues outside the nervous system and describe its association with plasma membrane lipid rafts in cultured human SH-SY5Y cells.

Studies have shown that Nrn1 is overexpressed in human astrocytoma [17, 18], gastric cancer [19] and Kaposi's sarcoma [20], which is correlated with tumor malignancy. In contrast, others have shown that Nrn1 can be a novel angiogenic factor [21] and inhibit tumor growth in glioma cells [22, 23]. But whether the distribution of Nrn1 in human normal tissues is associated with the development of these human cancers remains unclear. Here, we demonstrated that the expression of Nrn1 was significantly downregulated in 12 kinds of human cancers but only upregulated in DLBC. In addition, the Nrn1 expression level was associated with overall survival of the human cancer patients in which Nrn1 expression was downregulated.

Materials and methods

Human tissues

Human tissue specimens (n = 42) were obtained from the autopsy archives at the Department of Pathology, the First Affiliated Hospital of Shihezi University School of Medicine. Specimens were identified as normal by histopathological analysis. Samples included tissue from the brain (cerebellum, medulla, hippocampus, basal ganglia, and cortex), spinal cord, heart, liver, lungs, placenta, umbilical cord, small intestine, colon, ovary and glands.

Gene expression array

Nrn1 gene expression in human adult tissues was determined using the Clontech Multiple Tissue (CMT) cDNA panel (Mountain View, CA, USA). Specific primers were used to amplify a 430-bp product. The reaction conditions were as follows: 94°C for 3 min; 39 cycles of 94°C for 30 s, 37°C for 30 s, and 72°C for 90 s; and 72°C for 5 min. The glyceraldehyde 3-phosphate dehydrogenase gene (1 kb) was amplified as a positive control.

Immunohistochemistry

The tissues were formalin-fixed and paraffin-embedded and stored for 2-5 years. Specimens were cut into 4-μm sections and mounted on Polysine™ adhesion slides overnight at 60°C. Sections were deparaffinized with dimethylbenzene and rehydrated through a graded series of ethanol (100%, 95%, 90%, 80%, and 70%). After three washes with phosphate-buffered saline (PBS), the slides were boiled in an antigen retrieval buffer (0.01 M sodium citrate-hydrochloric acid, pH = 6.0) for 10-15 min in a microwave oven at 95°C-97°C. Endogenous peroxidase activity was blocked by incubation with 3% peroxide for 10 min followed by three washes in PBS for 5 min. Nonspecific binding was blocked by incubation with bovine serum albumin, and the sections were then incubated at 4°C overnight with polyclonal rabbit anti-Nrn1 (FL-142) antibody (cat. no. sc-25651; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:50). After washing with PBS, the sections were processed at room temperature for 30 min with the ChemMate EnVision/Horseradish peroxidase kit (GK500705; Dako, Glostrup, Denmark) according to the manufacturer's instructions. After washing in PBS, sections were developed with 3,3'-diaminobenzidine, washed under tap water, counterstained with hematoxylin, and mounted with coverslips. Negative control specimens were prepared by replacing the primary antibody with PBS. Cerebellum tissue was used as a positive control. All sections were reviewed by experienced pathologists.

Cell culture

Human SH-SY5Y neuroblastoma cells were cultured in Dulbecco's Modified Eagle's Medium containing 4.5 g/l glucose and 4 mM L-glutamine (HyClone, Logan, UT, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco,

