Biochemical and Biophysical Research Communications 433 (2013) 496–501

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Cell surface Nestin is a biomarker for glioma stem cells

Xiong Jin [a], Xun Jin [a], Ji-Eun Jung [a], Samuel Beck [b], Hyunggee Kim [a,*]

[a] School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea
[b] Section of Molecular Cell and Developmental Biology, University of Texas at Austin, Austin, TX 78712, USA

ARTICLE INFO

Article history:
Received 2 March 2013
Available online 21 March 2013

Keywords:
Glioblastoma
Glioma stem cells
Nestin
γ-Secretase

ABSTRACT

Cancer stem cells (CSCs) are the most aggressive cell type in many malignancies. Cell surface proteins are generally used to isolate and characterize CSCs. Therefore, the identification of CSC-specific cell surface markers is very important for the diagnosis and treatment of malignancies. We found that Nestin (a type VI intermediate filament protein), like the glioma stem cell (GSC) markers CD133 and CD15, exhibited different levels of expression in primary human glioblastoma specimens. Similar to our previous finding that cytoplasmic Nestin is expressed as a cell surface form in mouse GSCs, the cell surface form of Nestin was also expressed at different levels in human GSCs. We isolated cell surface Nestin-positive cell populations from human GSCs by fluorescence-activated cell sorting FACS analysis, and observed that these populations exhibited robust CSC properties, such as increased tumorsphere-forming ability and tumorsphere size. Mechanistically, we found that DAPT, a γ-secretase (a multi-subunit protease complex) inhibitor, reduced the proportion of cell surface Nestin-positive cells in human GSCs in a time- and dose-dependent manner, without significant changes in total Nestin expression, implying that a post-translational modification was involved in the generation of cell surface Nestin. Taken together, our data provides the first evidence that cell surface Nestin may serve as a promising GSC marker for the isolation and characterization of heterogeneous GSCs in glioblastomas.

1. Introduction

Glioblastoma multiforme (GBM, WHO grade IV) is the most aggressive malignancy of the central nervous system with a median survival time of only 12–15 months despite vigorous treatments such as surgical resection, radiotherapy, and chemotherapy [1]. Recently, glioma stem cells (GSCs), a subpopulation of glioma cells [2], were identified as the main cause of tumor propagation or tumor recurrence after anti-cancer therapy [3–5].

A number of cell surface markers are generally used to isolate and characterize cancer stem cells (CSCs) [6]. In GBM, 2 cell surface markers, CD133 [7] and CD15 [8], are generally used to isolate and characterize GSCs. CD133 is a glycoprotein that specifically localizes to the outer cellular membrane [9,10], and is expressed in hematopoietic stem cells [11], endothelial progenitor cells [12], neural stem cells, and brain tumors [10,13]. However, several recent reports have indicated that CD133 may not be a robust marker for GSCs [14–18]. CD15 is a carbohydrate adhesion molecule that is also known as stage-specific embryonic antigen 1 (SSEA1) [19], and is expressed in embryonic or adult central nervous system stem cells [20,21], leukemias [22], and GBM [23].

Many cellular factors, including transcriptional factors (e.g., Sox2, Nanog, and Oct3/4) [24], cytoskeletal proteins (e.g., Nestin) [25], post-transcriptional factors (e.g., Musashi 1) [25], and Polycomb transcriptional suppressors (e.g., Bmi1 and Ezh2) [26,27], are also considered GSC markers. However, in contrast to CD133 and CD15, these cellular factors are not useful for the isolation of live GSCs from tumor tissues given their intracellular localization, such as in the nucleus or cytoplasm.

During the early developmental stage of the central nervous system, Nestin is primarily expressed in neural progenitor/stem cells [28]. The Nestin protein is mainly localized in the cytoplasm and functions as a type VI intermediate filament with a high molecular weight (240 kDa) [29]. We have previously reported that an ~60-kDa N-terminal isotype of Nestin (hereafter, referred to as cell surface Nestin) is expressed on the outer cellular membrane of Id4-driven murine GSCs [30]. Here, we report the expression of cell surface Nestin in human GBM specimens and human GSCs, the isolation of live cell surface Nestin-positive GSCs, characterization of their self-renewal property, and a plausible mechanism underlying the generation of cell surface Nestin.

* Corresponding author. Address: School of Life Sciences and Biotechnology, Korea University, 5-ga, Anam-dong, Seongbuk-gu, Seoul 136-713, Republic of Korea. Fax: +82 2 953 0737.
E-mail address: hg-kim@korea.ac.kr (H. Kim).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.021

2. Materials and methods

2.1. Conditions and reagents for GSC suspension culture

All human GSCs (X01 and X02 [31], GSC3, GSC4, GSC5, GSC8, AC17, AC20, 84NS, 528NS, MD13, MD30, 1123NS [32,33]) were established from patients with GBM, except X03 GSCs derived from patient with WHO grade III oligoastrocytoma [31]. All GSCs were grown in DMEM/F12 medium (Lonza) supplemented with modified N2, B27, penicillin/streptomycin (1%; Lonza), epidermal growth factor (EGF, 20 ng/mL; R&D Systems), and basic fibroblast growth factor (bFGF, 20 ng/mL; R&D Systems). EGF and bFGF were replaced every 3 days, as described previously [34]. GSCs were treated with γ-secretase inhibitor, DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester) (LY-374973; Sigma–Aldrich).

