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Reconsidering Neurogenetic Indication in the Human Brain: Broad Expression of Doublecortin Transcript in the Hippocampal and Cortical Cell Populations

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Adult neurogenesis · Hippocampus · Prefrontal cortex · Degenerative disease · Neural stem cell · Single-nucleus RNA sequencing

Abstract
Introduction: Neurogenesis in the adult brain may play an important role in memory and cognition; however, knowledge of neurogenic markers in the human brain remains limited. We compared the single-nucleus transcriptome of the hippocampus with that of other cortical regions to identify hippocampus-specific neurogenic markers. ***Methods:*** We analyzed 26,189 nuclei from four human brains collected within 16 h of death. Clustering and annotation were performed to examine differential expression, gene ontology, and intercellular communication. DCX expression was validated by ddPCR. ***Results:*** Immature markers such as DCX, CALB2, NES, SOX2, PAX6, DPYSL3, and TUBB3 were expressed in both hippocampus and prefrontal cortex, with higher levels in the prefrontal cortex. ddPCR confirmed higher expression of DCX in the prefrontal cortex. DCX was involved in both neurogenesis and neuroprotection pathways. ***Conclusion:*** Neurogenic markers are not definitive indicators of adult neurogenesis as their roles are more complex than previously understood.

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Introduction

Adult hippocampal neurogenesis is known to be associated with memory, mood, and cognition [1–3]. Animal models have demonstrated that functional integration associated with adult neurogenesis fosters cognitive segregation by enhancing the excitability and plasticity of hippocampal circuits and modulating inhibitory feedback [4–11]. A landmark study by Moreno-Jiménez et al. [12] was the first to demonstrate the presence of doublecortin (DCX) in immature neurons in the human dentate gyrus (DG) [13–16] and subsequent research has confirmed its lifelong presence [12, 17–20]. Furthermore, decreasing numbers of putative neuronal markers have been correlated with cognitive decline, highlighting DCX expression as a marker of brain health during aging [4, 19]. However, whether previously identified neurogenesis markers are unique indicators of adult neurogenesis in the human brain has not been elucidated.

Chronic antidepressant use, epileptic seizures, and toxic accumulations of amyloid-β and kainic acid can drive mature neurons to revert to an immature state, as evidenced by the re-expression of developmental markers [4, 7, 8, 11]. While microglial cytokines, such as interleukin-6 and tumor necrosis factor-α, can inhibit neurogenesis, inflammation can also trigger neuronal dematuration and subsequent marker re-expression [21]. In particular, hippocampal single-nucleus transcriptome analysis could help determine whether neurogenic markers are exclusive to the DG. However, significant analytical challenges exist, including the susceptibility of neurogenic marker genes to RNA degradation owing to suboptimal sample collection, long postmortem intervals (PMIs), and unstable storage conditions. Herein, we constructed a comprehensive single-nucleus transcriptomic profile of the hippocampus and prefrontal cortex from the same brains, which were processed immediately after collection to improve transcript quality, to identify the neurogenic markers exclusive to the DG.

Materials and Methods

Postmortem Human Brain Tissue Harvesting

Human brain tissues were obtained within 24-h PMIs from four cadavers (two males and two females with a mean age of 68.8 years). The cadavers had been legally donated to the Surgical Anatomy Education Centre of the Yonsei University College of Medicine (Approval No. YSAEC 23-008). The Institutional Review Board approved the study (IRB number: 4-2020-0986). Any samples with a suspected or confirmed neurodegenerative or neurological disorder in the medical history were excluded. We carefully removed the calvaria and obtained the hippocampus and prefrontal cortex. All brain tissues were immersed in RNAlater solution (AM7021, Invitrogen, Carlsbad, CA, USA) and maintained at 4°C overnight following en bloc harvesting within 10-h PMIs.

Braak Staging of Tau Pathology in Alzheimer's Disease

Following immunohistochemistry for beta-amyloid, alpha-synuclein, and paired helical filament-tau protein (online suppl. materials 1; for all online suppl. material, see https://doi.org/10.1159/000540976), Braak staging was carried out using standard diagnostic criteria by evaluating the hippocampus (cornu ammonis subfields, DG, entorhinal cortex, and adjacent fusiform temporal neocortex) and prefrontal cortex [22, 23]. Based on the distribution of tau pathology marked by GT-38, each case was assigned a Braak stage ranging from B0 to B3.

RNA Isolation, cDNA Synthesis, and Droplet-Based Digital Polymerase Chain Reaction

The protocols for total RNA extraction, cDNA synthesis, and droplet-based digital polymerase chain reaction (ddPCR) are described in detail in the online supplementary materials 1. A ddPCR workflow was performed using a recently revised RNA quantification protocol [24]. The data were analyzed using the manufacturer's software (Bio-Rad Laboratories, Quanta Soft analysis software, version 1.7.4.0917).