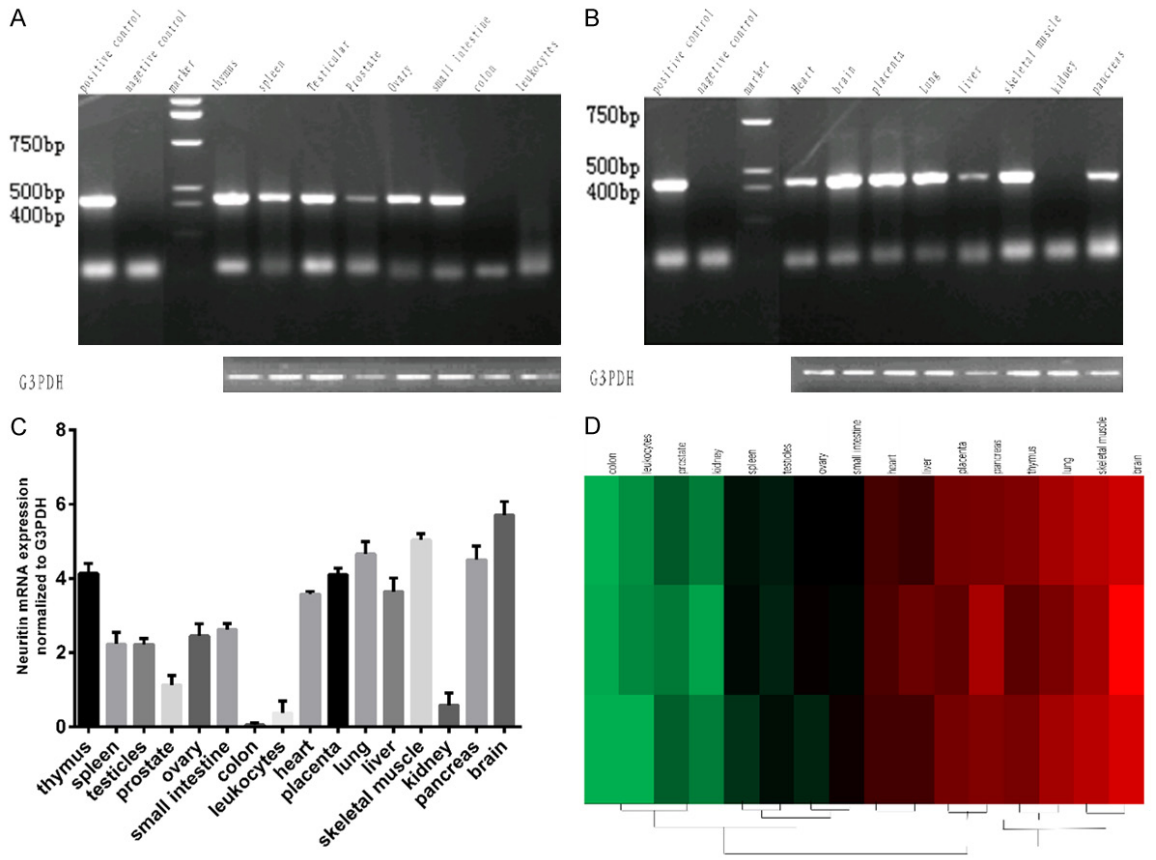

Figure 1. *Nrn1* mRNA expression in multiple tissues. *Nrn1* cDNA (426 bp) was detected in the thymus, spleen, testicles, prostate, ovary, small intestine, colon and leukocytes (A), as well as the heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (B). Glyceraldeyde 3-phosphate dehydrogenase (G3PDH) was used as a loading control. (C) Quantification of OD ratio for Nrn1 mRNA expression (n = 3). Each bar corresponds to mean ± Standard Deviation for individual tissues. (D) The clustering heat map analysis performed using R tools with the OD ratio of Nrn1 mRNA expression in each tissue.

Grand Island, NY, USA). Cells were maintained at 37°C in a humidified atmosphere of 95% air/5% CO_2 until 70%-80% confluence.

Immunocytochemistry

Cells were seeded on coverslips and incubated at 37°C until 50%-60% confluence, then fixed in 4% paraformaldehyde for 30 min at 4°C and blocked in 10% (w/v) normal goat serum in PBS for 30 min at room temperature. The cells were then incubated for 2 h with antibodies against Nrn1 (developed in our laboratory) and caveolin 1 (Abcam, Cambridge, UK) followed by fluorescein isothiocyanate-conjugated (Sigma, St. Louis, MO, USA) or rhodamine-conjugated (ZSBIO, Beijing, China) goat anti-mouse IgG. Digital images were acquired with an LSM 510 Meta laser scanning confocal microscope (Zeiss, Oberkochen, Germany) or an IX70 inverted fluorescence microscope (Olympus, Tokyo, Japan). Adobe Photoshop software was used to process the images.

Membrane protein extraction and western blotting

Plasma membrane proteins were extracted from cultured SY5Y cells using the MEM-PER mammalian membrane protein extraction kit (Pierce, Rockford, IL, USA), according to the manufacturer's instructions. For Western blotting, lysates were centrifuged at 15,616 × *g* for 10 min at 4°C, and the protein concentration in the supernatant was determined with the Super-Bradford Protein Assay kit (CWBIO, Beijing, China) and normalized. After electrophoretic separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA) as previously described [24], which was probed with