2.2. Immunofluorescence

Paraffin-embedded human GBM sections were blocked with 10% donkey serum, and then simultaneously stained with primary antibodies against CD133 (1:20, #AC133; MACS), CD15 (1:200, #559045; BD Pharmingen), or Nestin (1:200, #N5413, which recognizes an N-terminal epitope of Nestin; Sigma–Aldrich) for 12 h at 4 °C. After binding of fluorescent protein-conjugated secondary antibodies, green-fluorescent Alexa Fluor 488 goat anti-rabbit IgG (1:400, #A11008; Invitrogen) and red-fluorescent Alexa Fluor 594 goat anti-mouse IgG (1:400, #A11005; Invitrogen) for 2 h at 20 °C, nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, 1 μg/mL) for 5 min at 20 °C. Human glioma specimens were collected after protocol approval by the Institute for Biomedical Research Ethics Committee, Samsung Medical Center. Fluorescent images were obtained using a confocal laser scanning microscope. The levels of Nestin, CD15, and CD133 expression were quantified using the Zeiss LSM Image Browser software.

2.3. Flow cytometry and fluorescence-activated cell sorting

To detect the proportions of cell surface Nestin-positive cells, live GSCs were directly incubated with a primary antibody against Nestin (1:200, #N5413; Sigma–Aldrich) for 30 min at 4 °C. GSCs were fixed with 4% paraformaldehyde and permeabilized in 0.15% saponin for 30 min at 4 °C. The permeabilized GSCs were incubated with primary antibody against Nestin for 30 min at 4 °C to identify cells expressing total Nestin (cytoplasmic and cell surface form). After binding of biotinylated goat anti-rabbit IgG (1:400, #BA-1000; Vector) for 20 min at 4 °C, streptavidin–phycoerythrin (1:1000, #554061; BD Pharmingen) was added for 10 min at 4 °C before fluorescence-activated cell sorting (FACS) analysis (BD FACSCalibur and BD FACSAria).

2.4. Single-cell sphere formation assay

After sorting with an anti-Nestin antibody (#N5413), live GSCs were seeded at a density of 1 cell per well in 96-well plates and grown under suspension culture conditions, as described in the "*Conditions and reagents for GSC suspension culture*" section. At day 14, the tumorsphere numbers and sizes were determined with a light microscope.

2.5. Statistics

Data were analyzed using two-tailed Student's *t*-tests. *P* values <0.05 were considered statistically significant.

3. Results

3.1. Cell surface Nestin expression in human primary GBM specimens and GSCs

To assess whether Nestin was expressed at the surface of cells expressing the GSC marker CD133 or CD15 in primary human GBM specimens, we performed an immunofluorescence (IF) assay on paraffin-embedded tissue sections from 5 human GBM specimens using antibodies against Nestin, and CD133 or CD15. Four out of 5 GBM specimens exhibited varying proportions of cells co-expressing Nestin and CD133 or Nestin and CD15 (Fig. 1A). Interestingly, 1 GBM specimen only expressed cell surface Nestin, and no CD133 or CD15 (Fig. 1B). Quantification of IF images revealed that an average of 13% (range, 6–28%) cells were cell surface Nestin-positive, 22% (range, 0–58%) cells were CD15-positive, and 4% (range, 0–7%) cells were CD133-positive in the 5 GBM specimens (Fig. 1C). These data suggest that cell surface Nestin may be one of the reliable GSC markers and may serve as an unique GSC marker in GBMs that are devoid of CD133-positive and CD15-positive GSCs.

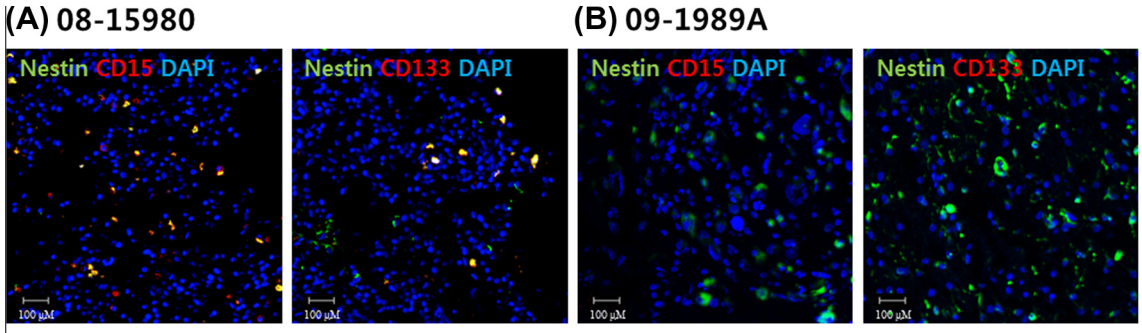
To determine whether cell surface Nestin was expressed in human GSCs, we performed flow cytometric analysis on 14 primary GSC lines—AC17, X02, 528NS, GSC4, GSC3, MD30, X03, 83NS, MD13, GSC5, GSC8, X01, 1123NS, and AC20—using an anti-Nestin antibody, and found that the proportion of cell surface Nestin-positive GSCs ranged from 1.4% to 70% (Fig. 2), which is similar to the proportions of CD15-positive and CD133-positive cells in human GSCs [8].