Nuclei Isolation, Library Preparation, and Single-Nucleus RNA Sequencing

Nuclei isolation protocol is described in the online supplementary materials 1 [25, 26]. The single-nucleus libraries were prepared from approximately 10,000 nuclei from each sample using the Chromium Single Cell 3' Reagent kit (PN-1000269; 10x Genomics, CA, USA). Droplet-based single-nucleus RNA-seq libraries were prepared using the Chromium Single Cell 3' kit (PN-1000128; 10x Genomics) according to the manufacturer's protocol. Quality control was applied to the libraries using the 5200 Fragment Analyzer (Agilent Technologies) before sequencing the libraries on a NovaSeq6000 device (Illumina, San Diego, CA, USA).

Sequencing Alignment and Quality Control

The CellRanger software (10x Genomics, version 6.0.1) was used to align the sequenced reads to *GRCh38* (GENCODE version 32/Ensembl98) and generate their

gene expression matrices. The R package Seurat (version 4.3.0.1) was then used to merge all matrices into a single SeuratObject class with tags for cadaver identification and region and processed for quality control and clustering. A filtered matrix of nuclei with >200 and <2,500 genes, <200 UMI, and <15% mitochondrial transcripts was obtained for downstream analysis.

Bioinformatics Analysis

All detailed bioinformatics protocols are shown in the online supplementary materials 1. Filtering data, clustering analysis, and annotation were performed. Additional analyses, such as differential gene expression analysis and functional annotation, cell-to-cell communication analysis, and more detailed information, were subsequently performed.

Results

Nuclei in the Prefrontal and Hippocampal Populations

The sample information and experimental design details are provided in Figure 1a and online suppl. Figure S1A. The transcriptional profiles were assessed by isolating single nuclei (online suppl. Fig. S1). After strict quality control (retaining 200–2,500 genes with >15% mtRNA), 26,189 of the 40,691 nuclei were used for further analysis (median number of UMI, 1,820; online suppl. Fig. S2). Applying unsupervised uniform manifold approximation and projection reduction to the total nuclei resulted in the identification of 21 clusters, which were subsequently annotated into 9 cell types using the cell marker genes established by Ayhan et al. (2021) [27] (online suppl. Fig. S3).

In total, 15,237 neuronal nuclei were categorized into 5 cell types (online suppl. Fig. S4) and 19 clusters (Fig. 1; online suppl. Fig. S5): pyramidal neuron (Pyr; marked by RBFOX3, SLC17A7, and GAP43), interneurons (GAD1 and SLC6A1), excitatory granule cells (GCes; CALB1, SLC17A7, and PROX1), inhibitory granule cells (GCis; GAD1, SLC6A1, and PROX1), and the residual group (bridge cells, Brds; PLP1 and GFAP). The GCe cluster was predominantly observed in the hippocampus (>99%), with unique expression of MAML2. The comparison of hippocampus-only subclusters with others (online suppl. Fig. S6) revealed distinct differentially expressed genes. REACTOME pathway analysis suggested that hippocampus-only Pyr and GCe are closely associated with the activation of developmental biology or axon guidance.

Neuronal Nuclei Expressing Immature Markers

A tentative immature signature $DCX^{+}calbindin^{-}$ (CALB1) was recruited for immature neurons, which were observed in 10.99% of all neuronal populations across all neurons, except for GCe (Fig. 2a). Surprisingly, DCX was more robustly expressed in the prefrontal cortices of all four brains (Fig. 2b and online suppl. Fig. S7A). REACTOME pathway analysis of upregulated genes in the immature neurons compared to others suggested that $DCX^{+}calbindin^{-}$ cells are closely associated with the activation of GABA synthesis or signaling, as well as the suppression of Rho GTPase signaling and receptor tyrosine kinase signaling (online suppl. Fig. S7D). Contrary to our expectations, GCe rarely expressed neurogenic markers. Instead, they expressed signals related to synaptic transmission or axonal reconnection, such as brain-derived neurotrophic factor (BDNF) and SEMA3 (online suppl. Fig. S8).

Other markers widely associated with immaturity, including calretinin (CALB2), NES, SOX2, PAX6, DPYSL3, and TUBB3, were extensively expressed across both regions (Fig. 3a; online suppl. Fig. S9). The relative expression of these genes was slightly higher in the prefrontal cortex than in the hippocampus. ddPCR of each DCX two-pair sample confirmed that DCX^{+} nuclei were more abundant in the prefrontal cortex than in the hippocampal population across all four brains (Fig. 3b).

Cell-to-Cell Interactions among Different Cell Types in the Prefrontal Cortex and Hippocampus

There were notable regional differences in cell interactions. The CellChat analysis suggests the hippocampus might have a more complex and dynamic cellular communication network. Also, oligodendrocyte precursor cell (OPC), astrocyte (Astro), and oligodendrocyte (Olig) showed strong outgoing and incoming interactions (Fig. 4a).