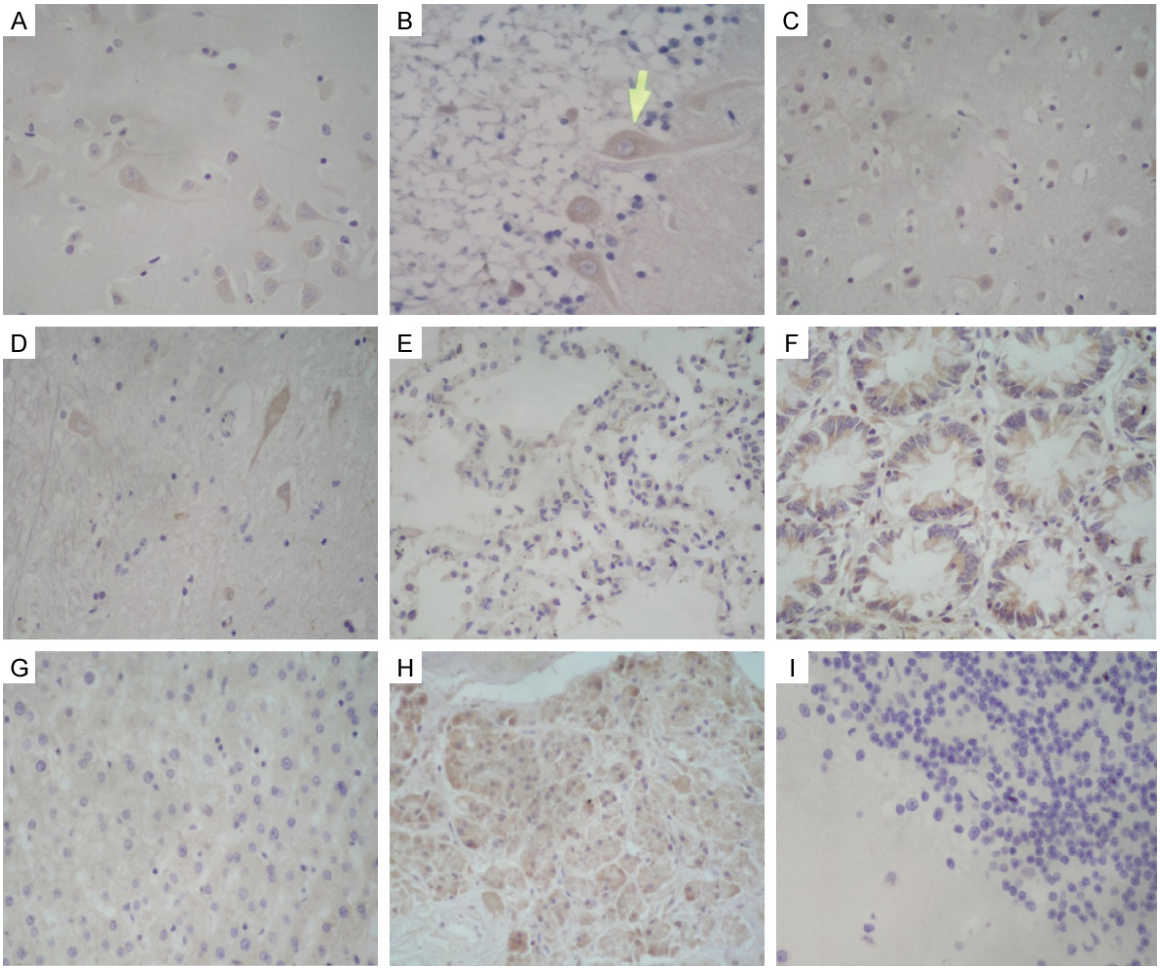

Figure 2. Detection of *Nrn1* in human tissues by immunohistochemistry. Areas of positive immunoreactivity are brown and indicated by arrows; nuclei are stained violet with hematoxylin. A. Hippocampus (positive control); B. Cerebellum; C. Cortex; D. Spinal cord; E. Alveolar epithelium; F. Colonic gland; G. Liver; H. Pancreatic epithelium; I. Negative control. Images are shown at 40 × magnification.

antibodies and visualized using Immobilon Western Chemiluminescent Horseradish Peroxidase Substrate (Millipore).

Correlation between Nrn1 and human cancers

The study on the correlation between Nrn1 and huaman cancers was performed using the online tools GEPIA (http://gepia.cancer-pku.cn/index.html). GEPIA is a web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline [25].

Statistical and bioinformatics analyses

Nrn1 mRNA expression was quantified with a Gel-Pro analyzer. Statistical analysis was performed using Prism 5 software (GraphPad, La Jolla, CA, USA). The clustering heat map analysis was performed using R tools with the OD ratio of Nrn1 mRNA expression. The Nrn1 gene sequence was analyzed using SignalP 4.1 and TMHMM software [26-28]. SignalP predicts secretory proteins in eukaryotes using truncated 70-amino acid protein sequences as filters. The standard was L = -918.235-123.455* (mean S score), +1983.44* (HMM score), and L > 0 for predicting signal peptide proteins. The protein sequence was then analyzed with TMHMM to identify transmembrane segments.