3.2. Cell surface Nestin-positive cells have robust tumorsphere-forming ability

To determine the biological significance of cell surface Nestin-positive GSCs, we first isolated cell surface Nestin-positive and -negative MD30, 528NS and GSC8 by FACS analysis using the anti-Nestin antibody. The green (P3) and blue (P4) dots in the FACS plot represent cell surface Nestin-positive and -negative cells, respectively. Representative images show single cell-derived tumorspheres of cell surface Nestin-positive and -negative GSC8 cells grown in serum-free medium containing EGF and bFGF for 14 days (Fig. 3A). Because GSCs can form floating clonal colonies (referred to as tumorspheres) and sustain their self-renewal property when grown in suspension in serum-free medium containing defined growth factors, such as EGF and bFGF [35], we incubated cell surface Nestin-positive and -negative GSCs under suspension culture conditions. As shown in Fig. 3B, cell surface Nestin-positive MD30, 528NS, and GSC8 GSCs formed significantly larger tumorspheres than cell surface Nestin-negative GSCs, implying higher proliferation ability of cell surface Nestin-positive GSCs. In addition, the single-cell sphere formation assay revealed that 43% (range, 31–57%) cell surface Nestin-positive GSCs were able to generate tumorspheres, whereas only 14% (range, 7–23%) cell surface Nestin-negative GSCs formed tumorspheres (Fig. 3C), indicative of a higher tumorsphere-forming ability in cell surface Nestin-positive GSCs. These findings suggest that cell surface Nestin is an useful GSC marker for GSC isolation and characterization.

3.3. γ-Secretase regulates the generation of cell surface Nestin in human GSCs

Nestin is a typical cytoplasmic intermediate filament protein. However, our previous study demonstrated that cell surface



(C)

Patient No.	Nestin	CD15
07-11259A	6.387665	8.14978
08-15980	13.47826	24.78261
08-21637A	8.356546	18.66295
08-19984A	12.2449	58.16327
09-1989A	26.90763	0
average	13.475	21.95172

Patient No.	Nestin	CD133
07-11259A	6.423983	5.139186
08-15980	10.90147	5.24109
08-21637A	5.87484	6.768838
08-19984A	13.19156	3.228782
09-1989A	27.88204	0
average	12.85478	4.075579

Fig. 1. Nestin expression in human primary glioblastoma specimens. (A) Immunofluorescence (IF) images (magnification, 20×; scale bar, 100 μm) showing co-localization of Nestin (green) with CD15 (red) and CD133 (red) in a human primary GBM specimen (Patient No. 08-15980). Nuclei were stained with DAPI (blue). (B) IF images (magnification, 20×; scale bar, 100 μm) showing expression of Nestin (green) without CD15 and CD133 in a human primary GBM specimen (Patient No. 09-1989A). Nuclei were stained with DAPI (blue). (C) Quantification of Nestin-positive, CD15-positive, and CD133-positive cells in 5 human primary GBM specimens was performed using the Zeiss LSM Image Browser software. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

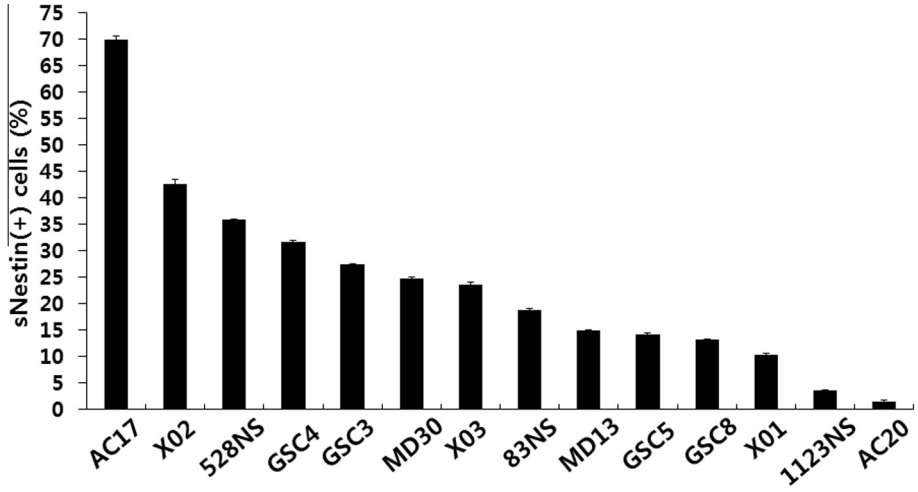


Fig. 2. Expression levels of cell surface Nestin (sNestin) in 14 human GSCs. The proportions of cell surface Nestin-positive cells in human GSCs were determined by flow cytometry using an antibody recognizing an N-terminal epitope of Nestin ($n = 3$).