Interactions were stronger for hippocampal than prefrontal clusters (Fig. 4b). Detailed patterns of these interactions and the associated expression levels of the ligand-receptor pairs are presented in Figure 4c. The hippocampal GCe received pleiotrophin, a known heparin-binding mitogen, from endothelial cell (Endo), Astro, and OPC (Fig. 4d). In return, GCe exported nerve growth factors such as BDNF (designated as neurotrophin signals in the CellChat database) to all neuronal clusters and some glial clusters (Astro, Olig, and OPC) (Fig. 4e; online suppl. Fig. S10). The Olig cluster exported semaphorin 3 (SEMA3) – related to cellular and axonal migration – to the GCe. Meanwhile, the hippocampal Brd cluster received pleiotrophin signals from the Endo and

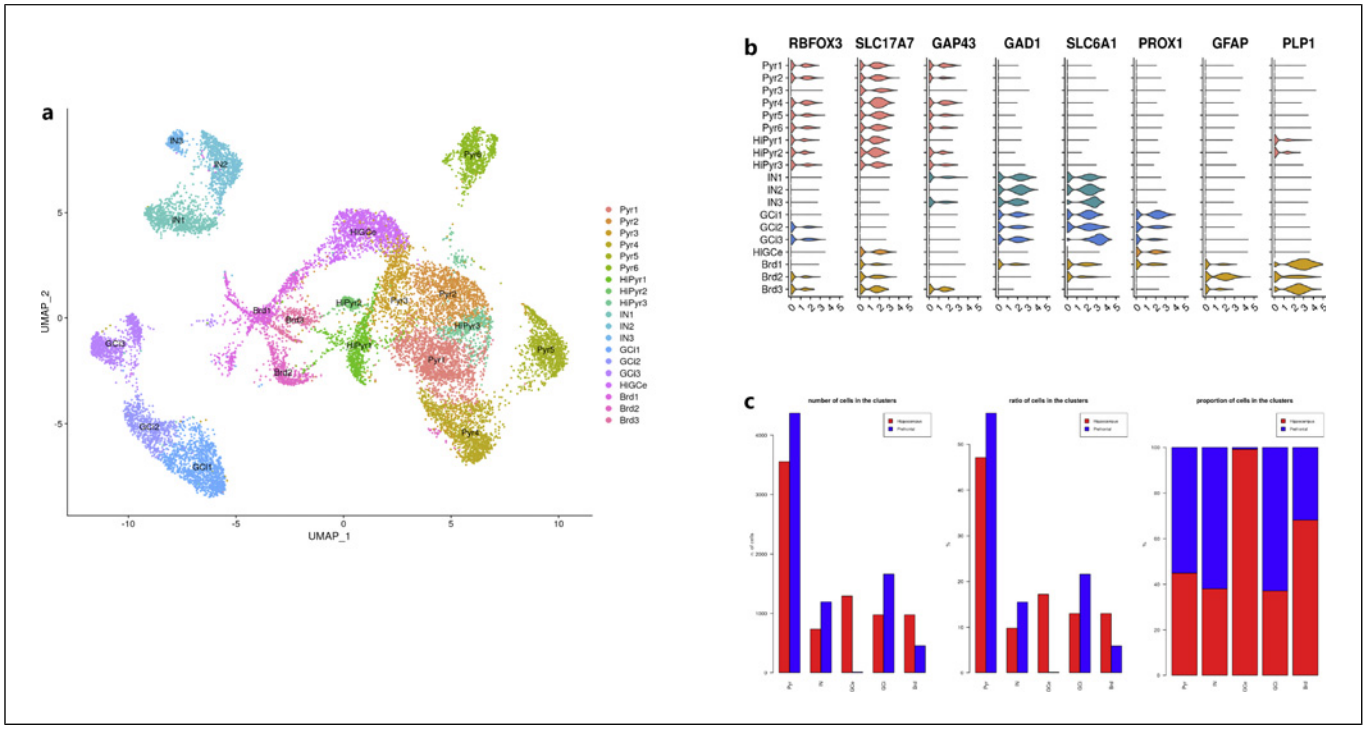

Fig. 1. Identification of hippocampal and prefrontal neuronal cells. **a** Uniform manifold approximation and projection (UMAP) plot demonstrating 19 annotated neuronal clusters. **b** Violin plot of marker genes in these neuronal clusters. **c** Bar charts show the numbers, ratios, and proportions of cells among cell types. Further details regarding the number of different cell types in the hippocampal and prefrontal populations are provided in online suppl. Figures S4 and S5. Pyr, pyramidal neuron; HiPyr, hippocampal-pyramidal neuron; IN, interneuron; GCi, inhibitory granule cell; GCe excitatory granule cell; HiGCe, hippocampal-excitatory granule cell; Brd, bridge cell.

Astro. Conversely, the prefrontal Brd cluster was implicated in the insulin pathway, receiving insulin-like growth factor signals from the GCi cluster and VISFATIN, an adipocytokine with antidiabetic properties, from the Endo cluster.