Results

Nrn1 is expressed in multiple tissues, with the highest expression detected in the brain

To analyze mRNA expression of human Nrn1 in human tissues, an analysis of Nrn1 expression

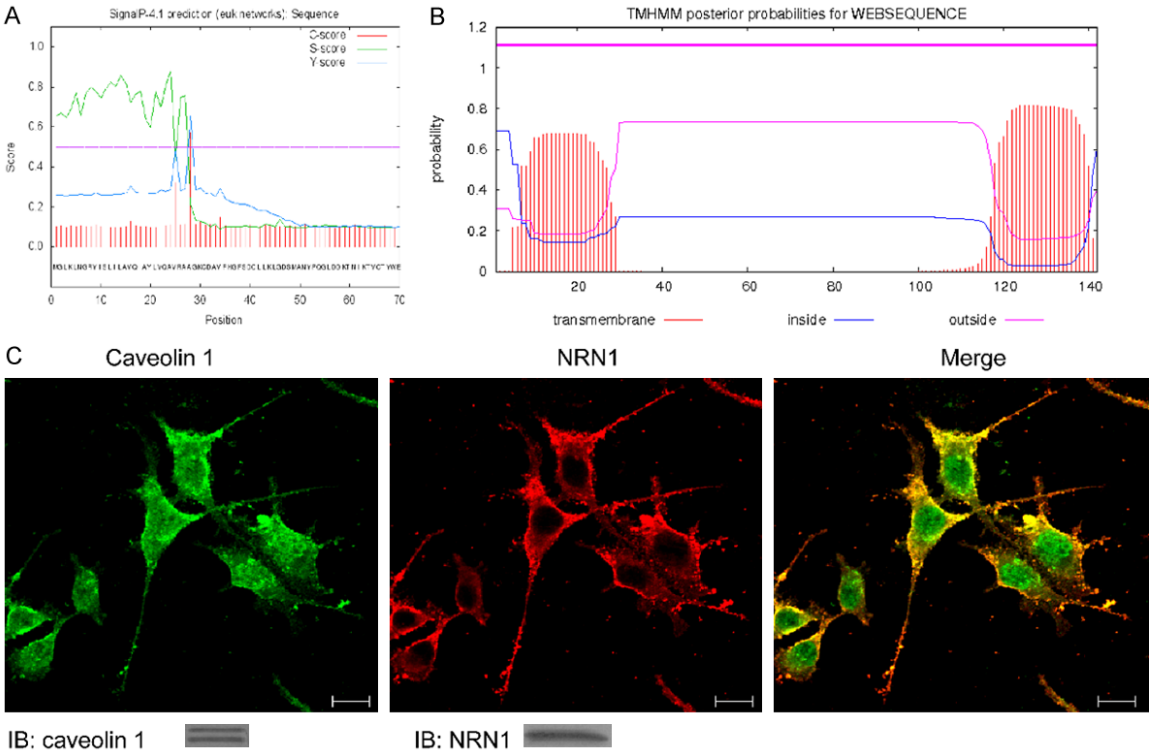

Figure 3. Subcellular localization of Nrn1 and its association with the lipid raft. A. SignalP results graphed by C-, S-, and Y-scores. Default (D-)cutoff was set to 0.5 (purple line). Genes with graphical intersections above the D-cutoff contain signal peptides. B. Transmembrane helices of Nrn1 predicted by TMHMM software. TMHMM plots are shown as probability (y-axis) of an amino acid (x-axis) residue sitting in a helix, inside, or outside summed over all possible model paths. The N-best prediction threshold was scored between 1 and 1.2 (purple line). Transmembrane, inside, and outside represent the probability of an amino acid being located within a transmembrane helix, on the cytoplasmic side of a membrane, and in the extracellular space, respectively. C. Nrn1 and caveolin 1 localization at the plasma membrane in SH-SY5Y cells. Scale bar = 50 μm. Detection of caveolin 1 and Nrn1 in SY5Y cell membrane protein extracts by Western blotting.

using the CMT panel comprising 16 normal human adult tissues was performed. The statistics and clustering heat map analysis revealed that Nrn1 mRNA was highly expressed in the brain, and the skeletal muscle, lung, thymus, pancreas, planceta, liver and heart tissues showed a similar level with it; lower levels were detected in the spleen, testicles, ovary, small intestine, but there was no detectable expression in the kidneys, colon, prostate or leukocytes (**Figure 1C**, **1D**). To analyze the Nrn1 protein expression in these human tissues, some of the tissues with Nrn1 mRNA expression were picked out for immunohistochemical staining. The results indicated that Nrn1 was expressed in multiple tissues and cell types, including hippocampal neurons (**Figure 2A**), Purkinje cells (**Figure 2B**), cortical neurons (**Figure 2C**), spinal cord neurons (**Figure 2D**), alveolar epithelial cells (**Figure 2E**), colonic gland cells (**Figure 2F**), liver tissue (**Figure 2G**), and intestinal gland cells (**Figure 2H**).