Nestin is also expressed in Id4-driven murine GSCs without additional genes encoding short isoforms of Nestin or post-transcriptional modifications, such as alternative splicing [30]. These data suggest that cell surface Nestin may be generated by a post-translational modification, such as proteolysis by intracellular proteases [36]. Among the numerous intracellular endopeptidases, γ-secretase is one of most well-known proteases involved in the processing of stem cell signals, such as the generation of active Notch-intracellular domain from intact Notch protein [37]. To determine whether γ-secretase was involved in the generation of cell surface Nestin in GSCs, we treated GSCs with DAPT (a γ-secretase-specific inhibitor) and examined the proportion of cell surface Nestin-positive cells. The proportion of cell surface Nestin-positive cells in GSCs was significantly reduced by 20% and 50% following 0.1 μM and 1 μM DAPT treatments, respectively, and gradually decreased in a time-dependent manner (Fig. 4A), suggesting that γ-secretase may regulate the generation of cell surface Nestin in GSCs. We then

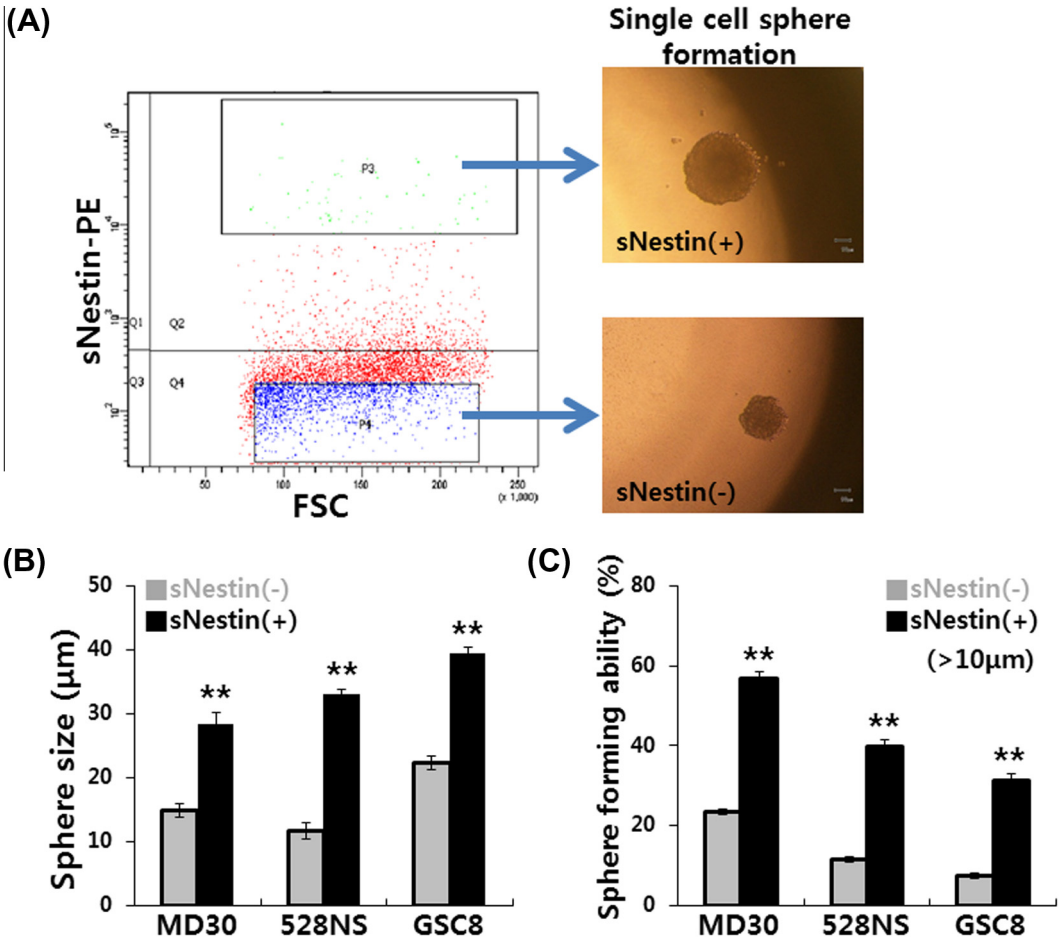


Fig. 3. Cell surface Nestin-positive cells have robust proliferation and tumorsphere-forming abilities. (A) Cell surface Nestin-positive (green dots) and -negative (blue dots) cells were isolated from MD30, 528NS and GSC8 by FACS analysis. Representative images (magnification, 10×; scale bar, 10 μm) show tumorspheres of cell surface Nestin-positive and -negative GSC8 grown in serum-free medium containing EGF and bFGF for 14 days. (B) Tumorsphere sizes (μm) of cell surface Nestin-positive and -negative cells grown under single-cell sphere formation culture conditions for 14 days ($n = 3$, $^{**}p < 0.01$). (C) Tumorsphere-forming ability (%) of cell surface Nestin-positive and -negative cells grown under single-cell sphere formation culture conditions for 14 days ($n = 3$, $^{**}p < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compared the proportions of cell surface Nestin-positive cells in 12 human primary GSCs—528NS, X02, GSC4, MD30, X03, 83NS, GSC5, 1123NS, AC17, GSC3, MD13, and GSC8—following treatment with 1 μM DAPT for 4 h. Eight of the 12 GSCs (528NS, X02, GSC4, MD30, X03, 83NS, GSC5, and 1123NS) exhibited greater than 20% reduction in the proportion of cell surface Nestin-positive cells, whereas AC17, GSC3, MD13, and GSC8 cells