Discussion

DCX, which has emerged as a widely accepted marker for newly generated granule cells in a variety of human neurons, has been found to be expressed in the adult human DG, as well as in cats and rodents. Moreno-Jimenez et al. [12] observed DCX expression in the subgranular zone of the adult human brain with very short PMI, even in individuals over 80 years old, using immunohistochemistry with a meticulously optimized antigen retrieval procedure. DCX declines with age, with a particularly pronounced decline observed in patients with Alzheimer's disease. Similarly, Zhou et al. [20] reported DCX transcript expression in the human hippocampus across the lifespan, from neonatal to old age, using single-nucleus RNA sequencing and suggested a lifespan presence of the immature granule cell. In addition, they reported that DCX was not present in the frontal, prefrontal, visual, and middle temporal cortices, despite its expression in the cortex of the neonatal brain. In contrast, Li et al. [28] found protein expression of DCX in the human cortex and amygdala using immunostaining and Western blotting, where it was expressed in the frontal, parietal, and occipital lobes up to 56 years old, and in the temporal and amygdala throughout life. Similarly, our transcriptomic study demonstrated DCX+ nuclei to be diffusely distributed in neuronal clusters and OPCs, including those not only in the hippocampus but also in the prefrontal lobe of the adult brain. Interestingly, we found DCX expression in the prefrontal lobe of individuals over 60 years old, even in those over 90 years old. Moreover, its prefrontal expression was more robust in the paired hippocampus of the same brain across individuals.

Although DCX appears to be involved in neuronal development, it is not certain that the presence of DCX+ is absolutely indicative of an immature neuron. Franjic et al. [14] convincingly argued that the DCX transcript is not a specific indicator of neuroblasts or immature granule

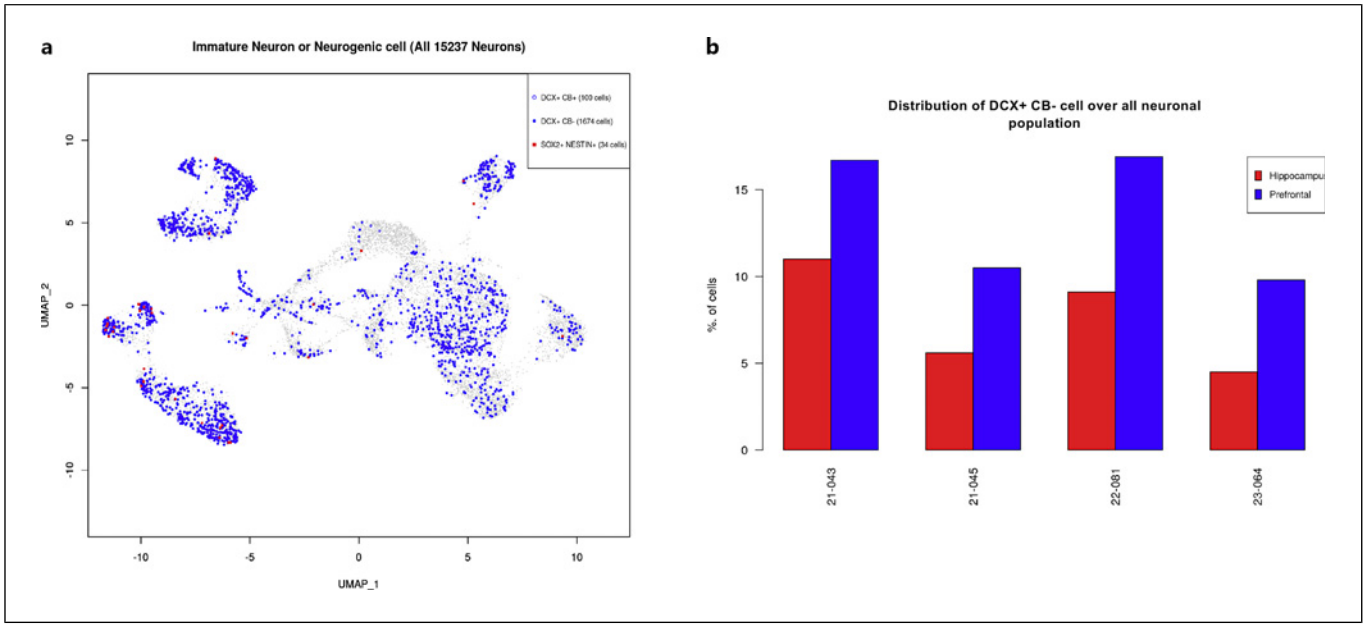

Fig. 2. Immature nuclei population expressing DCX+/CB–. **a** Uniform manifold approximation and projection (UMAP) expression plots for immature neuronal (DCX^+CB^-) populations. Doublecortin (DCX) is expressed by all cell types except excitatory granule cell (GCe). **b** Bar plot showing the distribution of DCX^+CB^- neuronal populations in each sample. DCX is more robustly expressed in the prefrontal cortex in all four brains. Further details regarding the number of immature neurons in the hippocampal and prefrontal populations are provided in online supplementary Fig. S7A. CB, calbindin (CALB1).