Nrn1 is localized in plasma membrane lipid rafts of mature neuronal cells

In order to investigate the subcellular localization of Nrn1, we performed a bioinformatics analysis and prediction including SignalP prediction and TMHMM v.2.0. The results of the SignalP prediction revealed that Nrn1 was a signal peptide (**Figure 3A**), while the TMHMM v.2.0 tool predicted that Nrn1 sequences were distributed outside the cell membrane without a transmembrane helix (**Figure 3B**). This evidence indicates that Nrn1 is a membrane-linked protein. Since the previous experiments indicated high levels of Nrn1 in brain tissue, we used SH-SY5Y human neuroblastoma cells to confirm the association of Nrn1 with cell mem-

Wide distribution of neuritin and human cancers

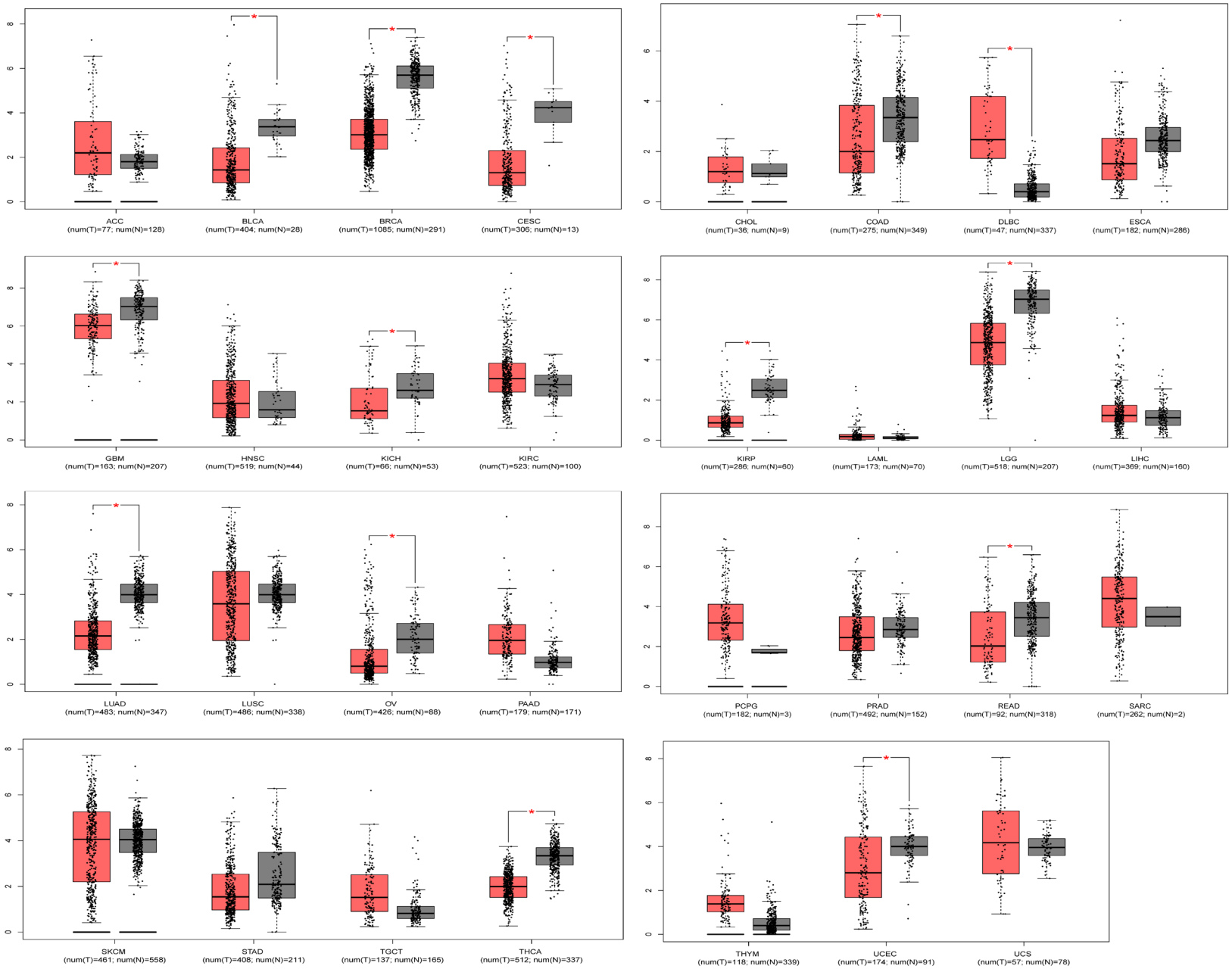

Figure 4. Expression level of Nrn1 in cancers including 31 kinds of cancers as indicated in the figure and paired normal tissues. T: tumor tissues, N: normal tissues, *P<0.05.