were insensitive to DAPT treatment (Fig. 4B), indicating that γ-secretase-driven proteolysis is not a common event and that additional mechanisms may be involved in the generation of cell surface Nestin. A previous study has shown that Notch signaling induces Nestin mRNA expression [38]. Thus, it is plausible that DAPT reduces cell surface Nestin levels by suppressing the expression of intact Nestin via inhibition of γ-secretase-mediated activation of Notch. We examined total and cell surface Nestin protein levels in the DAPT-sensitive GSCs—528NS, X02, MD30, GSC4, 83NS and X03 by incubating DAPT-treated cells with or without saponin (a cell-permeabilizing reagent), respectively. The proportion of total Nestin-positive cells was not dramatically decreased in 5 out of 6 permeabilized GSCs, whereas the proportion of cell surface Nestin-positive cells decreased in the all 6 non-permeabilized GSCs (Fig. 4C), implying that γ-secretase regulates the generation of cell surface Nestin in a Notch signaling-independent fashion.

4. Discussion

Although many studies have shown that GSCs can be isolated from primary human GBMs using an antibody against the GSC marker CD133 [7], we and others have demonstrated that 27–69% patients with GBM have CD133-negative cells that also possess GSC traits and give rise to aggressive gliomas [14–18]. Consistent with this, we demonstrated in this study that 1 GBM specimen contained cell surface Nestin-positive cells, but not CD133-positive cells, indicating that CD133 is not a reliable GSC marker for the isolation of GSCs from certain GBMs. Although CD15 is another cell surface marker used to isolate GSCs from primary human GBMs, and is considered a better marker than CD133, Son and colleagues demonstrated that 8% GSCs are CD15 negative that also possess GSC traits and give rise to aggressive gliomas [8]. Consistent with this, we showed that CD15-positive cells were not detectable in 1 out of 5 human GBM specimens. By contrast, cell surface Nestin-positive cells were observed in all the 5 human GBM specimens tested in the present study. Nestin is broadly expressed in CSCs isolated from various malignancies, such as prostate, head and neck, uterine/cervical, testicular, bladder, pancreatic, and ovarian cancers as well as GBMs [39–43].

During interphase, Nestin is extended from the perinuclear region to the cell surface through phosphorylation on threonine