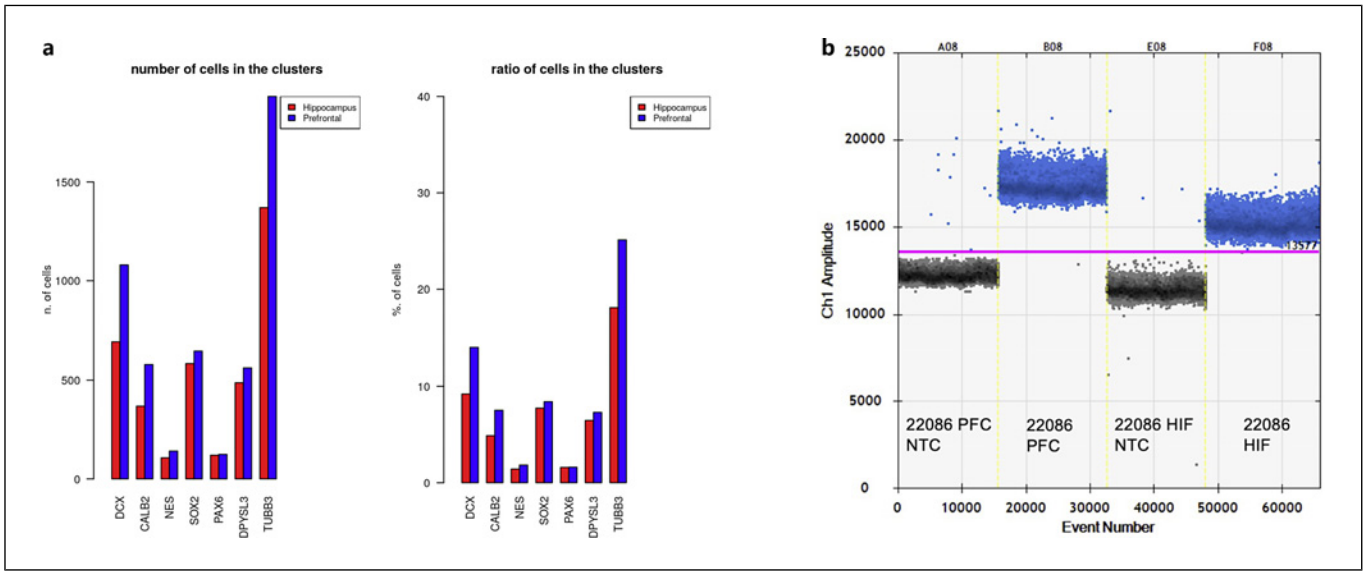

Fig. 3. Immature marker expression. **a** Bar plots showing numbers and proportions of cells in clusters and immature neuron-related genes (e.g., doublecortin [DCX], CALB2, NES, SOX2, PAX6, DPYSL3, and TUBB3) in the prefrontal cortex (blue bar, PFC) and hippocampus (red bar, HIF). **b** Results of the droplet-based digital polymerase chain reaction (ddPCR) for two paired samples (prefrontal cortex and hippocampus). Further details regarding the number of markers expressed nuclei in the hippocampal and prefrontal populations are provided in online supplementary Fig. S8.