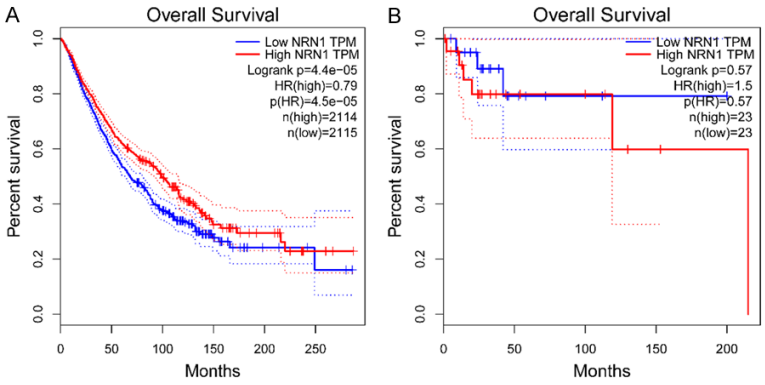

Figure 5. A. Integrity of prognostic value of Nrn1 in BLCA; BRCA; CESC; COAD; GBM; KIHC; KIRP; LGG; READ; UCEC; LUA and OV patients. B. Prognostic value of Nrn1 in DLBC patients. HR: hazard ratio, CI: confidence interval, logRank P<0.01.

branes and lipid rafts by immunofluorescence analysis. Samples were probed with antibodies against Nrn1 and the lipid raft marker caveolin 1. Nrn1 was mostly concentrated at the plasma membrane of SH-SY5Y cells and colocalized with caveolin 1 (**Figure 3C**). To further confirm these observations, membrane proteins were extracted from the cells and analyzed by western blotting. Caveolin 1 and Nrn1 were both present in the membrane fraction (**Figure 3C**).

Nrn1 expression level was disordered in 13 kinds of human cancers

Since in our previously study, the expression of Nrn1 was not only present in the nervous system but also in various normal human tissues. The function of Nrn1 in these tissues outside the nervous system remains unclear. Here, by exploring The Cancer Genome Atlas (TCGA) a database for human caner transcriptome, we investigated the expression level in 31 kinds of human cancers. Compared to the normal tissues, the results revealed that Nrn1 was significantly downregulated in BLCA; BRCA; CESC; COAD; GBM; KIHC; KIRP; LGG; READ; UCEC; LUA and OV (*P<0.05), but only upregulated in DLBC (*P<0.05) (**Figure 4**). Survival analysis is widely used in clinical and epidemiological research: in randomized clinical trials for comparing the efficacy of treatments and in observational (non-randomized) research to determine and test the existence of epidemiological association [29]. To explore the potential functions of Nrn1 in these human tumors, a survival analysis was performed. A combination of the overall survival analysis of the 12 kinds of human tumors with Nrn1 downregulation revealed that patients with high levels of Nrn1 present a long term survival (*P<0.01) (**Figure 5A**). But there is no significant effect on DLBC patients (**Figure 5B**).

Discussion

Nrn1/CPG15 is a neurotrophic factor and glycosylphosphatidylinositol (GPI)-anchored axonal protein that is mainly expressed in the brain and induced by neuronal activity. In this study, we demonstrated that *Nrn1* mRNA is expressed in various normal human tissues. Our results indicate that *Nrn1* is expressed in the placenta, heart, lungs, skeletal muscle, and spleen at a level similar to that in brain. Nrn1 mRNA was detected in the prostate, heart, small intestine, ovary, thymus, pancreas, liver, and testes with a lower level, but there was no detectable expression in the kidneys, colon, or leukocytes. These results suggest that Nrn1 may have varied roles in multiple tissues, not just in the brain. Nrn1 is acknowledged as a neurotrophic factor and its function in other tissues is unclear. Nrn1 may participate in various functions and biological processes depending on its tissue distribution. For example, the high level of *Nrn1* mRNA in placental tissue suggests that it may play an important role in early embryonic development [30].