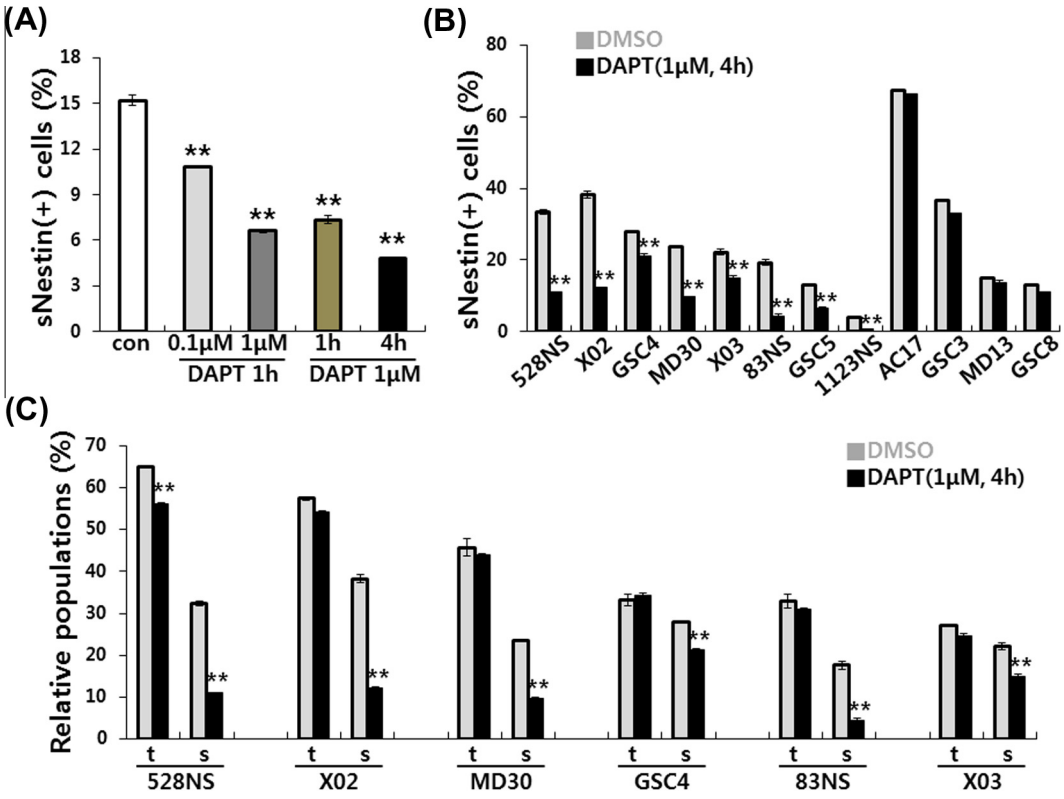


Fig. 4. γ-secretase regulates cell surface Nestin formation in GSCs. (A) The proportion of cell surface Nestin-positive cells in GSC5 cells was examined by treatment with different concentrations of DAPT, a γ-secretase inhibitor, for 1 h, or was determined at various time points after treatment with DAPT (1 μM). ($n = 3$, $^{**}p < 0.01$). (B) The proportions of cell surface Nestin-positive cells in 12 GSCs were determined after treatment with 1 μM DAPT for 4 h. ($n = 3$, $^{**}p < 0.01$). (C) The proportions of total (marked as t) and cell surface (marked as s) Nestin-positive cells in DAPT-sensitive 6 GSCs that were permeabilized (for total Nestin) or non-permeabilized (for cell surface Nestin) were examined by treating cells with or without 1 μM DAPT for 4 h ($n = 3$, $^{**}p < 0.01$).

316 by CDK5 [44], which may facilitate the formation of cell surface Nestin. Moreover, cell surface proteins usually contain a transmembrane domain with an alpha-helical structure. Interestingly, the N-terminus of Nestin contains 3 alpha-helical and internal loop structures [45], indicating that Nestin may have an intrinsic ability to localize in the cell membrane. Although several studies have reported that the expression of a truncated form of Nestin in myogenic stem cells [46] and neural stem cells [47], it is not known how the truncated form of Nestin is generated in certain types of adult stem cells. In this regard, our finding that γ-secretase regulates the generation of cell surface Nestin in human GSCs provides a novel reliable mechanism, because γ-secretase also activates Notch signaling, which plays a crucial role in activating "stemness" in various CSCs and a variety of tissue-specific stem/progenitor cells.

Acknowledgments

This work was supported by the National R&D Program for Cancer Control, Ministry of Health and Welfare, Republic of Korea (1020270) and a Korea University grant. We thank Dr. Akio Soeda for providing the X01, X02, and X03 GSCs, and Dr. Ichiro Nakano for providing the AC17, AC20, 84NS, 528NS, MD13, MD30, and 1123NS GSCs.

References

[1] P.Y. Wen, S. Kesari, Malignant gliomas in adults, N. Engl. J. Med. 359 (2008) 492–507.

[2] I. Nakano, H.I. Kornblum, Brain tumor stem cells, Pediatr. Res. 59 (2006) 54R–58R.

[3] T.C. Johannessen, R. Bjerkvig, B.B. Tysnes, DNA repair and cancer stem-like cells–potential partners in glioma drug resistance?, Cancer Treat Rev. 34 (2008) 558–567.

[4] A.J. Chalmers, Radioresistant glioma stem cells–therapeutic obstacle or promising target?, DNA Repair (Amst) 6 (2007) 1391–1394.

[5] S. Bao, Q. Wu, R.E. McLendon, Y. Hao, Q. Shi, A.B. Hjelmeland, M.W. Dewhirst, D.D. Bigner, J.N. Rich, Glioma stem cells promote radioresistance by preferential activation of the DNA damage response, Nature 444 (2006) 756–760.

[6] J.E. Visvader, G.J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions, Nat. Rev. Cancer 8 (2008) 755–768.

[7] S.K. Singh, C. Hawkins, I.D. Clarke, J.A. Squire, J. Bayani, T. Hide, R.M. Henkelman, M.D. Cusimano, P.B. Dirks, Identification of human brain tumour initiating cells, Nature 432 (2004) 396–401.

[8] M.J. Son, K. Woolard, D.H. Nam, J. Lee, H.A. Fine, SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma, Cell Stem Cell 4 (2009) 440–452.

[9] A.H. Yin, S. Miraglia, E.D. Zanjani, G. Almeida-Porada, M. Ogawa, A.G. Leary, J. Olweus, J. Kearney, D.W. Buck, AC133, a novel marker for human hematopoietic stem and progenitor cells, Blood 90 (1997) 5002–5012.

[10] D. Corbeil, C.A. Fargeas, W.B. Huttner, Rat prominin, like its mouse and human orthologues, is a pentaspan membrane glycoprotein, Biochem. Biophys. Res. Commun. 285 (2001) 939–944.