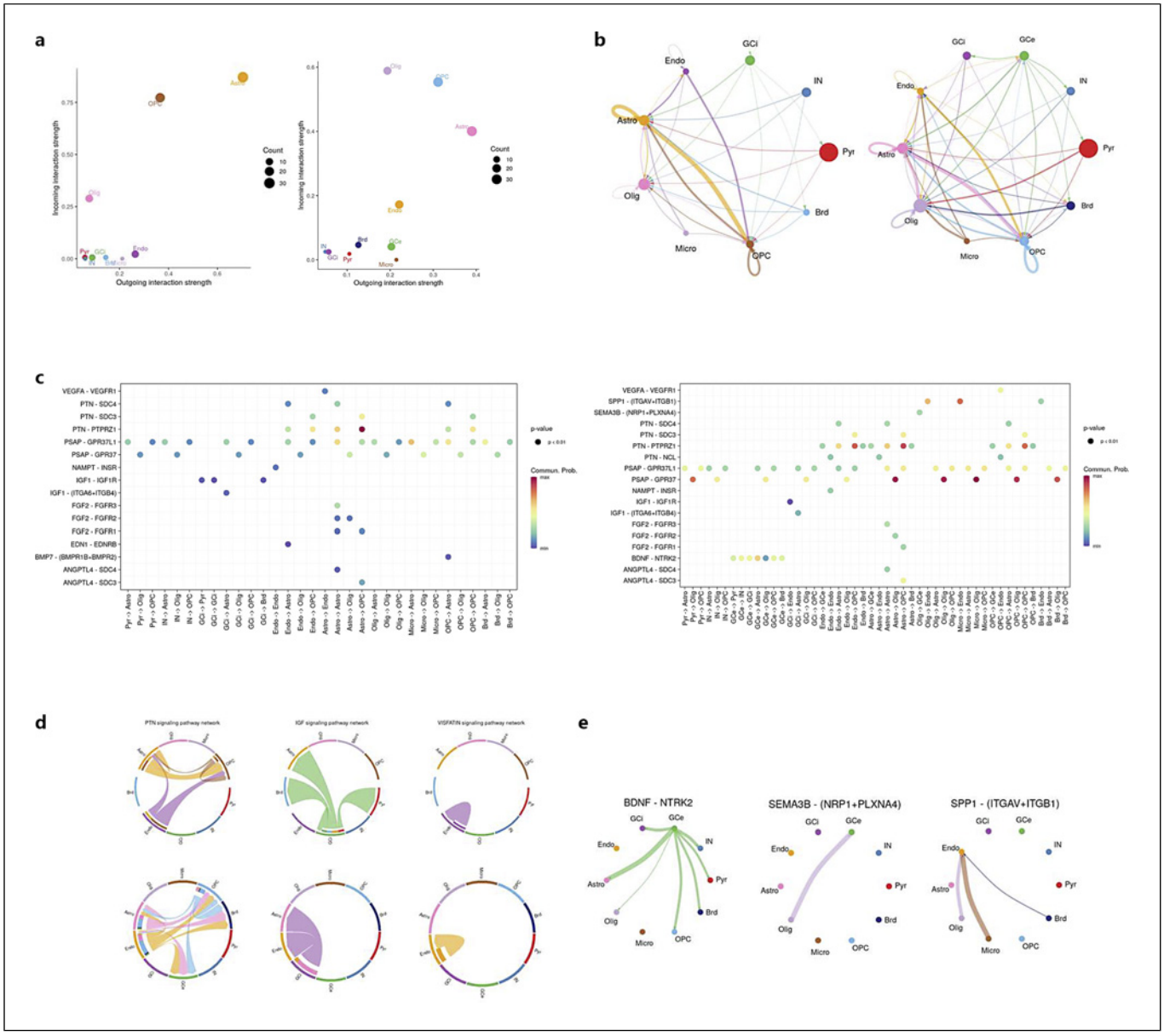

Fig. 4. Cell-to-cell communication (analyzed using CellChat) among all nucleus types in hippocampal and prefrontal populations. **a** Outgoing and incoming interaction strengths and expression levels of significant ligand-receptor pairs in the prefrontal (left) and hippocampal (right) populations. **b** Cell-to-cell interactions among all cell types in the prefrontal (left) and hippocampal (right) populations. **c** Ligand-receptor interactions among all cell types in the prefrontal (left) and hippocampal (right) populations. **d** Pleiotrophin (PTN), insulin-like growth factor (IGF), and VISFATIN signaling pathway networks in the prefrontal (upper) and hippocampal (lower) populations. **e** Cell-to-cell interactions (NGFR, SPP, and semaphorin 3 [SEMA3]) among all cell types of the hippocampal population. GC, granule cell; Endo, endothelial cell; Astro, astrocyte; Olig, oligodendrocyte; Micro, microglia; OPC, oligodendrocyte precursor cell; Brd, bridge cell; Pyr, pyramidal neuron; IN, interneuron; GCe, excitatory granule cell; GCi, inhibitory granule cell.

neurons per se, based on the fact that DCX expression was present outside neuroblasts or granule cells in all species analyzed in their study. In fact, DCX is very sensitive for identification that its presence in adult humans has been considered controversial until recently. Previous interpretations of DCX expression as an indicator of neurogenesis were based on regional differences in area or time of expression. There have been inconsistencies in the presence of DCX in previous studies due to prolonged PMI of the sample and tissue pre-processing that would cause denaturation and

degradation of the DCX protein or transcript. In our study, the hippocampus and prefrontal lobe of a short PMI brain were recruited and paired for transcriptomic analysis. DCX was not only found in the region outside the hippocampus but was more highly expressed in the frontal lobe.