Signal peptide is important for protein transportation and localization (26). Our bioinformatics analysis showed that Nrn1 is distributed outside the cell membrane, which depends on a signal peptide or GPI anchor but not on a transmembrane helix. We speculate that Nrn1 is a secreted protein or a membrane-linked protein with autocrine and paracrine functions. Nrn1 is a GPI-anchored axonal protein [1] that is associated with lipid rafts [31]. In our study,

we detected Nrn1 at the plasma membrane by immunocytochemistry and Western blotting and found that it colocalized with the lipid raft marker caveolin 1.

Nrn1 distribution in various normal human tissues indicated a novel biological process exploration of Nrn1. But these potential functions of Nrn1 are still unknown. Data have shown that the neurotrophin family is always involved in the regulation of tumorigenesis like nerve growth factor (NGF), which functions as either supporting or suppressing tumor growth depending on the tumor type [32]. Thus, we speculate that the effect of the wide distribution of Nrn1 in human tissues may be associated with tumor development. In this study, 31 categories of human cancer data were included to investigate the correlation of Nrn1 expression and tumorigenesis. The results showing that Nrn1 was significantly downregulated in a total of 12 kinds of human cancers and the survival analysis of these 12 kinds of human cancers indicated that Nrn1 may play an important role in suppressing tumor growth or development. One of the interesting findings was that Nrn1 was significantly upregulated only in DLBC, a kind of Lymphatic hematopoietic malignancy tumor, but there is no significant effect of Nrn1 on DLBC patients' survival.

In conclusion, this study demonstrated that Nrn1 was expressed in a variety of human tissues (brain, skeletal muscle, lung, thymus, pancreas, planceta, liver and heart tissues) and especially concentrated in the lipid raft of cell membranes. We also provide the first evidence that Nrn1 is associated with a variety human cancers including BLCA; BRCA; CESC; COAD; GBM; KIHC; KIRP; LGG; READ; UCEC; LUA; OV; DLBC, and can act as a tumor suppressor gene. This study provides some powerful evidence to make Nrn1 a new biomarker and a target of these cancers.

Acknowledgements

We extend our sincere gratitude to Dr. Jin Zhao, Dr. Xiaolin Pan, and Dr. Jiangmei Qin for their immense assistance during the study. We are grateful to the staff at the Department of Pathology, The First Affiliated Hospital of Shihezi University School of Medicine, for technical support. This work was supported by NSFC Grant 31060136 (Huang Jin), 307600633 (Huang Jin) and 30260029 (Huang Jin).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jin Huang, Department of Biochemistry, The Key Laboratory of Xinjiang Endemic & Ethnic Diseases, School of Medicine, Shihezi University, Shihezi 832002, Xinjiang, China. Tel: +86-0993 205-7882; E-mail: bio-huangjin@shzu.edu.cn

References

[1] Naeve GS, Ramakrishnan M, Kramer R, Hevroni D, Citri Y and Theill LE. Neuritin: a gene induced by neural activity and neurotrophins that promotes neuritogenesis. Proc Natl Acad Sci U S A 1997; 94: 2648-2653.

[2] Nedivi E, Wu GY and Cline HT. Promotion of dendritic growth by CPG15, an activity-induced signaling molecule. Science 1998; 281: 1863-1866.

[3] Cantallops I, Haas K and Cline HT. Postsynaptic CPG15 promotes synaptic maturation and presynaptic axon arbor elaboration in vivo. Nat Neurosci 2000; 3: 1004-1011.

[4] Choi Y, Lee K, Ryu J, Kim HG, Jeong AY, Woo RS, Lee JH, Hyun JW, Hahn S, Kim JH and Kim HS. Neuritin attenuates cognitive function impairments in tg2576 mouse model of Alzheimer's disease. PLoS One 2014; 9: e104121.

[5] Javaherian A and Cline HT. Coordinated motor neuron axon growth and neuromuscular synaptogenesis are promoted by CPG15 in vivo. Neuron 2005; 45: 505-512.

[6] Sharma TP, Liu Y, Wordinger RJ, Pang IH and Clark AF. Neuritin 1 promotes retinal ganglion cell survival and axonal regeneration following optic nerve crush. Cell Death Dis 2015; 6: e1661.

[7] Wang H, Li X, Shan L, Zhu J, Chen R, Li Y, Yuan W, Yang L and Huang J. Corrigendum: recombinant hNeuritin promotes structural and functional recovery of sciatic nerve injury in rats. Front Neurosci 2017; 11: 65.

[8] Gao R, Li X, Xi S, Wang H, Zhang H, Zhu J, Shan L, Song X, Luo X, Yang L and Huang J. Exogenous neuritin promotes nerve regeneration after acute spinal cord injury in rats. Hum Gene Ther 2016; 27: 544-554.