[11] D. Corbeil, K. Röper, A. Hellwig, M. Tavian, S. Miraglia, S.M. Watt, P.J. Simmons, B. Peault, D.W. Buck, W.B. Huttner, The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions, J. Biol. Chem. 275 (2000) 5512–5520.

[12] N. Sanai, A. Alvarez-Buylla, M.S. Berger, Neural stem cells and the origin of gliomas, N. Engl. J. Med. 353 (2005) 811–822.

[13] S.K. Singh, I.D. Clarke, M. Terasaki, V.E. Bonn, C. Hawkins, J. Squire, P.B. Dirks, Identification of a cancer stem cell in human brain tumors, Cancer Res. 63 (2003) 5821–5828.

[14] D. Beier, P. Hau, M. Proescholdt, A. Lohmeier, J. Wischhusen, P.J. Oefner, L. Aigner, A. Brawanski, U. Bogdahn, C.P. Beier, CD133(+) and CD133(−) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles, Cancer Res. 67 (2007) 4010–4015.

[15] S. Bidlingmaier, X. Zhu, B. Liu, The utility and limitations of glycosylated human CD133 epitopes in defining cancer stem cells, J. Mol. Med. (Berl.) 86 (2008) 1025–1032.

[16] C.E. Griguer, C.R. Oliva, E. Gobin, P. Marcorelles, D.J. Benos, J.R. Lancaster Jr., G.Y. Gillespie, CD133 is a marker of bioenergetics stress in human glioma, PLoS One 3 (2008) e3655.
[17] K.M. Joo, S.Y. Kim, X. Jin, S.Y. Song, D.S. Kong, J.I. Lee, J.W. Jeon, M.H. Kim, B.G. Kang, Y. Jung, J. Jin, S.C. Hong, W.Y. Park, D.S. Lee, H. Kim, D.H. Nam, Clinical and biological implications of CD133-positive and CD133-negative cells in glioblastomas, Lab. Invest. 88 (2008) 808–815.
[18] J. Wang, P.Ø. Sakariassen, O. Tsinkalovsky, H. Immervoll, S.O. Bøe, A. Svendsen, L. Prestegarden, G. Røsland, F. Thorsen, L. Stuhr, A. Molven, R. Bjerkvig, P.Ø. Enger, CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells, Int. J. Cancer 122 (2008) 761–768.
[19] M.A. Kerr, S.C. Stocks, The role of CD15-(Le(X))-related carbohydrates in neutrophil adhesion, Histochem. J. 24 (1992) 811–826.
[20] A. Capela, S. Temple, LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal, Neuron 35 (2002) 865–875.
[21] A. Capela, S. Temple, LeX is expressed by principle progenitor cells in the embryonic nervous system, is secreted into their environment and binds Wnt-1, Dev. Biol. 291 (2006) 300–313.
[22] C.H. Dunphy, L.J. Gardner, H.L. Evans, N. Javadi, CD15(+) acute lymphoblastic leukemia and subsequent monoblastic leukemia: persistence of t(4;11) abnormality and B-cell gene rearrangement, Arch. Pathol. Lab. Med. 125 (2001) 1227–1230.
[23] G. Reifenberger, P. Sieth, M. Niederhaus, W. Wechsler, Expression of CD15 in tumours of the nervous system, Histochem. J. 24 (1992) 890–901.
[24] P.B. Dirks, Brain tumour stem cells: the undercurrents of human brain cancer and their relationship to neural stem cells, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363 (2008) 139–152.
[25] T. Strojnik, G.V. Røsland, P.O. Sakariassen, R. Kavalar, T. Lah, Neural stem cell markers, nestin and musashi proteins, in the progression of human glioma: correlation of nestin with prognosis of patient survival, Surg. Neurol. 68 (2007) 133–143.
[26] M. Abdouh, S. Facchino, W. Chatoo, V. Balasingam, J. Ferreira, G. Bernier, BMI1 sustains human glioblastoma multiforme stem cell renewal, J. Neurosci. 29 (2009) 8884–8896.
[27] M.L. Suvà, N. Riggi, M. Janiszewska, I. Radovanovic, P. Provero, J.C. Stehle, K. Baumer, M.A. Le Bitoux, D. Marino, L. Cironi, V.E. Marquez, V. Clément, I. Stamenkovic, EZH2 is essential for glioblastoma cancer stem cell maintenance, Cancer Res. 69 (2009) 9211–9218.
[28] U. Lendahl, L.B. Zimmerman, R.D. McKay, CNS stem cells express a new class of intermediate filament protein, Cell 60 (1990) 585–595.
[29] H. Herrmann, U. Aebi, Intermediate filaments and their associates: multi-talented structural elements specifying cytoarchitecture and cytodynamics, Curr. Opin. Cell Biol. 12 (2000) 79–90.