DCX expression is also associated with various pathological conditions; it has been detected in the neocortex during epilepsy, in multipolar astroglial-like cells in the temporal lobe of focal cortical dysplasia, and even in cells within acute infarct areas. In this study, DCX expression in oligodendrocytes appears to play a role in cell migration and neuronal differentiation. While hippocampal pyramidal cells and granule cells did not express DCX, NES, or SOX as significantly as expected, they exhibited higher expression of ZBZT20 and SEMA5A, genes related to axon guidance. The data suggest that DCX expression in oligodendrocytes may be involved in cell migration and neuronal differentiation. Notably, BDNF, which promotes dendritic growth, complexity, and axonal reconnections, is exported from GCe to most other neuronal groups and glial cells, implying a potential role for GCe in neuronal reorganization and migration. Figure 4 presents molecular signaling data that, although not directly linked to DCX expression, explores the complex cellular communication network within the hippocampus, particularly in relation to neurogenesis. The strong signals observed between glial cells suggest significant interactions that may play a crucial role in cellular communication and neurogenesis, though the exact mechanisms are not yet fully understood. While these findings do not directly connect to DCX expression, they offer valuable insights into the broader context of neurogenesis-related cellular interactions within the hippocampus. Further analysis is needed to fully understand the implications of these glial signals and their potential relationship to DCX expression.

Further functional studies are warranted to clarify the role of DCX in brain function or its involvement in the degenerative disease. Nevertheless, our study provides insight that DCX is not only a neurogenesis marker expressed in immature GC neurons but also a factor that may be involved in the migration and axonal guidance expressed in a broader region including the frontal lobe.

This study successfully identified a unique transcriptomic profile in fresh samples with minimal PMI owing to RNA stabilization. We identified the expression of canonical neurogenic markers in cortical neurons, highlighting a previously unappreciated intricacy in adult neurogenesis. This transcriptomic landscape of the aged human brain yielded novel reference data for understanding the molecular complexity of neurogenesis. Specifically, DCX might not solely function within traditional neurogenesis, with it also possibly being instrumental in strengthening neuroprotective or recovery pathways.

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Statement of Ethics

All protocol in the present study were reviewed and approved by the Institutional Review Board of Yonsei University College of Medicine (IRB Approval No. 4-2020-0986). All study procedures were performed ethically in accordance with the World Medical Association Declaration of Helsinki. All cadaver donors for this study were informed in writing at the time of donation that their cadavers would be used for research, and they provided written consent. In addition, the research use of the cadavers was approved by the Surgical Anatomy Education Center, a legal body of Yonsei University College of Medicine that oversees the research and educational use of cadavers (Approval No. YSAEC 23-008).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Concept and design, drafting of the manuscript, and supervision: T.-H.C., M.K., and H.-M.Y.; acquisition, analysis, or interpretation of data and critical review of the manuscript for important intellectual content: all authors; statistical analysis: T.-H.C., M.K., S.H.K. – Shin Hyung Kim,

J.E.L., H.J.K., and H.-M.Y.; obtained funding: J.E.L. and H.-M.Y.; and administrative, technical, or material support: T.-H.C., M.K., S.-H.K. – Shin Hyung Kim, S.H.K. – Se Hoon Kim, J.E.H., I.-S.Y., and H.-M.Y. All authors have read and agreed to submitted version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