[9] Kalinski AL, Sachdeva R, Gomes C, Lee SJ, Shah Z, Houle JD, Twiss JL. mRNAs and protein synthetic machinery localize into regenerating spinal cord axons when they are provided a substrate that supports growth. J Neurosci 2015; 35: 10357-10370.

[10] Picard N, Leslie JH, Trowbridge SK, Subramanian J, Nedivi E and Fagiolini M. Aberrant development and plasticity of excitatory visual cortical networks in the absence of cpg15. J Neurosci 2014; 34: 3517-3522.

[11] Sharma N, Marzo SJ, Jones KJ and Foecking EM. Electrical stimulation and testosterone differentially enhance expression of regeneration-associated genes. Exp Neurol 2010; 223: 183-191.
[12] Seale P, Ishibashi J, Holterman C and Rudnicki MA. Muscle satellite cell-specific genes identified by genetic profiling of MyoD-deficient myogenic cell. Dev Biol 2004; 275: 287-300.
[13] Kojima N, Shiojiri N, Sakai Y and Miyajima A. Expression of neuritin during liver maturation and regeneration. FEBS Lett 2005; 579: 4562-4566.
[14] Nedivi E, Javaherian A, Cantallops I and Cline HT. Developmental regulation of CPG15 expression in Xenopus. J Comp Neurol 2001; 435: 464-473.
[15] Fujino T, Lee WC and Nedivi E. Regulation of cpg15 by signaling pathways that mediate synaptic plasticity. Mol Cell Neurosci 2003; 24: 538-554.
[16] Putz U, Harwell C and Nedivi E. Soluble CPG15 expressed during early development rescues cortical progenitors from apoptosis. Nat Neurosci 2005; 8: 322-331.
[17] Zhang L, Zhao Y, Wang CG, Fei Z, Wang Y, Li L, Li L and Zhen HN. Neuritin expression and its relation with proliferation, apoptosis, and angiogenesis in human astrocytoma. Med Oncol 2011; 28: 907-912.
[18] Yang Z, Zhao T, Liu Y, Gong Z, Cheng S and Yang Q. Identification of new HLA-A*0201-restricted cytotoxic T lymphocyte epitopes from neuritin. J Neurooncol 2013; 114: 51-58.
[19] Yuan M, Li Y, Zhong C, Li Y, Niu J and Gong J. Overexpression of neuritin in gastric cancer. Oncol Lett 2015; 10: 3832-3836.
[20] Raggo C, Ruhl R, McAllister S, Koon H, Dezube BJ, Fruh K and Moses AV. Novel cellular genes essential for transformation of endothelial cells by Kaposi's sarcoma-associated herpesvirus. Cancer Res 2005; 65: 5084-5095.
[21] Han D, Qin B, Liu G, Liu T, Ji G, Wu Y and Yu L. Characterization of neuritin as a novel angiogenic factor. Biochem Biophys Res Commun 2011; 415: 608-612.
[22] Yuan B, Shen H, Su T, Lin L, Chen T and Yang Z. A novel nanoparticle containing neuritin peptide with grp170 induces a CTL response to inhibit tumor growth. J Neurooncol 2015; 125: 23-32.
[23] Feng YA, Liu TE and Wu Y. microRNA-182 inhibits the proliferation and migration of glioma cells through the induction of neuritin expression. Oncol Lett 2015; 10: 1197-1203.
[24] Huang J, Yuan H, Lu C, Liu X, Cao X and Wan M. Jab1 mediates protein degradation of the Rad9-Rad1-Hus1 checkpoint complex. J Mol Biol 2007; 371: 514-527.
[25] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.
[26] Sonnhammer EL, von Heijne G and Krogh A. A hidden Markov model for predicting transmembrane helices in protein sequences. Proc Int Conf Intell Syst Mol Biol 1998; 6: 175-182.
[27] Krogh A, Larsson B, von Heijne G and Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 2001; 305: 567-580.
[28] Petersen TN, Brunak S, von Heijne G and Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 2011; 8: 785-786.
[29] Flynn R. Survival analysis. J Clin Nurs 2012; 21: 2789-2797.
[30] Wang X, Liu C, Xu F, Cui L, Tan S, Chen R, Yang L and Huang J. Effects of neuritin on the migration, senescence and proliferation of human bone marrow mesenchymal stem cells. Cell Mol Biol Lett 2015; 20: 466-474.
[31] Ikezawa H. Glycosylphosphatidylinositol (GPI)-anchored proteins. Biol Pharm Bull 2002; 25: 409-417.
[32] Retamales-Ortega R, Orostica L, Vera C, Cuevas P, Hernandez A, Hurtado I, Vega M and Romero C. Role of nerve growth factor (NGF) and miRNAs in epithelial ovarian cancer. Int J Mol Sci 2017; 18.