[30] S. Beck, X. Jin, J. Yin, S.H. Kim, N.K. Lee, S.Y. Oh, X. Jin, M.K. Kim, E.B. Kim, J.S. Son, S.C. Kim, D.H. Nam, S.H. Kim, S.K. Kang, H. Kim, Y.J. Choi, Identification of a peptide that interacts with Nestin protein expressed in brain cancer stem cells, Biomaterials 32 (2011) 8518–8528.
[31] A. Soeda, M. Park, D. Lee, A. Mintz, A. Androutsellis-Theotokis, R.D. McKay, J. Engh, T. Iwama, T. Kunisada, A.B. Kassam, I.F. Pollack, D.M. Park, Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1alpha, Oncogene 28 (2009) 3949–3959.
[32] M. Jijiwa, H. Demir, S. Gupta, C. Leung, K. Joshi, N. Orozco, T. Huang, V.O. Yildiz, I. Shibahara, J.A. de Jesus, W.H. Yong, P.S. Mischel, S. Fernandez, H.I. Kornblum, I. Nakano, CD44v6 regulates growth of brain tumor stem cells partially through the AKT-mediated pathway, PLoS One 6 (2011) e24217.
[33] T. Miyazaki, Y. Pan, K. Joshi, D. Purohit, B. Hu, H. Demir, S. Mazumder, S. Okabe, T. Yamori, M. Viapiano, K. Shin-ya, H. Seimiya, I. Nakano, Telomestatin impairs glioma stem cell survival and growth through the disruption of telomeric G-quadruplex and inhibition of the proto-oncogene, c-Myb, Clin. Cancer Res. 18 (2012) 1268–1280.
[34] S.M. Pollard, K. Yoshikawa, I.D. Clarke, D. Danovi, S. Stricker, R. Russell, J. Bayani, R. Head, M. Lee, M. Bernstein, J.A. Squire, A. Smith, P. Dirks, Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens, Cell Stem Cell 4 (2009) 568–580.
[35] J. Lee, S. Kotliarova, Y. Kotliarov, A. Li, Q. Su, N.M. Donin, S. Pastorino, B.W. Purow, N. Christopher, W. Zhang, J.K. Park, H.A. Fine, Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines, Cancer Cell 9 (2006) 391–403.
[36] X.S. Puente, L.M. Sánchez, C.M. Overall, C. López-Otín, Human and mouse proteases: a comparative genomic approach, Nat. Rev. Genet. 4 (2003) 544–558.
[37] B. De Strooper, W. Annaert, P. Cupers, P. Saftig, K. Craessaerts, J.S. Mumm, E.H. Schroeter, V. Schrijvers, M.S. Wolfe, W.J. Ray, A. Goate, R. Kopan, A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain, Nature 398 (1999) 518–522.
[38] A.H. Shih, E.C. Holland, Notch signaling enhances nestin expression in gliomas, Neoplasia 8 (2006) 1072–1082.
[39] S.K. Singh, I.D. Clarke, T. Hide, P.B. Dirks, Cancer stem cells in nervous system tumors, Oncogene 23 (2004) 7267–7273.
[40] S. Kasper, Exploring the origins of the normal prostate and prostate cancer stem cell, Stem Cell Rev. 4 (2008) 193–201.
[41] A. Bentivegna, D. Conconi, E. Panzeri, E. Sala, G. Bovo, P. Viganò, S. Brunelli, M. Bossi, G. Tredici, G. Strada, L. Dalprà, Biological heterogeneity of putative bladder cancer stem-like cell populations from human bladder transitional cell carcinoma samples, Cancer Sci. 101 (2010) 416–424.
[42] K. Okuno, S. Ohta, H. Kato, T. Taga, K. Sugita, Y. Takeuchi, Expression of neural stem cell markers in malignant rhabdoid tumor cell lines, Oncol. Rep. 23 (2010) 485–492.
[43] A. Jimeno, G. Feldmann, A. Suárez-Gauthier, Z. Rasheed, A. Solomon, G.M. Zou, B. Rubio-Viqueira, E. García-García, F. López-Ríos, W. Matsui, A. Maitra, M. Hidalgo, A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development, Mol. Cancer Ther. 8 (2009) 310–314.
[44] M. Bentahir, O. Nyabi, J. Verhamme, A. Tolia, K. Horré, J. Wiltfang, H. Esselmann, B. De Strooper, Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms, J. Neurochem. 96 (2006) 732–742.
[45] K. Michalczyk, M. Ziman, Nestin structure and predicted function in cellular cytoskeletal organization, Histol. Histopathol. 20 (2005) 665–671.
[46] D. Park, A.P. Xiang, L. Zhang, F.F. Mao, N.M. Walton, S.S. Choi, B.T. Lahn, The radial glia antibody RC2 recognizes a protein encoded by Nestin, Biochem. Biophys. Res. Commun. 382 (2009) 588–592.
[47] L.W. Kong, X.Y. Ding, H. Kitani, R. Shiurba, N.H. Jing, Evidence for a mouse brain-specific variant of alpha-tubulin, Cell Res. 9 (1999) 315–325.