References

1 Alam MJ, Kitamura T, Saitoh Y, Ohkawa N, Kondo T, Inokuchi K. Adult neurogenesis conserves hippocampal memory capacity. J Neurosci. 2018;38(31):6854–63. https://doi.org/10.1523/JNEUROSCI.2976-17.2018
2 Berger T, Lee H, Young AH, Aarsland D, Thuret S. Adult hippocampal neurogenesis in major depressive disorder and Alzheimer's disease. Trends Mol Med. 2020;26(9):803–18. https://doi.org/10.1016/j.molmed.2020.03.010
3 Kempermann G, Song H, Gage FH. Neurogenesis in the adult hippocampus. Cold Spring Harb Perspect Biol. 2015;7(9):a018812. https://doi.org/10.1101/cshperspect.a018812
4 Boldrini M, Santiago AN, Hen R, Dwork AJ, Rosoklija GB, Tamir H, et al. Hippocampal granule neuron number and dentate gyrus volume in antidepressant-treated and untreated major depression. Neuropsychopharmacology. 2013;38(6):1068–77. https://doi.org/10.1038/npp.2013.5
5 Catavero C, Bao H, Song J. Neural mechanisms underlying GABAergic regulation of adult hippocampal neurogenesis. Cell Tissue Res. 2018;371(1):33–46. https://doi.org/10.1007/s00441-017-2668-y
6 Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, et al. Neurogenesis in the striatum of the adult human brain. Cell. 2014;156(5):1072–83. https://doi.org/10.1016/j.cell.2014.01.044
7 Gonçalves JT, Schafer ST, Gage FH. Adult neurogenesis in the hippocampus: from stem cells to behavior. Cell. 2016;167(4):897–914. https://doi.org/10.1016/j.cell.2016.10.021
8 Lepousez G, Nissant A, Lledo P-M. Adult neurogenesis and the future of the rejuvenating brain circuits. Neuron. 2015;86(2):387–401. https://doi.org/10.1016/j.neuron.2015.01.002
9 Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference and indexing. Nat Neurosci. 2019;22(10):1565–75. https://doi.org/10.1038/s41593-019-0484-2
10 Vicidomini C, Guo N, Sahay A. Communication, cross talk, and signal integration in the adult hippocampal neurogenic niche. Neuron. 2020;105(2):220–35. https://doi.org/10.1016/j.neuron.2019.11.029
11 Restivo L, Niibori Y, Mercaldo V, Josselyn SA, Frankland PW. Development of adult-generated cell connectivity with excitatory and inhibitory cell populations in the hippocampus. J Neurosci. 2015;35(29):10600–12. https://doi.org/10.1523/JNEUROSCI.3238-14.2015
12 Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med. 2019;25(4):554–60. https://doi.org/10.1038/s41591-019-0375-9
13 Habib N, Avraham-Davidi I, Basu A, Burks T, Shekhar K, Hofree M, et al. Massively parallel single-nucleus RNA-seq with DroNc-seq. Nat Methods. 2017;14(10):955–8. https://doi.org/10.1038/nmeth.4407
14 Franjic D, Skarica M, Ma S, Arellano JI, Tebbenkamp AT, Choi J, et al. Transcriptomic taxonomy and neurogenic trajectories of adult human, macaque, and pig hippocampal and entorhinal cells. Neuron. 2022;110(3):452–69. e14. https://doi.org/10.1016/j.neuron.2021.10.036
15 Seki T, Hori T, Miyata H, Maehara M, Namba T. Analysis of proliferating neuronal progenitors and immature neurons in the human hippocampus surgically removed from control and epileptic patients. Sci Rep. 2019;9(1):18194. https://doi.org/10.1038/s41598-019-54684-z
16 Wang W, Wang M, Yang M, Zeng B, Qiu W, Ma Q, et al. Transcriptome dynamics of hippocampal neurogenesis in macaques across the lifespan and aged humans. Cell Res. 2022;32(8):729–43. https://doi.org/10.1038/s41422-022-00678-y
17 Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, et al. Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell. 2018;22(4):589–99. e5. https://doi.org/10.1016/j.stem.2018.03.015
18 Sorrells SF, Paredes MF, Zhang Z, Kang G, Pastor-Alonso O, Biagiotti S, et al. Positive controls in adults and children support that very few, if any, new neurons are born in the adult human hippocampus. J Neurosci. 2021;41(12):2554–65. https://doi.org/10.1523/JNEUROSCI.0676-20.2020
19 Tobin MK, Musaraca K, Disouky A, Shetti A, Bheri A, Honer WG, et al. Human hippocampal neurogenesis persists in aged adults and Alzheimer's disease patients. Cell Stem Cell. 2019;24(6):974–82. e3. https://doi.org/10.1016/j.stem.2019.05.003
20 Zhou Y, Su Y, Li S, Kennedy BC, Zhang DY, Bond AM, et al. Molecular landscapes of human hippocampal immature neurons across lifespan. Nature. 2022;607(7919):527–33. https://doi.org/10.1038/s41586-022-04912-w
21 Hagihara H, Murano T, Ohira K, Miwa M, Nakamura K, Miyakawa T. Expression of progenitor cell/immature neuron markers does not present definitive evidence for adult neurogenesis. Mol Brain. 2019;12(1):108. https://doi.org/10.1186/s13041-019-0522-8
22 Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 2006;112(4):389–404. https://doi.org/10.1007/s00401-006-0127-z
23 Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement. 2012;8(1):1–13. https://doi.org/10.1016/j.jalz.2011.10.007
24 Ferracin M, Salamon I, Lupini L, Miotto E, Sabbioni S, Negrini M. Circulating microRNA quantification using DNA-binding dye chemistry and droplet digital PCR. J Vis Exp. 2016;(112):e54102. https://doi.org/10.3791/54102
25 Foster DS, Griffin M, Januszyk M, Delitto D, Norton JA, Longaker MT. Optimized nuclei isolation from fresh and frozen solid tumor specimens for multiome sequencing. J Vis Exp. 2023;(200):e65831. https://doi.org/10.3791/65831
26 Narayanan A, Blanco-Carmona E, Demirdizen E, Sun X, Herold-Mende C, Schlesner M, et al. Nuclei isolation from fresh frozen brain tumors for single-nucleus RNA-seq and ATAC-seq. J Vis Exp. 2020;(162):e61542. https://doi.org/10.3791/61542
27 Ayhan F, Kulkarni A, Berto S, Sivaprakasam K, Douglas C, Lega BC, et al. Resolving cellular and molecular diversity along the hippocampal anterior-to-posterior axis in humans. Neuron. 2021;109(13):2091–105. e6. https://doi.org/10.1016/j.neuron.2021.05.003
28 Li Y-N, Hu D-D, Cai X-L, Wang Y, Yang C, Jiang J, et al. Doublecortin-expressing neurons in human cerebral cortex layer II and amygdala from infancy to 100 years old. Mol Neurobiol. 2023;60(6):3464–85. https://doi.org/10.1007/s12035-023-03261-7